BIOCHEMICAL TECHNOLOGY DIVISION

Dear Fellow BIOT Members:

On behalf of the BIOT Executive Committee, I would like to extend an enthusiastic welcome to all those who will be able to join us for the ACS Fall National Meeting in Boston. Program co-chairs Wilfred Chen, Bill Wang and Anurag Rathore have done an outstanding job of organizing the technical program, including over 40 sessions that comprise nearly 500 poster and oral presentations during the meeting. I want to thank Wilfred, Bill and Anurag for putting together a fantastic meeting, as well as thank the session organizers and speakers and attendees for making these meetings successful.

Highlights of the Boston meeting include a special set of sessions developed on topics related to several emerging areas such as Stem Cells, Bioenergy, and Nanobiotechnology. These sessions will supplement our historically strong program in the areas of fermentation, cell culture, purification, and product characterization in biotechnology. Another series of sessions will focus on issues particularly relevant to commercialization of biotechnology products.

For those of you interested in becoming involved in future meeting programming, I encourage you to attend the programming meeting on Tuesday. Remember that the organization and success of the meeting are based on a network of volunteers who give of their own personal time. We appreciate any time you can give to make the organization better for you and your colleagues.

The success of BIOT programming depends on the donations it receives from supporting companies. We thank all of the organizations that have contributed to BIOT financially. These contributions are used to partially subsidize meeting expenses such as speaker registrations and travel support. These registrations are also used for providing some registration support for students and for us to organize special events during the meeting.

Beyond programming, there are many ways you can contribute to BIOT. The business of BIOT is also volunteer-run and we welcome your help! The executive committee is responsible for ensuring the continuing success of BIOT. In particular, I want to acknowledge the hard work of Anne Robinson (Past Chair), Erik Fernandez (Chair-Elect), Nedim Altaras (Secretary), Hari Pujar (Treasurer), Weichang Zhou (Awards Coordinator), Kent Goklen (Communications Coordinator), Claire Komives (Newsletter Editor), Mark Marten (Long Range Program Chair), Arindam Bose, Fred Heineken, Eliana Clark, and Sharon Shoemaker (Councilors), David Block, Ilse Blumentals, Paul Mensah (Alternate Councilors), Collette Ranucci & Jayanth Sridhar (Membership). Find out more about BIOT at the Business meeting on Wednesday or visit www.acsbiot.org. I encourage you to consider becoming involved to meet new colleagues and help BIOT to continue to have a positive impact on our scientific community.

Best Regards,
Dana Andersen
BIOT Chair 2007

Acknowledgements from the Program Chairs

We would like to take this opportunity to thank all the volunteers who worked tirelessly and the industrial sponsors who supported the meeting in order to make this possible. We would like to acknowledge all the session chairs and our symposium coordinators (Dana Andersen and Sarah Harcum for the upstream area, Steve Cramer and Ajoy Velayudhan for the downstream area, John Carpenter and Jerry Sparks for the biophysical and biomolecular area, Wei-Shou Hu and Peter Zandstra for the stem cell area, Ranjan Srivastava and Huimin Zhao for the emerging technologies area, and Raghavan Venkat and Brian Kelley for the commercialization area). We also need to thank the support by Richard Love and Laura Melohn from ACS. Mostly importantly, we would like to thank Mark Marten, our long-range program coordinator, for his invaluable support throughout the whole process. As you enjoy the meeting, please take a moment to thank all those who made this BIOT program possible. We encourage you to continue your support of the BIOT program when Ranjan Srivastava and Anurag Rathore organize the Fall 2008 ACS meeting in Philadelphia.

Wilfred Chen, William Wang and Anurag Rathore
2007 BIOT Program Chairs

The Biochemical Technology Division of ACS gratefully acknowledges the financial support of these industry leaders in biotechnology.
(list is current as of time of publication)

Amgen
Biogen Idec
BioProcess International
Bristol-Myers Squibb
Centocor
EMD
GE Healthcare
Genentech
Genzyme
GlaxoSmithKline
HGS
Lilly
MedImmune
Merck
Novo Nordisk
PDL BioPharma
Pall Life Sciences
Pfizer
Tosoh
Wyeth BioPharma

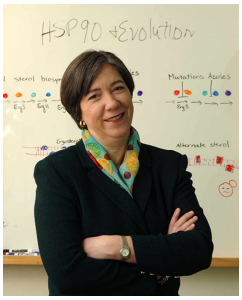
Table of Contents

BIOT Division Awards 2007

Weichang Zhou, ACS BIOT Awards Chair

The Biochemical Technology (BIOT) Division of the American Chemical Society (ACS) will present six division awards at the ACS national meeting in Boston, Massachusetts, August 19-23, 2007. These awards are the David Perlman Lecture, the Marvin J. Johnson Award, the James M. Van Lanen Distinguished Service Award, the Industrial Biotechnology Award, Alan S Michaels Award for the Recovery of Biological Products, and Young Investigator Award. In addition, the 2006 W.H. Peterson Award and Elmer Gaden Award will also be presented in this year's BIOT division program. The Awards selection process starts with nominations by our division members. Nomination packages are reviewed and judged by individual selection committees, which decide the winner for the Awards. Details about these Awards, their nomination and selection process can be found at our division website: http://membership.acs.org/b/biochem/awards.html.

The David Perlman Lecture

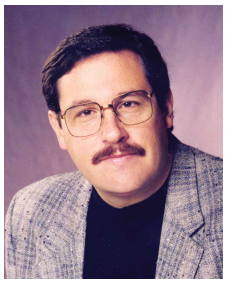


The BIOT division selected Susan Lindquist, Member of the Whitehead Institute for Biomedical Research, Professor of Biology at Massachusetts Institute of Technology (MIT), and a Howard Hughes Medical Institute investigator to deliver this year's Perlman Lecture. This award, which is sponsored by Genzyme Corp., recognizes many of Professor Lindquist's achievements including her contributions in the area of protein folding and nanotechnology. In particular, her research provided strong evidence for a new paradigm in genetics based upon the inheritance of proteins with new, self-perpetuating shapes rather than new DNA sequences. Her award lecture titled "Prion Proteins and How They Fold" is scheduled for August 19.

Professor Lindquist is a member, and former Director, of the Whitehead Institute for Biomedical Research. Previously she was the Albert D. Lasker Professor in the Department of Molecular and Cellular Biology at the University of Chicago. She received her PhD in Biology from Harvard in 1976 and was a postdoctoral fellow of the American Cancer Society. She was elected to the National Academy of Sciences in 1997 and the Institute of Medicine in 2006. Lindquist's honors also include the Dickson Prize in Medicine, the Sigma Xi William Procter Prize for Scientific Achievement, and designation by Scientific American as one of the top 50 leaders in business, policy, and research for 2006.

The Marvin J. Johnson Award in Microbial and Biochemical Technology

This year's winner of the Marvin J. Johnson Award in Microbial and Biochemical Technology is Jonathan S. Dordick, the Howard P. Isermann Professor of Chemical and Biological Engineering at the Rensselaer Polytechnic Institute (RPI). This award, which is sponsored by Pfizer, Inc., recognizes many of Professor Dordick's achievements leading to functional bioengineered materials, enzyme-based nanocomposites, and bioactive agents that impact human health and bioprocesses. Professor Dordick will receive the award and present the award lecture titled "Molecular Bioprocessing: From Design to Discovery to Dreams" on August 21.



Professor Dordick received a Ph.D. in Biochemical Engineering in 1986 from the Massachusetts Institute of Technology. He began his academic career at the University of Iowa in the Department of Chemical and Biochemical Engineering as an Assistant Professor and moved through the ranks to become department chairman in 1995. At Iowa he held a joint appointment in the College of Pharmacy's Medicinal and Natural Products Chemistry program, and he served as the founding Associate Director of the Center for Biocatalysis and Bioprocessing. He joined the Rensselaer faculty in 1998 and served as the Chemical and Biological Engineering Department chairman for four years. Professor Dordick also holds a joint appointment in the Department of Biology at RPI. Professor Dordick has received the 2003 International Enzyme Engineering Award, the Elmer Gaden Award honoring the best publication in 2006 in "Biotechnology & Bioengineering", and the 1998 Iowa Section Award from the ACS. He was elected a Fellow of the American Institute for Medical and Biological Engineering in 1996, and a Fellow of the American Association for the Advancement of Science in 2004. Professor Dordick has made numerous significant contributions to the field of biotechnology. He co-founded EnzyMed, Inc., a pharmaceutical discovery company now part of Albany Molecular, and also co-founded Solidus Biosciences, a venture-stage biotechnology company. Professor Dordick is currently an associate editor for Biotechnology & Bioengineering and also serves on the editorial boards of several other journals. He served as a chair of the BIOT Division in 1992 and was the BIOT Program Chair in 1989 and 1990.

The James M. Van Lanen Distinguished Service Award

This year's winner of the BIOT James M. Van Lanen Distinguished Service Award is Eleftherios Terry Papoutsakis, Walter P. Murphy Professor of Chemical and Biological Engineering at the Northwestern University and of July 1, 2007 Eugene DuPont Professor of Chemical Engineering at the University of Delaware, Newark, DE. The award recognizes Terry's dedication and leadership on many fronts, but especially as the division awards chair between 1998 and 2005 and as a very active contributor to the division's success over many years.

Professor Papoutsakis graduated with a Ph.D. in Chemical Engineering from Purdue University in 1980 and began his academic career at the Rice University. In 1987, he joined the Northwestern University as an Associate Professor in the Department of Chemical Engineering, and was later promoted to Professor and appointed Walter P. Murphy Professor of Chemical and Biological Engineering at the Northwestern University. On July 1, 2007, he joins the University of Delaware as Eugene DuPont Professor. Professor Papoutsakis has received many prestigious awards and honors including the Amgen Biochemical Engineering Award in 2005, Merck Cell Culture Engineering Award in 2004, Alpha Chi Sigma Award of the American Institute of Chemical Engineers in 2003, Marvin Johnson Award of the American Chemical Society, Biochemical Technology Division in 1998. He was elected as a Fellow of the American Association for the Advancement of Science in 1998 and a Fellow of The American Academy of Microbiology in 2005. Professor Papoutsakis served as Editor in Chief between 1990 and 1995 for Biotechnology & Bioengineering and later as an Associate Editor and Editorial Board member.

The Industrial Biotechnology Award

BIOT will present the Industrial Biotechnology Award to the Genentech Lucentis® Development CMC Team for the achievements in developing the manufacturing process for the potent vision restoring drug ranibizumab (Lucentis®). Dr. John Joly, Director of Early Stage Cell Culture, Process Development will accept the award and present the award lecture titled "A Vision Saving Therapy made by Biotechnology" on August 20.

Lucentis® is a humanized antibody fragment to Vascular Endothelial Growth Factor (VEGF) which is produced using *E coli* fermentation technology. Lucentis® is designed to bind to and inhibit VEGF, a protein that plays a role in angiogenesis (the formation of new blood vessel). Lucentis® offers vision improvement to patients with the devastating condition of wet age-related macular degeneration and received FDA approval in June 2006 for commercialization. Lucentis® is one of the most successful biotechnology commercial launches treating approximately 60,000 patients and generating $380 millions in product sales in the first six months of approval. The Genetech Lucentis® Development CMC Team developed a state-of-the-art manufacturing process for producing a highly homogenous protein containing very low impurities. Both drug product and drug substance are homogenous as assayed by size exclusion chromatography, ion exchange chromatography and capillary electrophoresis. The production process involves the use of *E. coli* fermentation in a 1000 L bioreactor and seven downstream processing steps including homogenization, centrifugation, four chromatographic steps: cation exchange, hydrophobic interaction, a mixed-mode ion exchange, and anion exchange, and ultrafiltration/diafiltration. The formulated drug product is stored as a liquid at 2-8 ^{o}C and shows excellent stability.

The Alan S Michaels Award for the Recovery of Biological Products

Maria-Regina Kula, Professor and Former Director of Institute of Enzyme Technology, Heinrich-Heine Dusseldorf, Germany is the second recipient of the Alan S Michaels Award for the Recovery of Biological Products. This award, established in 2006, is sponsored by the Recovery of Biological Products Conference Series, which Dr. Alan Michaels helped to start in 1981. This award recognizes Professor Kula's outstanding contributions for 40 years to the understanding and practice of enzyme-based chemical processes and protein separations for the recovery of biologicals, excellence in science, and to education and inspiration of a generation of bioprocessing scientists and engineers. She will present the award lecture titled "Early steps in protein recovery - the messy part of a clean industry" on August 19.

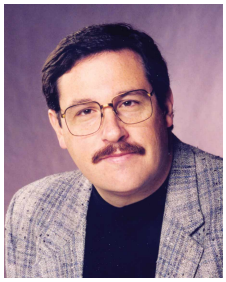
Professor Kula received a Ph.D. in Chemistry in 1962 from Ludwig Maximilians University Munich, Germany. With a continuing education scholarship from the German Research Council she studied Molecular Biology as a Post Doc at Johns Hopkins University, School of Medicine 1964-67. She was head of the Department of Enzyme Technology at the Society for Biotechnology Research in Braunschweig, Germany between 1969 and 1985. In the subsequent 16 years, she was Professor and Director of Institute of Enzyme Technology, Heinrich-Heine Dusseldorf, Germany. Professor Kula has received many prestigious awards and honors including National Medal 1st class in 1997 and Presidential "Future Award" in 2002 from the German government. She was elected into the US National Academy of Engineering as a Foreign Associate member in 2002 and acatech, the German National Academy of Technical Sciences in 2005.

The Young Investigator Award

Patrick S. Daugherty, an Associate Professor of Chemical Engineering (as of July 1) at the University of California at Santa Barbara is the second recipient of the ACS BIOT Young Investigator Award. This award, established in 2006 and sponsored by Genentech, intends to recognize an outstanding young contributor to the field of biochemical technology and active participant in the division programs. This year's award recognizes Professor Daugherty's outstanding contributions to the field of protein engineering including the development of novel peptide display methodologies, fluorescent protein sensors, and library screening methodologies. Daugherty has applied these new tools to investigate and engineer the specificity of protein-peptide interactions in complex biological environments for diagnostic and therapeutic applications. The award will be presented on August 22, followed by the award lecture titled "Building proteins with new therapeutic functions from peptide modules".

Professor Daugherty received a Ph.D. in Chemical Engineering in 1999 from University at Texas at Austin. After a postdoctoral research at Fred Hutchinson Cancer Center in Seattle, he joined University of California at Santa Barbara in 2001 as an Assistant Professor of Chemical Engineering. He also became an Assistant Professor of Biomolecular Science and Engineering in 2002. Daugherty is also a Team Leader for the Institute for Collaborative Biotechnologies and an affiliate member of the California NanoSystems Institute. Professor Daugherty has received many awards including a Cottage Hospital Research Award in 2003, National Science Foundation Career Award in 2005, and Camille Dreyfus Teacher Scholar Award in 2006.

The Elmer Gaden Award



Jonathan S. Dordick, the Howard P. Isermann Professor of Chemical and Biological Engineering at Rensselaer Polytechnic Institute (RPI) will receive the Elmer Gaden Award honoring his 2006 publication in "Biotechnology & Bioengineering". The award is sponsored by John Wiley & Sons and is presented for a paper of exceptional originality and likely impact. Professor Dordick will present the award lecture entitled "Directing the Assembly of Multifunctional Biomolecular Architectures" on August 22nd, which is based on a paper co-authored by Dr. Grazyna Sroga at RPI. In this paper, the authors present a paradigm for exploiting biological systems to control the molecular assembly of multiple biological and nonbiological architectures at nanoscale dimensions.

The W.H. Peterson Award

The W.H. Peterson Award is granted each year by the Division of Biochemical Technology to student members who present outstanding research work in sessions sponsored by the division at ACS national meetings. The 2006 Award was organized by Gargi Maheshwari and Katerina Kourentzi, and sponsored by Gibco Cell Culture, Invitrogen. The winner of the 2006 Peterson Award for the best oral presentation is Brian Timko, a Graduate Student from Professor Charles Lieber's lab at Harvard University for his presentation titled "Designing and implementing an electronic interface between nanowires and neurons" at the 2006 ACS meeting in San Francisco, California. No award was given for the best poster presentation given by a student in 2006.

Biochemical Technology (BIOT) Division: Events & Meetings

2007 Fall National Meeting – August 19 – 23 – Boston, MA

Sunday, August 19, 2007

Alan S. Michaels Award in the Recovery of Biological Products, Maria-Regina Kula, Professor and Former Director of Institute of Enzyme Technology, Heinrich-Heine Dusseldorf, Germany, "Early Steps in Protein Recovery: The Messy Part of a Clean Industry", BCEC 107 B, 11:30 am.

Perlman Lecture, Susan Lindquist, Member of the Whitehead Institute for Biomedical Research, Professor of Biology at MIT, "Prion Proteins and How They Fold", BCEC 107 B, 4:30 pm.

BIOT Reception - Dinner Cruise - "Spirit of Boston", Boarding 5:30/Dinner 6:00 – 8:30 pm.

Monday, August 20, 2007

Industrial Biotechnology Award, Genentech Lucentis® Development CMC Team, Dr. John Joly, "A Vision Saving Therapy made by Biotechnology", BCEC 107 B, 11:30 am.

Industrial Keynote, Bob Adamson, Vice President, Wyeth Biotech, "Role of Technology and Science in Manufacturing Economics", BCEC 107 B, 2:00 pm.

BIOT Executive Committee Meeting, BCEC 212, 6:00 – 10:00 pm.

Sci – Mix Poster Session, BCEC Exhibit Hall B2 **,** 8:00 – 10:00 pm.

Tuesday, August 21, 2007

Marvin J. Johnson Award. Professor Jonathan S. Dordick, RPI, "Molecular Bioprocessing: From Design to Discovery to Dreams", BCEC 107 A, 11:30 am.

Emerging Keynote. Lee R. Lynd, Dartmouth College, "Energy from biomass: Anticipating a revolution", BCEC 109 A, 2:00 pm.

Future Programming Meeting – Open Meeting – come join the discussion about ideas for future programming. Invite your colleagues, BCEC 253A, 5:30 – 6:30 pm.

Wednesday, August 22, 2007

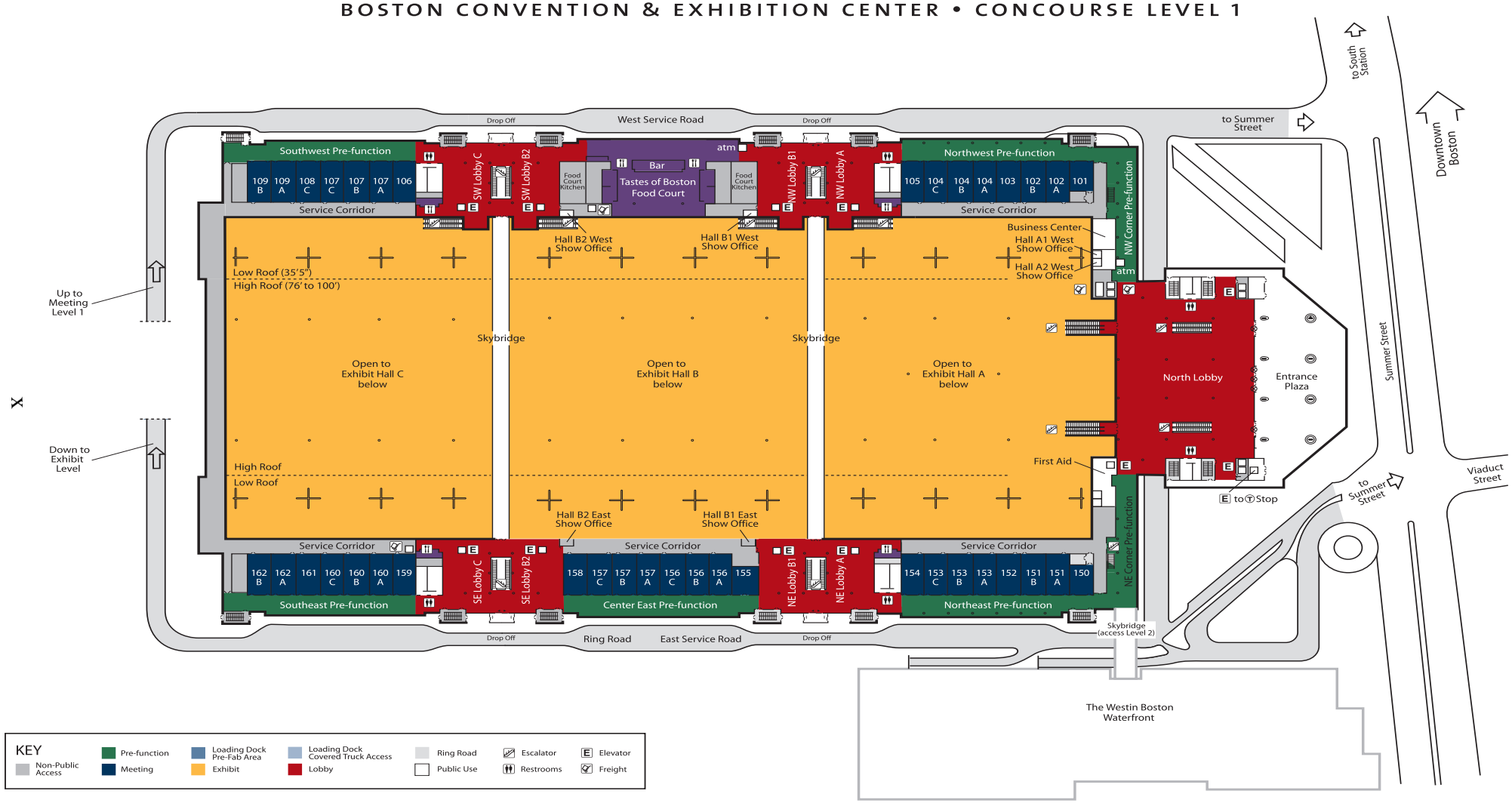
Elmer Gaden Award. Professor Jonathan S. Dordick, RPI, "Directing the Assembly of Multifunctional Biomolecular Architectures", BCEC 107 B, 11:30 am.

BIOT Young Investigator Award, Associate Professor Patrick S. Daugherty, University of California at Santa Barbara,"Building proteins with new therapeutic functions from peptide modules", BCEC 108, 2:00 pm.

Poster Session and Social Hour, BCEC Hall C**,** 5:30 – 7:30 pm.

Divisional Business Meeting, BCEC 260**,** 6:30 pm.

BOSTON CONVENTION & EXHIBITION CENTER • CONCOURSE LEVEL 1



Program by Day

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

SUNDAY MORNING

8:20 - 10:40 am	Stem Cells: Quantifying, Modeling and Controlling Stem Cell Fate	J. M. Piret, Organizer	Papers 1 - 7	BCEC 108
8:00 - 11:10am	Biophysical and Biomolecular Symposium: Protein Stability	E. J. Fernandez, N. Rathore, C. R. Middaugh, and A. Blake-Haskins, Organizers	Papers 8 - 15	BCEC 107 A/B
8:00 - 11:10 am	Biophysical and Biomolecular Symposium: Protein Folding & Characterization	K. Mallela and T. Cellmer, Organizers	Papers 16 - 23	BCEC 106
8:00 - 11:00 am	Upstream Processing: Advances in Biocatalysis	P. C. Cirino and L. Sun, Organizers	Papers 24 – 31	BCEC 107 C
11:30 - 12:35 pm	Alan S. Michaels Recovery Award Lecture	W. Zhou, Organizer	Paper 32	BCEC 107 B

SUNDAY AFTERNOON

2:00 - 3:45 pm	Stem Cells: Stem Cell Based Tissue Engineering	T. McDevitt, and P. W. Zandstra, Organizers	Papers 33 - 37	BCEC 108
2:00 - 4:15 pm	Downstream Processing: Primary Recovery - Membranes and Other Technologies	D. J. Roush and Y. Lu, Organizers	Papers 38 - 43	BCEC 107 B
2:00 - 4:15 pm	Biophysical and Biomolecular Symposium: Protein Folding & Characterization	K. Mallela and T. Cellmer, Organizers	Papers 44-49	BCEC 106
2:00 - 4:10 pm	Emerging Technologies: Nanobiotechnology	J. R. Cochran and L. Malmberg, Organizers	Papers 50 - 55	BCEC 107 C
2:00 - 4:20 pm	Commercialization of Biologics: Case Studies: Post-Approval Changes & Comparability Challenges	S. Lee and V. Paradkar, Presiding	Papers 56 - 61	BCEC 109 B
4:30 - 5:30 pm	Perlman Lecture	W. Zhou, Organizer	Paper 62	BCEC 107 B

SUNDAY EVENING

BIOT Reception	Spirit of Boston	6:00 – 8:30pm

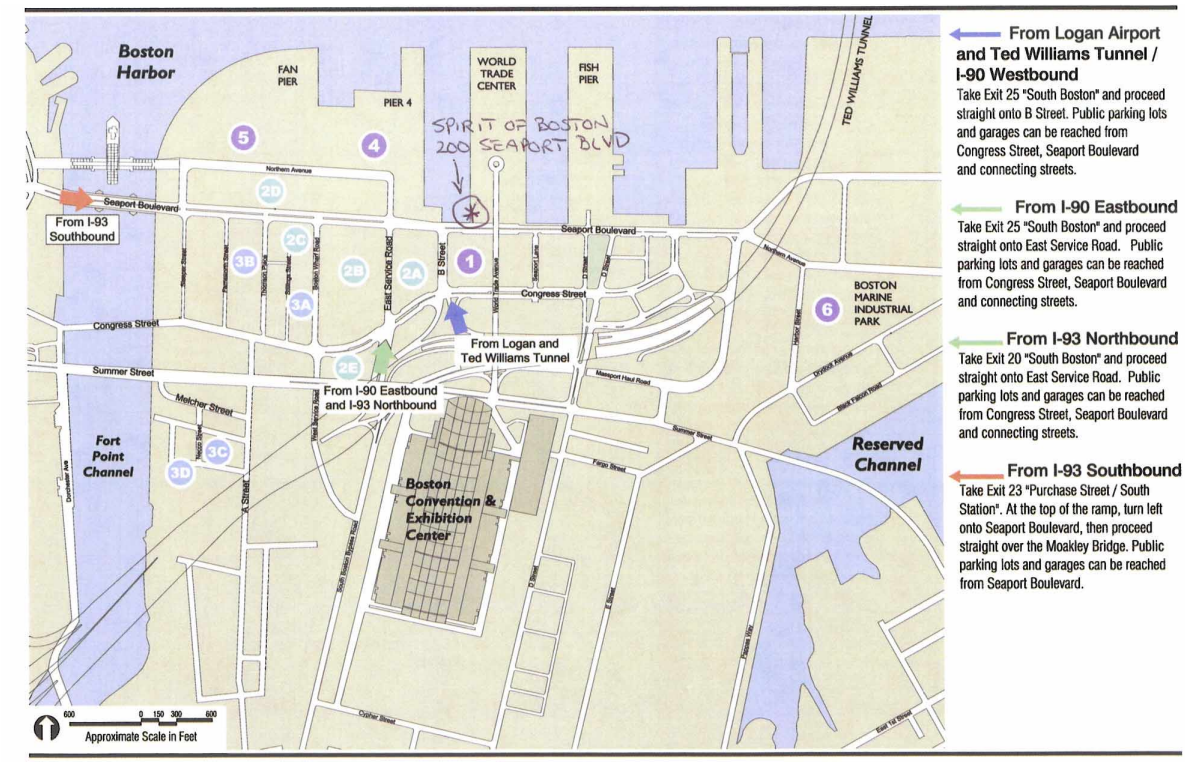
Sun

BIOT RECEPTION
Sunday, 6:00 – 8:00pm
Boarding at 5:30pm

Spirit of Boston

The BIOT reception will be a dinner cruise on the Spirit of Boston operated by Spirit Cruises sailing out of the Boston Harbor. BIOT will have exclusive use of two decks (weather permitting). Boarding will begin at 5:30 PM on Sunday August 19, 2007 with sailing time from 6:00- 8:00 PM.

The dock is within walking distance from the convention center. Exit the Boston Convention and Exhibition Center using the front entrance, walk three blocks toward the harbor on World Trade Avenue, turn left on Seaport Boulevard and the dock will be half a block on your right.



SUNDAY MORNING

8:20 – 10:40 am BCEC 108

Stem Cells: Quantifying, Modeling and Controlling Stem Cell Fate

J. M. Piret, Organizer Papers 1-7

BIOT 1 – Behavior-activated cell selection: Application to stem cell expansion

Brad Dykstra[1], John Ramunas[2], David Kent[1], Connie J. Eaves[1], and Eric Jervis[2]. (1) Terry Fox Laboratory, British Columbia Cancer Agency, 675 West 10th Avenue, Vancouver, BC V5Z 1L3, Canada, (2) Department of Chemical Engineering, University of Waterloo, 200 University Ave. West, Waterloo, ON N2L 3G1, Canada, ericjj@cape.uwaterloo.ca

The nondestructive nature of cellular behavior analysis using long-term imaging facilitates cell classification. To look for new indicators of self-renewing capacity, hematopoietic stem cells (HSCs) isolated from adult mouse marrow were individually micromanipulated into the wells of a microarray imaging chamber and then cultured for 4 days with each cell and its progeny being imaged using time-lapse photography. Individual clones were then harvested and assayed in mice for HSC compartment repopulating activity. Characteristics identified from image sequences were screened and 3 were found to be predictive of HSC potential: mitotic activity, longer cell cycle times, and uropodia. Clone selection criteria developed using these data allowed all HSC-containing clones to be detected and at a 2.4-fold greater efficiency in subsequent experiments. The BACS platform represents an advance towards the goal of dynamic single cell analysis in heterogeneous populations and demonstrates the potential of this technology for preparative cell isolation devices.

BIOT 2 - Tracking stem cell fate using a cell-based microscale platform

Tiago G Fernandes[1], Seok Joon Kwon[1], Moo-Yeal Lee[1], Margarida M Diogo[2], Cláudia Lobato da Silva[2], Joaquim MS Cabral[2], and Jonathan S. Dordick[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, 110 8th Street, Troy, NY 12180-3590, Fax: 518-276-2207, fernat2@rpi.edu, (2) IBB - Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Lisboa 1049-001, Portugal

Stem cells are potential sources for clinical applications. However, their therapeutic use is contingent upon precise control over the cell population during their *in vitro* expansion and differentiation. We have developed a miniaturized 3D cell-culture based chip for high-throughput screening. The cell chip consists of mouse embryonic stem (mES) cells encapsulated in 20nL alginate gels arrayed on a functionalized glass slide. Our results show that this platform is suitable for studying the expansion of mES cells, while retaining their pluripotent and undifferentiated state. Moreover, it provides a suitable system for the high-throughput screening of small molecules and their combinations that direct the fate of stem cells in a spatially addressable manner. This screening was aided by the development of a microarray in-cell Western technique that provides highly quantitative information on cell function. We expect this work to impact the design and control of stem cells for tissue engineering and biological studies.

BIOT 3 - Exploiting the synergy between lysate and acoustic standing wave fields to increase the retroviral transduction of suspension cells

Pascal R. Beauchesne[1], Venkata S. Tayi[1], Bruce D. Bowen[2], and James M. Piret[1]. (1) Michael Smith Laboratories & Department of Chemical and Biological Engineering, University of British Columbia, 2185 East Mall, Vancouver, BC V6T 1Z4, Canada, Fax: 604-822-2114, pbeauchesne@chml.ubc.ca, (2) Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

Recombinant retroviruses are effective therapeutic transgene delivery vehicles for stem cell-based gene therapy. However, the short half-life (~6 h) and low diffusivity (~6x10-8 cm2/s) of retroviruses often contribute to limited transduction efficiency. We have explored using whole cell lysate to improve the transduction efficiency of K-562 and TF-1 cells in MSCV-IRES-GFP virus-containing medium. Lysate addition increased transduction efficiency by 6-fold in t-flasks as well as in stirred suspension spinners and was a function of both lysate and target cell concentrations. When used in combination with a 1.95 MHz acoustic resonance filter, in which cells are retained within the pressure node planes while lysate and retroviral vectors are more readily displaced by fluid flow, transduction efficiency was increased by 2-fold more than for lysate alone leading to an overall increase of 12-fold. This approach utilizing the lysate-acoustic synergy can be readily scaled-up to transduce large numbers of cells.

BIOT 4 - Self-assembly of multipotent adult progenitor cells (MAPCs) and differentiation to the hepatic lineage

Kartik Subramanian[1], Karen Pauwelyn[2], Catherine Verfaillie[2], and Wei-Shou Hu[1]. (1) Department of Chemical Engineering and Materials Science, University of Minnesota, Amundson Hall , 421, Washington Ave S.E, Minneapolis, MN 55455, subramanian@cems.umn.edu, (2) Stem Cell Institute, Katholieke Universiteit Leuven, Leuven, Belgium

Multipotent adult progenitor cells (MAPCs) isolated from postnatal rat, mouse, and human bone marrow can be expanded in vitro without senescence and can differentiate into cells with morphological, phenotypic, and functional characteristics of hepatocytes. Several studies have shown that spheroidal aggregate (3D) culture of primary hepatocytes resulted in the maintenance of viability and enhancement of liver specific functions over a long culture period. We thus investigated the ability of MAPCs to self-assemble into 3D aggregates and explored the possibility of enhanced differentiation. MAPCs were successfully induced into 3D aggregates that exhibited good viability, morphology and differentiation potential based on expression of several endoderm markers like HNF3b, AFP, AAT, TTR and albumin. Advantages

of this approach are in obtaining more functionally mature differentiated cells, as a model system for studying nascent 3D development and in designing scalable culture systems that can be monitored and controlled to enhance differentiation.

BIOT 5 - 3-D differentiation model of ex vivo megakaryopoiesis

Younes Leysi-Derilou[1], Nicolas Pineault[2], Carl Duchesne[1], Jean-François Boucher[2], and **Alain Garnier**[1]. (1) Chemical engineering, Université Laval, Pouliot building, Cité Universitaire, Quebec city, QC G1K 7P4, Canada, Fax: (418) 656-5993, alain.garnier@gch.ulaval.ca, (2) R&D, Héma-Québec, Quebec city, QC G1V 5C3, Canada

Megakaryopoiesis (MKpoiesis) is a complex process by which hematopoietic stem cells (HSC) differentiate progressively into megakaryocytes (MK), which are large polyploïd cells, from which blood platelets shed. To better understand this process, we have developed a new 3-D dynamic mathematical model of MKpoiesis and have applied it to in vitro platelet production from cord blood stem cells. This model is based on an eight compartment representation of MKoiesis obtained by the division of three phenotypical dimensions into two levels each (-/+), the three dimensions being: 1) CD34+ cells (HSC), 2) CD41+ cells (megakaryocytes, MK), and 3) CD42+ cells (mature MK). One differential balance equation per compartment was stated, by considering differentiation, proliferation or death fluxes effects, and assuming logistic relations for the proliferation fluxes and constant rates for the other processes. The final nine differential equation model (eight cell- plus one platelet compartment) was solved numerically, and it's parameter evaluated by non-linear regression over experimental data obtained at two different temperatures (37 and 39 °C). Finally, the regression results have been validated by assessing the parameters identifiability and confidence intervals. Sensitivity analysis was also performed to evaluate the effect of the parameters on the responses. A clear effect of temperature on the different steps of MKoiesis was observed, and the relative importance of differentiation and proliferation on the overall cell expansion was also evaluated.

BIOT 6 - P53 tumor suppressor protein affects hematopoietic stem cell differentiation in the megakaryocyte compartment

Peter G. Fuhrken[1], Pani Apostolidis[1], Stephan Lindsey[1], William M. Miller[2], and E. Terry Papoutsakis[2]. (1) Department of Chemical and Biological Engineering, Northwestern University, 2145 N. Sheridan Road, Evanston, IL 60208, p-fuhrken@northwestern.edu, (2) Department of Chemical & Biological Engineering and The Interdepartmental Biological Sciences Program, Northwestern University, Evanston, IL 60208-3120

The molecular mechanisms underlying differentiation of hematopoietic stem cells (HSCs) into megakaryocytes is poorly understood. During megakaryopoiesis, committed progenitor cells undergo endomitosis resulting in polyploid, multi-lobated nuclei. Subsequently, a constitutive program of apoptosis is linked to proplatelet formation. Transformation-related protein p53 can act as a transcription factor affecting both cell cycle control and apoptosis. The up-regulation of several known p53 target genes during megakaryocyte differentiation led to a hypothesis that p53 activation is involved in regulating megakaryopoiesis. A p53-DNA binding activity assay showed increased p53 activity during megakaryocytic differentiation in a validated human model cell-line system (CHRF cells). We generated stable CHRF clones expressing microRNAs that knock down p53 expression. Phorbol-ester-induced megakaryocytic differentiation of these cells results in higher ploidy and viability than cells expressing scrambled controls. This work broadens our understanding of the p53 regulon's role in HSC differentiation and points to ways of manipulating stem cell fate *in vitro*.

BIOT 7 - Differentiation of adult human stem cells guided by mechano-sensing of matrix elasticity

Florian Rehfeldt, Adam J Engler, and Dennis E. Discher, Department of Chemical and Biomolecular Engineering, University of Pennsylvania, 112 Towne Building, 220 South 33rd Street, Philadelphia, PA 19104-6315, Fax: 215 573 2093, rehfeldt@sas.upenn.edu

Human mesenchymal stem cells (MSCs) from bone marrow are promising candidates for potential therapeutic applications since they can differentiate into various lineages. State of the art differentiation of these pluripotent cells is done biochemically using appropriate growth factors. But biochemical stimuli are just one part of the complex chemo-physical environment cells face in vivo. It is now well acknowledged that cells feel and respond to their physical environment and that these physical cues are as important as the biochemical ones. Recently, we demonstrated that substrate elasticity can even direct MSC differentiation to osteogenic, myogenic or neurogenic cells. While these experimental results are striking, understanding of the complex underlying molecular mechanisms of force sensing and transduction is only at its very beginning. A detailed insight to cell adhesion on tunable biomimetic substrates is given and the connection between cell adhesion, mechanical properties of the substrate and stem cell differentiation is highlighted.

SUNDAY MORNING

8:00 – 11:10 am BCEC 107 A/B

Biophysical and Biomolecular Symposium: Protein Stability

T. G. Fernandes, S. J. Kwon, M -Y. Lee, M. M. Diogo, C. Lobato da Silva, J. M. Cabral, J. S. Dordick, Organizer Papers 8-15

BIOT 8 - Use of biophysical techniques for manufacturability assessment of therapeutic proteins

Cynthia Li[1], Yijia Jiang[2], Juraj Svitel[1], Jie Wen[1], Ranjini Ramachander[2], Shengwu Wang[1], Jenny Li[1], and Linda Narhi[2]. (1) Amgen, Inc, Thousand Oaks, CA 93120, cynthial@amgen.com, (2) Global Cellular and Analytical Resources, Amgen Inc, Thousand Oaks, CA 91320

PURPOSE: The purpose of this study was to assess the manufacturability by comparing the effect of pH, storage and stress on the conformation, thermal stability and propensity for self-association of therapeutic proteins. METHODS: CD, fluorescence, FTIR, and Raman spectroscopies, dynamic light scattering (DLS), sedimentation velocity analytical ultracentrifugation (AUC-SV) and differential scanning calorimetry (DSC) RESULTS: The conformational stabilities of therapeutic proteins at different pH with different salt concentration or under different stress and storage conditions may affect the quality of the final protein products. This report describes the effect of low pH on the conformation and thermal stability of the proteins, the reversibility of any acid-induced changes in conformation, and also compares the solubility of the proteins at neutral pH. The secondary structure was assessed by far UV CD, FTIR and Raman spectroscopy, the tertiary structure was assessed with near UV CD, Raman and fluorescence spectroscopy, the surface hydrophobicity was compared using ANS binding, the conformational and thermal stability was assessed by DSC, and self-association was analyzed by both dynamic light scattering using a Zetasizer, and sedimentation velocity analytical ultracentrifugation.

CONCLUSIONS: Changes in conformation, self-association and thermal stability of the therapeutic proteins studied were observed at pH 4 and below. The changes in secondary structure induced by acid pH are mostly reversible for all proteins. The changes in tertiary structure that occur at pH 3.5 and above are also reversible for the proteins. Small irreversible changes in the tertiary structure of the proteins occur at pH 3. Different proteins unfold to a slightly different extent at pH 4 and below. Some of the differences in stability and solubility could result in difficulties during processing, formulation, and delivery. Manufacturability assessments of the therapeutic proteins by biophysical characterization techniques prove to be very useful during early stage development.

BIOT 9 - A novel bioseparation approach: Use of chaotropic agents for HIC and ion exchange chromatography to dissociate noncovalent multimers during purification of an Fc fusion protein

Allen B. Magill II, Pharmaceutical Development, Centocor, R & D, 145 King of Prussia Road, Radnor, PA 19085, Fax: 610-993-7864, amagill@cntus.jnj.com

An Fc- fusion protein formed over 30% non-covalently associated multimers in cell culture harvest. Following Protein A low pH elution, nearly 95% of the protein was multimeric. The protein was converted to an active monomeric state using chaotropic agents such as guanidine HCl (GuHCl) and urea with minimal effect on biological activity. A process was developed that included an HIC step utilizing the conductivity of GuHCl for binding. Binding and eluting in GuHCl and urea, respectively, was necessary to maintain the protein in a monomeric state. The HIC step significantly reduced aggregated and degraded protein. Additionally, a polishing anion exchange step was developed using urea to maintain the monomeric state of the fusion protein. Due to urea degradation in solution to isocyanate and ammonium, several steps were implemented to maintain less than 1% carbamylation of the protein by isocyanate. Purified Fc-fusion remained monomeric once dialyzed into formulation buffer.

BIOT 10 - Aggregation of a monoclonal antibody induced by adsorption to microparticle surfaces

Jared Bee[1], Jennifer Stevenson[2], Koustuv Chatterjee[2], Erwin Freund[2], John F. Carpenter[3], and Theodore W. Randolph[1]. (1) Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80309, jaredsbee@gmail.com, (2) Drug Product and device development, Amgen Inc, Thousand Oaks, CA 91320, (3) Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262

Purpose The purpose of this research was to determine the aggregation induced by adsorption of a monoclonal antibody to various microparticle surfaces relevant to the final fill, finish and storage of a biopharmaceutical product. We also investigated how formulation excipients can either aggravate or mitigate microparticle induced aggregation.

Methods Adsorption was performed by incubation of IgG (antistreptavidin, donated by Amgen Inc.) with microparticles. The suspensions were centrifuged and the protein in the supernatant quantified by UV at 280 nm or SEC and UV at 280 nm. Adsorbed and aggregated protein was then determined by mass balance.

Results The aggregation of IgG induced by adsorption was found to be strongly affected by both the surface type and the solution conditions. Tungsten microparticles caused dramatic IgG loss from solution. Addition of 0.01% Tween 20® to the buffer stabilized IgG from silica induced aggregation, but the addition of sodium chloride increased the observed aggregation. Conclusions Protein aggregation is increased in the presence of microparticles; however, solution conditions can dramatically alter the rate and extent of induced aggregation. Incubation studies with microparticles could be used for accelerated screening of formulation stability with respect to adsorption induced aggregation.

BIOT 11 - Assessment of protein stability during freeze-thaw process

Kapil Gupta[1], Nitin Rathore[2], Lorena Barron[2], Wenchang Ji[3], Feroz Jameel[3], and Keith Murphy[3]. (1) Drug Product and Device Development, Amgen, Mail stop 30W- 3-A , one amgen center drive, Thousand oaks, CA 91320, kgupta@amgen.com, (2) Global Drug Product & Device Development, Amgen Inc, Thousand Oaks, CA 91320, (3) Global Drug Product & Device Development, Amgen, Thousand Oaks, CA 91320

Biopharmaceutical industries often rely on frozen storage of the bulk material due to the increased physical and chemical stability in the frozen state compared to the liquid state. However, freezing and thawing at large scale may have impact on the quality of protein products due to cryoconcentration, ice-surface induced denaturation and cold denaturation. One potential way to minimize freeze-thaw induced protein instability is by controlling the rates of the freezing and thawing processes. This study evaluates the effect of controlling the freeze rate and freeze front velocity on the product quality. In this evaluation, Celsius-pak© disposable bag

technology is used for controlled freeze-thaw operation while uncontrolled freeze-thaw is performed in polycarbonate bottles.

BIOT 12 - Identifying optimal solution conditions for high concentration protein formulations: Use of self-interaction chromatography

Mark Cornell Manning[1], Charles S. Henry[2], Joseph J. Valente[2], Robert W. Payne[2], and W. William Wilson[3]. (1) Legacy BioDesign LLC, 1826 Monarch Circle, Loveland, CO 80538, Fax: 970-663-6006, manning@legacybiodesign.com, (2) Department of Chemistry, Colorado State University, Fort Collins, CO 80523, (3) Department of Chemistry, Mississippi State University, Mississippi State, MS 39762

Numerous studies have now demonstrated that the osmotic second virial coefficient (B22) of macromolecules is directly correlated to solubility, viscosity, and aggregation propensity. Control of these processes is critical to developing viable high concentration formulations of peptides and proteins. Historically, B22 has been measured using static light scattering. However, such determinations are labor-, material-, and time-intensive. Furthermore, B22 values cannot be obtained for peptides by light scattering methods due to their small size. Recently, the advent of self-interaction chromatography (SIC) has allowed B22 to be obtained directly for both peptides and proteins in a rapid fashion using conventional HPLC equipment. This approach allows a number of potential formulations to be screened in a short period of time using little material. We have used SIC to identify formulations with sufficient solubility, for biotherapeutics ranging in size from peptides to monoclonal antibodies, thereby allowing product development to proceed rapidly.

BIOT 13 - Stability of high concentration rhuMAb VEGF

Hong Liu and Mary E. M. Cromwell, Early Stage Pharmaceutical Development, Genentech, Inc, 1 DNA Way 96A, South San Francisco, CA 94404, hongliu@gene.com

The purpose of this study was to develop a high concentration rhuMAb VEGF formulation for subcutaneous (SC) delivery. A stability study was designed with 100mg/ml rhuMAb VEGF, based on rhuMAb VEGF's unique reversible self-association. The extent of self-association depends on pH, protein concentration and ionic strength. Formulations were tested over a narrow pH range to minimize aggregation. Protein stability in these formulations was evaluated at 2-8°C and –20°C storage by Size Exclusion HPLC and Ion Exchange HPLC. Due to concern of potential hydrolysis of sucrose under the lower pH conditions, a boronate affinity HPLC method was utilized to evaluate glycation on stability samples.
The study shows that lower pH can help to minimize the amount of aggregate at a rhuMAb VEGF concentration of 100mg/ml. As expected, sugars helped in freeze/thaw stability and storage at –20°C. There are significant differences in aggregate content between sucrose-containing and trehalose -containing liquid formulations stored at 2-8°C, and the differences are not due to glycation.

BIOT 14 - Stability of protein antigens in vaccine delivery formulations

James D Chesko[1], Jina Kazzaz[2], Padma Malyala[1], Kathryn Patton[1], Mildred Ugozzoli[1], Derek O'Hagan[1], and Manmohan Singh[1]. (1) Vaccines and Diagnostics, Novartis, 4560 Horton Street, M/S 4.3, Emeryville, CA 94608, Fax: 510-923-2586, James.Chesko@novartis.com, (2) Novartis, Emeryville, CA 94709

The molecular stability of proteins in association with a delivery system is a key consideration in the formulation of vaccines. The effect of adsorption and release of antigenic proteins from aluminum salts, PLG microparticles, MF59 suspensions can enhance the immunogenicity of the overall vaccine system due to adjuvant effects. We will examine the effects of formulating various proteins such as HIV-envelope, rPA for anthrax, hemagglutinin for influenza and Meningitis B on the overall stability and biophysical properties of the system. Spectroscopic and analytical methods including circular dichroism, fluorescence, light scattering, FTIR and chromatography were employed to measure changes in association, molecular structure, disposition and stability that occur to these important protein antigens in various delivery systems.

BIOT 15 - Tungsten, prefilled syringes and protein aggregation

Robert Swift[1], Yasser Nashed-Samuel[2], Wei Liu[3], Linda Narhi[4], and Janice Davis[2]. (1) Commercialization & Packaging, Amgen Inc, 1 Amgen Ctr Dr, Thousand Oaks, CA 91320, rswift@amgen.com, (2) Amgen Inc, Thousand Oaks, CA 91320, (3) Drug Product and Device Development, Amgen, Thousand Oaks, CA 91320, (4) Global Cellular and Analytical Resources, Amgen Inc, Thousand oaks, CA 91320

Amgen has learned recently that tungsten forming pins are used in the manufacture of glass pre-filled syringe barrels and that residual tungsten from the process can lead to protein aggregation in some therapeutic protein formulations. This paper discusses: 1) the source of tungsten in syringe manufacture, 2) how Amgen confirmed the link between aggregated proten and residual tungsten, 3) the effect of fill-finish processes on product exposure to tungsten, 4) development of methods to analyze residual tungsten in empty syringes, 5) the testing of drug products to determine sensitivity to tunsten, 6) the proposed aggregation mechanism, and 7) how this experience has changed Amgen's paradigm for managing relation supplier relationships.

SUNDAY MORNING

8:00 – 11:10 am BCEC 106

Biophysical and Biomolecular Symposium: Protein Folding & Characterization

K. Mallela and T. Cellmer, Organizers

Papers 16-23

BIOT 16 - Hidden folding intermediates in small proteins: Implications for the folding energy landscape, cooperativity, and evolution of protein structures

Yawen Bai, Laboratory of Biochemistry and Molecular Biology, NCI,NIH, 37 Convent Drive, Building 37, Room 6114E, Bethesda, MD 20892, Fax: 301-402-3095, yawen@helix.nih.gov

Using native-state hydrogen exchange and kinetic methods, we have demonstrated that partially unfolded intermediates can exist after the rate-limiting step in the folding of small proteins and become undetectable in the conventional kinetic folding experiments. Moreover, using a native-state hydrogen exchange-guided protein engineering approach, we have populated such hidden intermediates and solved their high resolution structures by multi-dimensional NMR method. These results and their implications on the folding energy landscape, cooperativity, and structure evolution of proteins will be presented.

BIOT 17 - Electrostatic properties of the unfolded state: What can we learn from modeling?

Nicholas C. Fitzkee and Bertrand García-Moreno E, TC Jenkins Department of Biophysics, Johns Hopkins University, Jenkins Hall 204, 3400 N Charles St., Baltimore, MD 21218, Fax: 410-516-4118, nfitzkee@jhu.edu

Current models for electrostatic interactions in the unfolded state operate on simplified representations of proteins instead of an ensemble of three-dimensional structures. Thus, existing models cannot investigate the effects of organization in the unfolded state. We developed a method for modeling these interactions that uses three-dimensional protein structures. Our method was used to calculate electrostatic properties of T62P Staphylococcal nuclease (Snase), a variant known to resemble unfolded wild-type Snase. Proton binding data measured potentiometrically were compared with our calculations. We find that an ensemble of highly disordered structures is consistent with the data, but we cannot rule out the possibility of some organization. Energetically, our calculations indicate that weak, non-specific Coulomb interactions are unavoidable in the unfolded state, and while these contribute minimally to organization, they are not negligible in calculations of protein stability. Therefore, the electrostatic properties of Snase are determined more by a background of fluctuating ions rather than a particular chain conformation.

BIOT 18 - Ultrafast folding of the villin subdomain

Troy Cellmer[1], William A. Eaton[2], and James Hofrichter[2]. (1) Laboratory of Chemical Physics, NIDDK,/NIH, 9000 Rockville Pike, Bethesda, MD 20892-0520, cellmert@niddk.nih.gov, (2) Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892-0520

The ultra-fast-folding subdomain of the chicken-villin headpiece (villin) is the smallest, naturally-occurring protein that folds autonomously, making it feasible to directly compare experimental folding kinetics with theoretical and computational studies. We have studied the folding kinetics of villin as a function of temperature and guanidinium chloride (GdmCl) concentration using nanosecond laser temperature-jump measurements. At low GdmCl concentrations, the observed relaxation is bi-exponential, consisting of a fast (~100ns) relaxation and a slow (~5micro) phase that corresponds to the unfolding/refolding transition. The relative amplitude of the fast phase decreases monotonically from > 50% in water to ~ 10% in 1M GdmCl. At high GdmCl concentrations there is only a single slow phase. The GdmCl dependence of the folding (slow) relaxation rate is very weak, indicating the presence of a very small barrier to folding. Various quantitative estimations of the barrier height will be presented, and the possibility of "downhill" or barrier-less folding discussed.

BIOT 19 - Simultaneous and systematic evaluation of multiple protein refolding conditions

Peter A. Leland, Purification & Protein Biochemistry, EMD Chemicals, Novagen Brand, 441 Charmany Drive, Madison, WI 53719, Fax: 608-238-1388, peter.leland@emdchemicals.com

Production of foreign proteins in E. coli often yields inclusion bodies – dense, insoluble, aggregates of mis-folded protein. Inclusion bodies, however, are easily purified, resistant to proteolysis and can be solubilized with chaotropes. Defining conditions that promote refolding of a chemically solubilized target protein into its native conformation is difficult. The chances of identifying an efficient refolding condition are increased by simultaneously and systematically evaluating multiple conditions. To enable this experiment, we developed a library of 96-well plate-based refolding screens. Individual components of the screens included established refolding additives and novel refolding additives. Fractional factorial methods were used to assemble the additives into a collection of refolding buffers with maximal diversity but fewer than 96 trials per screen. Using the refolding screens, we have defined optimal refolding conditions for several proteins. Additionally, results have allowed identification of additives that are efficacious for several proteins.

BIOT 20 – Driving forces for protein folding on the ribosome as a function of polypeptide chain elongation: Experimental and computational approaches

Silvia Cavagnero, Jamie P. Ellis, Bryan C. Mounce, and Nese Kurt, Department of Chemistry, University of Wisconsin-Madison, 1101 University Ave., Madison, WI 53706, Fax: 608-262-9918, cavagnero@chem.wisc.edu

The driving forces that contribute to protein folding in the cell are extremely important to understand the biology of living organisms. Yet, these key forces and the related underlying mechanisms are still poorly understood. The physical interactions responsible for conformational sampling in the cell start acting cotranslationally during ribosome-assisted protein biosynthesis, in the absence of any denaturing agents and under physiologically relevant temperature and solution conditions. We have investigated the conformation of a series of N-terminal protein fragments of increasing length on the ribosome by dynamic fluorescence depolarization and cone angle analysis. Sequences belonging to proteins capable of independent folding and intrinsically

unstructured proteins were analyzed. Nascent polypeptide dynamics in the low ns and sub-ns timescale regimes have been identified at specific chain lengths, showing how independent structure and motions evolve at different stages of chain elongation on the ribosome. In addition, computational approaches have been adopted as a complementary tool, to gather information of general applicability on the role of both short-/long-range interactions and nonpolar surface burial for the formation of native-like structure as a function of chain elongation (from N- to C-terminus).

BIOT 21 - Joining NMR and simulation to capture Alzheimer's Aβ monomer and fibril structural ensembles

Nicolas Lux Fawzi, UCSF / UC Berkeley Joint Graduate Group in Bioengineering, UC Berkeley, Berkeley, CA 94720, fawzin@berkeley.edu, and Teresa Head-Gordon, Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720

The molecular events that direct the misfolding and fibril formation of protein and peptides central to human disease such as Alzheimer's Disease are not well elucidated because no single experimental or computational technique is able to easily capture the relevant time and size scales with the necessary atomic detail. We propose to examine Aβ aggregation by combining the excellent solid state NMR data recently available on fibrils with our own solution NMR monomer studies, all atom and coarse grained simulations in order to develop a fuller picture of the steps from monomer to mature fibril. In an Aβ sub-peptide comprising residues 21 to 30, we find support for a diverse ensemble of conformations building on previous NMR results, and highlight the conformational transition necessary to go from the structure in the monomer to the structure in the fibril form of the full peptide. We conclude by examining the connection between structure and familial mutations in this region in both the monomer state through explicit water all atom molecular dynamics and in the fibril state through our coarse grained physical model.

BIOT 22 - Denaturant and isotopic effects on stability and folding of interleukin-1 receptor antagonist

Ramil F. Latypov[1], Dingjiang Liu[1], Tadahiko Kohno[2], David N. Brems[1], and Andrei A. Raibekas[1]. (1) Pharmaceutics Department, Amgen Inc, One Amgen Center Dr, Thousand Oaks, CA 91320, rlatypov@amgen.com, (2) Protein Science, Amgen Inc, Thousand Oaks, CA 91320

Structural properties and folding of interleukin-1 receptor antagonist (IL-1ra), a therapeutically important cytokine with a symmetric beta-trefoil topology, are characterized using optical spectroscopy, high resolution NMR and small angle X-ray scattering. Overall, equilibrium denaturation data is consitent with a two-state unfolding mechanism with small, but detectable structural changes in the pre-transition region. 1H-15N HSQC cross-peaks for the folded state show only limited chemical shift change as a function of denaturant concentration. However, the amide cross-peak of Leu31 demonstrates a significant urea dependence that can be fitted to a two-state binding model with a dissociation constant of 0.95 ± 0.04 M. This interaction has at least a five times higher affinity than reported values for non-specific urea binding to denatured proteins and peptides, suggesting that the structural context around Leu31 stabilizes the protein-urea interaction. A possible role of the denaturant binding in inducing the pre-transitional changes is discussed. Experiments on isotopically enriched preparations of IL-1ra show measurable isotopic effects on protein conformational stability in the absence of structural changes. Elucidation of the mechanism of the isotopic effects and their impact on protein folding is currently underway.

BIOT 23 - Protein folding principles: Foldons, sequential stabilization, and optional errors

Krishna Mallela and S. Walter Englander, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 1007 Stellar Chance Bldg., 422 Curie Blvd, Philadelphia, PA 19104-6059

Detailed structural information from hydrogen exchange and related experiments indicate the following three physical principles underlying protein folding pathways. (1) Proteins are made up of small cooperative unfolding/folding submolecular units known as foldons. (2) Proteins construct these foldon pieces to progressively build their final native states using the sequential stabilization principle where pre-formed foldons guide and template the subsequent foldons. (3) Ubiquitous optional misfolding errors can corrupt different naturally occurring on-pathway intermediates and cause intermediates to accumulate by inserting error-repair barriers at different points along the pathway. The first two principles dictate that the folding pathway of a protein gets predetermined by its component foldon substructure, and the order of steps is set by the way the foldon units are organized in the native structure. The third principle dictates whether the pathway appears to be kinetically 2-state or multi-state or heterogeneous. The integration of these three well-documented principles into a coherent mechanism provides a unifying explanation for how proteins fold and why they fold in that way.

SUNDAY MORNING

8:00 – 11:00 am BCEC 107 C

Upstream Processing: Advances in Biocatalysis

P. C. Cirino and L. Sun, Organizers
Papers 24-31

BIOT 24 - A blue fluorescent protein with oxidoreductase activity

Karen M Polizzi, School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Rd, Exeter EX4 4QD, United Kingdom, K.M.Polizzi@exeter.ac.uk, Desmond A Moore, School of Physical Science & Engineering, Morehouse College, Atlanta, GA 30314, and Andreas S. Bommarius, Schools of Chemical Engineering and Chemistry/Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0363

Fluorescent proteins revolutionized the study of proteins, organelles, and metabolic pathways by making it possible to visualize events within cells. In 2001, Su et al identified a blue fluorescent protein from Vibrio vulnificus (bfpvv) does not adopt a beta-can fold like traditional fluorescent proteins, but belongs to the short-chain dehydrogenase/reductase family and obtains its fluorescence by binding and magnifying the intrinsic fluorescence of NADPH. Since the fluorescence level of Bfpvv is very low, its natural role is unlikely to be as a fluorescent protein. However, a protein with both fluorescence and activity would be a useful addition to the toolbox for protein biophysical studies as it would allow the separate monitoring of binding and catalytic events.
Despite the diverse nature of the SDR family, we were able to identify redox substrates on which Bfpvv shows considerable activity. The enzyme is highly active in the reduction of aldehydic substrates, particularly those with high hydrophobicity (e.g. aromatic and long chain hydrocarbon). The activity with ketones is considerably lower, as is the activity in the oxidative direction. When oxidizing substrates, the enzyme shows more than 15-fold preference for the S-enantiomer. The substrate profile of the enzyme suggests that the active site is partially impaired and this is what likely led to the development of fluorescent properties. Mutants with increased fluorescence have accumulated mutations in areas that affect the protein activity, further corroborating that fluorescence comes at the expense of activity. The unique properties of Bfpvv allow for both visual levels of fluorescence to monitor binding of NADPH and measurable levels of redox activity to monitor catalytic turnover.

BIOT 25 - A novel laccase/redox mediator system increases space-time yields and operational stability in dehydrogenase reactions

Roland Ludwig, Department of Food Sciences and Technology, Research Centre Applied Biocatalysis, Muthgasse 18/2/71, 1190 Wien, Austria, Fax: +43-1-36006-6251, roland.ludwig@a-b.at, and Dietmar Haltrich, Institute of Food Technology, University of Natural Resources and Applied Life Sciences, Vienna, 1190 Wien, Austria

The use of FAD containing oxidases and dehydrogenases in biocatalysis is limited by the formation of reactive oxygen species (ROS) and their sometimes low activity with molecular oxygen as electron acceptor. Frequently, these FAD-containing oxidases show significant activity with electron acceptors other than oxygen. The engineering of a process using alternative electron acceptors circumvents the formation of ROS, but demands a regeneration system to avoid stoichiometric use of these substances. We coupled several artificial electron acceptors for oxidizing flavoenzymes to laccase as a regenerating enzyme to recycle the reduced form of the electron acceptor. Several tested flavoenzymes and laccase show complementary activity on most of these redox active substances, thus forming a regenerative cycle. For the study of the proposed regeneration system cellobiose dehydrogenase, and pyranose 2-oxidase were studied in coupled biotransformation experiments for the production of oxidized carbohydrate intermediates of industrial interest. The impact of several process parameters on specific productivity and operational stability were evaluated in batch reactions were found to have a significant influence on conversion rate and turnover stability. In conclusion it could be shown that the presented regeneration system works very well with different flavoenzymes, increased the specific productivity and enzyme stability significantly, and hence is applicable for a wide range of biotransformations employing flavoenzymes.

BIOT 26 - Alginate-derived nanovolume cytochrome P450 microarrays for high-throughput inhibition assays

Sumitra Meena Sukumaran1, Moo-Yeal Lee2, Douglas S Clark3, and Jonathan S. Dordick1. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, sukums@rpi.edu, (2) Solidus Biosciences Inc, Troy, NY 12180, (3) Department of Chemical Engineering, University of California at Berkeley, Berkeley, CA 94720

Cytochrome P450 inhibition represents a safety hurdle and a major expense in drug discovery. Previous *in vitro* assay technologies include well-plates and microfluidic platforms; however, these are not truly high-throughput, nanovolume assays.
We have developed a solid-phase, fluorescence-based microarray for high-throughput P450 inhibition assays. P450s immobilized in 10 nl alginate spots reproducibly exhibited reactivity comparable to that in solution, and increased storage stability and linear reaction time. We show that a single 1,080 or 2,856-spot array can simultaneously provide IC_{50} values for nine potential inhibitors with multiple P450s. IC_{50} values comparable to those in solution were obtained for P450 isoforms 3A4 and 2C9 with a number of compounds, including inhibitors like ketoconazole and sulphaphenazole. In addition, the P450 microarrays were interfaced to a wide-field, whole-slide imaging system to rapidly generate IC_{50} and K_i values. These advances represent steps toward an automated, high-throughput metabolism and toxicology assay directed towards personalized medicine.

BIOT 27 - Evolution in reverse: Engineering a xylose-specific xylose reductase

Nikhil Nair, Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, 600 S. Mathews Avenue, 215 Roger Adams Laboratory, Box C3, Urbana, IL 61801, nunair2@uiuc.edu, and **Huimin Zhao**, Department of Chemical and Biomolecular Engineering, University of Illinois, 215 RAL, Box C3, 600 S. Mathews Ave., Urbana, IL 61801, Fax: 217-333-5052, zhao5@uiuc.edu

Xylitol is a five-carbon sugar alcohol with many industrial applications. However, it is still relatively expensive to produce either by chemical synthesis or by fermentation. To address this limitation, we are using protein engineering techniques to create a xylose reductase (XR) mutant with decreased specificity toward L-arabinose, while maintaining its high activity toward D-xylose such that a mixture of pentose (D-xylose and L-arabinose) derived from renewable plant biomass can directly be used as substrates. The Neurospora crassa XR was chosen for protein engineering work due to several favorable properties over other XRs, in addition to its innate >2-fold catalytic efficiency toward D-xylose than L-arabinose. A directed evolution strategy was developed that consists of a combined structure-function based semi-rational design involving active site residue mutagenesis followed by random mutagenesis and selection for desired substrate specificity.

After the first round of evolution, a mutant was identified with fourteen-fold preference for D-xylose over L-arabinose. To our knowledge this is the most xylose-specific XR identified or engineered to date. Further engineering rounds are currently underway on this template to further reduce its promiscuity.

BIOT 28 - Directed evolution of LuxI for enhanced synthesis of OHHL

Pavan Kumar Reddy Kambam and **Lianhong Sun**, Department of Chemical Engineering, University of Massachusetts Amherst, 686 N. Pleasant Street, Amherst, MA 01003

As a bacterial cell-cell communication mechanism, quorum sensing plays a unique role for a bacterial population to synchronize its group behavior. As a result, quorum sensing has been identified in a variety of biological processes, including biofilm formation, bioluminescence, release of toxin, and plasmid conjugation. In particular, because quorum sensing is involved in the processes of many infectious diseases, developing drugs targeting quorum sensing has been proposed and analogs of the signal molecules have been examined for their effectiveness in inhibiting quorum sensing. In addition, quorum sensing systems have been increasingly used to construct complicated artificial genetic circuits with unique dynamic behaviors.

We are interested in engineering the LuxI-LuxR quorum sensing systems. Engineered quorum sensing systems with enhanced functionality can be used to construct complex genetic circuits or artificial ecosystems to understand the underlying principles of biological networks and systems. In addition, engineering quorum sensing also provides valuable insights into the quorum sensing mechanism, which are significant for designing novel broad-spectrum anti-bacterial drugs.

In this presentation, we will discuss our recent work on directed evolution of LuxI for enhanced activity. LuxI catalyzes the synthesis of 3-oxo-hexanyl homoserine lactone (OHHL) which functions as the signal molecule of the LuxI-LuxR quorum sensing system. We developed a high throughput genetic selection for identifying LuxI mutants with enhanced activity from random mutagenesis libraries. A semi-quantitative fluorescence method was developed to verify identified mutants. All mutants were then quantified using HPLC-MS by determining their OHHL production yields. Mutants with significantly increased activity have been identified and quantified. We will also discuss the amino acid substitutions and their putative effects on LuxI activity.

BIOT 29 - Biocatalytic systems for aromatic oxidations

Angela M. McIver, S V B Janardhan Garikapati, and Tonya L. Peeples, Department of Chemical and Biochemical Engineering, The University of Iowa, 4133 Seamans Center, Iowa City, IA 52242, amciver@engineering.uiowa.edu

The purpose of this research is to engineer a biocatalytic system to facilitate the production of oxidation products in an economic and environmentally benign fashion. Oxygenases are powerful stereoselective and regioselective catalysts that are useful in the preparation of valuable pharmaceutical and specialty chemical intermediates. The need for cofactor regeneration necessitates the use of whole-cells in such bioprocesses. We have selected to work with various organisms carrying mono- and dioxygenases. Immobilization of these microorganisms, including solvent tolerant host organisms, will result in more stable biocatalysts that will be more amenable to meet process requirements. Specifically, retention of the immobilized catalyst will facilitate isolation of valuable products. This effort is critical to producing environmentally beneficial biotransformation systems by providing an environmentally benign oxidation process with reaction and separation of products. This work highlights some results comparing the effectiveness of naphthalene and toluene dioxygenases expressed in *Escherichia coli* for aromatic oxidations. The use of biphasic reaction media is used to increase productivity and enhance the reaction. We will show methods of creating an environmentally benign system.

BIOT 30 - Design of an enzyme-chaperone chimera as a new approach to enzyme immobilization and self-renaturation

Lisa M Bergeron[1], Talar Tokatlian[1], and Douglas S Clark[2]. (1) Department of Chemical Engineering, University of California at Berkeley, 473 Tan Hall, Berkeley, CA 94720, LBergero@berkeley.edu, (2) Department of Chemical Engineering, University of California, Berkeley, Berkeley, CA 94720

The use of enzymes in the presence of organic solvents is often limited by enzyme stability, particularly in solutions containing high concentrations of water-miscible organic co-solvents. Immobilization has been used to overcome this challenge by providing the enzyme with a stabilizing support; however, this can result in a reduction of enzyme activity. We have shown that some molecular chaperones can function to maintain enzyme activity under solvent-denaturing conditions. A single subunit isolated from the thermosome of the hyperthermophile *Methanocaldococcus jannaschii* functions in the presence of several water-miscible organic co-solvents. We have designed a chimera in which enzyme is fused to this chaperone and immobilized. This allows for continuous use of the enzyme in a flow-through reactor and provides it with the protection of a molecular chaperone to resist loss of activity in the presence of organic co-solvents.

BIOT 31 - Protein dissection and shuffling for generating recombined proteins

Yong Chen, Tingjian Chen, Hui Hua, and **Zhanglin Lin**, Department of Chemical Engineering, Tsinghua University, 1 Tsinghua Yuan Road, Beijing, China, zhanglinlin@mail.tsinghua.edu.cn, zhanglinlin@mail.tsinghua.edu.cn

We have developed a simple method that allows for the facile recombination of distantly related proteins at multiple discrete sites. The approach relies on protein random dissection to search for possible breakage points, which involves the use of a folding reporter to identify soluble shorter peptides independently of protein function. These dissection points are then used as candidate sites for protein recombination. To evaluate this method, two variants of aminoglycoside-3'-phosphotransferase (APHI and APHII) were chosen as model proteins, which share 31.4% amino

acid identity. APH I and APH II were recombined at three sites, the library of 16 (24) chimeras was screened, among which 3/16 were found to show resistance to kanamycin, albeit at lower levels compared with the wild-type APH. We further applied random mutation to improve the activity of these chimeras. This protein shuffling method should be useful for creating artificial biocatalysts with sequences from different templates.

SUNDAY MORNING

11:30 – 12:35 am BCEC 107 B

Alan S. Michaels Recovery Award Lecture

W. Zhou, Organizer Paper 32

BIOT 32 - Early steps in protein recovery: The messy part of a clean industry

Maria-Regina A. Kula, Institute of Enzyme Technology, Heinrich Heine University Duesseldorf, Stetternicher Forst, D-52428 Juelich, Germany, Fax: -49 2461 612490, m.r.kula@fz-juelich.de

Abstract Not Available

SUNDAY AFTERNOON

2:00 – 3:45 pm BCEC 108

Stem Cells: Stem Cell Based Tissue Engineering

T. McDevitt, and P. W. Zandstra, Organizers Papers 33-37

BIOT 33 - Generation and characterization of keratinocytes from human embryonic stem cells

Christian M. Metallo[1], Lin Ji[2], Juan J. De Pablo[3], and **Sean P. Palecek**[1]. (1) Department of Chemical & Biological Engineering, University of Wisconsin - Madison, 1415 Engineering Drive, Madison, WI 53706, Fax: 608-262-5434, palecek@engr.wisc.edu, (2) University of Wisconsin-Madison, Madison, WI 53706, (3) Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, WI 53706

Primary epidermal keratinocytes are used in several tissue engineered skin products. These allogeneic tissues pose safety concerns and the risk of immunological rejection. In addition, cell survival rates following implantation are extremely low resulting from the lack of a functional, vascularized dermis. Embryonic stem cells offer the potential to provide more functional engineered skin constructs by incorporating numerous cell types comprising skin in the appropriate three-dimensional configuration. In addition, ESC-derived tissues can be generated from a single clonal source that potentially could be engineered to repress tissue rejection. Toward this goal, we have developed a protocol to generate pure populations of epidermal keratinocyte progenitors expressing keratin 14 (K14) from human embryonic stem cells (hESCs). We will describe how temporal presentation of multiple environmental factors, including soluble cytokines and differentiation factors, extracellular matrix, and cell-cell contact, affect yield of keratinocyte progenitors from hESCs. In addition, we will discuss efforts to terminally differentiate these keratinocyte progenitors to epithelial sheets with epidermal barrier properties.

BIOT 34 - Characterization of embryonic stem cells' differentiation into insulin-producing endocrines cells in 3-D cultures

Xiuli Wang, Department of Biomedical Engineering, College of Engineering, University of Arkansas, 700 Research Center Blvd, 3420 ENRC, Fayetteville, AR 72701, Fax: 479-575-7318, xxw005@uark.edu, and Kaiming Ye, Department of Biomedical Engineering, University of Arkansas, Fayetteville, AR 72701

Production of sufficient numbers of pancreatic endocrine cells that function similarly to primary islets is the premise of cell therapies for diabetes. To characterize the differentiation of embryonic stem (ES) cells into insulin-producing cell clusters (IPCCs) in three-dimensional (3D) environments, we cultured mouse ES Cells within collagen scaffolds and four-step differentiation protocol was developed. The cell differentiation was determined by the expression of a variety of islet-specific markers. Our data indicate that ES cells differentiated within 3D scaffolds and embryoid bodies (EBs) formed were similar to those in traditional two-dimensional (2D) cultures; however, unlike 2D differentiation, these EBs appeared embedded in a network of extracellular matrix and their sizes are more uniform. Most significantly, the differentiation of ES cells into IPCC on 3D collagen scaffolds gives rise to cells displaying morphological and functional activities characteristic of islets, which may provide a potential source of differentiated cells for the diabetes treatment.

BIOT 35 - A novel method to control differentiating human embryonic stem cell (hESC) aggregate size and composition: Effects on cardiomyocyte development

Céline L Bauwens[1], **Raheem Peerani**[1], Sylvia Niebruegge[1], Karolina Kolodziejska[2], Mansoor Husain[2], and Peter W Zandstra[1]. (1) Institute of Biomedical and Biomaterials Engineering, University of Toronto, Toronto, ON M5S 3E1, Canada, cbauwens@gmail.com, rpeerani@rogers.com, (2) Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON M5G 2C4, Canada

The effect of ESC differentiation methodology on cardiomyocyte yield is largely unexplored. Typically, hEBs are formed by dissociating attached hESC colonies into cell clumps and culturing these clumps in suspension. Using this method we achieve inefficient cell expansion and differentiation, with poor reproducibility due to large EB size distributions and variations in average EB size between runs. To resolve these issues, we have developed a micro-contact printing-based system for the robust generation of uniform size-controlled aggregates from size-controlled hESC colonies. Flow cytometric analysis of Oct4 reveals a decrease in expression in smaller colonies and either maintained or elevated expression in larger colonies. Quantitative RT-PCR has demonstrated upregulation of cardiac-associated

genes including Nkx2.5, Troponin-T and alpha-Actinin in patterned aggregates. Patterned aggregates exhibit spontaneous electrical activity as demonstrated by the appearance of contracting areas and optical mapping of membrane potential. Ongoing studies focus on the mechanism behind aggregate-size influenced cardiomyocyte yield.

BIOT 36 - Serum-free expansion of embryonic stem cells in a bioreactor for tissue engineering applications

Daniel E. Kehoe[1], Lye T. Lock[2], and Emmanuel S. Tzanakakis[2]. (1) Chemical and Biological Engineering, State University of New York at Buffalo, 910 Furnas Hall, Amherst, NY 14260, (2) Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, Buffalo, NY 14260

Large-scale production of embryonic stem cells (ESCs) will be essential for the realization of stem cell-based clinical applications. Expansion of mouse ESCs in stirred-tank bioreactors culture was recently demonstrated. However, the use of serum with its high compositional variability, cost and the possible presence of pathogens is problematic for large-scale ESC cultures. We investigated the use of serum-free medium for the propagation of ESCs in bioreactors. The growth rate of ESCs cultured in a stirred suspension with serum-free medium was constant and comparable to that of ESCs grown in dishes. An average cumulative increase of more than $2x10^5$-fold/2 weeks in live cell concentration was observed with above 85% cell viability. Notably, ESC pluripotency was not affected. Compared to static culture ESCs, bioreactor ESCs exhibited similar expression levels of stem cell genes and proteins. These cells were successfully directed to progeny expressing markers of neuronal cells, cardiac muscle and endoderm.

BIOT 37 - Hypoxic effect on hematopoietic development in human embryonic stem cell-derived embryoid bodies

C. M. Cameron, Department of Chemical Engineering and Materials Science, University of Minnesota, Twin Cities, 421 Washington Avenue SE, Minneapolis, MN 55455, cameron@cems.umn.edu, Wei-Shou Hu, Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455, and Dan S. Kaufman, Stem Cell Institute, Department of Medicine, University of Minnesota, Twin Cities, Minneapolis, MN 55455

Human embryonic stem cells (hESCs) provide an ideal resource to understand the earliest stages of blood cell formation. Translation to clinical applications will rely on the ability to control hESC expansion and differentiation in scalable and defined conditions. HESCs are capable of differentiating into mature hematopoietic cells through the formation of embryoid bodies (EBs). We have employed both static conditions and a closed stirred bioreactor to more effectively evaluate the effect of oxygen tension on EB-mediated survival, proliferation and differentiation. Here we show that exposing hESC-derived EBs to hypoxic conditions (1% and 5% oxygen) stimulates a transient accumulation of HIF-1α and HIF target genes, VEGF and GLUT1. Low oxygen conditions lead to increased apoptosis and decreased proliferation of cells in the EBs. However, the onset and duration of hypoxic exposure did not affect the outcome of hematopoietic differentiation nor modulate differentiation toward any one specific cell lineage or germ layer.

SUNDAY AFTERNOON

2:00 – 4:15 pm BCEC 107 B

Downstream Processing: Primary Recovery - Membranes and Other Technologies

D. J. Roush and Y. Lu, Organizers Papers 38-43

BIOT 38 - Engineering the adsorption of plasmid DNA

William J Kelly, Department of Chemical Engineering, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, william.j.kelly@villanova.edu, and Paul Butts, Chemical Engineering Department, Villanova University, Villanova, PA 19085-1681

The production of plasmid DNA (pDNA) for use in DNA vaccines and gene therapy is limited by the high costs of the process, especially the isolation and purification steps. The separation of the pDNA from the genomic DNA (gDNA) is a significant challenge, because of the chemical similarity of the molecules. In this study, the feasibility of engineering pDNA to contain a triplex forming region that allows for selective binding to some relatively inexpensive hydrophobic adsorbents is evaluated. Preliminary results have identified conditions (pH and ionic strength) that encourage binding of the pDNA while discouraging binding of gDNA.

BIOT 39 - Creating a robust and reproducible recovery process using centrifugation for the recovery of a soluble fusion protein in Saccharomyces cerevisiae

Kurt Herzog, John Barringer, and Ciaran Brady, Human Genome Sciences, 14200 Shady Grove Road, Rockville, MD 20850, Kurt_Herzog@hgsi.com

A fed-batch high cell density process was developed for clinical production of a recombinant therapeutic fusion protein in Saccharomyces cerevisiae. Harvest of the product-containing supernatant was achieved by Tangential Flow Filtration (TFF). Upon scale-up of the process to 750 L production scale in the cGMP clinical production suite, robustness and reproducibility issues were observed with the TFF harvest operation. Continuous centrifugation was investigated and implemented into the process in order to improve reproducibility, robustness, and yields for future production.

BIOT 40 - Ideal membrane cascades for downstream processing

Edwin N. Lightfoot and J. L. O'Dell, Department of Chemical and Biological Engineering, University of Wisconsin, 1415 Engineering Drive, Madison, WI 53706-1691, Fax: 608-262-5434, enlightf@wisc.edu

It is suggested here that properly designed counterflow cascades of permselective membranes can compete successfully with existing downstream processing techniques in a significant number of applications. They can produce much higher separation factors than the very simple configurations that still dominate membrane based separations. This claim will be supported using a combination of experimental data and process simulation. Effectiveness of HPTFF can be substantially increased by modifying individual modules and by incorporating them into an efficient counterflow cascade. Such cascades can be operated either batchwise or continuously. A primary practical problem of implementing such a process scheme in practice is solvent management, and strategies for dealing with this problem will be discussed. A major advantage of such a new technology is its relative insensitivity to diffusional limitations which become ever more stringent as we deal with larger molecules and even more complex moieties such as viruses, organelles and whole cells.

BIOT 41 - Optimization of cell culture harvest clarification using an ultrascale down model for centrifugation

Manoj Menon, **Andrea Hamilton**, and Frank Riske, Purification Development, Genzyme Corporation, 45 New York Avenue, Framingham, MA 01710, manoj.menon@genzyme.com, andrea.hamilton@genzyme.com

Recent work from our lab has shown that the use of flocculants, such as chitosan, can dramatically improve the efficiency of cell culture harvest clarification by continuous centrifugation. This presentation will focus on the effect of harvest characteristics (solid content and particle size distribution), flocculant (type, amount, and treatment time) and processing parameters (feed flow and shear) on the efficiency of clarification by centrifugation. An ultra-scale down model for centrifugation will be used to evaluate the efficiency of centrifugation. In this model, a shearing device is used to first expose a small volume (~30 mL) of flocculated cell culture harvest to shear levels typically observed in a continuous disk stack centrifuge. The sheared material is then centrifuged in a laboratory centrifuge and supernatant clarity is measured. The utility of the ultra-scale down model will be evaluated by comparing scale-down data to that obtained in a pilot-scale continuous disk-stack centrifuge.

BIOT 42 - Effect of solid ejection size on continuous centrifugation during mammalian cell culture product recovery

Xiaoyang Zhao and Joe Zhou, Process Development, Amgen, Inc, One Amgen Center Drive, M/S 18S-1-A, Thousand Oaks, CA 91362, xzhao@amgen.com

In recent years, monoclonal antibody expression levels in mammalian cell culture have been increased significantly. In the meantime, the cell culture clarification experienced more challenges mainly due to the increased cell mass and overall solid content in cell culture. Thus, as a preferred primary clarification technology, the continuous centrifugation is evolved rapidly to meet the challenges posted by high density cell culture harvest.
In this presentation, we will discuss the observation of high turbidity spike at the beginning of each centrifugation cycle during our development runs. We will also present the possible causes and the impact of these spikes to overall centrifugation and depth filtration clarification performance, as well as the mitigation strategies during process scale-up. With increased understanding of continuous centrifuge clarification and the arrival of very high density cell culture, an alternative solid ejection strategy is emerging.

BIOT 43 - Case study: Optimization of harvest stream clarification steps of a commercially-relevant fusion protein mammalian cell culture using charged depth filters

Ronald Bates, Junfen Ma, and Colleen Mason, Bioprocess Development, Bristol-Myers Squibb Company, 6000 Thompson Road, East Syracuse, NY 13057, ronald.bates@bms.com

Charged depth filters are widely used in the biopharmaceutical industry for primary and intermediate recovery steps following centrifugation or microfiltration (MF). By combining mechanical sieving and electrostatic adsorption, charged depth filters remove particles and debris as well as process-related impurities, such as host cell proteins (HCP) and DNA, which reduces the impurity burden on the downstream process. This paper evaluates depth filtration as a post-MF clarification step. Several commercially available depth filters were evaluated for filtrate turbidity, differential pressure, filtration capacity (L/m2) and product recovery. In addition, several 0.2 micron filters were evaluated with respect to capacity following depth filtration. The combined depth and polishing filtration steps were optimized with respect to throughput, impurity (host cell protein and DNA) clearance, product recovery and flush volumes. The optimized steps were then scaled up to manufacturing scale.

SUNDAY AFTERNOON

2:00 – 4:15 pm BCEC 106

Biophysical and Biomolecular Symposium: Protein Folding & Characterization

K. Mallela and T. Cellmer, Organizers
Papers 44-49

BIOT 44 - All-atom computer simulation of amyloid fibrils disaggregation

Chuck Tan, Hai-Feng Chen, and **Ray Luo**, Department of Molecular Biology and Biochemistry, UC-IRVINE, Irvine, CA 92697-3900, Fax: 9498248551, rluo@uci.edu

Amyloid-like fibrils are found in many fatal diseases, including Alzheimer's disease, type II diabetes mellitus, and the transmissible spongiform encephalopathies, and prion diseases. The kinetics of fibril formation is still hotly debated and remains an important open question. In this study, we have utilized the GNNQQNY crystal structure and high-temperature molecular dynamics simulation in explicit water to understand its aggregation mechanisms. A GNNQQNY hexamer model based on the crystal structure is adopted in our analysis. We have used both free energy landscape and kinetics analyses to study the high-temperature disaggregation process. The important findings from this work are: (a) dimer is not a thermodynamically stable state, (b) disaggregation of fibrils is more difficult than aggregation, (c) tetramer is found to be an intermediate state, but only meta-stable, and (d) trimer and pentamer are the transition states.

BIOT 45 - Dynamics of intramolecular contact formation in islet amyloid polypeptide (IAPP)

Sara Vaiana[1], William A. Eaton[2], Rodolfo Ghirlando[3], and James Hofrichter[2]. (1) Laboratory of Chemical Physics, NIDDK/NIH, 9000 Rockville Pike, Bethesda, MD 20892, sarav@niddk.nih.gov, (2) Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD 20892-0520, (3) Laboratory of Molecular Biology, NIH, NIDDK, Bethesda

Human islet amyloid polypeptide (hIAPP) is co-secreted with insulin in pancreas beta-cells and forms amyloid deposits in typeII Diabetes. We use tryptophan triplet quenching by cystine to measure the rates of intramolecular end-to-end contact formation in hIAPP and rIAPP (non-aggregating variant) and compare them to the model peptide C(AGQ)9W. This allows us to probe how sequence and solvent composition affect intra-chain interactions. From viscosity-dependent measurements on rIAPP we determine the reaction-limited and diffusion-limited rates as a function of denaturant concentration and temperature. These reveal a chain collapse in aqueous solvent, resulting from attractive effective intra-chain interactions. Interestingly these introduce kinetic traps that significantly slow down end-to-end diffusion. Observed rates for hIAPP in high denaturant are faster than for rIAPP, indicating a more collapsed state or less stiff diffusive dynamics. Sedimentation equilibrium experiments prove that the peptide is a monomer in all conditions studied, so this is not due to aggregation.

BIOT 46 - Folding of the β-helix domain of P22 tailspike protein

Michelle L. Spatara, Christopher J. Roberts, and Anne Skaja Robinson, Department of Chemical Engineering, University of Delaware, 150 Academy Street, Newark, DE 19716, spatara@udel.edu

The large, homotrimeric tailspike protein of the P22 bacteriophage has been studied extensively as a model system for folding and assembly of multimeric proteins. The main structural element of the tailspike protein is an elongated β-helix, which forms a stable monomer in solution when expressed as an isolated domain. Correct formation of this monomer in the full-length protein is implicated in the partitioning between productive folding and aggregation. Thus, we are studying the *in vitro* process of β-helix formation in detail using this monomeric truncation of tailspike (residues 109-544) comprising the β-helix domain (bhx). Equilibrium and stopped-flow chemical denaturation and refolding experiments were used to investigate the kinetics, thermodynamics, and intermediates in bhx folding. Our results identify at least one intermediate that inhibits productive folding due to proline isomerization.

BIOT 47 - Folding and insertion of melittin in membrane mimics

Matthew R. Hartings[1], Harry B. Gray[1], and Jay R. Winkler[2]. (1) Beckman Institute, California Institute of Technology, Pasadena, CA 91125, hartings@caltech.edu, (2) Beckman Institute, California Insitute of Technology, Pasadena, CA 91125

The peptide toxin mellitin, the primary component of bee venom, forms stable structures in both aqueous and membrane environments. We have investigated the energetics of structure formation and membrane insertion for the wild-type peptide, which is a random coil in aqueous solution, as well as for several mutants. We have found that single point mutations in the primary sequence induce helical structure in the peptide, even in aqueous environments. Analysis of fluorescence energy transfer from a tryptophan donor to a nitrotyrosine acceptor in a selectively labeled mutant indicates that the peptide adopts a bent helical structure when inserted into lipid vesicles.

BIOT 48 - Understanding the molecular mechanism of Hsp90: The role of nucleotide hydrolysis in the chaperone cycle

Kristin A. Krukenberg, Program in Chemistry and Chemical Biology, University of California, San Francisco, 600 16th St, GH, MC 2280, San Francisco, CA 94158-2517, Fax: 415-476-1902, kkruken@msg.ucsf.edu, Friedrich Foerster, Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158-2330, Andrej Sali, Department of Biopharmaceutical Sciences, University of California, San Francisco, San Francisco, CA 94158-2330, and David A. Agard, Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94158-2517

Hsp90 is an essential eukaryotic chaperone whose function requires the ATPase activity of the N-terminal domain. Crystal structures of the bacterial Hsp90 homolog htpG (Shaiu et al, Cell, 2006) and the yeast Hsp90 (Ali et al, Nature, 2006) reveal large domain rearrangements between the nucleotide-free and the nucleotide-bound conformations, giving insight into the possible mechanisms of Hsp90 function. However, new questions are also raised. What conformations are present in solution, and how do these structural reorganizations relate to Hsp90 function? To answer these questions we are investigating the structure of htpG in solution using small angle X-ray scattering (SAXS). Using SAXS and newly developed molecular modeling methods, we determined novel conformations of htpG in solution, and demonstrated that multiple conformations are in equilibrium. These results provide important new insights into the role of nucleotide in modulating Hsp90 conformation and have important implications for the molecular mechanism and regulation of Hsp90.

BIOT 49 - Breaking the symmetry of a homodimeric enzyme: Engineering and characterization of monomeric disulfide isomerases

Silvia A. Arredondo, Chemical Engineering, University of Texas at Austin, 1 University Station Stop C0400, Austin, TX 78712, silvia@che.utexas.edu, and George Georgiou, Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712

Protein disulfide isomerases are ubiquitous proteins that catalyze the proper formation of disulfide bonds during oxidative protein folding. The bacterial protein disulfide isomerase DsbC is a homodimeric V-shaped enzyme that consists of a dimerization domain, two α-helical linkers and two opposing thioredoxin fold catalytic domains. The functional significance of the two opposing catalytic sites is not well understood yet. To address this problem we fused the two polypeptides that compose the DsbC homodimer into a single protein. The monomeric DsbC (mDsbC) was shown to exhibit high catalytic activity. Mutants containing only one active site or catalytic domain were constructed and their catalytic properties, including their reduction by the membrane bound electron shuttle protein DsbD, resistance to oxidation by DsbB and, their ability to serve as a thiol oxidant and as a catalyst of disulfide bond rearrangement, were studied in detail and will be discussed. Apart from helping understand the catalytic cycle of disulfide isomerization, this study also provides insights onto the evolution of the eukaryotic enzymes (such as PDI) relative to their bacterial counterparts.

SUNDAY AFTERNOON

2:00 – 4:10 pm BCEC 107 C

Emerging Technologies: Nanobiotechnology

J. R. Cochran and L. Malmberg, Organizers Papers 50-55

BIOT 50 - Co-assembly of genetically-modified bacteriophages and inorganic nanoparticles into silica-stabilized nanoarchitectures

Carlee E Ashley[1], Eric C Carnes[1], Landon T White[1], Zhen Yuan[1], Darren R. Dunphy[2], Dimiter N Petsev[1], Plamen Atanassov[1], David Peabody[3], and C Jeffrey Brinker[2]. (1) Department of Chemical Engineering, University of New Mexico, Advanced Materials Laboratory, 1001 University Blvd. SE, Suite 100, Albuquerque, NM 87106, ceashley@unm.edu, (2) Sandia National Laboratories, Albuquerque, NM 87106, (3) Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, NM 87131

Due to their regular, symmetrical geometries, and the ease with which their protein capsids can be genetically engineered, bacteriophages are well suited for use as structure-directing agents in the spatially-defined self-assembly of inorganic nanoparticles within highly-ordered silica nanostructures. To this end, we have used phage display to engineer model icosahedral bacteriophages to express non-native peptides at precise surface locations that bind specific nanoparticles. We have used convective assembly, a deposition technique that enables rapid assembly of colloidal particles into mono- and multilayered structures, to self-assemble phage-nanoparticle conjugates within a silica nanostructure. Using a variety of characterization techniques, including x-ray scattering at a synchrotron source, we have demonstrated that phage concentration, deposition speed, and humidity can be controlled to tailor nanoparticle architectures within silica-stabilized phage nanostructures. Furthermore, we have patterned surfaces with alternating hydrophobic and hydrophilic regions; phage particles selectively wet hydrophilic regions, enabling us to ultimately construct patternable nanoparticle-based devices.

BIOT 51 - Biofabrication with genetically modified viral nanotemplates

Hyunmin Yi, Department of Chemical and Biological Engineering, Tufts University, 4 Colby St., Science & Technology Center Rm 147, Medford, MA 02155, Fax: 617-627-3991, hyunmin.yi@tufts.edu, Gary W. Rubloff, Department of Materials Science and Engineering, University of Maryland, College Park, College Park, MD 20742, Gregory F. Payne, Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742-4450, William E Bentley, Fischell Department of Bioengineering, Center for Biosystems Research, Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD 20742, and James N. Culver, Center of Biosystems Research, University of Maryland, Biotechnology Institute, College Park, MD 20742

Biological macro/supramolecules such as DNA, proteins and viruses hold great promise as templates for nanodevice fabrication due to the inherent advantages over inorganic materials such as selectivity, mild processing conditions and precise self-assembly mechanisms nature has provided. Despite recent developments in exploiting such advantages for various applications, reliable fabrication of nanodevices remains challenging due to the lack of facile assembly techniques that allow high spatial and orientational control. To address this we have developed biochemically driven patterned assembly of potent viral nanotemplates onto inorganic substrates. Specifically, Tobacco Mosaic Viruses that serve as nanotemplates via a genetically inserted cysteine on each coat protein were partially stripped and assembled onto capture DNA surfaces through hybridization with its own genomic RNA. Fluorescence and SEM images show high spatial, orientational and sequence specificity as well as stability. Simultaneous assembly of multiple TMVs onto microarray platforms via linker DNA hybridization based programming is also demonstrated.

BIOT 52 - Engineering stability and self-assembly in a caged protein scaffold

Sierin Lim, Merce Dalmau Mallorqui, Cesar I. Ruiz, and **Szu-Wen Wang**, Department of Chemical Engineering and Materials Science, University of California, Irvine, 916 Engineering Tower, Irvine, CA 92697-2575, wangsw@uci.edu

Due to the ability to precisely modify the architecture of protein structures, biological scaffolds are attractive templates upon which to build novel functions. The E2 subunit of pyruvate

dehydrogenase from *Bacillus stearothermophilus* is a protein complex which self-assembles to form a hollow 25-nm capsid-like structure. We have demonstrated that while this structure is unusually stable and amenable to modifications that are important for targeted delivery, it can also be engineered to enable reversible, triggered assembly. Using a synthetic gene optimized for *E. coli* expression, we can genetically modify the protein and generate nanocapsule templates. Physicochemical alterations to both the internal cavity and the outer surface show that the scaffold is remarkably tolerant of changes, with stability remaining up to approximately 80°C for the variants which we have investigated. Defined modifications at the inter-subunit interface have yielded nanocapsules that are stable at physiological pH but aggregate at low pH.

BIOT 53 - Nanoscale reservoirs for spatially and temporally controlled biointerfaces

Nick A. Melosh, Jules J. VanDersarl, Elizabeth Hagar-Barnard, and Erhan Yenilmez, Department of Materials Science and Engineering, Stanford University, 476 Lomita Mall, Stanford, CA 94305, nmelosh@stanford.edu

Directing the behavior of biological cells using nanoscale materials and devices has great promise for improved control and understanding of biological processes. In particular, translation of electronic stimuli into biochemical signals promises great improvements in our understanding of cell behavior by providing precise spatial and temporal signal localization. We report the fabrication and testing of a system of electrically addressable nano-scale reservoirs that can selectively release biological signaling molecules with spatial control of ~200 nanometers. This system is comprised of an array of hemispherical nano-scale cavities within a silicon chip, each of which can hold a specific signaling molecule within it. Different molecules can be held in each reservoir via a nanoscale electrochemical sealing process. Upon application of an electrical pulse, the signal is released from the reservoir. Release times are controlled by the geometry of the reservoir, and can vary from ~25-1000 ms. These devices can be arrayed such that multiple reservoirs could fit under a single cell, enabling site-selective stimulation. This chip represents a means to translate between electronics and the chemical communication of cells, allowing direct investigation of the effects of signal delivery time, location, and composition upon cell behavior.

BIOT 54 - Biological fabrication of nanostructured silicon-germanium photonic crystals possessing unique photoluminescent and electroluminescent properties

Gregory L. Rorrer[1], Clayton Jeffryes[1], DooHyoung Lee[1], Tian Qin[1], Chih-hung Chang[1], Timothy Gutu[2], Jun Jiao[2], and Raj Solanki[2]. (1) Department of Chemical Engineering, Oregon State University, 102 Gleeson Hall, Corvallis, OR 97331, Fax: 541-737-4600, rorrergl@engr.orst.edu, (2) Department of Physics, Portland State University, Portland, OR 97207

Diatoms are single-celled algae that make amorphous silica shells called frustules that possess intricately patterned, nanoscale features. We harnessed the biomineralization capacity of diatoms to biologically fabricate Si-Ge oxide nanoparticles that were self-assembled into frustules consisting of pore arrays that strikingly resembled 2D photonic crystals. Specifically, a two-stage photobioreactor cultivation process was used to metabolically insert germanium into the biosilica matrix of the living diatom cell. The frustules were then isolated by hydrogen peroxide treatment. Metabolic insertion of germanium altered the frustule self-assembly process. In the case of the diatom Pinnularia, the metabolic insertion of 0.25-1.0 wt% germanium into the frustule biosilica tuned the pore diameter to 150 nm, whereas in Nitzschia, the pore arrays fused into 100 nm slits. STEM-EDS analysis revealed that the germanium was uniformly distributed into the biosilica. However, thermal annealing of the frustules in 5% H2 gas at 900 C reduced the germanium oxides to 3-5 nm Ge nanocrystals imbedded in silica. Subsequent photoluminescence (PL) analysis revealed that the diatom frustules possessed strong blue photoluminescence, where the blue shift and PL intensity were controlled by the biological fabrication process. The diatom frustules were also fabricated into an electroluminescent (EL) device. The EL spectrum revealed three near-lasing signals at 340, 360, and 380 nm, as well as a band gap in the 500-620 nm range. This work suggests that living cells can biologically fabricate metal oxide photonic crystals that possess unique PL and EL properties on a massively parallel scale.

BIOT 55 - Biologically programmed synthesis of hybrid semiconductor nanocrystals

Shailendra Singh, Nosang Myung, Ashok Mulchandani, and Wilfred Chen, Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, CA 92521, ssing007@student.ucr.edu

Type II-VI semiconductor nanocrystals (e.g., ZnS, CdS, CdSe, CdTe) have been shown to possess unique optical, electrical and optoelectronic properties for a wide range of applications and devices fabrication. More importantly, the spectral properties of these semiconductor nanocrystals can be controlled effectively by tuning their sizes, compositions, and phases. Among different quantum dot materials, ZnS-shelled CdSe QDs have played a particularly important role because high-quality quantum dots with narrow emission bands can be created that span the whole optical spectrum. One of the most successful chemical routes for the synthesis of CdSe nanocrytals is based on high temperature organicmetallic process followed by a size-selective precipitation step. This approach, however, possesses several intrinsic disadvantages because of the high-temperature and extremely toxic, expensive, unstable, and explosive raw materials. Here we report for the first time a green chemistry room temperature method for core-shell semiconductor CdSe/ZnS quantum dot synthesis with a control over size and crystallinity. A bipeptide fusion selected by phage display for bulk semiconductor binding was used to achieve the goal. Highly monodisperse and photoluminescent nanocrystals were obtained, having an average size of 6 nm as observed under TEM. Nanoparticles had a defect free crystallinity as seen under HRTEM. Further we will report a one step purification of such synthesized quantum dots using Elastin Like Protein. The results are significant as this is the first report of core-shell semiconductor nanoparticle green chemistry room temperature synthesis using biological templates.

SUNDAY AFTERNOON

2:00 – 4:20 pm BCEC 109 B

Commercialization of Biologics: Case Studies: Post-Approval Changes & Comparability Challenges

S. Lee and V. Paradkar, Presiding Papers 56 - 61

BIOT 56 - Post approval changes for a commercial process: An historical look

Carole Heath, Amgen, 1201 Amgen Court West, Seattle, WA 98119-3105, and Hassan Madani, Purification Process Development, Amgen Corporation, Seattle, WA 98177

Although Enbrel® (etanercept) was approved nearly nine years ago, interest in its history remains strong because of the series of post-approval changes that have been filed with regulatory agencies. To meet the growing market demand since licensure, applications have included modifications to increase yield and improve robustness, as well as to add manufacturing sites (five to date). Key considerations throughout this life time have been to show process consistency with scale-up and transfer and to demonstrate product comparability. This talk will cover some of the process modifications and their rationales, as well as the strategy for rapid filing and approval at multiple manufacturing sites.

BIOT 57 - Case study: Downstream purification support of a cell culture raw material change for a licensed mAb product

Frank Maslanka, Purification Technology, Global Biologics Supply Chain, LLC/Centocor, 200 Great Valley Parkway, Malvern, PA 19355, fmaslank@cntus.jnj.com

International regulatory climate and supply chain pressures may result in the need to change a cell culture raw material. A change in media raw material, in turn, can impact the growth and productivity of the cells as well as the characteristics of the product produced by the cells. When the switch from bovine insulin to recombinant human insulin in the production media of an approved monoclonal antibody product was evaluated, various studies were performed to assess the comparability of the monoclonal antibody product produced from bioreactors using either bovine or recombinant human insulin. Both laboratory scale and manufacturing scale studies were used to show that the monoclonal antibody produced using recombinant human insulin was comparable to that produced using bovine insulin.

BIOT 58 - Rituximab post approval changes and comparability assessments

Robert Kiss[1], Srikanth R. Chary[1], Steven Meier[1], Jason C. Goodrick[1], and Dana C. Andersen[2]. (1) Process Development (LSCC), Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, kiss.robert@gene.com, (2) Late Stage Cell Culture, Bioprocess Development, Genentech, Inc, South San Francisco, CA 94080

Rituximab (Rituxan®) is a chimeric monoclonal antibody originally approved in the US for the treatment of Non-Hodgkins lymphoma in 1997. Market demand for the product has grown rapidly in the U.S. and the rest of the world since its approval, with 2005 U.S. sales exceeding $1.8B. The continued success of this product has triggered an ongoing need for expanded manufacturing capacity. This has been accomplished through a combination of process improvements and process transfers to additional manufacturing facilities. This presentation will review elements of the evolution of the rituximab manufacturing process as driven by factors including the need for increased supply, desires to enhance manufacturing safety and efficiency, and meeting emerging regulatory needs. The strategies followed in efficiently achieving increased manufacturing capacity will be described as will key lessons learned along the way in regards to process improvements, process transfers, and comparability assessments..

BIOT 59 - Demonstrating comparability for post approval CMC changes

Reed Harris, Late Stage Analytical Development, Genentech, 1 DNA Way, South San Francisco, CA 94080-4990, harris.reed@GENE.COM

Genentech and its partners have demonstrated post-approval comparability for numerous manufacturing site transfers, process changes, and new formulations. Guidelines have been developed for determining quantitative and qualitative acceptance criteria. A decision tree for physicochemical, biological, non-clinical, and/or clinical assessments has also been developed. Comparability case studies will be presented, including examples where physicochemical analyses were supplemented with in-vivo studies.

BIOT 60 - Comparability protocol to support a chromatography resin change

Peter J. Kramer, Manufacturing Sciences, Bayer HealthCare, LLC, 800 Dwight Way, Berkeley, CA 94701-1986, Fax: 510-705-4710, peter.kramer.b@bayer.com, Rob Frankenberg, Project Management, Bayer HealthCare, LLC, Berkeley, CA 94701, Ramadas Bhat, Quality Assurance, Purification and Plasma, Bayer HealthCare, LLC, Berkeley, CA 94701, Parris Burd, Regulatory Affairs, Bayer HealthCare, LLC, Berkeley, CA 94701, and Andrew Hetherington, Technical Operations, Purification and Plasma, Bayer HealthCare, LLC, Berkeley, CA 94701

Comparability protocol to support a chromatography resin change
Suppliers are subjected to many of the same regulatory pressures to upgrade manufacturing practices as are experienced by biopharmaceutical manufacturers. In the world of biological manufacturing; however, improved manufacturing practices at a supplier must not affect the critical process parameters or product quality attributes when the supplied material is introduced into a licensed pharmaceutical manufacturing process. This talk will discuss the complexity of assuring that a chromatography resin manufactured using an improved manufacturing process had no impact on the process or product purified using the new resin. All

stages of the project, from planning through final implementation will be discussed.

BIOT 61 - Investigation of an ultrafiltration yield problem for a conjugate vaccine

Shwu-Maan Lee, Bob Kruse, Amy Robinson, John Davis, and Chris Donaldson, Baxter Healthcare Corporation, 12140 Indian Creek Court, Beltsville, MD 20705, LEES4@BAXTER.COM

The meningitis vaccine (NeisVac-C) is comprised of the de-O-acetylated form of the group C meningococcal polysaccharide conjugated to tetanus toxoid. The polysaccharide purification process includes base treatment and subsequent diafiltration to remove hydrolysed cell impurities. During development, a 100K ultrafiltration membrane was used satisfactorily. Unfortunately, the yield from the first 12 production lots varied from 18 to 100%. Investigation revealed that the ultrafiltration membrane porosity varied with different manufacturer's lots. The corrective action included changing the membrane from 100K to 50K, just prior to our license application. To support the change, we spiked a lab-scale process stream with 200% of the cell impurities and tested the "tightest" 50K membrane available. Even under the worst-case conditions, the impurities were removed to meet the original specifications achieved by the 100K membrane. The change was incorporated into the license application and over 100 lots have since been produced with satisfactory yield and purity.

SUNDAY AFTERNOON

4:30 – 5:30 pm BCEC 107 B

Perlman Lecture W. Zhou,

Organizer Paper 62

BIOT 62 - Prion proteins and how they fold

Susan Lindquist, Whitehead Institute for Biomedical Research & Dept. of Biology, Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA 02142, lindquist_admin@wi.mit.edu

Proteins must fold into just the right shape to do their jobs. When proteins misfold, they are usually degraded by vigilant cellular quality-control mechanisms. However, some misfolded proteins persist in their altered shapes. In humans, the consequences can be deadly, leading to devastating illnesses such as Alzheimer's and Parkinson's diseases. In the special case of prion proteins, altered forms can template their change in shape to other proteins of the same type, leading to transmissible spongiform encephalopathies like "mad cow." In yeast cells, however, prions are not toxic, but can actually provide helpful new phenotypes. For example, one yeast prion is formed from an inactive, misfolded translation-termination factor. The altered form is passed from mother cells to daughters, acting as a "seed" to perpetuate the prion state. This results in changes in gene expression and many new phenotypes. Remarkably, in collaboration with Eric Kandel and Kausik Si, we have also found that a protein that plays an important role in synaptic plasticity behaves as a prion in yeast, and postulate that the self-perpetuating folding of its prion domain can act as a molecular memory. In both cases the prion is an amyloid, difficult to study by conventional methods. We are devising a variety of new tools to determine how prion proteins fold and template their own conformations.

Program by Day

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

MONDAY MORNING

8:00 - 10:55 am	Emerging Technologies: Nanobiotechnology	J. R. Cochran and L. Malmberg, Organizers	Papers 63 - 70	BCEC 108
8:00 - 11:00 am	Downstream Processing: Advances in Chromatography	A. M. Lenhoff and S. Ghose, Organizers	Papers 71 - 78	BCEC 107 B
8:00 - 10:15 am	Upstream Processing: Advances in Microbial Fermentation Process Development	D. Ren, M. Laird, and X. Yang, Organizers	Papers 79 - 84	BCEC 106
8:00 - 10:55 am	Upstream Processing: Advances in Biocatalysis	P. C. Cirino and L. Sun, Organizers	Papers 85 - 92	BCEC 107 C
11:30 - 12:30 pm	Industrial Biotechnology Award Lecture	W. Zhou, Organizer	Paper 93	BCEC 107 B

MONDAY AFTERNOON

2:00 - 3:00 pm	Industrial Keynote Symposium	W. Chen, Organizer, Presiding	Paper 117	BCEC 107 B
3:00 - 5:00 pm	Stem Cells: Engineering the Embryonic and Adult Stem Cell Niche	A. Khademhosseini and B. Rao, Organizers	Papers 94 - 99	BCEC 108
3:00 - 5:20 pm	Downstream Processing: Bioprocess Integration & Industrial Case-Studies	M. R. Ladisch and C. Liu, Organizers	Papers 100 - 105	BCEC 107 B
3:00 - 5:35	Upstream Processing: Advances in Microbial Fermentation Process Development	X. Yang, M. Laird, and D. Ren, Organizers	Papers 106 - 110	BCEC 106
3:00 - 5:20 pm	Commercialization of Biologics: Data Management & Process Modeling in Support of Commercial Processes	J. Prior and S. Ahuja, Organizers	Papers 111 - 116	BCEC 107 C

MONDAY EVENING

6:00 – 10:00pm	Exec. Comm. Meeting	
8:00 – 10:00pm	Sci-Mix Posters	

MONDAY MORNING

8:00 – 10:55 am BCEC 108

Emerging Technologies: Nanobiotechnology J. R. Cochran and L. Malmberg, Organizers Papers 63 – 70

BIOT 63 - Inhibiting bacterial biofilm formation by self-assembled monolayers of functional alkanethiols on gold

Shuyu Hou[1], Erik A Burton[2], Karen A. Simon[2], Dustin Blodgett[1], Yan-Yeung Luk[3], and Dacheng **Ren**[1]. (1) Biomedical and Chemical Engineering, Syracuse University, 121 Link Hall, Syracuse, NY 13244, dren@syr.edu, dren@syr.edu, (2) Department of Chemistry, Syracuse University, Syracuse, NY 13244, (3) Department of Chemistry; Department of Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY 13244

Bacterial biofilms cause serious problems, such as antibiotic resistance and medical-device-related infections. To further understand bacteria-surface interactions and to develop efficient control strategies, self-assembled monolayers (SAMs) of alkanethiols presenting different functional groups on gold films were analyzed for their resistance to biofilm formation. *Escherichia coli* was labeled with green florescence protein and its biofilm formation on SAM-modified surfaces was monitored by confocal laser scanning microscopy (CLSM). The three dimensional structure of biofilm was analyzed with the COMSTAT software to get information about biofilm thickness and surface coverage. The SAM presenting methyl, L-gulonamide (a sugar alcohol tethered with an amide bond) and tri(ethylene glycol) (TEG) groups were tested. Among these, TEG was the most resistant to *E. coli* biofilm formation; e.g., it repressed the biofilm formation of DH5α by 99.5±0.1% for one day compared to bare gold surface. By patterning surfaces with regions consisting of methyl-terminated SAMs surrounded by TEG-terminated SAMs, *E. coli* formed biofilms only on methyl-terminated patterns. Addition of TEG free molecules to growth medium at 0.1 and 1% also inhibited biofilm formation, while TEG at concentrations up to 1.5% did not have any noticeable effects on cell growth. The mechanistic study suggests that the reduction of biofilm formation on surfaces modified with TEG-terminated SAMs is a result of multiple factors, including the solvent structure at the interface, the chemorepellent nature of TEG, and inhibitory effect of TEG on cell motility.

BIOT 64 - Development of electrospun scaffolds for application to medical devices

R. Delaporte, H. Yesilalan, R. Skowyra, and **Gary Cadd**, Organogenesis, Inc, 150 Dan Road, Canton, MA 02021, GCadd@Organo.com

Recent breakthroughs in the area of electroprocessing have allowed – for the first time – the recapitulation of the extracellular matrix using collagen and other ECM proteins. Electroprocessing solutions of collagen produce dry non-woven mats of material containing fibers that closely mimic those found in the body. Injection of collagen into a charged electric field causes the collagen fibers to align in the staggered array seen in native systems; and is reflected in a banding pattern of 67 nm found in electrospun collagen nanofibers. Animal studies have shown collagen nano-scaffolds produced by electrospinning to be exceptionally well tolerated in vivo and may serve as a platform for delivery of cells or other therapeutics. Commercial application of electroprocessed collagen scaffolds to medical devices has been limited primarily by two factors: the strength of the resultant materials and the fluorinated alcohol solvents that must be used to electrospin proteins. Recent developments in our laboratory have allowed us to overcome these limitations. This presentation will focus on advances in the area of electroprocessed materials, including collagen, and the development work that is leading to the commercialization of this technology.

BIOT 65 - Polymersome encapsulated hemoglobin: A novel type of hemoglobin-based oxygen carrier

Andre F. Palmer, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, OH 43210, palmer.351@osu.edu

Hemoglobin (Hb) was encapsulated inside polymer vesicles (polymersomes) to form polymersome encapsulated Hb (PEH) dispersions. PEH particles are 100% surface PEGylated with longer PEG chains and possess thicker hydrophobic membranes compared to conventional liposomes. Polymersomes were self-assembled from poly(butadiene)-poly(ethylene glycol) (PBD-PEO) amphiphilic diblock copolymers with PBD-PEO molecular weights of 22-12.6, 5-2.3, 2.5-1.3, and 1.8-0.9 kDa. The first two diblock copolymers possessed linear hydrophobic PBD blocks, while the later possessed branched PBD blocks. The size distribution, Hb encapsulation efficiency, P-50, cooperativity coefficient, and methemoglobin (metHb) level of PEH dispersions were consistent with values required for efficient oxygen delivery in the systemic circulation. PEH dispersions exhibited average radii larger than 50 nm and exhibited oxygen affinities comparable to human erythrocytes. Polymersomes did not induce Hb oxidation. These results suggest that PEHs could serve as efficient oxygen therapeutics.

BIOT 66 - A novel, biomimetic hydrogel construct to repair the cornea: Molecular design and biological response

David Myung[1], Jennifer R. Cochran[2], Jaan Noolandi[3], Christopher N. Ta[3], and Curtis W. Frank[1]. (1) Department of Chemical Engineering, Stanford University, 381 North-South Mall, Stauffer III, Stanford, CA 94305, djmyung@stanford.edu, (2) Department of Bioengineering, Stanford University, Stanford, CA 94305, (3) Department of Ophthalmology, Stanford University, Stanford, CA 94305

Artificial corneas have potential to benefit millions worldwide who are blind due to corneal disease. However, the fabrication of polymers with the transparency, mechanical strength, nutrient permeability, and regenerative capacity of a human donor cornea remains a formidable challenge. We have developed an interpenetrating network hydrogel that incorporates these critical

properties in a single material. With water content (77%), tensile strength and modulus, and transparency that rival those of the natural cornea, this hydrogel is mechanically superior to conventional single network hydrogels because of strain-dependent, inter-network entanglements. Due to a sufficiently large mesh size, this hydrogel also facilitates ample transport of glucose, the essential nutrient for the cells of the cornea. Through application of photolithography and a photochemical surface modification technique, a "core-and-skirt" device prototype has been fabricated. The surface of the core component is tethered with biomolecules to promote epithelialization, while its periphery is patterned with an array of pores to enable stromal tissue integration. Ongoing work entails the optimization and evaluation of epithelialization and tissue integration of this device prototype toward the development of a sustainable artificial cornea.

BIOT 67 - Multifunctional titanium-binding peptides for rapid modification of implant surfaces

Xiaojuan Khoo, Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, MA 02215, xiao@bu.edu, Daniel J. Kenan, Department of Pathology, Duke University Medical Center, Durham, NC 27710, and Mark W. Grinstaff, Department of Chemistry, Boston University, Boston, MA 02215

Events that direct the integration and subsequent long-term performance of an implant take place largely at the tissue-implant interface. The development of an interfacial material with the ability to elicit the desired host response at this interface will have significant impact on a variety of biomedical applications. Towards this purpose, we have developed novel biomimetic peptides termed "Interfacial Biomaterials" (IFBMs) for rapid modification of implant surfaces. Peptide sequences with specific affinity to titanium ($K_{aff} \sim 10^5\ M^{-1}$) were identified using a phage display screening process. Real-time quartz crystal microbalance with dissipation (QCM-D) measurements revealed that IFBMs rapidly adsorbed ($t < 2$ min) onto titanium to form a thin (~ 2 nm) and rigid peptide film, in agreement with cross-sectional AFM profiles on mica. In addition, we demonstrate the utility of functionalized IFBMs (e.g. biotin or RGD terminated) in the controlled organization of biomolecules and cells on micropatterned titanium surfaces.

BIOT 68 - Effects of platinum and gold nanoparticles on osteoblast cells: A study of nanoparticles' cancer treatment potential

Yuan Sun[1], Daniel Katz[2], Tim Fraczak[1], **Nadine Pernodet**[1], **Miriam Rafailovich**[3], and **Jonathan Sokolov**[4]. (1) Department of Materials Science and Engineering, State University of New York at Stony Brook, Stony Brook, NY 11794, yuasun@ic.sunysb.edu, npernodet@notes.cc.sunysb.edu, (2) Hebrew Academy of the Five Towns and Rockaway, Cedarhurst, NY 11516, (3) Department of Materials Science and Engineering, State University of New York Stony Brook, Stony Brook, NY 11794, miriam.rafailovich@sunysb.edu, (4) Department of Materials Science and Engineering, State University of New York at Stony Brook, Stony Brook, NY 11794-2275, Fax: 631-632-8052, jonathan.sokolov@sunysb.edu

Platinum and gold nanoparticles are toxic to cells. However, if coated with specific targeting agents, the nanoparticle's destructive abilities can be utilized to destroy cancerous cells while leaving healthy cells unharmed. A simple, one-step method was first developed to synthesize platinum and gold nanoparticles coated with folic acid. Comparison experiments were then carried out in which healthy osteoblasts (Mc 3t3 cells) and cancerous osteoblasts (ros cells) were treated with the folate-coated particles. Confocal microscopy studies showed that the folate-coated particles destroyed the actin cytoskeleton of cancerous cells, causing the cells to swell and burst; while the particles had little noticeable effect on the healthy cells. Cancerous cells require exponentially greater amounts of folic acid than do normal cells. We believe that the folic acid not only serves as a particle stabilizer; it also becomes a targeting molecule as it hides the particles and thereby "tricks" the cancer cells into absorbing them.

BIOT 69 - Degradable nanoparticle gene carriers for efficient and versatile nonviral gene delivery

Shiwei Lu, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, shiwei.lu@case.edu, and **Young Jik Kwon**, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, young.kwon@case.edu

New acid-degradable cationic nanoparticles were synthesized using monomer-to-polymer approach which enabled highly flexible nanoparticle fabrication to achieve controlled size, surface charge, degradability, and conjugation with functional groups. The nanoparticles cause swelling and osmotic destabilization of the endosome to free them into the cytoplasm, while cationic branches holding anionic DNA are cleaved from the polymeric backbone of the nanoparticles and make plasmid DNA accessible for efficient gene expression. Structure of the nanoparticles, efficient release of plasmid DNA upon hydrolysis of the nanoparticles, and enhanced transfection efficiency at a very low DNA concentration was confirmed by nanoparticle characterizations and ex vivo studies. A selective uptake of the nanoparticles by phagocytic cells (e.g., macrophages) and non-phagocytic cells (e.g., fibroblasts) was also demonstrated, which implies tunable gene therapy and DNA vaccination using the nanoparticle system. Also results from pulmonary transfection promise a use of the nanoparticles for cyctic fibrosis gene therapy.

BIOT 70 - Assembly of PEI/DNA nanoparticles for gene delivery by microfluidic hydrodynamic focusing

Chee-Guan Koh[1], **Zhengzheng Fei**[1], Jingjiao Guan[2], Yubing Xie[2], and L. James Lee[3]. (1) Chemical and Biomolecular Engineering, Ohio State University, 140 W 19th Ave, Columbus, OH 43210, koh@chbmeng.ohio-state.edu, fei@chbmeng.ohio-state.edu, (2) NSF Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymer Biomedical Devices, Ohio State University, Columbus, OH 43212, (3) Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210

PEI/DNA nanoparticles has recently been used to make nonviral vectors for gene delivery. The conventional method of producing

this nanoparticles involve mixing the ingredients together in bulk followed by vortexing which result in large particle size, low cytotoxicity, and poor gene transfection at low N/P ratios whereas small particle size, high cytotoxicity, and better gene transfection at high N/P ratios. Recently, the use of microfluidic channels with hydrodynamic focusing has generated well-defined and enhanced diffusional mass-transfer at the interfacial region between two fluids between two fluids for forming liposome particles. In this study, we used a microfluidic hydrodynamic focusing (MHF) device to achieve small nanoparticle size with narrow size distribution, and lower cytotoxicity for more efficient and consistent in vitro and in vivo gene transfection. The nanoparticles were characterized by atomic force microscopy (AFM), dynamic light scattering, and zeta potential. We have successfully demonstrated that nanoparticles produced by MHF yielded better gene expression in addition to smaller nanoparticle size and narrower size distribution at N/P=3.3.

MONDAY MORNING

8:00 – 11:00 am BCEC 107 B

Downstream Processing: Advances in Chromatography A. M. Lenhoff and S. Ghose, Organizers Papers 71 - 78

BIOT 71 - Are high capacity and high selectivity mutually exclusive? New approaches to the chromatographic resin game

Jerry Rasmussen, 3M, Minneapolis, MN, jkrasmussen@mmm.com

Abstract not available.

BIOT 72 - Microstructure and retention properties of dextran-grafted agarose media

Harun Koku, Brian D Bowes, and Abraham M Lenhoff, Department of Chemical Engineering, University of Delaware, Newark, DE 19716

Stationary phases in which the base matrix is modified by addition of secondary polymers can differ significantly in their adsorption and transport properties from unmodified media. In this work, electron microscopy, confocal microscopy, chromatographic measurements, and additional techniques are employed to attempt to relate the chemical composition and microstructure of Sepharose XL to its chromatographic performance. This agarose-based adsorbent is modified with dextran, and a study of its structure and function compared to that of the dextran-free Sepharose FF is used to probe the role of the dextran. TEM and SEM images are used to examine the distribution of dextran within the XL media as well as regions of protein retention. Chromatographic characteristics that are correlated with these observations include retention characteristics, binding capacities, and thermodynamic driving forces. Confocal microscopy is used to examine the uptake rate, which has been reported previously to be anomalously fast under some conditions.

BIOT 73 - Use of displacement chromatography for proteomic applications

Steven Taylor Evans[1], Alexander S. Freed[1], Mark Platt[2], and Steven M. Cramer[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, evanss3@rpi.edu, (2) Core Director, Proteomics: Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY

Proteomics provides one of the most important bioanalytical challenges of our time. Displacement chromatography has significant potential for the simultaneous concentration and purification of complex biological mixtures. Model studies were carried out using high affinity, low molecular mass displacers for the ion exchange displacement chromatography of proteins and peptides to determine the limits of this approach. Parametric studies with various displacers, displacer concentrations, stationary phases, and mobile phase conditions were performed to optimize these difficult separations. Results indicate high affinity displacers employed at relatively low concentrations can effect high resolution separations amenable to the identification of low abundance solutes. Displacement ion exchange systems were then used in concert with nano-flow reversed phased liquid chromatography and mass spectrometry to evaluate the utility of this combined approach for the analysis of complex biological mixtures with a wide dynamic range of feed concentrations.

BIOT 74 - High performance chromatofocusing as a novel analytical method to support process comparability and product characterization

Xuezhen Kang[1], Joseph Kutzko[1], Michael Hayes[1], and Douglas Frey[2]. (1) Purification and Process Research, Genzyme Corporation, One the Mountain Rd, Framingham, MA 01701, xuezhen.kang@genzyme.com, (2) Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, Baltimore, MD 21228

Chromatofocusing separates proteins by taking advantage of the focusing effects of a retained pH gradient produced inside a weak anion- or cation-exchange chromatographic column. It was shown that proteins with closely related pI values can be separated and collected for further identification and characterization. Novel high performance chromatofocusing methods have been developed as a complimentary tool to IEF to support product characterization and process comparability. Novel buffer systems were also developed to replace polyampholytes to improve method ruggedness. The method can also be easily scaled up to semi-preparative scale. As a case study, a semi-preparative method was used to separate a monoclonal antibody into individual pI variants. Coupled with mass spectrometry, the method was used to identify the nature of the charge heterogeneity of monoclonal antibodies. Data will be presented on how chromatofocusing can effectively be used as a stability indicating analytical method to monitor deamidation.

BIOT 75 - Membrane adsorber technology for trace impurity removal applications

Michael W. Phillips[1], Christopher Teske[2], Rachel Specht[2], Mikhail Kozlov[3], and Wilson Moya[1]. (1) Research and Technology Development, Millipore Corporation, 80 Ashby Road, Bedford, MA 01730, Fax: 781-533-3143, michael_phillips@millipore.com, (2) Late Stage Purification, Genentech, South San Francisco, CA 94080, (3) Millipore Corporation, Bedford, MA 01730

For trace impurity removal applications, membrane adsorbers provide an attractive alternative technology to traditional bead-based chromatographic separations. Since the adsorption sites of membrane adsorbers are within the convective flow path of the fluid stream, mass transfer rates are often several-fold higher than with conventional porous beaded resins, allowing for high efficiency, high throughput separations.
Current membrane adsorbers are extremely effective in removing trace levels of nucleic acid, but have proven to be less effective in the removal of residual host cell protein and mammalian virus. A new membrane adsorber technology has been developed that eliminates many of these shortcomings. In this presentation, data are presented showing the robustness of this next generation membrane adsorber for removing trace levels of host cell protein and mammalian virus from monoclonal antibody feedstreams.

BIOT 76 - Validation of host cell protein removal in purification processes of biopharmaceutical manufacturing

Canping Jiang[1], Junfen Ma[1], Michael Rubacha[1], Steven Lee[2], and Abhinav A Shukla[1]. (1) Bioprocess Engineering, Bristol-Myers Squibb, PO BOX 4755, Syracuse, NY 13221, canping.jiang@bms.com, (2) Technical Operations, Bristol-Myers Squibb, East Syracuse, NY 13057

Purification of protein biopharmaceuticals often consists of multiple chromatography steps. Evaluating the capacity of each chromatography step for removal of process related impurities including host cell proteins (HCP) is essential in assurance of process consistency and product safety. Host cell derived proteins are a highly complex mixture of a variety of proteins. A common approach to demonstrating HCP clearance ability is to spike in high levels of HCP from upstream process steps and then demonstrate log clearance of these impurities as is done to demonstrate viral clearance ability. As will be detailed in this presentation, this approach can be erroneous as a large proportion of the HCPs cleared are not relevant to that particular downstream unit operation. This presentation will explore alternative strategies to demonstrate robustness of a downstream process for demonstrating clearance of relevant HCP species. Information obtained from process characterization studies will be used towards an improved approach to HCP clearance validation. A case study involving three chromatography steps will be presented.

BIOT 77 - Profiling metal binding *E. coli* proteins: Its use in purification process development

Rajaramesh Varakala[1], Ryan Haley[2], Ralph Henry[3], Mohammad M Ataai[4], and Robert R. Beitle[1]. (1) Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, AR 72701, Fax: 479-575-7926, rvaraka@uark.edu, (2) University of Arkansas, Department of Chemical Engineering, Fayetteville, AR 72701, (3) Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, (4) Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15261

Immobilized metal affinity chromatography (IMAC) is the most widely used technique for in purification of recombinant proteins. Although its use in bioseparation is pervasive, the true bottleneck with IMAC now lay with the presence of native *E. coli* proteins that show a high affinity for divalent cations like nickel, cobalt or zinc. Naturally occurring histidine and cysteine rich regions in the proteins of the host cell result in unwanted protein binding during the capture step. Profiling these host proteins which have affinity to IMAC columns will be helpful to understand and control contaminants that elute with target proteins.
We identified several proteins of *E. coli* bind to IMAC columns under different conditions. This data helps to choose best combination of support, metal and binding conditions for a target protein to be expressed. Selection of intermediate and polishing purification steps can be done based on the properties of contaminants to achieve high purities (e.g. 99.5 or 99.8%). A three fold strategy has been developed to improve purification processes: (1) mutation of genes corresponding to essential proteins, (2) deletion of genes corresponding to non essential proteins, and (3) rational affinity tail design to move the target elution into a region of less contaminant. During this presentation we will discuss the use of this information to design *E. coli* strains and use them during the expression of a recombinant protein.

BIOT 78 - Affinity adsorption of viruses using small peptide ligands

Caryn L Heldt[1], Patrick V. Gurgel[2], Lee-Ann Jaykus[3], and Ruben G Carbonell[1]. (1) Department of Chemical and Biomolecular Engineering, North Carolina State University, 911 Partner's Way, Raleigh, NC 27695, clheldt@ncsu.edu, (2) ProMetic Life Sciences, Mont-Royal, QC H4P 2L7, Canada, (3) Department of Food Science, North Carolina State University, Raleigh, NC 27695

The capture of viruses from complex sample matrices is necessary for many different applications, including the removal of viruses from process streams, concentration of viral vectors for gene therapy, and molecular surface detection for use in a sensor. Small peptides can be used to create specific recognition surfaces and are a novel approach for the capture of viruses. For example, peptide sequences as small as three amino acids in length have been identified for the capture of porcine parvovirus (PPV). These peptides have been used in chromatographic columns for removal of PPV from complex mixtures containing human blood plasma. This approach is a promising alternative to virus capture using antibodies because it eliminates the risk of viral contamination that may be found in antibody preparations. Furthermore, peptides are inexpensive to produce and more stable than antibodies when used in a continual processing and regeneration environment.

MONDAY MORNING

8:00 – 10:15 am BCEC 106

Upstream Processing: Advances in Microbial Fermentation Process Development D. Ren, M. Laird, and X. Yang, Organizers Papers 79 - 84

BIOT 79 - Alleviation of extracytoplasmic stress for improving cell physiology and recombinant protein production in Escherichia coli

C. Perry Chou, Department of Chemical Engineering, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1, Canada, cpchou@uwaterloo.ca

The advent of recombinant DNA technology has revolutionized the strategies for protein production. Escherichia coli is still the most common workhorse for recombinant protein production. Recently, targeting recombinant proteins in the extracytoplasmic compartment has gained much attention. Periplasm has a special environment suitable for the formation of eukaryotic proteins and an isolated compartment significantly facilitating downstream protein purification. On the other hand, displaying polypeptides on E. coli cell surface has been extensively studied due to its potential for various novel biotechnological and industrial applications. Nevertheless, targeting polypeptides in the extracytoplasmic compartment often induces local stresses, resulting in physiological deterioration and poor expression performance, and developing the strategies to overcome this technical hurdle warrants more systematic exploration. A prerequisite to physiological improvement is that cell physiology needs to be properly monitored. In the research area of recombinant protein production, cell physiology is recognized as a vague term generally in correlation with cell's health, viability, metabolic activity, and protein-producing ability. The presence of an excess amount of the foreign gene products and the environmental impact can impose physiological challenges on the protein-overproducing cells. The response to the extracytoplasmic stress is driven by the synthesis of a variety of stress-responsive proteins expressing protease and/or chaperone activities. As a practical application, these proteins can be used not only as 'sensors' for monitoring cell physiology but also as suppressors for alleviating physiological stress. In this presentation, alleviation of the extracytoplasmic stress to improve both cell physiology and recombinant protein production will be demonstrated as an exploration of the natural defense system for industrial applications. Monitoring the extracytoplasmic stress using the fusions of a selection of stress-responsive promoters with a reporter gene will be described in two cases of recombinant protein targeting in the periplasm and on the outer membrane.

BIOT 80 - Metabolic activities of novel inducible fermentations for recombinant therapeutic proteins

Mark Berge and Radu Georgescu, Cell Science and Technology, Amgen, One Amgen Center Drive, 18S-1-A, Thousand Oaks, CA 91320, Fax: 805-499-6819, mberge@amgen.com

Gaining an understanding of a fermentation process can take many forms, one of which is the study of metabolic process parameters. This study investigates the metabolic heat released with regards to oxygen uptake characteristics during the varied stages of fermentation processes used for the production of recombinant therapeutic proteins. This data set builds on previous metabolic heat determinations for batch processes, expanding the metabolic heat evolution data into fed and induction stages of the fermentation which have not been well represented in literature. The relationship of carbon consumption with regards to the oxygen consumption and heat evolution are also detailed.

BIOT 81 - Fast track first-in-human development of novel therapeutic proteins with a robust microbial fermentation platform

Xiaoming Yang, Process Development, Amgen Inc, 18S-1-A, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-499-6819, xyang@amgen.com, and Bob Pendleton, Cell Science & Technology, Amgen, Inc, thousand oaks, CA 91320

Traditionally process development for therapeutic proteins uses a "molecule-specific" approach. Recently mammalian platform technology for antibody production has been considered a major advancement to enable an accelerated First-In-Human (FIH) pipeline development. In addition to the mammalian platform, Amgen has also developed an integrated microbial platform technology producing therapeutic proteins for FIH. The upstream part of the platform consists of a productive E. coli host cell and expression system, and a scale-up ready fermentation process tailored to the physiological characteristics of the recombinant E. coli system. The upstream platform has been integrated into a downstream purification platform process that was designed to create a boundary of unit operations and conditions based on the diverse character of the class of Fc-fusion proteins.

We will report on the rationale for the platform design and provide selected molecule data comparing the platform approach to the previous "molecule-specific" approach. A case study demonstrates that the platform strategy significantly minimized the resources and time from the initiation of process development to GMP production.

BIOT 82 - Using the two population theory to design an inducer feeding schedule to maximize recombinant DNA protein expression in Escherichia coli

Jesus M Gonzalez and James T. Hsu, Department of Chemical Engineering, Lehigh University, 111 Research Drive, Bethlehem, PA 18015, jegh@lehigh.edu

Metabolic constraints during the production of recombinant DNA protein in Escherichia coli impede the efficient utilization of resources by the cells thus reducing their production potential. In order to minimize these adverse effects we have proposed to segregate the cells into two groups, one not induced growing at a high specific growth rate and rapidly contributing cells to the system, and the other fully induced, growing slowly but using the

cell machinery to express the target protein. An adequate balance between these two populations should maximize the protein expression in a given system. The segregation model is based on the "all or none" phenomenon previously described by other authors and in which at inducer sub saturated conditions the cells are either fully induced or fully un induced. Our experimental findings demonstrate that the cells used in this study exhibit such behavior. Based on this two population theory we have developed a mathematical model in which the cell culture in batch fermentations is segregated into two groups, one fully induced and the other not induced. The parameters that characterize the model were determined experimentally and used to simulate different induction strategies in which the fraction of induced cells was varied with time according to several pre-determined schemes. It was found that the linear increase of this fraction ending at maximum induction in the final fermentation time would give the best results. The slope of this line varies with the particular conditions of the fermentation and a method to determine this "best" slope is discussed. Finally these results were validated experimentally finding that the results closely match the mathematical simulation.

BIOT 83 - Mixture design for optimization of fermentation medium for cutinase production from Colleotrichum lindemuthianum

Vishal Shah, Department of Biology, Dowling College, Oakdale, NY 11769, ShahV@dowling.edu, and Fred J Rispoli, Department of Mathematics, Dowling College, Oakdale, NY 11769

Culture medium optimization is a critical step in the fermentation process development and often continues throughout the production life of the fermentation product. Two of the most widely used statistical experimental designs are Plackett-Burman design, and fractional factorial design. In the current study, we would describe the use of mixture design experiments towards optimization of medium for cutinase production from the fungi Colletotrichum lindemuthianum. In the first stage of optimization, we screened whether starch, glucose, ammonium sulfate, yeast extract, magnesium sulfate and potassium phosphate have any influence on cutinase production. In the experimental design, we imposed the constraints that exactly one factor must be omitted in each set of experiments and no factor can account for more than 1/3 of the mixture. Thirty different sets of experiments were designed. Results obtained showed that while starch is found to have negative influence on the production of the enzyme, yeast extract and potassium phosphate have a strong positive influence. Magnesium sulfate, ammonium sulfate and glucose have low positive influence on the enzyme production. In the second stage, we used mixture design experiments to find the optimal concentration of glucose, yeast extract and potassium phosphate for maximum enzyme production and develop a model that illustrates the interaction amongst the media ingredients.

MONDAY MORNING

8:00 – 10:55 am BCEC 107 C

Upstream Processing: Advances in Biocatalysis P. C. Cirino and L. Sun, Organizers

Papers 85 – 92

BIOT 84 - Efficient antibody production with suppressing O-Glycosylation in yeast

Kazuo Kobayashi[1], Kousuke Kuroda[1], Haruhiko Tsumura[1], Toshihiro Komeda[2], Yasunori Chiba[3], Yoshihumi Jigami[3], Kimihisa Ichikawa[4], Koichi Nonaka[5], and Takeshi Suzuki[5]. (1) CMC R&D Laboratories, Kirin Brewery Co., Ltd, 100-1, Hagiwara-machi, Takasaki-shi, Gunma 370-0013, Japan, Fax: 81-27-353-7400, kkobayashi@kirin.co.jp, (2) 2, Central Laboratories for Frontier Technology, Kirin Brewery Co., Ltd, Takasaki-shi, Gunma 370-0004, Japan, (3) Cell Engineering Department, National Institute Advanced Industrial Science and Technology, Tukuba-chi, Ibaraki 305-8566, Japan, (4) Core Technology Research Laboratories, Sankyo Co., Ltd, Shinagawa-ku, Tokyo 140-8710, Japan, (5) Process Development Laboratories, Sankyo Co., Ltd, Iwaki-shi, Fukushima 971-8183, Japan

Antibodies for pharmaceuticals are produced by mammalian cells as conventional host. However several approaches have been investigated to reduce the cost of production by mammalian cells, little method is proposed to overcome the problem. When antibody was produced in fungi, abnormal O-glycosylation (mannosylation) was detected in secreted antibody. This modification was catalyzed by protein-O-mannosyltransferases (Pmtps) which localize in endoplasmic reticulum (ER) but not Golgi apparatus in mammalian cells. Because O-linked sugar chains might have immunogenicity against human, reduce stability and binding to antigens and Fc receptors, it is necessary to produce the antibody without the O-linked sugar chains for pharmaceuticals in yeast. In this study, we have examined to suppress the O-glycosylation in the antibody and developed a novel system for antibody production using methylotrophic yeast O. minuta.

BIOT 85 - Efficient synthesis of simvastatin using whole-cell biocatalysis

Yi Tang and Xinkai Xie, Department Chemical and Biomolecular Engineering, University of California, Los Angeles, 5531 Boelter Hall, 420 Westwood Plaza, Los Angeles, CA 90095, yitang@ucla.edu

Simvastatin is a semisynthetic derivative of the fungal polyketide lovastatin and is an important drug for lowering the cholesterol levels in adults. We have developed an one-step, whole cell biocatalytic process for the synthesis of simvastatin from monacolin J. Using an Escherichia coli strain overexpressing the previously discovered acyltransferase, LovD , we were able to achieve >99% conversion of monacolin J to simvastatin without the use of any chemical protection steps. The key finding was a membrane permeable substrate, alpha-dimethylbutyryl-S-methyl-mercaptopropionate (DMB-S-MMP), that was efficiently utilized by LovD as the acyl donor. The process was scaled up for gram-scale synthesis of simvastatin. We also demonstrated that

simvastatin synthesized via this method can be readily purified from the fermentation broth with >90% recovery and >98% purity as determined by HPLC. Bioconversion using high cell density, fed-batch fermentation was also examined. The whole cell biocatalysis can therefore be an attractive alternative to the current, multi-step semisynthetic transformations.

BIOT 86 - Enzyme-carbon nanotube conjugates in room temperature ionic liquids

Bilge Eker[1], Prashanth Asuri[1], Saravanababu Murugesan[1], Robert J Linhardt[2], and Jonathan S. Dordick[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, 110 8th Street, Troy, NY 12180, ekerb@rpi.edu, (2) Departments of Chemistry and Chemical Biology, Chemical and Biological Engineering, Biology, Rensselaer Polytechnic Institute, Troy, NY 12180

Room temperature ionic liquids (RTILs) are intriguing solvents, which are recognized as "green" alternatives to volatile organics. Although RTILs are nonvolatile and can dissolve a wide range of charged, polar, and nonpolar organic and inorganic molecules, there remain substantial challenges in their use, not the least of which is the solvents' high viscosity that leads to potential mass transfer limitations. In the course of this work, we discovered that the simple adsorption of the bacterial protease, proteinase K, onto single-walled carbon nanotubes results in intrinsically high catalytic turnover. The high surface area and the nanoscopic dimensions of SWNTs offered high enzyme loading and low mass transfer resistance. Furthermore, the enzyme-SWNT conjugates displayed enhanced thermal stability in RTILs over the native suspended enzyme counterpart and allowed facile reuse. These enzyme-SWNT conjugates may therefore provide a way to overcome key operational limitations of RTIL systems.

BIOT 87 - Exploiting phenotypic diversity through global transcription machinery engineering

Daniel Klein-Marcuschamer, Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave., Bldg 56-422, Cambridge, MA 02139, Fax: 617-253-7181, kemd@mit.edu, and Gregory Stephanopoulos, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

Strain optimization has been markedly constrained by our inability to map and manipulate multigenic traits, which has in turn limited the applications of metabolic engineering. To overcome these limitations, we have successfully delivered a method that introduces global transcriptomic changes, thereby generating diversity at the phenotypic level. In this study, we present novel methods for quantifying population diversity introduced by mutating a single gene, which is a good indicator of the value and efficacy of this technology. In addition, we extend this study to Lactobacillus plantarum for overcoming its poor resistance to high lactic acid conditions, especially at low pH. With a single round of mutagenesis, the best strain increased its growth rate in high lactic acid by 2.6-fold, the L-lactate production at low pH (initial pH=3.87) by 90% and the intracellular lactic acid tolerance by 2.3-fold with respect to the wild-type control. As such, this system has great potential for its use in the production of polylactic acid, a promising biodegradable polymer.

BIOT 88 - High-throughput zcreening for methionyl-tRNA synthetases that enable residue-specific incorporation of noncanonical amino acids into recombinant proteins in bacterial cells

Tae Hyeon Yoo and David A. Tirrell, Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, tae@caltech.edu

We report here a high-throughput method of screening aminoacyl-tRNA synthetase (aaRS) libraries for global incorporation of noncanonical amino acids. The activity of aaRS toward amino acid analogs can be monitored by translation of model proteins in media depleted of the corresponding canonical amino acids. Green fluorescent protein (GFP) was engineered to be foldable and fluorescent regardless of analogues incorporated into GFP Met codons, yielding GFPrm_AM. Using GFPrm_AM as a translational reporter, a saturation mutagenesis library of E. coli methionyl-tRNA synthetase (MetRS) was screened for activity toward 6,6,6-trifluoronorleucine (Tfn) by fluorescence activated cell sorting. Through several rounds of positive screening (with 19 amino acids (-Met) and Tfn) and negative screening (with 19 amino acids), we identified one MetRS variant which was very efficient in expressing recombinant proteins containing Tfn. The screening method described here is simple and efficient, and is directly applicable to Met analogs other than Tfn.

BIOT 89 - What controls enantioselectivity for carbonyl reductase

Ling Hua, Dunming Zhu, Yan Yang, and Thoris Hsin-Yuan Pan, Chemistry, Southern Methodist University, 3215 Daniel Ave, Fondren Science Building Rm231, Dallas, TX 75275, Fax: 214-768-4089

Enantiometrically pure alcohols are important and valuable intermediates in the synthesis of pharmaceuticals and other fine chemicals. Enzymatic reduction of prochiral ketones is the method of choice because of its environmentally benign reaction conditions, broad substrate scope, and high stereo- and regioselectivity. Many alcohol dehydrogenases reduce prochiral ketones to chiral alcohols following Prelog rule, only a handful of dehydrogenases give anti-Prelog alcohol products. We have studied the synthetic application of several alcohol dehyddrogenases, the carbonyl reductase from *Sporobolomyces salmonicolor* (SSCR) shows different enantiopreference for similar substrates as shown in equation (1) and (2). We have identified the residues in contact with the substrates and changed one or more of these residues caused the enantiopreference reversal of acetophenone reduction (equation (3) and (4)). The docking studies are engaged to understand what controls the enantioselectivity of this enzyme in the enantioselective reduction of ketones, a and b-ketoesters to their corresponding chiral alcohols.

BIOT 90 - Hydration effects on the active site and catalytic performance of organic-soluble subtilisin in octane

Elton P. Hudson[1], Jonathon S. Dordick[2], Jeffrey A. Reimer[1], and Douglas S. Clark[1]. (1) Department of Chemical Engineering, University of California, Berkeley, Berkeley, CA 94720, paulsimv@berkeley.edu, (2) Department of Chemical Engineering, Rennselaer Polytechnic Institute, Troy, NY 12180-3590

The potential of novel enzymatic reaction pathways in organic solvent systems is hindered by reduced biocatalyst efficiency in these solvents compared to aqueous media. The serine protease subtilisin Carlsberg can be extracted into octane using surfactants; the resulting organic soluble biocatalyst shows much higher turnover numbers (ca. 200-fold) than the insoluble form, yet is not as active as its aqueous counterpart. The low dielectric of octane and other solvents can restrict the enzyme's catalytic performance via de-polarization of the active site and decreased conformational dynamics. We have found that both of these limitations may be partially overcome if the biocatalyst is adequately hydrated. Hydration of soluble subtilisin in octane has been investigated by monitoring 19F NMR chemical shifts and relaxation dispersion profiles of an active site inhibitor. Clear trends in reaction rate with water activity, active-site polarity, and chemical exchange rates were observed and will be discussed.

BIOT 91 - Synthesis of anti-obesity stilbene derivatives using engineered microbial biocatalysts: Structure-function analysis of α-glucosidase inhibition potency

Effendi Leonard, Kok-Hong Lim, Chin-Giaw Lim, Joseph Chemler, and Mattheos Koffas, Department of Chemical and Biological Engineering, university, 303 Furnas Hall, The State University of New York, Buffalo, NY 14260, eleonard@buffalo.edu

Resveratrol is a type of stilbene polyketite found in small quantities in plants. Recently, resveratrol has been demonstrated to increase the life-span of many organisms and is being tested as new therapeutic against diseases associated with aging, such as diabetes and obesity. The minute quantities of resveratrol in plant resources hinder the development of inexpensive pharmaceuticals. Escherichia coli has been engineered for resveratrol biosynthesis. In the present work, E. coli biocatalyst was used to synthesize resveratrol derivatives and unnatural analogs that do not exist in nature. The capacity of synthesizing the unnatural analogs was initially low. To accelerate the in vivo reaction rate, fluxes toward the malonyl-CoA backbone was increased. Introduction of a foreign malonate assimilation pathway increased product synthesis. However, the highest productivity was only achieved after partial repression of a central enzyme in fatty-acid biosynthetic pathway. Through this strategy, the E. coli biocatalyst was capable of producing fluorinated stilbenes up-to 22 mg/L, and aminated stilbenes up-to 33 mg/L, an increase of up-to 633% and 6500% over the parental strain. Robust synthesis of the various derivatives allowed the structure-function analysis of inhibitory properties against α-glucosidase, an enzyme responsible of glucose assimilation in digestive track. The results showed that enzyme inhibition increased with the number of B-ring hydroxyl group. Replacement of the 4'-hydroxyl group in resveratrol with fluorine reduced inhibition. However, inhibition of α-glucosidase was increased 2 folds over resveratrol, by the addition of a fluorine atom at the 2'-B-ring position.

BIOT 92 - Biotransformations on solid-supported substrates

Sarah Brooks[1], Lydie Coulombel[1], Umar Akbar[2], Douglas S Clark[2], and Jonathan S. Dordick[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110, 8th Street, Biotech Center, Room 4117, Troy, NY 12180, brooks@rpi.edu, (2) Department of Chemical Engineering, University of California at Berkeley, Berkeley, CA 94720

Solid-phase combinatorial biocatalysis represents an alternative approach to combinatorial chemistry and exploits the exquisite enantio-, regio-, and chemoselectivity of enzymes that operate under mild reaction conditions. We employed a range of oxidative enzymes to perform alcohol oxidation (alcohol oxidase and alcohol dehydrogenase), phenolic coupling (soybean peroxidase), and aromatic halogenations, alkene halohydration, and alkene epoxidation (all via chloroperoxidase catalysis) reactions on solid-supported substrates, thus expanding the array of biotransformations that can be carried out on the solid phase. Model substrates were attached to controlled-pore glass (CPG) beads through an α-chymotrypsin-cleavable peptidic linker. Following oxidative biotransformations, the products were cleaved and analyzed via LC-MS. These reactions represent the first report of enzymatic oxidation and halogenation on solid phase substrates. This approach was expanded to complex lead compounds by attaching the flavonoid bergenin via an enzyme-cleavable linker onto CPG. Using both aqueous and nonaqueous reaction media, multiple transformations were performed to demonstrate the potential of solid-phase biocatalysis in the combinatorial optimization of complex lead molecules for pharmaceutical candidates.

MONDAY MORNING

11:30 – 12:30 pm BCEC 107 B

Industrial Biotechnology Award Lecture W. Zhou, Organizer Paper 93

BIOT 93 - Genentech Lucentis® Development CMC team: "A vision saving therapy made by biotechnology"

John C Joly, Cell Culture Process Development, Genentech, Inc, 1 DNA Way, MS32, South San Francisco, CA 94080, Fax: 650-225-2006, joly@gene.com

Abstract not available.

MONDAY AFTERNOON

2:00 – 3:00 pm BCEC 107 B

Industrial Keynote Symposium

W. Chen, Organizer, Presiding

Keynote Address

Role of Technology and Science in Manufacturing Economics

Bob Adamson, Vice President, Wyeth BioPharma Global Process Development, 181 Ballardvale Street, Wilmington, MA 01887, Fax: 978-247-4719, badamson@wyeth.com

"…many of today's most exciting and "disruptive" innovations now tend to occur at the intersection of market insight and technology know-how, making technology an input to the strategy process rather than an after-the-fact enabler." (Berman and Hagan, 2006). With the emergence of Follow-on Biologics and changing health care initiatives Biotechnology has reached the stage of development where cost-effective manufacture of products is as important to a company's success as product discovery. Thus, more than ever, enabling technologies will differentiate products from each other through improvements in cost, patient accessibility and convenience. The presentation will focus on the emerging central role of manufacturing technology in Biotech and more specifically Wyeth's Strategy. 1. How technology-driven business strategy can spur innovation and growth. S.J.Berman & J Hagan in Strategy and Leadership vol. 34#2 2006, pp28-34.

MONDAY AFTERNOON

3:00 – 5:00 pm BCEC 108

Stem Cells: Engineering the Embryonic and Adult Stem Cell Niche

A. Khademhosseini and B. Rao, Organizers
Papers 94 - 99

BIOT 94 - Matrix and environmental control of stem cell outcomes for tissue engineering

David L. Kaplan, Hyeon Joo Kim, Xiaoqin Wang, Xianyan Wang, Charu Vepari, Amanda Murphy, and Irene Georgakoudi, Department of Biomedical Engineering, Biotechnology & Bioengineering Center, Tufts University, 4 Colby Street, Medford, MA 02155, David.Kaplan@tufts.edu

Stem cells play a vital role in functional tissue engineering due to their plasticity. In particular, human bone marrow derived mesenchymal stem cells (hMSCs) are central to many strategies in tissue engineering and regenerative medicine. In light of this role, critical issues associated with the maintenance and fate of these cells becomes important in terms of tissue specific goals. Relevant issues include the role of matrix chemistry on cellular outcomes, the role of cell-cell signaling on cell functions and the role of selective environments on the fate of the cells. The complexity of stem cell and matrix interactions, referred to as the niche, offer tremendous challenges as well as opportunities to improve both the rate and extent of tissue specific outcomes under controlled laboratory conditions. We have focused some of our efforts on these topics, including: the role of specialized or functionalized protein scaffold systems to direct stem cells toward selective outcomes, the role of stem cell–differentiated cell signaling with regard to cell recruitment, and the role of selective bioreactor environments on stem cell fate. For example, mineralized protein-composite scaffolds seeded with hMSCs and cultured in vitro under osteogenic conditions demonstrated improved osteoconductive outcomes correlated with initial content of apatite in the porous scaffolds. In a parallel study, spinner flask bioreactor systems with improved mass transport when compared to static culture, resulted in improved osteogenic outcomes from hMSCs seeded on porous protein scaffolds under osteogenic conditions. These and other results provide insight into the role of scaffold and environmental signals on osteogenic outcomes related to bone tissue engineering. These studies have been extended to new modes to control the delivery of essential cell signaling factors to gain further control of regional differentiation of the hMSCs on such scaffold systems.

BIOT 95 - The effect of cell density and oxygen level on culture for rodent multipotent adult progenitor cells (MAPCs)

Yonsil Park[1], Fernando Ulloa[2], Catherine Verfaillie[2], and **Wei-Shou Hu**[1]. (1) Department of Chemical Engineering and Materials Science, University of Minnesota, 421 Washington Ave. SE, Minneapolis, MN 55455, Fax: 612-626-7246, parkx320@umn.edu, wshu@cems.umn.edu, (2) Stem Cell Institute, Katholieke Universiteit Leuven, Leuven, Belgium

Multipotent adult progenitor cells (MAPCs) can be expanded in vitro without obvious senescence, and give rise to cell types of mesoderm, endoderm, and ectoderm. They are maintained at low cell densities in order to maintain pluripotency. Isolation by culturing under hypoxic condition (5% O2) has yielded MAPC expressing high levels of the embryonic stem cell specific transcription factor Oct-4, which is associated with greater potency. In this study the growth rate and pluripotency of rat (r)MAPCs, as measured by Oct-4 mRNA and protein expression level, were shown to be unaffected by different cell densities. Their differentiation towards endothelium-like and hepatocyte-like cells as indicated by mRNA levels of endothelial and hepatocyte markers was also unaffected. Changing ambient oxygen from 5% to 21% did not affect the proliferation, Oct-4 levels and differentiation ability of high Oct-4 MAPC. The results provide evidences that rMAPCs isolated under hypoxic conditions and express high levels of Oct4 can be readily cultured at high density. The findings, if extended to human MAPC, will open the possibility for easy scale up and facilitate the potential clinical application of MAPC.

BIOT 96 - cAMP induced neural differentiation of mesenchymal stem cells: Real differentiation or pseudo-differentiation?

Linxia Zhang and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, 2527 Engineering Building, Michigan State University, East Lansing, MI 48824, zhanglin@egr.msu.edu

Mesenchymal stem cells (MSCs) have been shown to give rise to a number of cell lineages. It is reported that cAMP induces MSCs to differentiate into neural cells. Such conclusion is mostly based on morphological changes and expression of neural markers. Our present study suggests other considerations such as apoptosis should also be taken into account along with the differentiation behavior. An increase in cAMP level inhibits MSCs proliferation by blocking G1/S transition, which drives a portion of the cells towards apoptosis. These apoptotic cells have disrupted cytoskeleton structures and feature a neurite-like appearance due to the retraction of the cell body towards the center. Since many of the neuron markers are cAMP target genes, it is possible that while cAMP also may up-regulate the expression of a subset of neuron markers. Therefore, further study is on-going to determine whether cAMP indeed induces differentiation of MSCs into mature neural cells.

BIOT 97 - Effects of scaffold porosity and pore size on cell entrapment, attachment, distribution and proliferation in 3-D cell culture system

Robin Ng, Department of Chemical Engineering, Ohio State University, 140 West 19th Ave., Koffolt Lab, Columbus, OH 43210, Fax: 614-292-3769, ng.128@osu.edu, and Shang-Tian Yang, Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210

Current progress in three-dimensional culture produced a new culture system for biologic manufacturing in which scaffold's pore size and porosity are important parameters. The scaffold used in this study was nonwoven fibrous PET. Scaffolds with different porosities, ranging from 93% to 97%, and pore sizes, ranging from 27 μm to 70 μm, were prepared through hydrolysis in 1M NaOH at 65˚C. Our model predicted the effect of treatment time on pore size. We assessed the cell entrapment and distribution kinetics in the scaffolds using GFP-transfected ES cells and CHO-K1 cells. Cells distributed differently in scaffolds with different pore sizes: cells adsorbed on the fiber surface at a lower pore size and were entrapped in the void space between fibers at a higher. This difference in cell distribution led to the difference in cell attachment rate and cell proliferation. The pore sizes did not affect the growth rate of the cells.

BIOT 98 - Ex vivo expansion of human hematopoietic stem/progenitor cells in a human stromal-based culture system

C. Lobato da Silva[1], RM. Goncalves[1], MA. Lemos[1], F. Lemos[1], G. Almeida-Porada[2], and JMS. Cabral[1]. (1) IBB-Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Tecnico, Av. Rovisco Pais, Lisboa 1049-001, Portugal, Fax: +351218419062, claudia_lobato@ist.utl.pt, (2) Department of Animal Biotechnology, University of Nevada, Reno, Reno, NV 89557

The role of a human stromal-based (hu-ST) serum-free culture system on the ex-vivo expansion/maintenance of hematopoietic stem/progenitor cells from adult bone marrow (BM) and umbilical cord blood (CB) was investigated. Significant expansion of BM and CB cells occurred in the hu-ST system. In stroma-free cultures, BM cells showed a significant lower expansion, whereas CB cells simply could not be expanded. A simple kinetic modeling study using two parameters (expansion rate and death rate), allowed to conclude that total cell expansion is similar for hematopoietic cells from both BM and CB, whereas cell death seemed to be related with the presence/absence of hu-ST ex vivo. A predictive model was also developed accounting for hematopoietic cell expansion, differentiation, and death, which estimated adequately total cell numbers and relative amounts of the different phenotypes (e.g. CD34+, CD34+CD38-, CD33+), allowing the prediction of expansion/differentiation pathways and identification of key steps in the hematopoiesis scheme.

BIOT 99 - Multiparameter flow cytometric assay to monitor the activation of cytokine signaling pathways in specific subsets of primary erythroid cells

Weijia Wang and Julie Audet, Institute of Biomaterials and Biomedical Engineering, University of Toronto, Terrence Donnelly Centre for Cellular and Biomolecular Research, 160 College Street, 11th floor, Toronto, ON M5S 3E1, Canada, Fax: 416-978-2666, weijia.wang@utoronto.ca

Cytokine synergism is a common theme in tissue and cellular engineering. However, an understanding of the cellular and population mechanisms at play is often lacking. Stem Cell Factor (SCF) and Erythropoietin (EPO) have a synergistic effect on erythroid cell output in cultures of bone marrow cells. Previous studies have demonstrated that these cytokine effects were mainly sequential, with each cytokine acting on different cell populations. We are now interested in determining if a specific cell subset can integrate both SCF and EPO signals during erythroid development. Since such study requires single-cell measurements and the ability to distinguish different cell populations, we have developed a flow cytometric assay that enables the simultaneous detection and quantitation of intracellular phospho-proteins (ERK and STAT5) and cell-surface markers (c-kit and CD-71). A better understanding of the mechanisms underlying the synergistic interaction between EPO and SCF can facilitate the large-scale production of erythrocytes for transfusion.

MONDAY AFTERNOON

3:00 – 5:20 pm BCEC 107 B

Downstream Processing: Bioprocess Integration & Industrial Case-Studies

M. R. Ladisch and C. Liu, Organizers
Paper 100 - 105

BIOT 100 - CSAF: Constant shear affinity filtration technology

Patrick Francis[1], Eric von Lieres[1], Fariborz Taghipour[2], Bruce D. Bowen[2], and C. A. Haynes[1]. (1) Michael Smith Laboratories, University of British Columbia, 301 2185 East Mall, Vancouver, BC V6T 1Z4, Canada, pfrancis@chml.ubc.ca, (2) Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

Controlled shear affinity filtration (CSAF) is an integrated mammalian cell separation and product capture technology that can increase product yields by up to 20% while reducing total processing costs. CSAF positions a conical rotor directly above an affinity membrane chromatography column to permit affinity capture and purification of a secreted protein directly from cell culture. A prototype CSAF device used to capture tissue-type plasminogen activator (tPA) directly from recombinant CHO cell culture produced a 100% cell-free eluate with a purified product yield of 86% and a purification factor of 16.7. The design of this prototype device was then optimized using computational fluid dynamics, allowing for a scale up that provides more than a six-fold increase in volumetric throughput. Custom synthesis of PALL Ultrabind® affinity membranes for the capture of tPA, and development of a novel affinity membrane chromatography model have allowed for further optimization of CSAF operation.

BIOT 101 - Production of enantiopure molecules by integration of SMB technology and biocatalysis

Matthias Bechtold, Stefan Makart, and **Sven Panke**, Bioprocess Laboratory, Institute of Process Engineering, ETH Zurich, Universitaetsstrasse 6, 8092 Zurich, Switzerland, bechtold@ipe.mavt.ethz.ch, panke@ipe.mavt.ethz.ch

Integration of biocatalysis and simulated moving bed technology constitutes an attractive option to overcome a broad set of constraints frequently encountered in enantioselective catalysis such as thermodynamic limitations, compromised reaction yields by long reaction times, product inhibition and product instability (Fig. 1). Another attractive variant of such a process concept is the integrated operation of mild enzymatic racemisation and chiral simulated moving bed technology (SMB) for the transformation of racemates into single enantiomers in 100% yield [1].
In this work we present the development, implementation and optimization of two hybrid processes for the production of enantiopure amino acid: (i) Integration of asymmetric synthesis of L-*allo*-threonine from glycine and acetaldehyde catalyzed by an aldolase (reaction yield 40% at equimolar substrate conc.) and SMB separation of threonine and glycine based on an ion-exchanger. (ii) Integration of enzymatic racemisation catalyzed by an amino acid racemase and SMB enantioseparation of the racemic amino acid based on the Chirobiotic TAG column (Astec,Whippany, NJ, USA).
[1] M. Bechtold, S. Makart, M. Heinemann, S. Panke, J Biotechnol 124 (2006) 146.

Figure 1:By coupling a continuously operated bioreactor to an SMB that provides efficient separation of the substrate/product mixture of the reactor effluent and subsequent recycling of the substrate Ðtheoretically- 100% chemical yield can be achieved

BIOT 102 - Considerations for large scale extraction of monoclonal antibodies targeted to different subcellular compartments in transgenic tobacco plants

Sally Hassan[1], Craig van Dolleweerd[2], Julian Ma[2], Wei Liu[3], Colin R Thomas[3], and Eli Keshavarz-Moore[1]. (1) Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom, Fax: +44 (0)20 7209 0703, sally.hassan@ucl.ac.uk, (2) Department of Cellular and Molecular Medicine, St.George's University of London, London SW17 0RE, United Kingdom, (3) Department of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, United Kingdom

Large scale purification of monoclonal antibodies from transgenic tobacco plants offers many advantages over commonly used host production systems. However, there is limited information on purification of pharmaceuticals from transgenic plants.

Methods for extraction of recombinant monoclonal antibodies from transgenic tobacco were investigated. Parameters for optimal extraction have been established, using techniques at small scale that will provide information for large scale purification. Three targeting strategies for antibodies in transgenic tobacco have been investigated in order to compare extraction from different subcellular compartments.
We have identified that antibody yield is dependent upon plant age, leaf position, pre-harvest wounding, extraction methodology, and extraction buffer composition, e.g. pH and detergent. These factors all influence early decisions on the best strategy for extraction of antibodies from plants. In addition, we have demonstrated that antibody yield from GM plant roots is similar to that from leaves, and is therefore a feasible alternative to leaves.

BIOT 103 - Evaluating and minimizing water usage and wastewater ceneration in integrated bioprocesses using process simulation and scheduling tools

Demetri Petrides, INTELLIGEN, INC, 2326 Morse Avenue, Scotch Plains, NJ 07076, dpetrides@intelligen.com, and Charles Siletti, INTELLIGEN, INC, Mt. Laurel, NJ 08054

Water of various qualities is consumed in large quantities in the biopharmaceutical industries. It is used for preparing media and buffer solutions and for equipment cleaning. It is also used for generating steam of various qualities. The supply of purified water and the treatment of wastewater water is a common bottleneck during throughput increase projects. This presentation shows how process simulation and scheduling tools may be applied to evaluate the demand for water at various levels of purity and easily size water supply and wastewater treatment systems. The study also examines the effect of moving portions of the process to disposable vessels that do not require cleaning and sterilization.

BIOT 104 - Development and implementation of a manufacturing scale virus filtration step for monoclonal antibody production

Judy F Hsii and Chris Dowd, Bioprocess Development - Late Stage Purification, Genentech, Inc, 1 DNA Way, South San Francisco, CA, CA 94080

Biopharmaceutical products manufactured from mammalian cells must demonstrate that they are safe and free of endogenous retroviruses. Regulators require at least two orthogonal methods of clearing these retrovirus particles, whether it be physically removed or inactivated as part of the manufacturing process. The virus filter functions as a physical barrier to putative retroviruses while allowing the antibody product to flow through. Development of the virus filtration step for the production of a monoclonal antibody was carried out at small scale to define operating parameters in manufacturing. Parameters examined included flow rate, concentration, temperature and the use of a guard filter. Scale-up of the virus filtration step from lab-scale to pilot scale to manufacturing scale will be discussed.

BIOT 105 - Humira after launch: Opportunities and challenges in downstream process

Min Wan, Frank Gaibor, Marc Schrader, R. Michael Boychyn, Edwin Lundell, and George Avgerinos, Technical Operations, Abbott Bioresearch Center, 100 Research Drive, Worcester, MA 01605-4314, min.wan@abbott.com

Humira (Adalimumab) was successfully launched in 2002 and is currently manufactured at both 3,000L and 6,000L scales. During the past three years of manufacturing, there has been a continuous effort to: (1) increase process robustness and ensure high quality, (2) satisfy and harmonize divergent global regulatory demands, and (3) ensure that an identical process could be launched at the 12,000L scale.

First generation process improvements were developed in 2003 and implemented in 2004 to improve process robustness and harmonize the processes at different scales. Process mapping studies were undertaken in 2004 to refine our knowledge of process attributes like intermediate purity, yield and the dynamic performance of each unit operation. As a result, a fine-tuned revised adalimumab downstream process is currently submitted for approval and planned for 12,000L start up. Data from at scale validation demonstrating improved product quality, HCP clearance, and yield will be presented.

MONDAY AFTERNOON

3:00 – 5:35 pm BCEC 106

Upstream Processing: Advances in Microbial Fermentation Process Development

X. Yang, M. Laird, and D. Ren, Organizers
Papers 106 - 110

BIOT 106 - "Seeing the light" with cell-free protein synthesis

Aaron R. Goerke[1], Andreas M. Loening[2], Sam Gambhir[3], and James R. Swartz[1]. (1) Department of Chemical Engineering, Stanford University, Stauffer III, Room 113, Stanford, CA 94305-5025, argoerke@stanford.edu, (2) Department of Bioengineering, Stanford University, Stanford, CA 94305, (3) Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA 90095-1735

Bioluminescence imaging has made great progress in recent years due to improvements in the design of the imaging agents and detection techniques. Due to its small size and high activity, the luciferase from Gaussia princeps (GLuc) is ideal for such applications. However, this protein has been poorly characterized and has seen limited use, primarily because of the difficulty in producing GLuc in appreciable quantities using traditional expression methodologies. We discovered that cell-free protein synthesis (CFPS) is a vastly superior technology for the production of GLuc. A production protocol was established by improving cell strains, extract preparation procedures, CFPS reaction conditions, protein maturation methodologies, and by simplifying the purification scheme. The ability to control the folding conditions, lower the translation rate, and dilute the folding environment results in a 175-fold increase in GLuc yields and provides an economical source of a luciferase that is 100-fold brighter than the optimized Renilla luciferase.

BIOT 107 - Enhancing extracellular protein secretion in *Escherichia coli* by translation engineering

Prateek Gupta, Pat S. Lee, and Kelvin H. Lee, School of Chemical and Biomolecular Engineering, Cornell University, 120 Olin Hall, Ithaca, NY 14853-5201, Fax: 607-255-9166, pg76@cornell.edu

One approach to reduce costs associated with recombinant protein production is to have the protein secreted. Here, we describe series of experiments to enhance extracellular secretion in *E. coli* using the hemolysin secretion pathway. Based on observations from mRNA and protein expression profiling methods, we hypothesized that the translation rate of heterologous protein may be related to the ability to secrete higher levels of product via this pathway. We tested this hypothesis by making synonymous codon changes in different regions of the gene of interest and observed improved secretion of hemolysin and other proteins. Intracellular protein levels in the mutants suggest that increase in protein secretion may be a combination of decrease in protein aggregation and degradation. A mathematical model of secretion has been proposed and the model predictions correlate with experimental results. This study highlights the importance of -omics technologies coupled with a systems biology approach in bioprocess development.

BIOT 108 - Developing high throughput screening methods for multiple phenotype evaluation

Benjamin Wang, Yongchao Zhang, and Gregory N. Stephanopoulos, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave., 56-422, Cambridge, MA 02139, ben_wang@mit.edu

Inverse metabolic engineering involves generating a library of mutant strains and screening through these mutants to identify genes that are responsible for a particular phenotype. Since these libraries can be large, a high throughput screening method is necessary. In the area of metabolic engineering, one typically screens for the concentrations of the metabolite of interest secreted into the culture media and the cell culture nutrients which remain. Commonly used tools for measuring metabolites, such as HPLC, are slow. Here, we present the development of a fast approach for measuring tyrosine and glucose using enzyme electrode technology and our measurements of tyrosine overproducing strains.

BIOT 109 - Metabolic and process engineering of Propionibacterium acidipropionici for enhanced propionic acid fermentation

An Zhang and Shang-Tian Yang, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140W. 19th Ave. , Koffolt Laboratories, Columbus, OH 43210, zhang.510@osu.edu

A propionic acid fermentation process using metabolically engineered Propionibacterium acidipropionici immbolized in a fibrous bed bioreactor (FBB) was developed for propionic acid production from waste sugars and glycerol. An engineered mutant with reduced acetate production was obtained by inactivation of acetate kinase (ack) gene via homologous recombination and integrational mutagenesis. This mutant, after further adaptation in the FBB, was able to produce high concentrations of propionic acid (>100 g/L) with high propionic acid yields (>0.54 g/g glucose and 0.71 g/g glycerol). The mutant showed a 16-fold increase in its tolerance to propionic acid, which was attributed to proteome changes identified by two-dimensional electrophoresis (2-DE) and mass spectroscopy. The increased propionic acid production by the mutant in the FBB fermentation process allows propionic acid to be economically produced from waste sugars and glycerol.

BIOT 110 - Production of sophorolipid from cooking oil waste through fermentation

Vishal Shah, Department of Biology, Dowling College, Oakdale, NY 11769, ShahV@dowling.edu

Huge amounts of oil are generated per week as waste from restaurants around the country. Because of the health, environmental and economical reasons current methods of disposal are ineffective for disposal of the restaurant oil wastes. In this study we have investigated the ability of Candida bombicola to fermentatively transform the restaurant oil waste into glycolipids called Sophorolipids. Batch and Fed-batch studies were carried out using oil waste as the lipid feedstock in Erlenmeyer flasks and in a fermentor. Batch fermentation in a fermentor gave the highest yield of 34 g l-1 of Sophorolipids. Fermentation using oleic acid as control feedstock were also carried out. Batch fermentation in fermentor using this pure fatty acid gave the highest yield of 42 g l-1. The difference in the Sophorolipid yield was attributed to the fatty acid composition of restaurant oil waste. The decrease in Sophorolipid production during fed-batch studies is attributed to the formation of gel like structures during purification step.

MONDAY AFTERNOON

3:00 – 5:20 pm BCEC 107 C

Commercialization of Biologics: Data Management & Process Modeling in Support of Commercial Processes J. Prior and S. Ahuja, Organizers Papers 111 – 116

BIOT 111 - Multivariate analysis of transition analysis calculations applied to evaluate trends and changes in chromatography column packed bed integrity at BiogenIdec

Benjamin Gilbert, Manufacturing Sciences, Biogen Idec Inc, PO Box 14627, 5000 Davis Drive, Research Triangle Park, NC 27709, benjamin.gilbert@biogenidec.com

With increasing values of biologic products and higher cost chromatography media, improved methods of evaluating chromatography bed integrity are needed to minimize the risk of substantial loss which might accompany a packed bed failure. Traditional HETP pulse test methods used in the industry do not provide the sensitivity to trend or predict bed failures. BiogenIdec is using Transition Analysis calculations in Principal Component Analysis to trend chromatography bed integrity.

BIOT 112 - Application of multivariate analysis toward biotech processes: Case study of a cell-culture unit operation

Anurag S. Rathore and Alime Kirdar, Amgen, Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, arathore@amgen.com

This talk will focus on examination of the feasibility of using multivariate data analysis (MVDA) for supporting some of the key activities that are required for successful manufacturing of biopharmaceutical products. These activities include process scale-up, comparability, characterization and fault diagnosis. Multivariate data analysis and modeling were performed using representative data from small-scale batches (2L) and large-scale batches (2000L) manufactured with a cell-culture process. Several input parameters (pCO2, pO2, glucose, pH, lactate, ammonium ions) and output parameters (purity, viable cell density, viability, osmolality) were evaluated in this analysis. Score plots, loadings plots and VIP plots were utilized for assessing scale-up and comparability of the cell-culture process. Batch control charts were found to be useful for fault diagnosis during routine manufacturing. Finally, observations made from reviewing VIP plots were found to be in agreement with conclusions from process characterization studies demonstrating the value of MVDA as a tool for extracting process knowledge.

BIOT 113 - Production planning, scheduling, and debottlenecking practices in the biopharmaceutical industries

Charles Siletti, INTELLIGEN, INC, 700 Walton Avenue, Mt. Laurel, NJ 08054, Fax: (856) 235-1438, casiletti@intelligen.com, and Demetri Petrides, INTELLIGEN, INC, Scotch Plains, NJ 07076

This paper presents industrial experience with a resource-constrained batch process scheduling tool. The batch process representation is partially based on the ISA S88 batch process standard. This representation allows the import of batch process information from other software, e.g. batch process simulators. The scheduling algorithm is a non-optimization approach that proceeds in two steps. First a bottleneck analysis is done to determine a lower bound on the process cycle time, and all the batches are scheduled accordingly. Second, if conflicts remain, they are resolved by applying progressively aggressive modifications to the schedule. This approach to scheduling was tested on several biotech processes. These processes consist of a sequence of batch steps performed with dedicated equipment. The scheduling challenges in biotech processes lie in the ancillary operations: media and buffer preparation, vessel and line cleaning, and chromatography column preparation. Such operations may use shared resources that can to couple process suites with otherwise dedicated equipment. These considerations are further complicated by variability in process durations. Three case studies, which are based on a process for the manufacture of monoclonal antibodies (MABs), illustrate the value of a constrained-resource scheduling tool for biotech processes. In the first case study, the scheduling tool shows that auxiliary cleaning equipment can limit batch production. A second case study shows how scheduling tools can be used to identify and eliminate bottlenecks associated with utilities. A third case study illustrates how to use scheduling tools to mitigate the effects of process variability.

BIOT 114 - Case study: Applying the Six Sigma methodology to a commercial cell culture process

Ritchie Davis[1], Sanjeev Ahuja[2], Matthew Herwig[3], and Kenneth Hwang[2]. (1) Operational Excellence, MedImmune, Inc, One MedImmune Way, Gaithersburg, MD 20878, DavisDR@medimmune.com, (2) Process Cell Culture, MedImmune, Inc, Gaithersburg, MD 20878, (3) Technical Services, MedImmune, Inc, Frederick, MD 21703

Six Sigma can be described as a rigorous, disciplined, data-driven methodology for eliminating defects and reducing variability in any process. The method can be characterized as having five stages: define, measure, analyze, improve and control (DMAIC). The Six Sigma methodology is designed and expected to deliver real, tangible financial results by improving the performance of processes. This talk will describe how this methodology was applied to understand the variability and identify the process drivers associated with a commercial cell culture process.
The team was comprised of scientists, engineers and a Six Sigma Blackbelt who employed the DMAIC approach. Initially stakeholders and process owners were identified and the proper support was provided to the project team. A rigorous analysis of historical data was performed to determine process baselines, develop the correct metrics and determine the financial impact of the project. The validity of measurement systems was scrutinized, ensuring data accuracy. Detailed statistical analysis was performed, focusing primarily on special cause variability and the associated root causes. Root cause analysis led to the examination of process drivers, the variables that were found to "drive" the process and have the most direct impact on overall variability and process performance.

BIOT 115 - Strategies for integrating spectral data from raw materials to control biopharmaceutical manufacturing

Julie Wei[1], Mia Kiistala[1], **Maureen Lanan**[1], and Joydeep Ganguly[2]. (1) Analytical Development, Biogen Idec, 14 Cambridge Center, B8-4, Cambridge, MA 02142, Fax: 6176793476, (2) Manufacturing Sciences, Biogen Idec, Research Triangle Park, NC 27709-4627

Consistent production of biopharmaceuticals relies on control of cell culture conditions. This talk will focus on ways to include timely spectral analysis of raw material characteristics into a larger multivariate control strategy. Availability of spectral data from NMR, MS, and DAD-HPLC adds new insights and challenges to the integration and interpretation of data to predict and control cell culture. Comparison of data preprocessing techniques for complex analytical data will be presented and the benefits and challenges of including this data on an overall manufacturing control strategy will be discussed.

BIOT 116 - Use of mathematical models to determine the effect of various raw materials on mammalian cell culture performance at commercial-scale

Siddhartha Jain[1], Bernhard Schilling[1], Abhinav A. Shukla[2], and Steven Lee[3]. (1) Biotechnology Development, Bristol-Myers Squibb Company, P.O. Box 4755, Syracuse, NY 13221-4755, (2) Bristol-Myers Squibb, East Syracuse, NY 13057, (3) Biotechnology Development & Operations, Bristol-Myers Squibb Co, Syracuse, NY 13221-4755

Commercial-scale manufacturing of various therapeutic recombinant proteins involve mammalian cells cultured in bioreactors using a combination of chemically-defined serum-free components and complex medium components. Mammalian cells are sensitive to their hydrodynamic and chemical environment, and a variability in raw materials can significantly impact protein production by the cells. During commercial production of the recombinant proteins, it is often difficult to identify the effects of individual raw materials on cell culture performance due to interactions between components of different raw materials and use of multiple lots of the same raw material during production. We developed and used mathematical models in combination with various statistical tools to simulate the observed titers of a recombinant protein manufactured at different manufacturing facilities. Results from the simulations were used to predict the contribution of individual raw materials and of specific raw material lots, and helped us identify the critical raw material in our manufacturing process. We propose that mathematical tools such as the one presented in this work can be used to identify the critical raw materials and increase understanding of the recombinant protein manufacturing process.

Program by Day

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

TUESDAY MORNING

8:00 - 11:00 am	Emerging Technologies: Bioenergy	M. R. Ladisch and C. E. Wyman, Organizers	Papers 118 - 125	BCEC 109 A
8:00 - 11:00 am	Downstream Processing: Advances in Chromatography	A. M. Lenhoff and S. Ghose, Organizers	Papers 126 - 132	BCEC 107 A
8:00 - 11:05 am	Biophysical and Biomolecular Symposium: Protein Chemical Instability	E. M. Topp and M. Cromwell, Organizers	Papers 133 - 140	BCEC 106
8:00 - 11:15 am	Upstream Processing: Advances in Metabolic Engineering	J. March and I. S. Aldor, Organizers	Papers 141 - 148	BCEC 108
11:30 - 12:30 pm	Marvin J. Johnson Award Lecture	W. Zhou, Organizer	Paper 149	BCEC 107 A

TUESDAY AFTERNOON

2:00 - 2:50 pm	Emerging Technology Keynote Lecture	H. Zhao and R. Srivastava, Organizers	Paper 150	BCEC 109 A
3:00 - 5:25 pm	Upstream Processing: Advances in Cell Culture Process Development	S. T. Sharfstein, I. Blumentals, and G. Maheshwari, Organizers	Papers 151 - 156	BCEC 107 A
3:00 - 5:20 pm	Downstream Processing: Molecular Interactions and Modeling Approaches	J. Thommes and K. M. Lacki, Organizers	Papers 157 - 162	BCEC 106
3:00 - 5:20 pm	Commercialization of Biologics: Impact of Design Space Philosophy & Emerging Regulations on Process Validation	G. Knipp, K. Webber, and A. Kataria, Organizers	Papers 163 - 168	BCEC 108
3:00 - 5:35 pm	Upstream Processing: Advances in Metabolic Engineering	J. March and I. S. Aldor, Organizers	Paper 169 - 175	BCEC 109 A

TUESDAY EVENING

5:30 – 6:30pm	Future Programming Meeting	
	Awardee Dinner	

TUESDAY MORNING

8:00 – 11:00 am BCEC 109 A

Emerging Technologies: Bioenergy M. R.

M. R. Ladisch and C. E. Wyman, Organizers Papers 118 - 125

BIOT 118 - Commercialization of biomass processing: A consolidated approach

Mohammed Moniruzzaman, BioEnergy International, LLC, 99 Longwater Circle, Norwell, MA 02061, mmoniruzzaman@bioenergyllc.com

BioEnergy International is a new generation biotechnology company committed to develop and deliver advanced technologies to produce clean, environmentally beneficial fuels and specialty chemicals from renewable sources. The emergence of lignocellulosic biomass as a renewable energy resource is eagerly anticipated in many countries around the world as a means to decrease dependence on imported oil, reduce air pollution, and quickly and effectively impact greenhouse gas emissions. Recent growth of the fuel ethanol industry has established the U.S. Midwest, with starch from corn as the main feedstock, as a leading ethanol-producing region. Lignocellulose, however, constitutes the world's most widely available low-cost renewable resource. Biomass-based technologies to produce fuels & chemicals are rapidly evolving and bottlenecks are being identified that need to be overcome to achieve widespread commercialization. Current research is driven by the need to reduce the cost of production. The preferred method is to thermochemically pretreat the biomass material and subsequently, enzymatically hydrolyze the pretreated material to fermentable sugars that can then be converted to fuels & chemicals. Pretreatment research is focused on developing processes that would result in reduced capital cost, reduced bioconversion time, lower cellulase enzyme usage, and/or higher ethanol yields. Cellulase research efforts are focused on developing a cost-effective, synergistically acting enzyme mixture that would meet the end user's needs. Robust fermentation microorganisms are also being developed for conversion of biomass sugars to ethanol and other bioproducts. This presentation will provide a perspective on biomass processing by highlighting the key elements required for commercializing lignocellulosic biomass conversion, with particular emphasis on system integration.

BIOT 119 - Comparative hydrolysis, fermentation, and economic information for application of leading pretreatment technologies to corn stover and poplar

Charles E. Wyman, Chemical and Environmental Engineering, University of California, Riverside, CA 92521, cewyman@engr.ucr.edu, Bin Yang, Center for Environmental Research and Technology, University of California, Riverside, CA 92507, Bruce E. Dale, Dept of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824, Richard Elander, National Renewable Energy Laboratory, Golden, CO 80401, Mark Holtzapple, Chemical Engineering Department, Texas A&M University, College Station, TX 77843, Michael R Ladisch, Laboratory of Renewable Resources Engineering Department, Purdue University, West Lafayette, IN 47907-2022, Y. Y. Lee, Department of Chemical Engineering, Auburn University, Auburn University, AL 36849, Colin Mitchinson, Genencor International, Palo Alto, CA 94304, and John N. Saddler, Dean of Forestry, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

Pretreatment is essential to high yields and low costs for biological processing of cellulosic biomass to fuels and chemicals. A team experienced in biomass hydrolysis formed a Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) to develop the first comparative data for the promising pretreatment options of ammonia expansion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, lime, and sulfur dioxide steam explosion using shared feedstocks, enzymes, procedures, and analytical methods. Corn stover was initially employed, and material balances were developed. In addition, comparative data were developed on the digestibility of the pretreated solids. The fermentability and conditioning requirements of the pretreated hydrolyzates and solids were also assessed. Finally, material and energy balances were developed based on this data and used to project the impact of each pretreatment on the minimum ethanol selling price. All pretreatments were effective in making cellulose in corn stover accessible to enzymes with high yields, with trends slightly better for high pH technologies. Xylose recovery yields were high for all these pretreatments, although hemicellulase activity was vital to recover the substantial amounts of xylan left in the residual solids for pretreatments at high pH. However, yields were much more variable for applications of the same technologies to poplar wood, and significant performance differences were observed among these pretreatments for the same poplar variety from different locations. Overall, these results show the importance of linking selection of pretreatment technology with feedstock choice.

BIOT 120 - Mechanisms of plant cell wall deconstruction as a function of pretreatment and enzyme hydrolysis

Meijuan Zeng[1], Nathan Mosier[2], Charles E. Wyman[3], and **Michael R Ladisch**[2]. (1) Laboratory of Renewable Resources Engineering, Purdue University, Potter Engineering Center, 500 Central Drive, West Lafayette, IN 47907-2022, Fax: 765-494-7023, mzeng@purdue.edu, (2) Laboratory of Renewable Resources Engineering Department, Purdue University, Potter Engineering Center, 500 Central Drive, West Lafayette, IN 47907-2022, Fax: 765-494-7023, ladisch@purdue.edu, (3) Chemical and Environmental Engineering, University of California, Riverside, CA 92521

Corn stover consisting of stalks and leaves that remain after corn has been harvested may be collected as a feedstock for production of cellulose ethanol. We report studies that combine extensive compositional analysis and ultrastructural imaging of tissues from corn stalks and leaves as a function of aqueous pretreatment in hot liquid water for identifying mechanisms leading to enhanced enzyme hydrolysis. Synergistic effects of pretreatment and enzyme hydrolysis change the characteristics of plants cell walls by forming pores that increase accessible and susceptible surface

area in cell wall structures and which become progressively more numerous as hydrolysis proceeds. Mechanisms that explain how a soluble enzyme complex interacts with and modifies insoluble cellulose in lignified plant cell walls are discussed. Methods for pretreating and studying changes in corn stover are being extended to poplar. Preliminary data for enzyme hydrolysis of poplar shows that utrastructural changes in its cell wall structures are similar to ones observed for corn stalks and leaves. Plant cell wall characteristics that may lead to efficient combinations of pretreatments and enzyme hydrolysis are proposed. Evidence for the importance of surface area for enhanced hydrolysis is discussed.

BIOT 121 - Efficient ethanol production with engineered microorganisms

Cong T. Trinh, Chem. Eng. and Matl. Sci. and BioTechnology Inst, University of Minnesota, Minneapolis/St.Paul 55455/55108, trin0063@umn.edu, and **Friedrich Srienc**, Chemical Engineering and Materials Science, and BioTechnology Institute, University of Minnesota, 1479 Gortner Ave, Minneapolis/St. Paul, MN 55455/55108, srienc@umn.edu

An economic cellulosic ethanol production requires efficient conversion of hexoses and pentoses into ethanol at a high rate. We have used elementary mode analysis to rationally design strains that operate according to well defined pathways. Specifically, these strains are able to convert the sugar precursors into ethanol according to the best possible pathway options. We have experimentally implemented the predicted pathways using genetic engineering manipulations and characterized the resulting strains. Measurement of cell growth, of the nutrient consumption and of the metabolite secretion profiles indicates that the cells function close to the theoretically designed pathways. The ethanol yield is significantly improved in comparison to wildtype strains. Moreover, the sugar conversion can be accomplished in a significantly shorter time. Both effects contribute to a more economic realization of the production process.

BIOT 122 - Two-stage continuous fermentation of cellulose to ethanol

Chaogang Liu[1], John Bardsley[1], and Charles E. Wyman[2]. (1) Thayer School of Engineering, Dartmouth College, Hanover, NH 03755, (2) Chemical and Environmental Engineering, University of California, Riverside, CA 92521

High processing costs present an important barrier to commercialization of biomass-to-ethanol processes, and the biological steps of enzymatic hydrolysis and fermentation account for the major fraction of these costs. Combining both hydrolysis and fermentation in one vessel via the simultaneous saccharification and fermentation (SSF) process can significantly reduce capital investment and operating costs, and realize high ethanol yields and concentrations at lower enzyme loadings. Continuous fermentation can further reduce ethanol costs for the increased biomass conversion, ethanol concentrations, ethanol productivity, and enzyme effectiveness. In this study, an automatically-controlled two-stage continuous fermentation system was applied to evaluate the effects of flow rates and enzyme loadings on performances of yeast and enzymes in continuous ethanol process with pure cellulose as the substrate. An important finding is that ethanol concentration and yield for both stages decreased with dilution rate. When the dilution rate was increased from 0.02 to 0.08 h-1 for continuous SSF of 8wt% Avicel at 15 FPU/g glucan (FPU/Beta-g = 1:1), the ethanol concentration decreased from 24.3 to 14.7 g/L for the first tank, and 32.6 to 19.8 g/L for the second at steady state, respectively. A similar trend was observed for continuous operation at a lower enzyme loading of 10 FPU/g glucan. When the dilution rate was increased to 0.12 h-1, yeast was washed out from the first tank after 6 days of continuous operation, resulting in accumulation of glucose in the first tank and an increase in ethanol concentration in the second. In addition, glucose concentrations oscillated in the first tank, particularly for runs at high enzyme loadings and high dilution rates, though cellobiose accumulation was not observed in any of the runs. These results suggest that more fundamental issues should be addressed to develop a continuous process for production of cellulosic ethanol.

BIOT 123 - Butanol fermentation by Clostridium acetobutylicum immobilized in a fibrous bed bioreactor

Wei-Lun Chang and **Shang-Tian Yang**, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Ave., Columbus, OH 43210, Fax: 614-292-3769, vaylen@gmail.com, yangst@chbmeng.ohio-state.edu

The acetone-butanol-ethanol (ABE) fermentation by Clostridium acetobutylicum was one of the oldest and largest biotechnological processes. Butanol is an important industrial solvent and potentially a better fuel source than ethanol. Because of the high toxicity of butanol, its microbiological productivity via fermentation is limited to less than 20 g/L. In this work, C. acetobutylicum cells are immobilized in a fibrous bed bioreactor (FBB) to adapt cells to tolerate higher butanol concentrations. The FBB is an effective reactor device that can not only maintain high cell densities but also help cell to be more tolerant to solvent toxicity, thus yield higher product concentration. Several process parameters, including concentrations of glucose and butyric acid were found to affect butanol production. Butanol yield increased by 50% to 100% when butyric acid was used as a cosubstrate with glucose. The metabolic engineering of the bacterium to enhance the butanol production will also be discussed.

BIOT 124 - Enzymatic synthesis of biomass-based fuel methanol

Ping Wang, Department of Bioproducts and Biosystems Engineering, University of Minnesota, 2004 Folwell Ave, St Paul, MN 55108, Fax: 612-625-6286, ping@umn.edu, F. Suhan Baskaya, Bioproducts and Biosystems Engineering, University of Minnesota, St Paul, MN 55108, and Michael C Flickinger, Department of Biochemistry, Molecular Biology and Biophysics; BioTechnology Institute, University of Minnesota, St. Paul, MN 55108

Like ethanol, methanol is viewed as an easy-to-handle liquid fuel which can be immediately used in internal combustion engines (ICE) and existing fueling stations. Methanol can also be further converted to DME, another useful liquid transportation fuel.

Currently methanol is most efficiently produced starting from natural gas in petrochemical plants at a very large scale. Herein we will present the development of a co-immobilized biocatalyst system for efficient synthesis of biomass-based liquid fuel methanol. Specifically we will discuss a multienzyme synthetic pathway for methanol production from CO2, a gasification product of biomass. The biosynthesis of methanol is the reverse process of a biological metabolic pathway catalyzed by thermotolerant dehydrogenases. The expression and production of these enzymes in Bacillus, as well as the experimental results on reaction kinetics and equilibrium of the multistep biotransformation at different conditions including pH, substrate concentration and temperature will be presented.

BIOT 125 - Long-term hydrogen production from robust nongrowing *Rhodopseudomonas palustris* coatings and strategies for increased hydrogen production

Jimmy L. Gosse[1], Brian J. Engel[1], Amer S. Al-Homoud[2], Caroline S. Harwood[3], and Michael C. Flickinger[1]. (1) Department of Biochemistry, Molecular Biology and Biophysics; BioTechnology Institute, University of Minnesota-Twin Cities, 240 Gortner Lab, 1479 Gortner Avenue, St. Paul, MN 55108, goss0052@umn.edu, (2) BioTechnology Institute, University of Minnesota-Twin Cities, St. Paul, MN 55108, (3) Department of Microbiology, University of Washington-Seattle, Seattle, WA 98195

A thin, translucent, nano-porous latex coating micro-photobioreactor has been created to uniformly distribute light to immobilized non-growing *Rhodopseudomonas palustris* CGA009 for the optimization of anoxic hydrogen (H_2) production. *Rps. palustris* can utilize numerous electron donors for H_2 production including aromatic compounds derived from lignin. *Rps. palustris* contains three functional nitrogenase enzymes and a single nonfunctional uptake hydrogenase. H_2 production from latex coatings utilizing acetate was stably maintained for over 3000 hours in a repeated batch experiment. The pH dependence of the H_2 production rate from non-growing *Rps. palustris* coatings was evaluated as well as the optimal acetate concentration. Utilizing the optimal repeated batch process parameters a bench scale 1:100th m^2 continuous flow model photobioreactor has been constructed and the hydrogen production rate was investigated.

TUESDAY MORNING

8:00 – 11:00 am BCEC 107 A

Downstream Processing: Advances in Chromatography

A. M. Lenhoff and S. Ghose, Organizers

Papers 126 - 132

BIOT 126 - Post Protein A removal of contaminants from monoclonal antibodies with a multimodal anion exchanger

Kjell Eriksson, GE Healthcare Bio-Sciences, Björkgatan 30, Uppsala 751 84, Sweden, Fax: +46-18-6121844, kjell.eriksson@ge.com, Anders Ljunglöf, GE Healthcare Bio-Sciences, Uppsala SE-751 84, Sweden, and Gustav Rodrigo, GE Healthcare Bio-Sciences, Uppsala, Sweden

By using the newly introduced multimodal anion exchange resin, Capto™ adhere, it is possible to remove residual HCP, Protein A, DNA and dimers/aggregates post Protein A in a purification process to levels acceptable for formulation, often in a two-step process. Applications will be shown that illustrate the performance of Capto adhere in flow-through as well as in bind-elute mode. We will discuss important loading parameters and how they affect antibody yield and clearance of key contaminants. The presented results are based on the use of Design of Experiments and High Throughput Process Development Technologies. In addition the excellent virus clearance performance for MuLV and MMV will be presented.

BIOT 127 - Removal of HMW species using AEX in the weak partitioning mode for antibodies and related proteins

Scott A Tobler, Aaron Noyes, Paul R Brown, Mary Switzer, and Brian Kelley, Purification Process Development, Wyeth BioPharma, 1 Burtt Rd., Andover, MA 01810, stobler@wyeth.com

A platform process for monoclonal antibody (mAb) purification has been established using Protein A and anion exchange (AEX) chromatography operated in the weak partitioning (WP) mode. The success of a two-column process relies on the ability of the AEX step to remove several impurities, including high molecular weight (HMW) species. The WP mode can help maximize the selectivity between the product (monomer) and HMW species, and has reduced HMW levels to <1% in several processes. High throughput AEX batch binding methods will be described which predict the success of this step in providing sufficient HMW reduction. Special considerations must be given to the process robustness, including effects of load challenge, load volume, and strength of product binding on step recovery and pool purity. Development of WP-AEX steps for mAbs and related proteins will be discussed, including those in which a third column step was necessary to reduce HMW to acceptable levels.

BIOT 128 - Advances in membrane affinity chromatography for the recovery of antibodies

Cristiana Boi, Simone Dimartino, and Giulio C. Sarti, Dipartimento di Ingegneria Chimica Mineraria e delle Tecnologie Ambientali, Università di Bologna, viale Risorgimento 2, Bologna 40136, Italy, Fax: +39-051-6347788, cristiana.boi@mail.ing.unibo.it

Recovery of antibodies with Protein A affinity chromatography columns has become the standard for the biotechnology industry. Membrane affinity chromatography has not yet experienced extensive application due to the lower capacity of membrane supports compared to chromatographic beads. In this work new affinity membranes endowed with high capacity for IgG will be presented. These membranes have been experimentally tested and characterized by integrating experiments and mathematical modelling of the affinity membrane process. All the relevant parameters like static and dynamic binding capacity, selectivity and purity of the recovered antibody have been evaluated and will

be discussed. Indications towards scale-up and comparison with the state of the art of affinity chromatography will be addressed.
Acknowledgement This work has been performed as part of the "Advanced Interactive Materials by Design" (AIMs) project, supported by the Sixth Research Framework Programme of the European Union (NMP3-CT-2004-500160).

BIOT 129 - The use of modeling tools for the efficient purification process development

Dennis Dong, Thomas Gervais, Duc Tran, Nicole Quinlan, Ajoy Velayudhan, Patricia Alred, and Pedro Alfonso, Pharmaceutical Development, Centocor Research & Development Inc, 145 King of Prussia Road, Radnor, PA 19087, Fax: 610-993-7864, ddong@cntus.jnj.com

Protein-A based affinity chromatography is a key platform step in the manufacture of therapeutic monoclonal antibodies and antibody derived proteins. This affinity step removes host cell proteins, DNA, and other contaminants from a clarified cell harvest.
Here we describe the use of modeling tools for Protein-A chromatography to achieve high step yield and reduce product related aggregate in the shortest amount of time. Several tools were utilized during the development of the elution conditions for a Protein-A chromatography process. These include DOE, mathematical models, and an efficient method for process optimization, the MultiSimplex model. The MultiSimplex model is a sequential method for arriving at a process optimum for systems that possess multiple input and output variables. By using this method, an optimal elution condition was achieved after only 8 experiments, resulting in 85% process yield with the reduction of product related aggregate from about 25% to1.34%.

BIOT 130 - Modeling-based rational approach to purify proteins: A case study

Beckley K. Nfor[1], Tangir Ahamed[1], Marcel Ottens[1], Emile J. A. X. van de Sandt[2], Michel H. M. Eppink[3], Gijs W. K. van Dedem[1], and Luuk AM. van der Wielen[4]. (1) Department of Biotechnology, Delft University of Technology, Julianalaan 67, Delft 2628 BC, Netherlands, Fax: 0031 (0)15 27 82355, b.kungahnfor@tudelft.nl, (2) DSM Anti-infectives B.V, Delft 2600 AK, Netherlands, (3) Biotechnology Operations, N.V. Organon, Oss 5340 BH, Netherlands, (4) Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft 2628 BC, Netherlands

The design of protein purification processes is currently limited by two main factors, namely: (1) the general lack of the relevant thermodynamic properties of proteins and (2) the lack of a rational approach for selecting and sequencing protein purification techniques. Consequently, protein purification process synthesis is mostly carried out qualitatively, based on substantial experience and heuristics, often resulting in overall suboptimal downstream processing. In our recent study, the first problem above was addressed through the development of generalized thermodynamic models of macromolecules based on the second virial coefficient concept and applicable to a number of purification techniques [1]. Here, we address the second problem above by innovating protein purification process synthesis through the use of a modeling-based rational approach. In this approach, feasible unit operations for a given separation are rigorously modeled and the product cost minimized. The necessary input data are acquired by means of a micro-scale fractionation set-up for quickly characterizing the crude mixture. The use of the purification strategy is demonstrated with a relevant case study, the purification of a bulk industrial enzyme from E. coli.
Acknowledgement: This project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public private NWO-ACTS program.
[1] T. Ahamed, M. Ottens, B. K. Nfor, G. W. K. van Dedem, L. A. M. van der Wielen. Fluid Phase Equilibria, 241, 268-282 (2006).

BIOT 131 - Investigation of protein binding in ion exchange systems with protein charge ladders and homologous protein libraries

Wai Keen Chung[1], Ying Hou[1], James J. Keba[1], George Makhatadze[2], and Steve M. Cramer[1]. (1) Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, chungw@rpi.edu, (2) Department of Biochemistry and Molecular Biology, Penn State University College of Medicine, Hershey, PA 17033
It has been a longstanding goal to be able to understand the fundamental relationships between charge density, charge distribution and protein adsorption on ion exchange resins. Using protein charge ladders in an ion exchange gradient experiment, we examined the elution order of a heterogeneous, yet homologous protein mixture. The protein mixture was first passed through a cation exchange system and the eluent fractions were then subjected to analysis using capillary electrophoresis and direct-infusion mass spectrometry. Tryptic digest LC-MS was subsequently carried out to determine the exact sites of modification on the surface of the protein. It was observed that some protein variants were eluted in an order contrary to conventional thinking. Variants with different amounts of surface charge were also seen to co-elute in the same fractions. A library of Cold Shock Protein B (CspB) protein variants with single site mutations were also employed in ion exchange retention time studies. The results showed that mutations being made to some charge sites on the protein resulted in significant changes in binding affinity, indicating that these sites may play key roles in the adsorption of the protein on the ion exchange surface. A QSPR model was generated for this homologous mutant library for the a priori prediction of retention time. Correlation of retention time data with surface charge modification has enabled us to gain insight into how surface charge affects protein binding orientation and affinity in ion exchange systems.

BIOT 132 - Analysis and prediction of protein adsorption isotherms from interaction data

Xuankuo Xu and Abraham M Lenhoff, Department of Chemical Engineering, University of Delaware, Colburn Laboratory, 150 Academy Street, Newark, DE 19716, Fax: 302-831-1048, xkxu@udel.edu

A mechanistically based isotherm model is used to model the equilibrium adsorption of proteins on ion exchangers so as to facilitate isotherm prediction using minimal experimental data. The model explicitly considers the contributions of protein-surface and

protein-protein interactions, and decoupling them allows them to be correlated with different experimental measurements. Specifically, protein-surface interactions, which affect the low-concentration linear limit of the isotherm, are related to isocratic retention factor (k′) data, while protein-protein interactions are analyzed based on high-coverage isotherm data on an arbitrary stationary phase. Analysis of experimental data within this framework reveals a high level of consistency for isotherms of three small basic proteins on four cation exchangers under different solution conditions. The model is also used to facilitate prediction of adsorption isotherms on other ion exchange media using isotherms on one adsorbent.

TUESDAY MORNING

8:00 – 11:05 am BCEC 106

Biophysical and Biomolecular Symposium: Protein Chemical Instability

E. M. Topp and M. Cromwell, Organizers Papers 133 - 140

BIOT 133 - Chemical stability of proteins in injectable drug delivery systems

Franklin Okumu, Biopharmaceutical R&D, DURECT Corporation, 2 Results Way, Cupertino, CA 95014, Fax: 408-777-3577

Multiple formulation approaches have been evaluated to integrate proteins with injectable drug delivery systems. In summary these approaches fall into three major categories. Formulations of proteins for use with hydrated systems (hydrogels, liposomes, other), dehydrated particulate systems (microspheres, nanospheres, other) and injectable liquids (polymer and non polymer based). In all cases it is critical for the formulator to preserve the proteins chemical, physical and structural integrity during the manufacturing process. In most cases the only way to achieve sufficient stability of the therapeutic protein is the removal of water. This is normally accomplished by dehydration. In general this process slows down or eliminates chemical and physical changes that could reduce potency or lead to immunogenicity. However chemical and physical changes still occur and these changes must be monitored to assess impact of formulation variables on the chemical stability of proteins. The impact of formulation composition, manufacturing and storage on chemical stability of proteins in injectable drug delivery systems will be discussed.

BIOT 134 - Fragmentation within human IgG1 hinge region: Two distinct mechanisms, two different outcomes

Josef Vlasak, Merck Research Laboratories, West Point, PA 19486, josef_vlasak@merck.com, Colleen E. Price, Merck & Co, and Steven L. Cohen, Bioprocessing and Bioanalytical Research, Merck Research Laboratory, West Point, PA 19486

Therapeutic monoclonal antibodies represent a rapidly growing field with sales projected to reach $30B by 2010. Detailed analytical characterization and understanding of the stability of monoclonal antibodies is a fundamental part of their successful development for human use. Fragmentation within the hinge region is commonly observed in recombinant human IgG1 monoclonal antibodies. The fragmentation results from of a gradual non-enzymatic cleavage within the heavy chain upper hinge sequence SCDKTHTC and leads to the generation of Fc-Fab and Fab fragments. Using bioanalytical methods combined with mass spectrometry we provide evidence that two distinct cleavage mechanisms are responsible for human IgG1 hinge-region fragmentation. Our data confirms previously proposed hydrolysis of peptide bonds within the CDKTHTC sequence. Such cleavage leads to the generation of Fab fragment, designated as “covalent Fab”, which contains the disulfide linkage between the light chain and the N-terminal heavy chain fragment. In contrast, the adjacent heavy chain S-C bond is cleaved by a different mechanism. The outcome of this cleavage is Fab fragment, designated as “non-covalent Fab”, which lacks the heavy chain-light chain disulfide bridge. The molecular mechanism leading to the formation of the non-covalent Fab is presented.

BIOT 135 - Gamma irradiation-induced damage in proteins and peptides

Sheng-Xue Xie[1], Todd D. Williams[2], Dru Willey[3], and Elizabeth M. Topp[1]. (1) Department of Pharmaceutical Chemistry, University of Kansas, 2095 Constant Ave., Lawrence, KS 66047, Fax: 785-864-5736, sxie@ku.edu, (2) Department of Chemistry, University of Kansas, Lawrence, KS 66045, (3) Clearant, Inc, Los Angeles, CA 90025

Gamma rays inactivate all known blood-borne viruses, and have been used to sterilize blood products. Gamma irradiation may affect the integrity and function of plasma proteins, however. Gamma irradiation-induced damages were studied using a monoclonal IgG antibody (38C2, Sigma-Aldrich), its heavy chain, bovine serum albumin (BSA), lysozyme or a small peptide (tocinoic acid). Model compounds were irradiated in frozen form. SDS-PAGE demonstrated that aggregates and clipping fragments were generated in proteins after irradiation, and was attributed to the scrambling of disulfide bonds. The inclusion of various free radical scavengers influenced irradiation-induced damage. Antioxidants suppressed aggregate formation of and fragmentation of proteins and the scrambling of disulfide bonds of peptides, enhancing peptide stability.

BIOT 136 - Impact of degradations on bioactivity: A reflection from a monoclonal antibody

Bryan L Yu, Alona Vizel, Meagan Young, Ashley Morando, and Bing He, Pharmaceutics, Amgen Inc, One amgen center dr, Thousand oaks, CA 91320, Fax: 805-375-5794, leiy@amgen.com

Recombinant therapeutic monoclonal antibodies are subject to a variety of physical and chemical modifications that may affect their bioactivities. Protein degradation reactions include but not limited to aggregation, clipping, oxidation, deamidation, proteolysis cleavage, disulfide-bond scrambling, glycosylation and cyclization. These degradation reactions create size, charge, or

preferred conformation heterogeneity, which may further influence their binding or neutralizing capability against their targets. The potential impact of these modifications depends on not only their nature but also the site. Our characterization works on various degradation products revealed the locations and the extent of the degradations. Together with corresponding bioassay results, the findings allowed us to attain a better appreciation of their relationships. These data were collected throughout the commercial formulation development process of an IgG2.

BIOT 137 - Selective protein oxidation through reactive oxygen species: Catalysis by cysteine

Christian Schoneich, Department of Pharmaceutical Chemistry, University of Kansas, 2095 Constant Avenue, 104 McCollum Labs, Lawrence, KS 66047, Fax: 7858644880, schoneic@ku.edu

Oxidation represents a major degradation pathway for protein formulations in solutions and solids. The parameters controlling protein oxidation are not as well understood as it would be desirable for the rational design of oxidation-resistant formulations. An important feature of the reaction of a protein with a reactive oxygen species/free radical is that the initial site of attack may not necessarily represent the final location of an oxidation site. In this presentation, we will demonstrate the potential of Cys residues to serve as a point of primary attack with intermediary Cys thiyl radicals subsequently attacking other amino acids within the protein sequence. Thiyl radicals can either involve in reversible hydrogen transfer reactions, electron transfer, or in addition reactions to aromatic amino acid residues. Experimental evidence will be provided for each of these pathways.

BIOT 138 - Using solution NMR to probe the effects of excipients on protein stability

Jennifer S. Laurence, Depts. of Pharmaceutical Chemistry and Chemical & Petroleum Engineering, The University of Kansas, Multidisciplinary Research Building, 2030 Becker Dr, Lawrence, KS 66047, Fax: 785-864-5736, laurencj@ku.edu

Oxidation, deamidation and aggregation are common maladies affecting the integrity of protein molecules. Many biophysical and analytical tools are used commonly to empirically identify conditions that protect against degradation or contribute to it. The information generated from the standard methods has provided a basis for understanding the mechanisms by which these events occur. Nonetheless, increased resolution, in the form of residue-specific information, would further enable assessment and determination of how protein stabilization is accomplished. To address this issue, we developed a simple and rapid method in which heteronuclear, multidimensional solution NMR is applied to identify chemical and structural changes in proteins, which influence both its chemical and physical degradation. The effects of individual and combinations of excipients can be assessed quickly to determine which compounds promote protein stability. Combining the screening assay with addition NMR and/or other experiments generates mechanistic information to determine how stabilization is accomplished.

BIOT 139 - Effect of residual moisture content on the stability of dried Apo2L/TRAIL crystals

Heather Flores[1], Ana Sofia Ressurreiçao[2], Boyan Zhang[3], Bruce Kabakoff[1], Zahra Shahrokh[4], and Mary E. M. Cromwell[1]. (1) Early Stage Pharmaceutical Development, Genentech Inc, 1 DNA Way, South San Francisco, CA 94080, hae@gene.com, (2) Portugal, (3) Early Stage Analytical Development, Genentech Inc, South San Francisco, CA 94080, (4) Transkaryotics Therapies, MA

Reducing the moisture content of crystalline Apo2L/TRAIL, a non-covalent homotrimer, below 5% may decrease the stability of the protein, a result significantly different than observed for proteins lyophilized in a glassy state. The primary degradation product at low moisture content is one that does not occur in solution or in non-crystalline lyophilized formulations. A concurrent increase in hexamer content by native SEC and non-reducible dimer under denaturing conditions suggests that the degradation product is a non-disulfide, covalent bond between monomers in adjacent trimers. This is further supported by the apparent ratio of one covalent dimer to four monomers in purified hexamer. Increasing the residual moisture can decrease the formation rate of this covalent hexamer but increase the rate of intramolecular disulfide bonds within the trimer. Further investigation into the degradation mechanisms of Apo2L/TRAIL crystals is ongoing.

BIOT 140 - Protein stability at solid substrates: Influence of chemical and thermal excursions

Gaurav Anand, Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, anandg@rpi.edu, and Georges Belfort, Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590

There is great interest in understanding and controlling the structural behavior of adsorbed and covalently-bound proteins. Applications include biosensors, protein microarrays, single molecule spectroscopy and atomic force microscopy (AFM) with proteins and other biophysical studies. Adsorbed proteins on surgical instruments are a potential source of transmission of conformational diseases like Alzeheimer's, CJD and type II diabetes. Misfolded proteins adhere to surfaces more strongly than their natively folded analogs. Also, proteins adsorb on hydrophobic surfaces to a greater extent than on hydrophilic surfaces. Surface properties, such as exposed functional groups, surface restructuring and surface topology, affect protein folding thermodynamics. Here, we report on the stability of chemically-immobilized hen egg lysozyme (Lys) onto a gold substrate using AFM in force-mode (i.e. a 10 μm diameter borosilicate sphere attached to the cantilever tip) so as to measure the adhesion energy between the protein and various functionalized surfaces. The structure of the immobilized Lys was thermally and chemically perturbed during the force measurements and the protein's binding energy with the different chemical moieties measured. Adhesion energies were plotted against the forcing function i.e. concentration of chemical denaturant or temperature. Results of thermal and chemical denaturation of immobilized lysozyme were then compared with those in solution phase. Effects of both stabilizing and destabilizing osmolytes on immobilized protein were also analyzed.

TUESDAY MORNING

8:00 – 11:05 am BCEC 108

Upstream Processing: Advances in Metabolic Engineering J. March and I. S. Aldor, Organizers Papers 141 - 148

BIOT 141 - Application of metabolic flux and logarithmic sensitivity analysis for optimization of *A. niger medium*

Reza Gheshlaghi, Jeno M. Scharer, and Murray Moo-Young, Department of Chemical Engineering, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L-3G1, Canada, Fax: 519-746-4979, rgheshla@engmail.uwaterloo.ca

A detailed metabolic network of more than 280 biochemical reactions and transport processes for 137 metabolites distributed among three compartments (cytoplasm, mitochondrion and peroxisome) was developed for Aspergillus niger. A unique aspect of the metabolic network was that it included carbohydrates and amino acids in both degradative and biosynthetic reactions. The general applicability of the methodology was evaluated by establishing commonality to optimize recombinant HEWL production. Experimental results indicated that HEWL production was growth associated. Linear programming was used for the optimization of the specific growth rates in combination with 37 measured input and output fluxes of the key metabolites to evaluate corresponding intracellular flux distributions throughout the batch fermentations. The importance of the implemented experimental results as constraints was proved to be necessary for having realistic results. Logarithmic sensitivity analysis and the experimental observations revealed that the addition of proline, alanine, glutamate, and tyrosine benefited growth in the defined media. In a series of preliminary nutritional experiments with extra amount of the identified amino acids contribution to growth enhancement was found to be 46% for proline, 23% for glutamate, and 22% for tyrosine. The addition of four amino acids (proline, glutamate, alanine, and tyrosine) in batch and fed-batch fermentations in a 7 liter bioreactor resulted in a 44% improvement in biomass and 41% improvement in recombinant protein production. The experiments also confirmed the model prediction that extra amount of amino acids besides the identified ones would not significantly enhance biomass and the recombinant protein production. These observations revealed that when the important organic nitrogen sources were supplied in excess, the higher concentration of glucose had a positive significant effect both in biomass and recombinant protein production. The experimental observations and simulation results presented could be used for some genetic manipulation processes.

BIOT 142 - Proteome map of *Aspergillus nidulans* during osmoadaptation

Yonghyun Kim, M. P. Nandakumar, and Mark R. Marten, Department of Chemical & Biochemical Engineering, University of Maryland, Baltimore County (UMBC), 1000 Hilltop Circle, Baltimore, MD 21250, yk1@umbc.edu

We report a comparative proteomic analysis (via 2DE and MALDI-TOF) to assess how intracellular proteins from *Aspergillus nidulans*, an industrially relevant model filamentous fungus, systematically change in a sub-optimal medium condition. Fungi grown in the presence of moderate salt concentration (0.6 M KCl) showed differential expression of 90 intracellular proteins, yet growth and morphology were not significantly affected. A total of 30 proteins were identified, of which 21 had not been previously annotated. These were assigned probable function using sequence homology and conserved domains. Identified proteins included glyceraldehyde-3-phosphate dehydrogenase, aldehyde dehydrogenase, and enolase. Together, these suggest a change in metabolic flux toward increased glycerol biosynthesis and decreased use of the TCA cycle. Other identified proteins included those involved in protein turnover, as well as five novel osmoadaptation proteins with unknown function. These insights may allow future molecular modifications to improve strains for the bioprocess industry by developing increased capacity to accommodate osmotic stress.

BIOT 143 - Nanofactories for synthesis and delivery of signaling molecules: A tool for engineering metabolism

Rohan Fernandes, Fischell Department of Bioengineering and Center for Biosystems Research, UMBI, University of Maryland, College Park, 5115 Plant Science Building # 036, College Park, MD 20742, Fax: 301-314-9075, rohan@umd.edu, Chen-Yu Tsao, Center for Biosystems Research, UMBI, Department of Chemical Engineering, University of Maryland, College Park, MD 20742, Chong Wing Yung, Chemical Engineering, University of Maryland, College Park, College Park, MD 20742, and William E. Bentley, Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742

Nanofactories for the localized synthesis and delivery of small molecules to targeted cells are demonstrated. Nanofactories comprise the dual-ability of being able to selectively attach to the target cell surface and to synthesize small molecules at the target cell surface thereby triggering their native phenotypic response, but with user-specified control. In this work, our nanofactories synthesize and deliver the 'universal' bacterial quorum sensing signal molecule autoinducer AI-2 to bacterial cell surfaces. Quorum sensing is a process that mediates intra- and inter-species bacterial communication resulting in coordinated multicellular response as observed in diverse phenomena such as biofilm formation, bioluminescence, pathogenicity. The nanofactories, made up of AI-2 synthases, Pfs and LuxS, expressed on a fusion protein attached to the target cell binding moiety, locally synthesize and deliver AI-2 from precursor SAH to the target cells thereby potentially enabling *ex vivo* fine-tuning of cellular response thus facilitating an understanding of harmful multicellular phenomena.

BIOT 144 - Engineering signal transduction for treating type 1 diabetes

John March and Faping Duan, Biological and Environmental Engineering, Cornell University, 220 Riley-Robb Hall, Ithaca, NY 14853-570

Interactions between organisms populating the human intestinal epithelia (IEC) space and human hosts are increasingly being resolved at the molecular level. As we learn more about these interactions, we gain a better understanding of how these relationships evolved and are governed. IECs together with dendritic cells (DCs) sample physically the bacterial community to maintain and update adaptive immunity. This environment of interspecies cross-communication has tremendous potential for therapeutic applications. If cross talk between IECs and bacteria can be engineered to mediate or inhibit specific signaling cascades, they could be used as *in vivo* relay stations: accessing the bloodstream, and thereby the entire host with highly specific signaling targets.
We examined the use of commensal enteric bacteria for the controlled expression of the proteins GLP-1 and PDX-1 into the intestinal epithelial cell (IEC) space. Both GLP-1 and PDX-1 have been shown to stimulate the synthesis of insulin in IECs. The use of *E. coli Nissle* 1907 for controlled release of PDX-1 or constitutive release of GLP-1 into or near the mucosal lining of the gut may mediated surrogate glucose regulatory mechanism. The fusion of insulin to a cell penetrating peptide (CPP) facilitates the uptake of insulin into human epithelial cells *in vitro*. We combined these elements and secrete both GLP-1 and/or a PDX-1-CPP fusion proteins from EcN in response to glucose by using the glucose promoter P0 from *E. coli* K12 strains. We report here on the engineered EcN's secretion rates, the rate of absorption of PDX-1 into IEC, and the level of insulin production in IEC following glucose stimulation of reconfigured EcN.

BIOT 145 - Development of a melanin-based screen for tyrosine production in *Escherichia coli*

Christine Nicole S. Santos and Gregory N. Stephanopoulos, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Building 56-422, Cambridge, MA 02139, cnsantos@mit.edu
Although many genetic methods are currently available for the manipulation of microorganisms, their application to inverse metabolic engineering is oftentimes hindered by the absence of an adequate screen capable of detecting a phenotype of interest. In order to facilitate target identification from these large combinatorial libraries, a simple, nontoxic, and high throughput assay is absolutely required. For the case of microbial production, it would be particularly advantageous to employ a metabolite-responsive assay capable of distinguishing mutants with enhanced product yields. Here, we present the development of a novel screening tool that allows for the high throughput identification and isolation of recombinant *Escherichia coli* optimized for tyrosine production. Through the introduction of a heterologous gene encoding for a bacterial tyrosinase, we are able to select for desirable mutants by visual detection of the dark and diffusible pigment melanin.

BIOT 146 - Engineering synthetic pathways for production of butanol as biofuels

Shota Atsumi, Department of Chemical and Biomolecular Engineering, UCLA, 5531 Boelter Hall, 420 Westwood Plaza, Los Angeles, CA 90034, Fax: 310-206-4107, atsumi@ucla.edu, and James C. Liao, Chemical Engineering, UCLA, Los Angeles, CA 90024
Global energy and environmental problems has stimulated increased effort in synthesizing biofuels from renewable resources. Compared to the common biofuel-ethanol, higher-order alcohols may offer advantages as gasoline substitutes because of higher energy contents and higher hydrophobicity. Here we present a metabolic engineering approach to produce butanol in a user-friendly microorganism. We built a synthetic pathway to convert glucose to butanol. Moreover, we improved the productivity by combining gene deletion and overexpression techniques. This work demonstrates the metabolic engineering principles in producing biofuels beyond those naturally accumulated to high quantities in microbial fermentation.

BIOT 147 - Intracellular enzyme crosslinking: Toward multifunctional enzyme machines for the production of R-1,2-propanediol

Robert J Conrado, Department of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853, and Matthew DeLisa, Cornell, Ithaca, NY 14853

Metabolic channeling, long established in bacterial and plant metabolism, enables cells to effectively synthesize specific products without metabolic interference, diffusion limitations, or inhibition from intermediate steps. Nature's metabolic channels employ both static and dynamic interactions to spatially organize cooperating enzymes into highly efficient and organized enzyme ensembles. The bacterial production of R-1,2-propanediol (R-1,2-PD) presents a unique opportunity for engineered channeling since this pathway includes both a bacteriocidal intermediate and undesired side reactions from competing pathways. Though unnatural, we hypothesize that metabolic channeling within this 3-enzyme network in E. coli will yield increased commercial titers of R-1,2-PD using renewable feedstocks. However, despite several computational arguments for channeling, few genetic techniques have been explored for intentionally engineering enzyme channels in vivo. We have developed and characterized a genetic selection capable of bestowing native E. coli enzymes (e.g., thioredoxin-1) with highly specific crosslinking functionality. Our preliminary results demonstrate that engineered crosslinking enzymes are active in both reducing (e.g., cytoplasm) and oxidizing (e.g., periplasm) environments, which dramatically broadens the utility of this tool. We will discuss our recent efforts to employ this post-translational crosslinking strategy for efficient production of R-1,2-PD.

BIOT 148 - Toward microbial synthesis of glucaric acid

Tae Seok Moon[1], Amanda Lanza[1], Leah Octavio[2], and Kristala Jones Prather[3]. (1) Department of Chemical Engineering, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, tsmoon@mit.edu, (2) Computational and Systems Biology, MIT, Cambridge, MA 02139, (3) Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

The growing interest in a “biomass-based” economy has led to new efforts to construct and improve microorganisms capable of producing new chemicals. While the current focus is largely on liquid biofuels, a successful “biorefinery” is likely to be a mixed-product facility, with many compounds produced from a single,

biomass-derived feed. One such "value-added" compound is glucaric acid, a molecule with potential uses ranging from human therapeutics to polymer synthesis. Glucaric acid is a natural product, found in fruits, vegetables, and mammals, but there is no known microbial pathway. We are working towards the construction of two different routes for the synthesis of glucaric acid from glucose in E. coli. The first is a three-step pathway consisting of a series of enzyme reactions derived from yeast, mammalian, and bacterial sources. While the combination of these three steps into a single organism constitutes a "novel" pathway, each step is a naturally-occurring reaction, demonstrated in the original species. In contrast, the second pathway proposes putative transformations based on the selection of enzymes primarily according to generalized enzyme reactions and not specific substrate-enzyme pairs. This latter method follows a framework of synthetic biology for "retro-biosynthetic design" of novel pathways and requires that new activities consisting of enzymes with altered substrate specificity be created. We will present progress on the construction of these pathways towards the microbial synthesis of glucaric acid.

TUESDAY MORNING

11:30 – 12:30 pm BCEC 107 A

Marvin J. Johnson Award Lecture

W. Zhou, Organizer

BIOT 149 - Molecular bioprocessing from design to discovery to dreams

Jonathan S. Dordick, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 4005 Center for Biotechnology and Interdisciplinary Studies, 110 8th Street, Troy, NY 12180-3590, Fax: 518-276-2207, dordick@rpi.edu

Abstract Not Available

TUESDAY AFTERNOON

2:00 – 2:50 pm BCEC 109 A

Emerging Technology Keynote Lecture H. Zhao and R. Srivastava, Organizers Paper 150

BIOT 150 - Energy from biomass: Anticipating a revolution

Lee R. Lynd, Thayer School of Engineering, Dartmouth College, 8000 Cummings Hall, Hanover, NH 03755, Fax: 603-646-3856, lee.lynd@dartmouth.edu

No Abstract Available

TUESDAY AFTERNOON

3:00 - 5:25 pm BCEC 107 A

Upstream Processing: Advances in Cell Culture Process Development

S. T. Sharfstein, I. Blumentals, and G. Maheshwari, Organizers Papers 151 – 156

BIOT 151 - Leveraging process characterization and validation data for continuous process improvement

Minh Luu, Robert Kiss, and Steven Meier, Process Development (LSCC), Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, luu.minh@gene.com

Post-approval changes to a process present many challenges and opportunities. In this case study, significant changes were made to enhance yield and consistency. Characterization and validation studies were undertaken in order to increase process understanding and ensure that current validation standards were met. As a result, the entire cell culture process from seed train to harvest was examined, even though some stages were unchanged. Documentation, resource requirements, and the benefits of such studies will be discussed. In addition to providing documentation to support deviation resolution for manufacturing operations, a good characterization and validation strategy can facilitate multi-facility transfers, allow for robustness-enhancing optimization, and identify key parameters for next generation process versions. The knowledge gained can also be leveraged in strategies for other products. Specific examples will be given along with some of the lessons learned.

BIOT 152 - Inhibitory threshold values of lactate, ammonia, carbon dioxide, and osmolality in CHO cell culture for a therapeutic protein production

Zizhuo Xing[1], Zhengjian Li[2], Vincent Chow[3], and Steven S. Lee[1]. (1) Biotechnology Development, Bristol-Myers Squibb Company, PO Box 4755, Syracuse, NY 13221-4755, zizhuo.xing@bms.com, (2) Process Development, Technical Operations, Bristol-Myers Squibb, Syracuse, NY 13057, (3) Michael Smith Laboratories & Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

Scale-up of a cell culture process for manufacturing a therapeutic protein is a challenge in the biopharmaceutical industry. In a case of CHO cell culture for a therapeutic protein production, higher levels of lactate, ammonia, osmolality, and carbon dioxide were observed at the manufacturing scale than the laboratory scale. The elevation of these four major metabolic by-products to inhibitory levels resulted in poor cell growth and low product concentration. Principal Component Analysis was applied to identify inhibitory threshold values of these by-products using the historical data. Our results demonstrated the following inhibitory threshold values: (1) ammonia level at greater than 5 mM to inhibit cell growth; (2) lactate and osmolality levels at greater than 58 mM and 382 mOsm/kg, respectively, to induce cell death; and (3) carbon dioxide level at greater than 111 mmHg to inhibit post-translation

modifications. Those threshold values were validated with the data from laboratory through large scales. Those threshold values were then used as guidelines to improve cell growth and the protein production in pilot scale bioreactors.

BIOT 153 - Scale-up of mammalian cell cultures controlled by automated flow cytometry

Greg Sitton and **F. Srienc**, Department of Chemical Engineering and Material Science, and BioTechnology Institute, University of Minnesota, Minneapolis/St. Paul, MN 55108, sitton@cems.umn.edu, fried@cbs.umn.edu

A significant problem in mammalian cell culture is the direct assessment of the concentration and composition of the biomass. It is well recognized that considerable single-cell variability exists in a cell culture and that this heterogeneity affects culture performance. Therefore, an automated flow cytometry system was developed to further understand how culture heterogeneity changes. Automated flow cytometry can provide online cell number and single-cell data for which a flow cytometry stain exists. We show that this device can be used to detect the onset of apoptosis which can predict the onset of stationary phase by approximately 20 hours. Therefore, by passaging the culture at the onset of apoptosis, cell death is minimized and subsequently, culture scale-up is accomplished in an automated and efficient manner. Furthermore, online cell cycle staining can be accomplished, which yields very detailed information of the cell growth dynamics that can be used for subsequent control strategies.

BIOT 154 - Metabolic process engineering applied to cell culture production processes

Bernhard M. Schilling, Biotechnology Development, Bristol-Myers Squibb, P.O. Box 4755, Syracuse, NY 13221-4755, Fax: 315 432-2930, bernhard.schilling@bms.com, Abhinav A. Shukla, Bristol-Myers Squibb, East Syracuse, NY 13057, and Steven Lee, Biotechnology Development & Operations, Bristol-Myers Squibb Co, Syracuse, NY 13221-4755

There are increasing demands placed on the understanding of cell culture based production processes for recombinant therapeutic proteins. Drivers include the expectation of a high level of operational robustness, a low level of process variability, and a high level of scientific process knowledge that can be demonstrated to regulatory agencies. The limited time available to develop or improve a cell culture based manufacturing process requires therefore a critical evaluation of the applied strategy. The primary expectation to achieve high productivities may be realized by empirical experimentation. However, such strategy will likely not lead to the generation of process 'know-why' or scientific process knowledge. The here presented approach applies metabolic process engineering to cell culture based manufacturing processes. The understanding behind targeted manipulations to the cell metabolism in the manufacturing process becomes especially critical for the production of complex antibody fusion proteins. Case studies will be presented in this contribution.

BIOT 155 - Investigating limitations during process development at reduced perfusion rates

Hans Drouin[1], Stéphane Lanthier[2], Amine Kamen[2], James M. Piret[1], and Yves Durocher[2]. (1) Michael Smith Laboratories & Department of Chemical and Biological Engineering, University of British Columbia, 2185 East Mall, Vancouer, BC V6T 1Z4, Canada, Fax: 604-822-2114, (2) Technologie des cellules animales, CNRC - Institut de Recherche en Biotechnologie, Montreal, QC H4P2R2, Canada

To increase perfusion culture product titres the strategy of operation at decreased dilution rates with fortified media was explored. HEK293 cells producing interferon-α2b were cultivated in perfusion mode until the system reached a steady state at 5.3 x 10^6 viable cells/mL with a specific productivity of 26 pg/cell-day. The dilution rate was then decreased every 5 days while the feed was enriched to maintain the nutrient feed rate. However, the cell specific rates decreased such that the product titre did not increase. Batches cultivated in fresh medium, spent medium (harvested during perfusion) and nutrient-enriched spent medium were compared to identify the major limitation between metabolite accumulation and nutrient depletion on the growth rate and productivity. Specific growth rates were 25% lower with spent medium cultures indicating a predominant growth inhibition effect that should explain the decreased productivity during the perfusion culture. Dilution rate should be reduced according to inhibition level while bleed rate maximized for growth sensitive cells.

BIOT 156 - High-throughput cell culture process development using 3-D microbioreactors and online quantifications of cell growth and GFP expression

Xudong Zhang, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Ave., Columbus, OH 43210, zhangx@chbmeng.ohio-state.edu, Yuan Wen, Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210, and Shang-tian Yang, Dept. of Chemical Engineering, Ohio State University, Columbus, OH 43210

A high-throughput, real-time, bioactivity assay based on three-dimensional (3D) cultures of GFP-expressing cells can provide accurate, parallel, non-invasive, and long-term cell bioactivity assays. The 3D culture assays were developed on two different microbioreactor platforms for high-throughput cell culture process development. The first platform contained 40 batch microbioreactors on a 384-well plate, which was used to optimize media components for antibody production by CHO cells. The second platform contained 4 x 4 microfluidic bioreactors with the capability of continuous perfusion. This scaled-down perfusion system can be used to quickly optimize a bioprocess with minimal reagent consumption. Finally, an online fluorescence probe was developed and used for monitoring and quantifying cell growth and antibody production in the FBB. A strong correlation between fluorescence signals and antibody production was observed, which can be used to study the effects of external stimuli on antibody production, and to guide process operation and optimize its productivity.

TUESDAY AFTERNOON

3:00 – 5:20 pm BCEC 106

Downstream Processing: Molecular Interactions and Modeling J. Thommes and K. M. Lacki, Organizers Papers 157 - 162

BIOT 157 - Custom chromatography: Optimizing surface functionalization to meet specific separation needs

Charles A. Haynes, Biotechnology Laboratory, University of British Columbia, 237 Wesbrook Bldg, Vancouver, BC V6T 1Z3, Canada, Fax: (604) 822-2114, israels@chml.ubc.ca

Rising downstream processing costs and speed-to-market pressures for recombinant protein therapeutics have recently led industry to develop high-throughput platforms to optimize selection of chromatographic media and suitable operating conditions. Most of these platforms are based on pipetting-robot screening of media from commercial suppliers, a strategy limited by the fact that these media are designed to provide acceptable purification performance for a wide range of feedstocks, rather than optimal resolving power for a particular feedstock. In this talk, I report on our ongoing efforts to develop tools for custom design and synthesis of tentacle-phase chromatography media to achieve a desired separation on the basis of protein size. Our technology, called entropic interaction chromatography (EIC), is based on the concept that protein partitioning into a grafted polymer brush decreases the entropy of both the protein ( ) and the terminally attached polymer chains ( ) displayed within the separation media. Surface-initiated atom-transfer radical polymerization (ATRP) is used to prepare preparative EIC media covering a wide range of grafted-chain densities and molecular weights. When informed by a molecular thermodynamic model designed to optimize brush properties, ATRP allows one to precisely modulate the dependence of both and on solute molecular weight, providing a powerful new strategy to fine-tune size-based separations to meet specific needs.

BIOT 158 - Molecular dynamic investigation of the interaction of supported affinity ligands with monoclonal antibodies

Laura Zamolo, Valentina Busini, Davide Moiani, Davide Moscatelli, and Carlo Cavallotti, Dept. Chimica, Materiali e Ingegneria Chimica, G. Natta, Politecnico di Milano, Via Mancinelli, 7, Milano, Italy, laura.zamolo@polimi.it

Monoclonal antibodies are gaining an increasing importance in diagnostic and therapeutic treatment of acute diseases such as cancer. However, the production and purification of these pharmaceuticals is still extremely expensive. Among the different methods for MAB purification, affinity chromatography is one of the most employed, despite its cost. In this work we present our computational investigation aimed at obtaining some guidelines for the rational design of affinity ligands, through the study of their interactions with both the antibody (IgG) and a model support material (agarose). This analysis was carried out performing MD simulations of the support-spacer-ligand-IgG system in explicit water. Two commercial ligands and several spacers, which differ for hydrophobicity and chemical structure, were considered. Binding energies were determined with the LIE and MM-PBSA approaches. The results were compared with experimental data and revealed that the interaction of the ligand with spacer and support can significantly affect the binding process.

BIOT 159 - Construction and study of porous polymer media for ion-exchange adsorption by molecular dynamics modeling and simulations

E. Riccardi, J.-C. Wang, and **A. I. Liapis**, Department of Chemical and Biological Engineering and Biochemical Processing Institute, University of Missouri-Rolla, Rolla, MO 65409-1230, ail@umr.edu

The MD modeling and simulations performed in this work provide detailed information about the structure of the porous dextran layers, the distribution of the pore surface area of the porous polymer structure, the distribution of the selected affinity groups/ligands on the surface of the porous polymer structure, as well as the effect of the density of the affinity groups/ligands on the state of the porous polymer structure before the start of the interaction of the analyte with the immobilized ligands. Furthermore, the transport in the pores and the interaction of the molecules of a selected analyte with the immobilized ligands is studied, and these physicochemical mechanisms have a dynamic effect on the pore structure of the dextran layer as well as on the transport and further adsorption of the analyte. Such studies can result in the appropriate selection and design with respect to length and side branching of dextran polymer chains, so that the resulting porous polymer structures are appropriate, after the chosen affinity group/ligand has been immobilized on the surface of the pores, for the effective separation of a biomolecule of interest by ion-exchange adsorption.

BIOT 160 - Investigation of ligand-protein binding affinity in ion exchange chromatography

Ting Yang[1], Wai Keen Chung[1], Scott A. McCallum[2], James Kempf[3], and Steve M. Cramer[1]. (1) Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, Fax: 518-276-4030, yangt3@rpi.edu, (2) Director, NMR Core Facility, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, (3) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180

A library of cationic ligands that exhibited different binding affinity to proteins at high salt conditions was studied using NMR techniques. Descriptors were calculated based on the ligand structures and Quantitative Structure Property Relationship (QSPR) models were developed. The selected features from the models were used to gain insight into the physicochemical properties of these ligands for binding proteins under high salt conditions. The NMR experiments were performed using radioisotope-labeled RNAse A and lysozyme as model proteins to investigate the effect of buffer conditions (salt concentration and mobile phase pH) on the binding behavior of these ligands to the proteins. The regions of interaction on both protein and ligand were identified and the binding kinetics was obtained. Docking calculations were also carried out to investigate the binding of

these ligands. The approaches developed in this work will facilitate future design of novel mixed mode ligands for complex bioseparations.

BIOT 161 - Molecular level inspection of viral clearance and membrane adsorbers

William T. Riordan and Mark R Etzel, Department of Chemical and Biological Engineering, University of Wisconsin, 1415 Engineering Drive, Madison, WI 53706-1607, wtriordan@wisc.edu

Viral clearance using disposable membrane adsorbers has evolved into a method of choice for many in protein therapeutics manufacture. However, little work has been done to understand this separation at the molecular-level, and no mechanistic model exists to predict viral clearance based on system parameter values. In this work, we used surface plasmon resonance to measure directly the adsorption kinetics between the immobilized ligand used in membrane adsorber products and the impurity molecules in solution such as virus and DNA. Next, we measured breakthrough curves and log reduction values for DNA and model bacteriophage viruses using a small-scale membrane adsorber. We compared these experimental observations to predictions from two diametrically opposed mathematical models: (1) adsorption kinetics controls performance, and (2) equilibrium sorption controls performance. The results of these studies indicate that the adsorption kinetics are not the rate-limiting factor, and that impurity-ligand solution equilibrium may be an important factor. However, not all observations can be explained perfectly using these models, and there is much we still do not understand about the science of viral clearance operations using membrane adsorbers.

BIOT 162 - Modeling of protein monomer/aggregate separation using Hydrophobic Interaction Chromatography: Effects of column scale

Justin T McCue, Process Biochemistry, Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142, Fax: 617-679-3408, justin.mccue@biogenidec.com

Hydrophobic interaction chromatography (HIC) is commonly used to separate monomer and aggregate species in the large-scale manufacturing of therapeutic proteins. Despite its ability to resolve monomer and aggregate forms, HIC often suffers from relatively low yields (< 80%) and is often limited to relatively low column loadings (< 20 mg protein/mL resin), in order to achieved acceptable monomer purity levels. Additionally, aggregate removal using HIC can be highly sensitive to the column operating conditions, including the fluid velocity and column packing quality (HETP). During column scale-up, the packing quality and flow distribution may change, which could potentially increase aggregate levels to above acceptable levels, potentially resulting in the loss of the batch. In this work, a theoretical model was formulated to describe the monomer/aggregate separation process for a therapeutic protein using HIC. The adsorption isotherms and binding kinetics of the individual monomer and aggregate species were evaluated in laboratory scale studies. Using the experimental parameters, the model was used to predict the effects of the column operating conditions and column packing quality (HETP) on column performance. Such predictions were used to quantify acceptable levels for the HETP for large scale columns and what impact the scale-up process could have on the performance of the HIC step.

TUESDAY AFTERNOON

3:00 – 5:30 pm BCEC 108

Commercialization of Biologics: Impact of Design Space Philosophy & Emerging Regulations on Process Validation

G. Knipp, K. Webber, and A. Kataria, Organizers
Papers 163 - 168

BIOT 163 - Efficient approach to setting validation acceptance criteria for a biopharmaceutical process

Tom Gleason[1], Rick Burdick[2], Steve Rausch[1], and James E Seely[3]. (1) Process Development Dept, Amgen, 4765 E. Walnut St., Boulder, CO 80301, tgleason@amgen.com, (2) Quality Engineering, Amgen, Longmont, CO 80503, (3) Manufacturing Science and Technology, Amgen Colorado, Longmont, CO 80503

To ensure that a process is working as expected, intermediate process steps typically have validation acceptance criteria (VAC) that must be met to provide assurance that the product quality attributes will meet final specifications. VAC based on only full or pilot scale manufacturing data can be problematic because operating parameters are typically run at their set points; therefore, little information can be gained regarding how the process is affected due to variations within these parameters' operating ranges. Including bench-scale characterization data from qualified bench-scale models in the analysis provides a way to better and more rapidly predict future process performance. We describe statistical approaches to analyzing bench-scale characterization data (including testing at the edges and 3X outside the normal operating ranges) combined with large-scale data, to set VAC. This allows the establishment of appropriate VAC in instances where there are limited large-scale data due to time or resource constraints.

BIOT 164 - A systematic method for design space definition

Roy D. Hegedus, Process Sciences Department, Abbott Laboratories, 100 Research Drive, Worcester, MA 01605, Fax: 508-793-4885, roy.hegedus@abbott.com

A pharmaceutical product has many quality attributes. Each attribute of each dose of the product must meet the specification. The definition of how to operate the manufacturing process in order to produce acceptable product is called the design space. International Conference on Harmonisation document Q8 provides guidance on the requirements for a design space but does not impose limits on how to determine or define the design space. A systematic method for the determination of the design space is

helpful because of the complexity of manufacturing processes and the large number of specifications to be met. A table which mathematically relates each attribute to the appropriate steps in the process can be used to define the design space in order to achieve a desired process capability index (Cpk) for that attribute. The table can also be used to organize the laboratory experimentation for the definition of design space. This talk will describe how to connect the design space to Cpk with this systematic method and includes examples from the purification of products from cell culture.

BIOT 165 - Apply design space philosophy in the establishment of process validation parameters and acceptance criteria

Xiangyang Wang[1], Jean Harms[1], Pim Van Hoek[1], and Anurag S. Rathore[2]. (1) Process Development, Amgen Inc, One Amgen Center Drive, Mail Stop 30W-2-A, Thousand Oaks, CA 91320, wangx@amgen.com, (2) Amgen, Inc, Thousand Oaks, CA 91320

Significant time and resource are invested in conducting process characterization studies by biotech industry to determine appropriate parameters and acceptance criteria for process validation and to define the "design space", within which the process produces acceptable product consistently. This is in trend with the recent release from FDA emphasizing streamlined regulatory oversight to quality by design and process understanding. This talk will use specific examples from multiple products that have recently gone through commercialization at Amgen to demonstrate the critical role of process understanding and statistical analysis in defining the design space and in setting up meaningful and defendable criteria for process validation. The key and critical process parameters and product quality attributes are continuously verified post process validation through process monitoring and evaluation of in-process materials during commercial production. Process improvement after initial implementation within design space should require minimum process validation and regulatory filing.

BIOT 166 - Quality by design and process analytical technology for freeze drying

Michael Pikal, School of Pharmacy, University of Connecticut, Storrs, CT 06269, pikal@uconnvm.uconn.edu

Successful applications of "Quality by Design" require at least a minimal knowledge of the basic physics of the formulation and process. Freeze drying is a unit operation for which the fundamentals are relatively well understood, and the fundamental formulation science critical for bio-stabilization has seen considerable advancement over the past decade. Thus, we argue that freeze dried dosage forms present an ideal opportunity to practice "Quality by Design". It is our thesis that formulation development can be effectively guided by the knowledge and general rules developed over the past decade, and much (perhaps most) of the process development and robustness testing can effectively carried out by calculation, provided that key physical parameters are evaluated by the appropriate experiments. Examples of such formulation and process development/optimization will be discussed. Further, for optimal design and control, the use of Process analytical technology (PAT) offers an opportunity for improved consistency in product quality as well as significant reductions in the cost of manufacturing. Ironically, although PAT technology does exist for freeze-drying, PAT has been grossly underutilized in this important unit operation. This presentation will review current freeze drying PAT as well as discuss several new technologies: (1) mass flux and product temperature measurement via Manometric Temperature Measurement, and (2) Doppler shift Tunable Diode Laser Absorption Spectroscopy (TDLAS) measurement of mass flux and gas flow velocity in freeze dryers.

BIOT 167 - Critical process parameters and design space

Peter W. Wojciechowski, Process Technology Department, Global Biologics Supply Chain, 200 Great Valley Parkway, Malvern, PA 19355, pwojcie2@gbsus.jnj.com

Design Space and Critical Process Parameters (CPPs) are familiar terms but have yet to take on a precise, universally understood meaning in our industry. This presentation is an exploration of one company's challenges in identifying CPPs, positioning them in regulatory submissions and understanding the risks and benefits of doing so. The CPP identification process begins during process development where Design of Experiments is used to understand the sensitivity of Critical Quality Attributes (CQAs)to wide ranges of critical and non-critical Process Parameters. Scale-up and technology transfer require a CPP risk assessment using the principles of Quality Risk Management to prioritize those Process Parameters that require the tightest control. Process validation then demonstrates the ability to consistently control CPPs at the commercial scale. Reduced scale process validation studies demonstrate the ability to manufacture product within specified CQA ranges when CPPs vary over their proven acceptable ranges. The validated CPP ranges may then be used to help define a Design Space for regulatory submissions.

BIOT 168 - Use of online Process Analytical Technology (PAT) to monitor Maytansinoid conjugation to modified antibodies: Implications in establishing robust processes for manufacturing disulfide-linked immunoconjugates

Ian M. Schwartz[1], Sanjiv Gohain[1], Dana C. Pentia[1], Godfrey Amphlett[2], Deborah H. Meshulam[1], and Gennady Malin[1]. (1) Process Science and Engineering, ImmunoGen, Inc, 128 Sidney Street, Cambridge, MA 02139, Fax: 617-995-2544, ian.schwartz@immunogen.com, (2) Process and Product Development, ImmunoGen, Inc, Cambridge, MA 02139

Process Analytical Technologies (PATs) are used primarily in the biotechnology industry during cell culture, filtration and column chromatography. A new application of PAT was established for the development and manufacture of immunoconjugates. Our PAT monitors reaction progression during disulfide exchange between a bispecific linker on the modified antibody and small molecule cytotoxic agent (Maytansinoid, DM4) by absorbance of released thiopyridine at 343 nm. The absorbance reaches a plateau upon saturation of available conjugation sites on the antibody. The presented approach will not only facilitate the process development

for protein conjugates by determining time to reaction completion, reagent excess, and effects of environmental variables including pH and temperature, but will also improve process robustness by interfacing online monitoring with automated reagent addition. This methodology will establish a more robust "self tuning" process that will respond in real time to variations in raw materials and manufacturing variables during protein conjugation.

TUESDAY AFTERNOON

3:00 – 5:35 pm BCEC 109 A

Upstream Processing: Advances in Metabolic Engineering G. Knipp, K. Webber, and A. Kataria, Organizers Papers 169 - 175

BIOT 169 - Incorporation of thermodynamics and metabolomics data into constraint-based model of Methylobacterium extorquens AM1

Robin E. Osterhout[1], Mariet van der Werf[2], and Steve Van Dien[1]. (1) Division of Metabolic Engineering, Genomatica, Inc, 5405 Morehouse Dr, Suite 210, San Diego, CA 92121, Fax: 858-824-1772, (2) Division of Analytical Biosciences, TNO Quality of Life, 3700 AJ Zeist, Netherlands

With the advent of high-throughput methods for "omics" technology, large datasets are rapidly being generated for a number of different organisms. Equal in importance is the development of methods for the analysis and interpretation of such data. To address this need, Genomatica has developed a set of computational tools for interpreting various data types within the constraint-based modeling framework. The goal of this effort is to utilize experimental data in conjunction with physicochemical properties to improve the quality of metabolic models and the accuracy of their predictions. Several computational methods have recently been developed to implement thermodynamic feasibility constraints in metabolic flux analysis. Metabolomics data can provide additional constraints, but currently there is a lack of high-quality data that provides good coverage of the network. Using a non-targeted approach, 134 metabolites in *Methylobacterium extorquens AM1* were identified and quantified in two different growth conditions: growth on methanol and succinate. We used this data to refine and validate a genome-scale metabolic model. Computational methods incorporating thermodynamic feasibility and quantitative metabolomics data were then applied to the network to test the predicted flux distribution. Using this approach, we were able to further constrain the flux space of the model and identify bottlenecks and potential regulatory sites under different growth conditions. This work has implications not only for the use of *M. extorquens* for the production of chemicals from methanol, but also improves our overall ability to utilize a model-driven approach for metabolic engineering.

BIOT 170 - Metabolic engineering of natural and unnatural flavonoid biosynthesis in microorganisms and their application for diabetes treatments

Joseph Chemler, Mattheos Koffas, Emmanuel S. Tzanakakis, Yajun Yan, Effendi Leonard, Zacchary Fowler, and Lye T. Lock, Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, 914 Furnas Hall, Buffalo, NY 14260, Fax: 716-645-3822, jchemler@buffalo.edu

Flavonoids are a diverse group of secondary metabolites found ubiquitously in the plant kingdom. Many flavonoids are used as nutraceutical supplements but a growing number of unnatural flavonoids are being investigated as therapeutic agents. Our group has been focusing on utilizing metabolic engineering to synthesize natural plant flavonoids (isoflavones, flavanones, flavones, dihydroflavonols, stilbenes, flavonols, catechins and anthocyanins) in microorganisms and their use for diabetes treatment. Our progress has covered the reconstruction of the plant biosynthetic pathway within both Escherichia coli and Saccharomyces cerevisiae. We have been able to increase flavonoid production yields by either introducing additional enzymes or inhibit competing pathways to increase intercellular pools of necessary auxiliary compounds such as malonyl-CoA and UDP:glucose for biosynthesis of specific flavonoids. In addition, we have explored the synthesis of novel flavonoid analogs using a mutasynthesis approach. Studies conducted in our lab as well as in collaboration efforts have shown that these compounds have significant biological activity towards digestive enzyme inhibition and up regulation of insulin secretion from pancreatic beta-cells.

BIOT 171 - Metabolic engineering of Saccharomyces cerevisiae for enhanced uptake and sequestration of arsenic

Michael Shen[1], Shailendra Singh[2], Dhawal Shah[1], Wonkyu Lee[2], Wilfred Chen[2], and **Nancy A. Da Silva**[1]. (1) Chemical Engineering and Materials Science, University of California, Irvine, 916 Engineering Tower, Zot 2575, Irvine, CA 92697-2575, (2) Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, CA 92521

Arsenic (As) is an extremely toxic metalloid that adversely affects human health and is currently ranked number one on the EPA's priority list. Increasingly restrictive federal regulation of allowable levels of arsenic in water (10 ppb) has prompted the development of novel remediation technologies. Biological methods are gaining momentum because of their potential in providing a cost effective and environmentally benign technology for arsenic remediation. A promising strategy is to engineer a "designer biosorbent" with enhanced arsenic uptake and accumulation capability. In this research, we are engineering a yeast strain optimized for both arsenic uptake and sequestration, culminating in the design of a resting cell system capable of the continuous removal of arsenic from contaminated water. Three major strategies were adopted: (1) overexpression of transporters to increase uptake of As(III) and As(V), (2) elimination of the Acr3p extrusion pathway to retain As inside the cell, and (3) introduction of new proteins for ATP-independent As sequestration. Potential limiting steps such as arsenate reduction and glutathione synthesis have also been addressed. Overexpression of the As(III) transporter Fps1p or Hxtp, the As(V) transporter Pho84p, or the *Arabidopsis thaliana* phytochelatin synthase gene (for As sequestration) each resulted in a 2-10 fold increase in intracellular As content. Similarly, the introduction of metallotheins and the highly specific *Escherichia*

coli ArsRp resulted in enhanced accumulation. The *acr3* knockout strain (no extrusion pathway) gave the highest levels of internal arsenic. The individual strategies also allowed the removal of *trace* arsenic (<50 ppb) in a buffer solution; *PHO84* overexpression resulted in nearly instantaneous uptake of trace arsenate. Current efforts are aimed at combining these independent strategies. We are also optimizing the system by integrating uptake and sequestration genes into the host chromosomes. The goal is to balance all pathways and select the best gene combination for maximal metalloid uptake.

BIOT 172 - Metabolic flux elucidation for large-scale models: 13C labeled isotope experiments and experimental design

Patrick F. Suthers[1], Anthony P. Burgard[2], Madhukar S. Dasika[1], Farnaz Nowroozi[3], Stephen Van Dien[2], Jay D. Keasling[3], and Costas D. Maranas[1]. (1) Department of Chemical Engineering, The Pennsylvania State University, 147 Fenske Lab, University Park, PA 16802, suthers@engr.psu.edu, (2) Genomatica, Inc, San Diego, CA 92121, (3) Department of Chemical Engineering, University of California - Berkeley, Berkeley, CA 94720-1462

One of the key considerations in metabolic engineering is determining fluxes of metabolites within the cell, which provides an unambiguous description of metabolism before and/or after engineering interventions. Here, we present a computational framework that combines a constraint-based modeling framework with isotopic label tracing on a large-scale. This model includes 350 fluxes, 183 metabolites, balances on cofactors such as ATP and NADH as well as the electron transport chain and a detailed biomass equation. Experimental results are presented for an *Escherichia coli* strain engineered to produce amorphadiene, a precursor to the anti-malarial drug artemisinin. Subsequently, we introduce a degrees of freedom based optimization method to exhaustively identify all combinations of isotope labeling experiments and flux measurement that completely resolve all flux values in the network. We also address how labeled substrate choice impacts the ability and ambiguity of elucidation of fluxes in large-scale metabolic reconstructions.

BIOT 173 - Biosynthesis of fosfomycin

Zengyi Shao, Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, 600 S. Mathews Avenue, 222 Roger Adams Laboratory, Box C3, Urbana, IL 61801, zshao@uiuc.edu, Ryan Woodyer, Department of Chemistry, University of Illinois, Urbana, IL 61801, and **Huimin Zhao**, Department of Chemical and Biomolecular Engineering, University of Illinois, 215 RAL, Box C3, 600 S. Mathews Ave., Urbana, IL 61801, Fax: 217-333-5052, zhao5@uiuc.edu

Phosphonic acids are a group of C-P bond containing natural products, many of which show important biological activities such as antibacterial, antiviral, pesticidal, and anti-cancer properties. However, very few biosynthetic pathways of phosphonic acids have been cloned, heterologously expressed, and biochemically characterized. Here we report the cloning of the fosfomycin gene cluster from Streptomyces wedmorensis, and the synthesis of fosfomycin in heterologous hosts including Streptomyces lividans and E. coli. The synthesis of fosfomycin in Streptomyces lividans was confirmed by an enzyme inhibition assay, NMR, and high resolution FTMS. Gene knockout and deletion experiments showed that a previously unknown gene, orfC plays a crucial role for the formation of fosfomycin. Detailed characterization of the enzyme encoded by orfC showed that it catalyzes a NADPH-dependent dehydrogenation step. In addition, phnDCE genes encoding an ABC type transporter were identified from the cluster, which might assist the transport of phosphonates between the organisms and the environment. Heterologous expression of the fosfomycin biosynthetic pathway in E. coli was also achieved recently and further engineering of E. coli for cost-effective production of fosfomycin is in progress.

BIOT 174 - Probing the supply-side attributes of different metabolically engineered host backgrounds

Drew Cunningham, Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, dcunning@andrew.cmu.edu, Nathand Domagalski, Bristol Meyers Squibb, Clinton, NJ, Richard Koepsel, Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15232, Mohammad M Ataai, Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15261, and **Michael M. Domach**, Department of Chemical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Doherty Hall, Pittsburgh, PA 15213, Fax: 412-268-7139, md0q@andrew.mu.edu

The net production of a heterologous protein depends on the balance struck between synthesis and protease-mediated degradation. Via network analysis (METABOLOGICA) and experimentation, we have probed the performance of different engineered E coli and B subtilis strains from the synthesis-side standpoint. That is, we explored how host metabolic engineering affects (i) excess redirectable metabolic capacity and (ii) positive transcription factors. We will show that when made deficient in pyruvate kinase (pyk-), theory indicates that more heterologous protein synthesis can be tolerated. Molecular biology evidence also suggests that when growth occurs on glucose, pyk- hosts behave less as catabolite-repressed cells and more transcription occurs from cAMP responsive genes. The expression of a protease-resistant protein will be shown to concur with these predictions; both pyk- E. coli and B. subtilis make much more heterologous protein per cell as compared to wild types, while growth kinetics is not significantly altered. Extension to folate synthesis will then be summarized to show a small molecular product result.

BIOT 175 - Growth-phase accumulation of poly-3-hydroxybutyrate in *Escherichia coli*: A prospectus for continuous production

Keith E. Tyo and Gregory N. Stephanopoulos, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave., Room 56-422, Cambridge, MA 02139, ktyo@mit.edu

While biological routes to desired products have mainly been applied to high value materials, an increasing demand for production of high-volume, low-margin chemicals is being driven by uncertainty in petroleum-based feedstocks. In such cases, continuous production of the desired product can have economic advantages over traditional batch processes. Important parameters

for continuous culture are yield, volumetric productivity, and genetic stability of the production strain. In the case of recombinant poly-3-hydroxybutyrate (PHB) in *Escherichia coli*, high metabolic demands for PHB set up an intricate tradeoff between specific growth and productivity which can be controlled genetically. In this study, we examine the effects of systematic perturbations to the recombinant PHB pathway (expressed from both plasmid and genomic integration), on growth rate, growth phase accumulation of PHB, and stability of expression. Experimental data and stoichiometric models will be used to evaluate the most desirable modes of metabolism for continuous PHB production.

Program by Day

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

WEDNESDAY MORNING

8:00 - 11:00 am	**Emerging Technologies: Systems Biology**	**C. Chan and C. Cho, Organizers**	**Papers 176 - 183**	**BCEC 108**
8:00 - 11:00 am	**Biophysical and Biomolecular Symposium: Targeted Delivery of Proteins & Nucleotides**	**C. Meares, W. Liu, and A. Blake-Haskins, Organizers**	**Papers 184 - 191**	**BCEC 107 A**
8:00 - 11:00 am	**Commercialization of Biologics: Characterization & Improvement of Platforms to Aid Commercialization of Biologics**	**S. Gadam and F. Olsen, Organizers**	**Papers 192-199**	**BCEC 106**
8:00 - 10:30 am	**Upstream Processing: Advances in Cell Culture Process Development**	**S. T. Sharfstein, I. Blumentals, and G. Maheshwari, Organizers**	**Papers 200-205**	**BCEC 107 C**
11:30 - 12:30 pm	**Elmer Gaden Award Lecture**	**W. Zhou, Organizer**	**Paper 206**	**ECEC 107 B**

WEDNESDAY AFTERNOON

2:00 - 2:50 pm	**BIOT Young Investigator Award Lecture**	**W. Zhou, Organizer**	**Paper 207**	**BCEC 108**
3:00 - 5:20 pm	**Emerging Technologies: Systems Biology**	**C. Chan and C. Cho, Organizers**	**Papers 208-213**	**BCEC 107 B**
3:00 - 5:20 pm	**Biophysical and Biomolecular Symposium: Targeted Delivery of Proteins & Nucleotides**	**C. Meares and W. Liu, Organizers**	**Papers 214-219**	**BCEC 106**
3:00 - 5:20 pm	**Commercialization of Biologics: Characterization and Improvement of Platforms to Aid Commercialization of Biologics**	**S. Gadam and F. Olsen, Organizers**	**Papers 220-225**	**BCEC 107 C**
3:00 - 5:20 pm	**Biophysical and Biomolecular Symposium: Protein Aggregation**	**C. J. Roberts and A. Grillo, Organizers**	**Papers 226-231**	**BCEC 108**

WEDNESDAY EVENING

5:30 – 7:30 pm	**Poster Session**	**Y. Tang, S. Ozturk, and S. Subramanian, Organizers**	**Papers 232-428**	**BCEC HALL C**
6:30 PM	**Divisional Business Meeting**			

WEDNESDAY MORNING

8:00 – 11:00 am BCEC 108

Emerging Technologies: Systems Biology

C. Chan and C. Cho, Organizers Paper 176 - 183

BIOT 176 - Scaffold proteins tune the regulatory properties of protein kinase cascades

Jason W Locasale, Department of Biological Engineering, Massachusetts Institute of Technology, 40 Ames St E19-534, Cambridge, MA 02139, locasale@mit.edu

The ubiquity of scaffold proteins in cell signaling pathways suggests that scaffold proteins are likely to have many regulatory functions. Here, we present Monte Carlo simulations of a model kinase cascade to investigate how characteristics of signaling cascades are influenced by the presence of scaffold proteins. Our results indicate that scaffold proteins can have a dual-regulatory function by amplifying and attenuating the amplitude of incoming signals depending on the physiological context. We also study how scaffold proteins can alter the temporal characteristics of a signal propagating along a kinase cascade. These simulations suggest that scaffolds can influence signal propagation by changing the duration of signaling as well how the output signal is distributed over time. Such behavior illustrates one of the many control properties that scaffold proteins may confer to a cellular signaling process. It is our hope that these simulations provide a roadmap for future experiments – several key predictions obtained from our model can be tested with single-molecule technologies.

BIOT 177 - Molecular systems biology via multiscale modeling and high-performance computing

Jeremy Purvis, Yingting Liu, Andrew Shih, Neeraj Agrawal, and **Ravi Radhakrishnan**, Department of Bioengineering, University of Pennsylvania, 240 Skirkanich Hall, 210 S 33 Street, Philadelphia, PA 19104, Fax: 215 573 2071, purvis@seas.upenn.edu, rradhak@seas.upenn.edu

Recent biochemical and epidemiological studies have shown that the signaling through the epidermal growth factor receptor (EGFR) can be sensitive to various tyrosine kinase inhibitors (TKIs) depending on the receptor's expression level and whether or not the tyrosine kinase domain harbors any somatic mutations. We describe a hierarchical multiscale computational approach based on molecular dynamics simulations, free energy based molecular docking simulations, deterministic network-based kinetic modeling, and hybrid discrete/continuum stochastic dynamics protocols to study the dimer-mediated receptor activation characteristics, signal transduction, and inhibition of the Erb family receptors, specifically the epidermal growth factor receptor (EGFR). By modeling signal flows through branching pathways of the EGFRTK resolved on a molecular basis, we are able to transcribe the effects of molecular alterations in the receptor (e.g., mutant forms of the receptor) to differing kinetic behavior and downstream signaling response. Our simulations reveal molecular mechanisms for receptor kinase activation and show that the drug sensitizing mutation (L834R) of EGFR signals preferentially to evoke a downstream Akt response and is also preferentially susceptible for its inhibition explaining the hyper-sensitivity of the tyrosine kinase inhibitor erlotinib in cell-lines carrying the mutation. These results are consistent with qualitative/quantitative experimental measurements reported in the literature. We believe that our model driven approach will in the long-term significantly impact the optimization of future small molecule therapeutic inhibition strategies as well as the formulation of drug-resistance models.

Y. Liu, J. Purvis, A. Shih, J. Weinstein, N. Agrawal, R. Radhakrishnan, 2007, Annals of Biomedical Engineering, in press; J. Purvis, Y. Liu, V. Ilango, and R. Radhakrishnan, to be submitted to IEE Sys Biol.

BIOT 178 - Predicted points of cell-cycle fragility are consistent with known malfunctions in solid and hematological cancers

Satyaprakash Nayak[1], Saniya Salim[2], and Jeffrey D. Varner[1]. (1) School of Chemical and Biomolecular Engineering, Cornell University, 244 Olin Hall, Cornell University, Ithaca, NY 14853, sn248@cornell.edu, (2) Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853

Cell division or cell cycle is one of the most fundamental and highly regulated processes in biology. Malfunctions in cell cycle, as evidenced by uncontrolled proliferation underlie many cancers. Our working hypothesis is that a single-cell mathematical model of cell-cycle, which mechanistically describes the molecular events controlling cell division, could be used to computationally determine points of network fragility that correspond to known, clinically observed, cell-cycle malfunctions. We analyzed three different published models of cell-cycle progression and compare our computationally identified fragile points with literature. A third-order backward difference scheme was used to solve the sensitivity equations which gave a 3-fold speedup over the traditional finite difference method. The top 15 fragile points matched with clinical observations of malfunctioning points and included global elements like translational efficiency, programmed proteolysis of cyclins by Ubiquitin Proteasome System (UPS), phosphorylation /dephosphorylation of CDC25 in G1/S and synthesis of 14-3-3 sigma proteins in G2/M phase.

BIOT 179 - Transcription analysis with gene expression and network connectivity data

Mark P. Brynildsen[1], Linh M. Tran[1], Tung-Yun Wu[2], Shi-Shang Jang[2], and James C. Liao[1]. (1) Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, 420 Westwood Plaza, 5806 Boelter Hall, Los Angeles, CA 90025, Fax: 310-206-4107, mbrynild@ucla.edu, (2) Department of Chemical Engineering, National Tsing-Hua University, Taiwan

DNA microarray and ChIP-chip binding assays are technologies that provide complimentary, genome-wide measurements of transcriptional regulation. These data sources have been used individually and in conjunction to define key features of transcription regulation, namely the transcription network and transcription factor activities. This is due to the difficulty

associated with measuring these features directly. Reliance on DNA microarray and ChIP-chip binding data to determine the transcriptional response to external and internal stimuli has become commonplace. However, we have shown that noise and environment-specific transcription network variation can significantly impact transcription analyses. The extent of this impact can be drastic, sometimes resulting in ChIP-chip derived networks performing as well as random networks. Here we present two methods aimed at mitigating the adverse impact with which noise and transcription network variation affect the ability of transcription analyses to deduce transcription networks and transcription factor activities.

BIOT 180 - Genome-scale metabolic analysis of bacteriophage infected Escherichia coli

Rishi Jain and Ranjan Srivastava, Department of Chemical, Materials, & Biomolecular Engineering, University of Connecticut, 191 Auditorium Rd., Unit 3222, Storrs, CT 06269, rishijain@engr.uconn.edu

RNA viruses are responsible for a variety of diseases. The goal of this research was to develop a model RNA viral system for identifying potential drug targets and optimizing therapeutic strategies. To accomplish this, we utilized the MS2 bacteriophage as an RNA virus model system. MS2 is an RNA virus and a lytic bacteriophage that infects F+ Escherichia coli bacterial cells. The effects of phage infection on the metabolism of its host at the genome-scale were studied using a combination of in silico and experimental techniques. Due to the host-pathogen interactions, a viral infection of the host brings a new dimension to the original metabolic network. To account for this affect, a new metabolic subnetwork representing the viral compartment of the infection process was introduced. Starting with a traditional E. coli flux balance model, new reactions were added to the host metabolic network, corresponding to the synthesis of viral components. To solve the model an objective function maximizing the viral fluxes was utilized. Differences in the metabolic fluxes were analyzed prior to and after infection of the cell. Post-infection, it was noted that cell growth was halted. Additionally, cell envelope biosynthesis reactions and the membrane lipid metabolism reactions were also completely deactivated. The glycolysis pathway was downregulated by two-fold. The TCA cycle was downregulated by four-fold. The host cell turned solely to ATP generation and shut down all the biomass production reactions. On the other hand, the amino acid synthesis reactions leading to the production of viral components were upregulated by more than two-fold. Further experiments are currently underway to validate these results.

BIOT 181 - Elucidating and filling gaps in genome-scale metabolic networks

Vinay Satish Kumar[1], Madhukar S. Dasika[2], and Costas D. Maranas[2]. (1) Department of Industrial Engineering, The Pennsylvania State University, 147A Fenske Building, University Park, PA 16802, vsk111@psu.edu, (2) Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802

All genome-scale metabolic reconstructions (e.g., *E. coli*, *S. cerevisiae*, etc.) are inherently incomplete with some functionalities missing due to the lack of experimental and/or homology information. A key challenge is to automatically identify the gaps in these networks and subsequently generate hypotheses to bridge them. Here we describe optimization-based procedures (GapFind, GapFill) to identify *disconnected* metabolites and subsequently restore their *connectivity* using a variety of mechanisms. We find that about 10% of the metabolites in the latest model of *E. coli* are disconnected from the rest of metabolism. This percentage goes much higher for multiple-compartment models such as *S. cerevisiae* (30%) and *H. sapiens* (30%). In addition to the imperative of connectivity we are also imposing restrictions in the hypotheses generated based on the prediction of growth phenotypes consistent with experimental data for *E. coli* and *S. cerevisiae* and metabolomics data for *H. sapiens*.

BIOT 182 - Metabolic network inference based on probabilistic modeling of metabolic profiles

Jeongah Yoon and **Kyongbum Lee**, Department of Chemical and Biological Engineering, Tufts University, 4 Colby street, Science and Technology Center, Medford, MA 02155, Jeongah.Yoon@tufts.edu, Kyongbum.Lee@tufts.edu

In this paper, we describe an analysis framework for characterizing the directed interactions between the enzymes of a metabolic network following a physiological perturbation. This framework combines modularity and Bayesian analysis to infer causality relationships within systematically detected metabolic sub-networks. This framework supports the use of prior biochemical knowledge and efficient heuristic search algorithms for structure learning; moreover, it avoids the limitations of models that only consider pair-wise correlations. Applied to metabolic flux data describing the time course of inflammation-mediated liver hypermetabolism, our analysis discriminated between flexible, i.e. physiological state dependent, and conserved pathway structures. A similar result was obtained for adipocytes undergoing differentiation and subsequent lipid loading, where highly conserved and directed interactions were found for enzymes of both lipogenesis and lipolysis. Maximum likelihood estimates of the node parameters predicted dependencies between the enzymes consistent with the known metabolic biochemistry of liver and adipocyte metabolism.

BIOT 183 - Isotopically nonstationary flux analysis using an elementary metabolite unit (EMU) framework

Jason L Walther, Jamey D Young, Maciek R Antoniewicz, Hyuntae Yoo, and Gregory Stephanopoulos, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Building 56 Room 422, Cambridge, MA 02139, jwalther@mit.edu

Nonstationary metabolic flux analysis (NMFA) is at present very computationally intensive, especially for large reaction networks. We applied elementary metabolite unit (EMU) theory to NMFA, dramatically reducing computational difficulty. We also introduced block decoupling, a new method that systematically divides EMU systems of equations into smaller subproblems. These improvements led to a 1500-fold reduction in computational times,

enabling an entirely new and more complicated set of problems to be analyzed with NMFA. We simulated nonstationary GC/MS measurements for a large reaction network representing E. coli metabolism that were then used to estimate parameters and their confidence intervals. We found that nonstationary measurements made concentration estimation possible for several metabolites even in the absence of metabolomic data. We applied EMU-based NMFA to experimental nonstationary brown adipocyte measurements and successfully estimated fluxes and some metabolite concentrations. By using NFMA instead of traditional MFA, the experiment required 6 hours instead of 50.

WEDNESDAY MORNING

8:00 – 11:00 am BCEC

Biophysical and Biomolecular Symposium: Targeted Delivery of Proteins & Nucleotides

C. Meares, W. Liu, and A. Blake-Haskins, Organizers
Papers 184 - 191

BIOT 184 - (R-Ahx-R) 4 cell-penetrating peptide enhances the in vivo delivery of antisense morpholino oligomers

Hong M. Moulton[1], Natee Jearawiriyapaisarn[2], Sue Fletcher[3], Benjamin W Neuman[4], Ryszard Kole[5], Wilton Steve[3], Michael Buchmeier[4], and Patrick L. Iversen[1]. (1) Biology, AVI BioPharma Inc, 4575 SW Research Way Suite 200, Corvallis, OR 97333, Fax: 541-754-3545, moulton@avibio.com, (2) University of North Carolina, Chapel Hill, NC 27599, (3) University of Western Australia, Nedlands, Australia, (4) The Scripps Research Institute, La Jolla, CA, (5) Lineberger Comprehensive Cancer Center and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599

Morpholino oligomers (PMO) are uncharged steric-blocking antisense compounds that interfere with protein translation, pre-mRNA splicing and viral RNA synthesis. An arginine-rich cell penetrating peptide (CPP), (R-Ahx-R)4, enhances the nuclear/cytosolic delivery of PMO and has generated exciting reports in the Duchenne muscular dystrophy (DMD) and antiviral fields. Several highlights of our findings with the (R-Ahx-R)4 PMO conjugates will be presented including 1) the functional biodistribution in a splice-correction mouse model, 2) altering pre-mRNA splicing in a DMD model and 3) inhibiting the replication of coronavirus in mice. We attribute the success of this CPP to its greater ability to escape entrapment and its better enzymatic stability compared to Tat or polyarginine CPPs. Further optimization of the CPP sequence may reduce its endosomal trapping and toxicity. These studies show that, if dosed appropriately, this CPP has broad applications for delivery of therapeutic PMO.

BIOT 185 - Mutant glycosyltransferases assist in linking glycoconjugates via glycan chains: Development of a targeted drug delivery system and contrast agents for MRI

Pradman Krishen Qasba, Structural Glycobiology Section, Nanobiology Program, Center for Cancer Research, NCI-Frederick, NCI, NIH, P.O Box B, Bldg. 469, Rm 221, NCI-Frederick, Frederick, MD 21702-1203, Fax: 301-846-7149, qasba@helix.nih.gov, Elizabeth Boeggeman, Structural Glycobiology Section, Nanobiology Program, Center for Cancer Research, SAIC, Inc., NCI-Frederick, NCI, NIH, Frederick, MD 21702-1203, and Boopathy Ramakrishnan, Structural Glycobiology Section, Nanobiology Program, Center for Cancer Research, SAIC, Inc. NCI-Frederick, NCI, NIH, Frederick, MD 21702-1203

The structural information of glycosyltransferases has revealed that the specificity of the sugar donor in these enzymes is determined by a few residues in the sugar-nucleotide binding pocket of the enzyme, conserved among the family members from different species. This in turn has made it possible to reengineer the existing glycosyltransferases with broader sugar donor specificities. Mutation of these residues generates novel glycosyltransferases that can transfer a sugar residue with a chemically reactive functional group to N-acetylglucosamine (GlcNAc), galactose (Gal) and xylose residues of glycoproteins, glycolipids and proteoglycans (glycoconjugates). The potential of mutant glycosyltransferases to produce glycoconjugates carrying sugar moieties with reactive groups which can be used subsequently in the assembly of bio-nanoparticles is making it possible to develop a targeted-drug delivery system and contrast agents for MRI. This project has been funded in whole or in part with federal funds from the NCI, National Institutes of Health, under contract N01-CO-12400.

BIOT 186 - A new gene delivery method based on yeast cell wall particles

Ernesto Soto and **Gary Ostroff**, Pediatrics Department, University of Massachusetts Medical School, Worcester Foundation 222 Maple Avenue, Rose Reed & Gordon Building, Shrewsbury, MA 01545, Ernesto.Soto@umassmed.edu, Gary.Ostroff@umassmed.edu

Current gene delivery approaches are based on viral and non-viral mediated delivery. The efficiency of non-viral gene delivery is dependent on 4 factors: protection of the nucleic acid payload, delivery across the cytoplasmic membrane, release from the endosome, and nuclear uptake. We have developed a new delivery technology based on the layer by layer (LbL) synthesis of DNA containing nanomaterials within hollow yeast cell wall particles (YCWP). The YCWP delivery system has allowed for the methodical evaluation of encapsulated nanostructured materials capable of overcoming these hurdles to facilitate efficient nucleic acid delivery and transient phagocytic cell transfection. The YCWP carriers provide protection and in vivo oral and systemic receptor-targeted delivery of nucleic acid payloads to phagocytic cells and tissues containing these cells. A range of polymeric components have been evaluated to construct the outer layers of the YCWP encapsulated nucleic acid containing nanomaterials to

both protect the payload and facilitate its release from the endosome into the cytoplasm. Low levels of cationic polymers, such as polyethylenimine (PEI) or less toxic derivatives such as acetylated PEI, cationic surfactants and cationic peptides have proven effective. Inner layers of the nucleic acid containing nanomaterials have been synthesized with components designed to enhance endosomal release and promote DNA nuclear uptake to maximize phagocytic cell transfection efficiency at low DNA levels.

BIOT 187 - Reversing the nucleophile and electrophile for covalent affinity capture

Diment D. Singh, Mark R. McCoy, Zheng Miao, Oliver L. Hsu, and **Claude Meares**, Department of Chemistry, University of California, 1 Shields Avenue, Davis, CA 95616, cfmeares@ucdavis.edu

Engineering the permanent formation of a receptor-ligand complex has a number of potential applications in chemistry and biology. Previously, we have used the site-directed incorporation of cysteine nucleophiles at the periphery of an antibody's binding site, paired with the chemical synthesis of weakly electrophilic ligands, to produce receptor-ligand pairs that conjugate specifically and permanently. After protein expression in Drosophila S2 cells we found that the engineered cysteine was reversibly blocked by disulfide linkage to a small thiol. Here we report that this can be used to advantage by treating the small thiol as a leaving group. Ligands bearing thiol side chains were synthesized and incubated with the antibody, with the finding that the ligands became covalently attached to the antibody within a few minutes under physiological conditions. This rapid, specific conjugation is particularly interesting for biomedical applications. Supported by NIH research grants CA016861 and CA098207.

BIOT 188 - D-galactose receptor-targeted in vivo spectral fluorescence molecular imaging of peritoneal metastasis

Hisataka Kobayashi[1], Yukihiro Hama[1], Andrew J Gunn[1], Yoshinori Koyama[1], Yasuteru Urano[2], and Peter L Choyke[1]. (1) Molecular Imaging Program, CCR, NCI/NIH, Building 10, Room 1B40 MSC 1088, 10 Center Drive, Bethesda, MD 20892-1088, Fax: 301-402-3191, Kobayash@mail.nih.gov, (2) Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

The D-galactose receptor is a promising molecular target for imaging of peritoneal carcinomatosis. The ligands to the D-galactose receptor have high binding capacity yet also have rapid clearance pharmacokinetics from the peritoneal cavity and are bound to D-galactose receptors on hepatocytes. Optical agents targeted to the D-galactose receptor could help surgeons identify peritoneal metastases. A serum albumin conjugated with 23 galactosamine and 2 rhodamine green molecules (GmSA-RhodG) was designed as a clinically feasible alternative to a successful avidin-FITC reagent, which targets the same D-galactose receptor but is made from a non-immunogenic source, and has more favorable binding and isoelectric point characteristics than avidin and includes Rhodamine green which has better imaging features. GmSA-RhodG showed greater than 10-fold uptake by SHIN3 ovarian cancer cells than either avidin or serum albumin without galactosamine conjugation (BSA-RhodG.). Sensitivity and specificity of GmSA-RhodG were 100%/99% (n = 566), respectively for ~1 mm lesions *in vivo*.

BIOT 189 - Design of novel non-viral gene vector based on head-tail type polycation block copolymer

Atsushi Harada, Masanori Kawamura, Yuka Kimura, Chie Kojima, and Kenji Kono, Department of Applied Chemistry, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan, Fax: +81-72-254-9328, harada@chem.osakafu-u.ac.jp

The progress in clinical gene therapy has significantly motivated the development of safe and efficient non-viral gene delivery systems, and various kinds of cationic compounds including lipids or polymers have been applied as non-viral gene vector through the complexation with negatively charged DNA. We have investigated the head-tail type polycation block copolymer, which composed of poly(L-lysine) as tail block and polyamidoamine (PAMAM) dendron as head block, as non-viral gene vector. The molecular balance between head and tail blocks strongly influenced to not only physicochemical properties of vector including average size and zeta-potential to but also in vitro transfection efficiency. Also, by introducing poly(ethylene glycol) (PEG) hairs to the periphery of PAMAM dendron, the stability of vector in serum was remarkably improved and the transfection efficiency to cultured cells with serum increased compared with head-tail type polycation vector without PEG hairs.

BIOT 190 - In vivo RNAi: New concepts and applications of siRNA-based therapeutic agents

Tariq M. Rana, Biochemistry and Molecular Pharmacology, UMASS Medical School, 364 Plantation Street, Worcester, MA 01605, Fax: 508-856-6696

Development of siRNA-based therapies faces three challenges: (1) a better understanding of RNAi mechanism in humans, (2) identification of stable and effective siRNA sequences, and (3) efficient and specific tissue-targeted delivery in vivo. We have designed and created non-toxic water soluble nanoparticles and nanotubes to deliver siRNA into cells and silence specific genes in animals. To identify stabilized siRNAs, diverse sets of chemical modifications were created in siRNAs to determine the biochemical properties required for sustained RNAi in vivo. Results showing chemically modified siRNAs combined with our nanotechnologies to silence a variety of endogenous genes involved in neurodegenerative disease, cholesterol metabolism, and in regulating HIV replication will be presented.

BIOT 191 - Potent immunoconjugates for cancer therapy

Peter D. Senter, Seattle Genetics, 21823 30th Dr SE, Bothell, WA 98021, psenter@seagen.com

A great deal of interest has surrounded the use of monoclonal antibodies (mAbs) for the selective delivery of cytotoxic agents to tumor cells. Although the approach is conceptually appealing,

several limitations have been identified, including the physiological barriers to mAb extravasation and intratumoral penetration, conjugate immunogenicity, non-specific conjugate uptake, low drug potency, and inefficient release of active drug. Several of these limitations were overcome in developing mAb-valine-citrulline-MMAE (mAb-vc-MMAE) conjugates. These are comprised of the highly potent antimitotic agent monomethyl auristatin E (MMAE), attached to mAbs through a cathepsin B cleavable vc linker. This lecture will surround how this technology has been further optimized with newer generation drugs, linkers, and delivery agents. The results illustrate many of the key parameters in achieving high potency, pronounced therapeutic indices, bystander effects, and high intratumoral drug concentrations over sustained time periods. An overview of this therapeutic approach to cancer therapy will be provided.

WEDNESDAY MORNING

8:00 – 11:00 am BCEC 106

Commercialization of Biologics: Characterization & Improvement of Platforms to Aid Commercialization of Biologics

S. Gadam and F. Olsen, Organizers

Papers 192 - 199

BIOT 192 - A systems approach to characterization, validation and monitoring of commercial processes

Abhinav A Shukla, Bioprocess Engineering, Bristol-Myers Squibb, 6000 Thompson Road, East Syracuse, NY 13057, Fax: 315-432-2343, abhinav.shukla@bms.com, and Steven Lee, Technical Operations, Bristol-Myers Squibb, East Syracuse, NY 13057

Systems biology views an organism through an integrated and interacting network of complex biochemical pathways. This provides a framework for combining detailed micro-level understanding of individual pathways to create a better understanding of the entire organism. Analogous to biological systems, commercial manufacturing processes for biologics are complex combinations of unit operations that interact and integrate with each other and ultimately produce a complex product with significant microheterogenity. This presentation highlights the relevance of taking a systems approach towards understanding the relevant details of each unit operation and how they combine together to create a well controlled process that operates at multiple commercial manufacturing sites to produce consistent and high quality drug substance.

Characterizing the operational design space for each unit operation is an important first step. Even more important is understanding the interplay between various process steps and how they combine together to influence key product attributes. Several case studies of process characterization and validation study designs to highlight this interplay will be presented for both upstream and downstream processes.

BIOT 193 - Systematic and logical strategies to reduce process scale-up and technology transfer challenges from process development to manufacturing

Tsu-shun Lee, Monica McGill, and Peter Thompson, Manufacturing Technology, sanofi pasteur, Discovery Drive, Swiftwater, PA 18370, Fax: 570-895-2922, Tsushun.Lee@sanofipasteur.com

Robust production at manufacturing facility is the ultimate goal for process scientists and engineers. To reach the goal, biopharmaceuticals face lots of challenges during the process development, process scale-up, process transfer, and final process demonstration at manufacturing scale, which would become more complicated to deal with under budget limitation, short project timeline, and disconnected organization communication situations. Overcoming these barriers has grown into a critical task for a biopharmaceutical company to survive in the current highly competitive environment. This presentation will focus on integrated and standardized approach to reduce the barrier for process transfer and scale-up. Integration involves process scientists and engineers to realize the "big picture" of tasks and to foresee the potential issues at the early stage. Standardization helps organization increase the efficiency and reduce the required resources. It can shorten project delivery time and save the invested capitals from the same or similar issues over and over again. Examples will be addressed to demonstrate the feasibility of these principles.

BIOT 194 - Evaluation of process and product impact of UVC treatment on a MAb process

R. Scott Rosenthal, Purification Process Development, Amgen, 4000 Nelson Rd, AC-24B, Longmont, CO 80503, Fax: 303-401-4401, rrosenth@amgen.com

Viral clearance is a critical component of the adventitious agent risk mitigation strategy employed in the protein therapeutics industry. The number of robust viral clearance technologies is currently limited and as such, new removal or inactivation techniques could prove to be extremely valuable to the industry. Short wavelength ultraviolet (UVC) light has been know to inactivate viruses and microbes for over half a century. While UVC is not a new technology per se, it has yet to find widespread application in the biotechnology industry for the purpose of viral inactivation. This stems at least in part from concern over potential process and product impact. To that end, we have investigated the impact of UVC treatment on a monoclonal antibody process. Process performance was evaluated using various intermediate pools treated with varying UVC stringency. Product impact was evaluated from the perspective of UVC induced product variants and stability issues as a function of UVC stringency. The ability of the various process steps to remove UVC induced product variants was also evaluated. A summation of the study results will conclude with a proposed platform approach to a robust 3 step viral clearance strategy that could potentially eliminate the need to make viral clearance claims across chromatography steps.

BIOT 195 - Viral clearance strategies using improved membrane adsorbers

William T. Riordan and Mark R Etzel, Department of Chemical and Biological Engineering, University of Wisconsin, 1415 Engineering Drive, Madison, WI 53706-1607, wtriordan@wisc.edu

Clearance of endogenous and potential adventitious viruses is a challenging and highly regulated processing step during the manufacture of cell line-derived biopharmaceuticals. In this work, we evaluated the ability of a strong anion exchange membrane adsorber to clear model bacteriophage across a range of operating conditions. We observed a large log reduction value (LRV) for a highly acidic phage independent of salt concentration, but for a more neutral phage LRV dropped from a high value to near zero after addition of only 50 mM salt. Many therapeutic proteins must have salt present to prevent aggregation. To overcome the limitation of salt intolerance for viral clearance operations, new salt-tolerant ligands must be found. We developed a protocol to characterize the salt tolerance and overall performance of new ligand candidates for membrane adsorbers. Our goal was to find a ligand that maintained a large LRV for a neutral virus independent of salt concentration. Overall, the results of this study should aid in the design of improved membrane adsorber systems and ultimately lead to increased product safety.

BIOT 196 - Standardization of purification process development for mAbs & Fc-fusion proteins

Ranga Godavarti[1], Glen Bolton[1], James Booth[2], Paul R Brown[3], Jon Coffman[3], Connie Esenther[1], Chris Gallo[4], T Iskra[5], Shujun Sun[4], Mary Switzer[3], Scott A Tobler[3], Richard Wright[4], and Brian Kelley[1]. (1) Wyeth BioPharma, One Burtt Road, Andover, MA 01810, rgodavarti@wyeth.com, (2) Drug Substance Development, Wyeth BioPharma, Andover, MA 01810, (3) Purification Process Development, Wyeth BioPharma, Andover, MA 01810, (4) Wyeth Biopharma, (5) Wyeth Biopharma, Andover, MA 01810

The development strategy for a mAb platform purification process using two chromatographic steps will be described. Following capture on Protein A, a single polishing step using anion-exchange chromatography is developed for removal of residual impurities prior to virus-removal filtration and ultrafiltration. The anion-exchange step is run under weak-partitioning conditions, which provides excellent impurity clearance and high loading capacity. High throughput screening (HTS) rapidly defines conditions for both chromatography steps. The definition of operating conditions for multiple mAbs using standard unit operations has revealed a 'platform space', akin to the design space for a single product. For Fc-fusion proteins, a second polishing step is often required; HTS screening of multiple chromatographic modes focuses development efforts on the most effective third chromatographic step. The minimum development package necessary to support Phase I cGMP processes will be described, together with practical tools for harmonizing development efforts across multiple labs. Emerging opportunities may allow further streamlining of IND filings through modular virus validation.

BIOT 197 - Characterization of a reversed phase chromatography step

Xiaochun Hu[1], Alice Wang[2], Yuefeng Lu[1], and Oliver Kaltenbrunner[1]. (1) Process & Analytical Sciences, Amgen Inc, 30W-2-A, One Amgen Center Drive, Thousand Oaks, CA 91320, (2) Process Development, Amgen, Thousand Oaks, CA 91320

Process characterization of a production process of a human therapeutic protein is a critical step before process validation. Using reversed phase chromatography as the example, this presentation will outline the characterization strategy for classifying operational and performance parameters and setting operating ranges and acceptance criteria. The necessary initial preparation including methods of risk analysis and scale-down model qualification will be shown. The experimental design of and results from robustness, edge of range, and worst-case studies will be presented. Finally, the path from experimental data to parameter classification and setting of validation ranges and in-process control limits will be discussed.

BIOT 198 - Strategies for maximizing the clearance of impurities across an anion exchange flow through step in the context of an integrated antibody purification process

Amitava Kundu[1], Amy Miller[1], Jerrod Einerwold[1], Michelle Xia[2], Robert Duffy[2], and Weichang Zhou[3]. (1) Purification Process Engineering, PDL BioPharma, 9450 Winnetka Ave N, Brooklyn Park, MN 55445, Fax: 763-3837058, amitava.kundu@pdl.com, (2) Purification Process Development, PDL BioPharma, Fremont, CA 94555, (3) Process Sciences Engineering, PDL BioPharma, Fremont, CA 94555

A commonly used platform process for purification of monoclonal antibodies involves the use of Protein A affinity chromatography as an initial capture step. This is typically followed by a couple of ion exchange steps as polishing steps. In this case study, we present a purification process that comprises of an anion exchange flow through (non binding) followed by a bind and elute cation exchange as polishing steps. While clearance of DNA and virus can be easily accomplished under a wide range of operating conditions, clearance of other process related impurities such as host cell proteins and leached Protein A is relatively more sensitive to operating conditions. This presentation will focus on obtaining an understanding of how different salt counter ions, in addition to pH and conductivity of the mobile phase impacts the clearance of these process related impurities. The robustness of the anion exchange flow through step with respect to the process parameters will be presented. Finally, the implications of an optimized anion exchange process in the context of a three-step versus a two-step purification process will be discussed.

BIOT 199 - Platform ultrafiltration process development: Charge and conductivity effects on ultrafiltration performance of monoclonal antibodies

Amy S Len, Late Stage Purification, Genentech, Inc, One DNA Way, South San Francisco, CA 94080, Fax: 650-225-4049, len.amy@gene.com, and Robert van Reis, Late Stage Purification, Genentech Inc, South San Francisco, CA 94080

Current antibody processes target high titer, high concentration formulations, often nearly doubling throughput through the same manufacturing facility. To enable these processes, recent investigations into differing process performance focus on the effect of protein charge and conductivity on mass transfer coefficient and wall concentration of two monoclonal antibodies. Further investigation focused on linking diffusion coefficient and osmotic pressure to performance. Using a greatly simplified approach to data analysis, mass transfer and wall concentration values can be quickly determined and performance easily compared. Additionally, the use of lab-scale equipment decreased the material resource requirement for UF/DF process development by 10-fold and the labor requirement by 4-fold. Insight into parameters effecting performance, combined with lab-scale equipment and simplified data analysis, allows for a platform approach to ultrafiltration process development, enabling highly efficient formulations that meet high titer and high concentration needs.

WEDNESDAY MORNING

8:00 – 10:30 am BCEC 107 C

Upstream Processing: Advances in Cell Culture Process Development

S. T. Sharfstein, I. Blumentals, and G. Maheshwari, Organizers Papers 200 - 205

BIOT 200 - High-producing CHO-GS and NS0-GS cell line generation and characterization using Cell Xpress(TM) technology

Nan Lin, Genova Richardson, Lynn Davis, Kimberly Lacy, Misa Gray, Jennifer Cresswell, Anne Dennett, Mark Gerber, Matthew Caple, and Kevin Kayser, Cell Sciences and Development, SAFC Biosciences, 2909 Laclede Avenue, Saint Louis, MO 63103, nlin@sial.com

In the present study, we generated thirteen CHOK1SV and NS0-GS (licensed from Lonza Biologics) transfectant populations that stably express recombinant human IgG using the Glutamine Synthetase (GS) expression system. We analyzed these transfectants using Cell Xpress™, a software module on the LEAP™ (Laser-Enabled Analysis and Processing) platform, for secretion heterogeneity. These transfected populations exhibited significantly different secretion heterogeneity on a per-cell basis. We demonstrated that correlations of average cell secretion and volumetric productivity yielded R^2 values of greater than 0.8.

In addition, we single-cell cloned four representative CHOK1SV and NS0-GS transfectant populations with different secretion heterogeneity, using both limiting dilution and Cell Xpress™. Cell Xpress ™ allows single-cell isolation by combining in situ imaging of secretion and laser manipulation to ablate poor secretors. The frequencies of high-producing clones and productivity were compared between the two single-cell cloning methods. We also compared secretion heterogeneity of the single-cell clones generated by the two methods. The gene copy numbers and mRNA levels of IgG heavy and light chain as well as GS were also determined in order to study their correlation with IgG secretion.

Compared to limiting dilution, Cell Xpress™ permits screening a greater number of cells, therefore increasing the likelihood of isolating high-producing clones. Moreover, secretion heterogeneity analysis can serve as a supplementary parameter for clone selection. This method also enables convenient monitoring of IgG secretion heterogeneity during clone expansion.

BIOT 201 - Developments in using the ACE System to generate high MAb expressing, stable CHO cell lines

Malcolm L. Kennard, Cell Line Engineering, Chromos Molecular Systems Inc, 8081 Lougheed Highway, Burnaby, BC V5A 1W9, Canada, Fax: 604-415-7151, mkennard@chromos.com

The ACE System was developed to rapidly generate stable high MAb expressing CHO cell lines. This platform technology is based on pre-engineered artificial chromosomes with recombination acceptor sites that allow for targeted transfection of multiple copies of genes into cells. Case studies will be presented where cell lines were generated in less than 2 months from transfection to produce research material and in less than 3.5 months to generate stable production cell lines. Cells were cultured in non-optimized, non-fed batch shake flasks and express MAb titres of > 1 g/L. Data will be presented on stability (expression and genetic), fed-batch development, scalability and performance in small scale bioreactors as well as auditioning different CHO cell lines. Finally, improvements to the ACE System to increase final titres and reduce cell line generation times will be discussed. The ACE System provides an attractive alternative to conventional methods of cell line generation.

BIOT 202 - Proteomic and genomic studies of mammalian cell physiology to optimize production of therapeutic and diagnostic proteins

Duan Shen[1], Thomas R. Kiehl[2], and Susan T. Sharfstein[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, shend2@rpi.edu, (2) Multidisciplinary Science, Rensselaer Polytechnic Institute, Troy, NY 12180

Hyperosmotic stress has been shown to increase specific productivity of monoclonal antibodies and other recombinant proteins in many mammalian cell lines including murine hybridomas and Chinese hamster ovary (CHO) cells. However, the adverse effects of hyperosmolarity on cell growth and/or induction of apoptosis lead to only modest increases in overall productivity.

We previously studied the overall transcriptional response using DNA microarrays and found three major families of genes, metabolism, and transcriptional regulation, and signaling pathways, were extensively involved in the transcriptional response to osmotic stress. Here we present a detailed analysis of the signal transduction pathways and the cellular defense responses in industrially relevant mammalian cell lines. We previously demonstrated (in a different cell line from those described in this study) that the all members of the mitogen activated kinase (MAPK) superfamily were activated in response to hyperosmotic stress, although p38 and ERK1/2 to a much lesser extent than SAPK. In this study, we measured the activity of the MAPK superfamily as well as the activation of a variety of kinases known to be activated in response to hyperosmotic stress (p38, Fyn, PKAc) in murine hybridoma OKT3 and two CHO cell lines. To study the protective response of OKT3 towards hyperosmolarity, the aldose reductase activity and accumulation of organic osmolytes including betaine, inositol, sorbitol and glycerophosphocholine were also investigated.
Gauging the osmolyte accumulation and mapping the signal transduction pathways that are activated in response to hyperosmolarity will provide a fundamental understanding of the changes in cellular physiology and lead to rational strategies for cellular and process engineering to improve overall productivity for monoclonal antibodies produced in cultured mammalian cells.

BIOT 203 - Impact of scale and bioreactor type on cell growth, productivity, product quality, and global gene expression

Michael A. Hanson[1], Jose Vallejos[1], Xudong Ge[1], Kurt Brorson[2], Antonio R. Moreira[1], and Govind Rao[1]. (1) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, TRC 227, 1000 Hilltop Circle, Baltimore, MD 21250, Fax: 410-455-6500, m.hanson@umbc.edu, (2) Division of Monoclonal Antibodies, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20903

During cell culture process development many experiments must be carried out to select and genetically engineer production organisms, develop appropriate growth media, and optimize bioreactor parameters. These experiments are often carried out in tissue culture or spinner flasks which generally are not equipped with the capability to monitor environmental flask conditions. This lack of knowledge hinders scale-up to larger pilot- and production-scale vessels. We have developed a high-throughput bioreactor (HTBR) system composed of twelve 30 ml stirred cell vessels, each equipped with optical-based sensors for pH and dissolved oxygen. Here we compare cell culture, product quality, and gene expression data obtained using the HTBR with data obtained in liter-scale bench-top and disposable bag bioreactors. The effectiveness of using the liquid mass transfer coefficient (kLa) as a scale-up parameter across the different mixing modes is assessed.

BIOT 204 - Sodium butyrate stimulates mAb over-expression in CHO cells by improving gene accessibility

Zhou Jiang and Susan T. Sharfstein, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, Fax: 518-276-4030, jiangz@rpi.edu

Sodium butyrate treatment can increase the specific productivity of recombinant proteins in mammalian cells; however, it dramatically decreases cell growth and frequently leads to apoptosis. We have studied the responses of several CHO cells lines with different specific productivities (qP) to a time course of sodium butyrate treatment. We observed that a two-day treatment in batch culture maximized volumetric productivity, and after two days, the productivity declined. In addition, cell lines with lower productivities exhibited greater enhancement from butyrate treatment than cells with higher productivities.
As we have observed previously in cell line characterization, heavy chain (HC) mRNA levels correlate very well with specific productivity and are amplified by butyrate treatment, indicating that sodium butyrate regulates the HC transcription. Sodium butyrate is an inhibitor of histone deacetylation, and possibly, increases gene transcription by enhancing gene accessibility to transcription factors. In this study, we have applied DNaseI footprinting to probe the HC and LC gene accessibility. We determined that more HC and LC gene copies are accessible by DNaseI in sodium butyrate-treated CHO cells than in of untreated controls, demonstrating that sodium butyrate regulates gene transcription by improving gene accessibility.

BIOT 205 - Improved analytical technique for high throughput analysis of glycoforms

Michael P. Gillmeister[1], Noboru Tomiya[2], Scott J. Jacobia[3], Yuan C. Lee[2], Stephen F. Gorfien[3], and Michael J. Betenbaugh[1]. (1) Department of Chemical and Biomolecular Engineering, (2) Department of Biology, Johns Hopkins University, 3400 North Charles Street, Maryland Hall Room 221, Baltimore, MD 21218, Fax: 410-516-5510, mpg@jhu.edu, (3) Cell Culture Research and Development, Invitrogen Corporation, Grand Island, NY 14072

An established large scale three dimensional HPLC technique to identify the total profile of glycan structures from glycoproteins was adapted to a two-step identification for smaller sample size while maintaining detailed structural and isomeric information. After sample preparation and one-dimensional HPLC analysis, samples are spotted onto a MALDI-TOF mass spectrometry plate and analyzed for molecular weight. A correlation was found between the 2mm column of the new system and the 6mm column of the standard method using more than 20 standard glycoforms. This adaptation allows a 100-fold reduction in sample starting material and can be carried out in 2-3 days running samples in parallel. Verification of the method was accomplished by analyzing the glycan profile from CHO cell cultures expressing tissue plasminogen activator (tPA) under varying culture conditions. Comparing the overall glycan profiles, these culture conditions showed both positive and negative affects on the total oligosaccharide structure complexity.

WEDNESDAY MORNING

11:30 – 12:30 pm BCEC 107 B

Elmer Gaden Award Lecture W. Zhou, Organizer, Paper 206

BIOT 206 - Directing the assembly of multifunctional biomolecular architectures

Jonathan S. Dordick, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 4005 Center for Biotechnology and Interdisciplinary Studies, 110 8th Street, Troy, NY 12180-3590, Fax: 518-276-2207, dordick@rpi.edu

Abstract text not available.

WEDNESDAY AFTERNOON

2:00 – 2:50 pm BCEC 108

BIOT Young Investigator Award Lecture W. Zhou, Organizer, Paper 207

BIOT 207 - Building proteins with new therapeutic functions from peptide modules

Patrick S. Daugherty, Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA 93106

We have developed a family of new molecular diversity tools for the discovery and optimization of peptides with improved functions. Modular peptide components designed for cell-specific targeting, enzymatic activation, membrane penetration, and highly-specific protein inhibition can be assembled piecewise to build multifunctional proteins. Collectively, these and related methodologies create exciting new opportunities for the design and engineering next generation biopharmaceuticals.

WEDNESDAY AFTERNOON

3:00 – 5:20 pm BCEC 107 B

Emerging Technologies: Systems Biology C. Chan and C. Cho, Organizers Papers 208 - 213

BIOT 208 - Dissection of the HIV promoter and possible contribution of NFκB and Sp1 to phenotypic bifurcation and latency

John C. Burnett[1], Adam P. Arkin[2], and David V. Schaffer[1]. (1) Department of Chemical Engineering and the Wills Neuroscience Institute, University of California at Berkeley, 201 Gilman Hall, College of Chemistry, Berkeley, CA 94720, burnett@berkeley.edu, (2) Department of Bioengineering and the Howard Hughes Medical Institute, University of California at Berkeley, Berkeley, CA 94710

We have previously reported that stochastic fluctuations in Tat lead to phenotypic diversity in HIV gene expression, a phenomenon that can be observed experimentally and predicted computationally with stochastic modeling. We have hypothesized that stochastic delays in the onset of viral gene expression may "buy time" for an infected T-cell to relax into a memory state, thus contributing to the formation of latent viral infections. Building on our previous discovery that stochastic fluctuations in Tat lead to phenotypic diversity in a small, but significant, portion of HIV infected cells possessing native NFκB and Sp1 complexes, we have found that the mutation of one of the three Sp binding sites increases the frequency of phenotypic diversity in clonal populations. This phenotype suggests that the transcriptional regulatory contributions of all three Sp1 sites dampen noisy HIV-1 transcription, which may reduce the probabilities of proviral latency. Thus, we show that despite the stochastic effects in transcriptional regulation of HIV, the virus minimizes the frequency of such events. Our current hypothesis explains this dampening effect by cooperative recruitment of Tat and p300, the primary cofactor of Sp1. Thus, stochastic fluctuations in Tat are dampened by the cooperative interactions of p300. This work was supported by the University of California Mentored Research Award (JB), the Howard Hughes Medical Institute (APA), and NIH R01-GM073058-01 (DVS).

BIOT 209 - A new kinetic model reveals the synergistic effect of E-, P- and A-sites on +1 ribosomal frameshifting

Pei-Yu Liao[1], Prateek Gupta[1], Alexey N. Petrov[2], Jonathan D. Dinman[2], and Kelvin H. Lee[1]. (1) School of Chemical and Biomolecular Engineering, Cornell University, 120 Olin Hall, Ithaca, NY 14853-5201, Fax: 607-255-9166, pl248@cornell.edu, (2)
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD 20742

Programmed ribosomal frameshifting (PRF) is the process by which ribosomes produce two different polypeptides from the same mRNA. Previously, we proposed a mechanism of +1 PRF by incorporating the effects of tRNA:mRNA and tRNA:ribosome interactions in the E-, P- and A-sites. In the current study, three extended kinetic models of +1 PRF are proposed. Specifically, the timing of E-site tRNA dissociation is discussed within the context of the kinetic proofreading mechanism of aminoacylated tRNA (aa-tRNA) selection. In addition, by using a dual fluorescence reporter system in *E. coli*, we study the effect of different E-site codon: anticodon interactions on +1 PRF. In terms of kinetic rate constants, we show that destabilization of deacylated tRNA in the E-site, rearrangement of peptidyl-tRNA in the P-site, and availability of cognate aa-tRNA corresponding to the A-site act synergistically to promote efficient +1 PRF.

BIOT 210 - Global assessment of protein turnover rates in *Streptomyces coelicolor* using a multitagging proteomic strategy

Karthik P. Jayapal[1], Siguang Sui[1], Robin J. Philp[2], Yee-Jiun Kok[2], Miranda GS. Yap[2], Timothy J. Griffin[3], and Wei-Shou Hu[1]. (1) Department of Chemical Engineering and Materials Science,

University of Minnesota, Minneapolis, MN 55455, (2) Bioprocessing Technology Institute, Agency for Science, Technology and Research, Singapore 138668, Singapore 138668, Singapore, (3) Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, Minneapolis, MN 55455

Diversity in protein turnover rates contributes profoundly to the apparent disparity between transcriptome and proteome data. In a previous time-course study, we identified ~1100 *Streptomyces coelicolor* proteins several of which displayed discordant or contrasting mRNA-protein profiles. We present here a mass spectrometry based multi-tagging strategy for proteome-wide assessment of protein half-lives in this antibiotic-producing microorganism. Exponentially growing and stationary phase *S. coelicolor* cells harvested from metabolically labeled (SILAC) cultures were transferred to an unlabeled medium resulting in immediate cessation of labeled protein synthesis. Protein degradation was then monitored by estimating the relative levels of labeled proteins in successive time-delayed sample lysates using iTRAQ^{TM} reagents. Results indicate that turnover rates vary substantially among different proteins and in addition, might display temporal growth-phase dependence. The novel use of multiple stable isotope tags also enables one to evaluate the stability of dynamic proteins whose abundance levels change rapidly.

BIOT 211 - Universal Sequence Tag ARray (U-STAR) platform: A universal platform for the quantitative analysis of transcriptomes

Austin P. So and C. A. Haynes, Michael Smith Laboratories, University of British Columbia, Rm 273, 2185 East Mall, Vancouver, BC V6T 1Z4, Canada, Fax: 604-822-2114, aphs@interchange.ubc.ca

Numerous technologies have been developed over the past decade to monitor gene expression on a global scale, and have demonstrated their effectiveness in providing very reproducible information on relative changes in gene expression. However, for systems biology to realize its goal of providing a predictive model of the transcriptome, technologies that enable the precise measurement of gene expression on an absolute scale are required. Here, we describe a novel platform which we term the Universal Sequence Tag Array (U-STAR) platform, which harnesses the principles and strengths of SAGE technology with the massively parallel analysis afforded through microarray technology to provide a quantitative evaluation of the transcriptome on an absolute scale. Utilizing a combinatorial array of LNA-DNA mixmers to create a uniform hybridization window, this platform is able to capture less than 2-fold changes in transcript abundances across a 4-order of magnitude range of concentrations (0.1 pM to 1 nM) from 100 ng of starting mRNA material without the requirement for amplification. As this platform can be applied to any organism with polyadenylated mRNA, the U-STAR platform promises to provide a robust and cost-effective means to obtain quantitative information about the transcriptome.

BIOT 212 - Proteome-wide identification of member-specific natural substrate repertoire of caspases and other proteases

Rihe Liu and C. Alexander Valencia, School of Pharmacy and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, rliu@email.unc.edu

Caspases are proteolytic enzymes that are essential for apoptosis. Understanding the many discrete and interacting signaling pathways mediated by caspases requires the identification of the natural substrate repertoire for each caspase of interest. Employing an amplification-based protein selection technique called mRNA-display, we developed a high throughput screen platform for member-specific caspase substrates on a proteome-wide scale (Figure 1). A large number of both known and novel caspase-3 substrates were identified from the human proteome. The proteolytic features of these selected substrates, including their cleavage sites and specificities, were characterized. Many substrates that were cleaved only by caspase-8 or granzyme B but not by caspase-3, were also successfully selected. The method reported here is systematic and high throughput, and can be broadly applied to all the members of human caspases, caspases in other organisms, and to numerous other proteases with high specificity. The availability of such information will greatly facilitate our understanding of apoptotic signaling pathways at the level of systems biology.

This work was supported by start-up funds from the Carolina Center for Genome Sciences and School of Pharmacy at the University of North Carolina at Chapel Hill and NIH grants R01-NS047650 and R21-DK067480 to R.L.

BIOT 213 - Cellular scale qualitative shotgun proteomic analysis of *Nostoc* sp. PCC 7120 isolated heterocysts and filaments under nitrogen deficient conditions using 8-plex isobaric peptide tags

Saw Yen Ow[1], Karin Stensjö[2], Phillip C. Wright[1], and Peter Lindblad[2]. (1) Department of Chemical and Process Engineering, The University of Sheffield, Mappin Street, Sheffield, S1 3JD, United Kingdom, Fax: +441142227501, s.ow@sheffield.ac.uk, (2) Department of Physiological Botany, Uppsala University, Uppsala SE-751 20, Sweden

The heterocyst is a specialized cell for N_2 fixation in the cyanobacterium *Nostoc* sp. PCC 7120. It differentiates from vegetative cells every 20th cell along the filament upon prolonged nitrogen starvation. This investigation furthers the understanding of the metabolic balance between heterocysts and adjacent vegetative cells through the use of quantitative shotgun proteomics. Relative comparisons between enriched heterocysts and filaments were achieved using an 8-plex peptide-iTRAQ workflow coupled with SCX prefractionation and LC/ESI-QTOF-MS techniques. Preliminary data shows significant downregulation of the phycobilisomes and PSII proteins in the heterocysts. Key indicators like the nitrogenase and heterocyst specific ferredoxins, and anabolic pathways such as OPP were found highly upregulated. Findings obtained from this investigation contribute to a better understanding of the iTRAQ methodology and data acquisition for systems biology research. Furthermore, it adds to

the understanding of heterocyst specific metabolism important for downstream research for H_2 bio-fuel production from heterocystous cyanobacteria.

WEDNESDAY AFTERNOON

3:00 – 5:20 pm BCEC 106

Biophysical and Biomolecular Symposium: Targeted Delivery of Proteins & Nucleotides C. Meares and W. Liu, Organizers Papers 214 - 219

BIOT 214 - N-ethoxybenzylimidazoles (NEBIs) as potential pH-sensitive linkers for drug delivery

Alice Luong[1], Seong Deok Kong[1], Gerald Manorek[2], Stephen B. Howell[2], and **Jerry Yang**[1]. (1) Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Dr, MC 0358, La Jolla, CA 92093-0358, jerryyang@ucsd.edu, (2) Department of Medicine and the Rebecca and John Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093

This presentation will describe the development of a new class of N-linked imidazoles as potential pH-sensitive, cleavable linkers for controlled release of therapeutics from drug delivery vessels. We will discuss kinetic studies on the rates of cleavage of N-ethoxybenzylimidazoles that exhibit accelerated hydrolysis in mild aqueous acidic solutions compared to in solutions at normal, physiological pH. We demonstrate methods to tune the rates of hydrolysis of N-ethoxybenzylimidazoles under mild acidic conditions with half-lives ranging from minutes to months. We will also present some initial studies towards the development of N-ethoxybenzylimidazoles as linkers to conjugate cytotoxic agents to drug delivery systems for potential cancer therapy applications.

BIOT 215 - Selective antitumor aptamer-photodynamic agent conjugates as a tool in photochemo therapy

Prabodhika Mallikaratchy and Weihong Tan, Department of Chemistry, University of Florida, PO Box 117200, Gainesville, FL 32603, pmall@chem.ufl.edu

The key step in increasing selectivity in chemotherapeutic drugs is creating effective platforms that could target cancer cells but not normal cells. Recently, we have developed a strategy via cell-SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to obtain cell specific aptamers using intact viable cells as targets to select aptamers that can recognize cell membrane proteins with high selectivity and affinity. In our recent studies with B-cell leukemia, we have identified an aptamer TD05 that only recognizes Ramos cells, a Burkitt's lymphoma cell line. As a proof of concept, we have combined the high specificity of aptamers to develop a highly selective aptamer-photosensitizer (PS) conjugate to effectively destroy aptamer specific cancer cells. Introduction of aptamer-PS conjugates followed by irradiation of light effectively destroyed target Ramos cells but not acute lymphoblastic leukemia and myeloid leukemia cell lines. This study demonstrates that use of cancer specific aptamers conjugated to a PS will enhance the selectivity of photodynamic therapy.

BIOT 216 - Synthesis, phototoxicity and metabolic stability of porphyrins conjugated to bifunctional peptide sequences

Maria Graca H. Vicente and Martha Sibrian-Vazquez, Department of Chemistry, Louisiana State University, 433 Choppin Hall, S. Tower Dr, Baton Rouge, LA 70803, Fax: 225-578-3458, vicente@lsu.edu

We have been investigating the conjugation of photosensitizing agents for the photodynamic therapy of cancer to certain peptide sequences in order to improve their specific delivery to targeted cells and organelles. The peptide signaling sequences investigated include cell penetrating peptides (CPP) and nuclear or mitochondria localization sequences (NLS, MLS). Our studies show that conjugates of photosensitizers to CPP or NLS/MLS peptides have enhanced photoactivity in vitro compared with the non-conjugated photosensitizers. The biological efficacy of the conjugates depends on the nature and sequence of the amino acids, the porphyrin peripheral substituents, the linker, their conformation(s) in aqueous solutions, amphiphilic character and metabolic stability. We will report our most recent results on the synthesis and properties of photosensitizer conjugates to one or more linear bifunctional peptide(s) composed of CPP and NLS or MLS peptide chains. Their uptake, subcellular localization, phototoxicity and enzymatic stability were investigated in human carcinoma HEp2 cells.

BIOT 217 - Optical antisense imaging of tumored mice using a fluorescent DNA duplex

Xinrong Liu[1], Yi Wang[1], Kayoko Nakamura[2], Guozheng Liu[1], Shuping Dou[1], Atsushi Kubo[2], Mary Rusckowski[1], and Donald J Hnatowich[1]. (1) Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, Fax: 508-856-4572, xinrong.liu@umassmed.edu, (2) Department of Radiology, Keio University School of Medicine, Tokyo, Japan

For optical antisense imaging, we have designed a Cy5.5 conjugated antisense 25 mer DNA hybridized with a BHQ3 quencher conjugated 18 mer cDNA(i.e. DNA25-Cy5.5/cDNA18-BHQ3) duplex to inhibit fluorescence but not after duplex dissociation in the presence of its target mRNA. As evidence of specific binding in culture, fluorescence was lower in control cells compared to study cells incubated with the study DNA25-Cy5.5/cDNA18-BHQ3 duplex but not with the control cDNA18-Cy5.5/DNA25-BHQ3 duplex with reversed fluorophores. The tumor/normal thigh fluorescence ratio was clearly positive by 30 min but not at any time in mice receiving the control duplex. Fluorescence was much higher in tumor sections from mice that had received the study rather than the control duplex. Thus combining a fluorophore-conjugated antisense DNA with an inhibitor-conjugated shorter complementary cDNA inhibited fluorescence both in cell culture and in tumored mice except in the presence of the target mRNA.

BIOT 218 - Specific cellular delivery of quantum dot-peptide and quantum dot-polymer nanoassemblies

James B. Delehanty[1], Dorothy Farrell[2], Igor L. Medintz[1], and Hedi Mattoussi[2]. (1) Center for Bio/Molecular Science and Engineering, US Naval Research Laboratory, 4555 Overlook Ave SW, Washington, DC 20375, jbd@cbmse.nrl.navy.mil, (2) Optical Sciences Division, US Naval Research Laboratory, Washington, DC 20375

Luminescent semiconductor quantum dots (QDs) possess unique optical and spectroscopic properties that make them attractive fluorophores for use in many biological applications. Chief among these properties are their high quantum yields, broad absorption spectra coupled to narrow symmetric, size-tunable emissions allowing large achievable Stokes shifts, and exceptional resistance to photo- and chemical degradation. These properties suggest that QDs will be instrumental in the development of the next generation of highly efficient biosensors for the simultaneous monitoring of multiple intracellular molecular processes in both fixed and living cells. We are developing robust and facile delivery schemes for the selective intracellular delivery of QD-based nanoassemblies. These schemes are based upon the self-assembly and subsequent cellular uptake of QD-peptide and QD-polymer conjugates. The peptide-based structures are generated by self-assembly of the peptide onto CdSe-ZnS core-shell QDs via metal ion coordination between the peptide's polyhistidine motif and the Zn-rich QD shell. The polymer-based assemblies are formed via the electrostatic interaction of aqueous cationic liposomes with available carboxylate moieties on the QD surface ligands. We will highlight cellular delivery experiments utilizing both delivery schemes and discuss the advantages and disadvantages of each approach.

BIOT 219 - Transencapsidation of Semliki Forest virus RNA with tobacco mosaic virus coat protein: Evaluation of antigen expression, antigen delivery and vaccine efficiacy

Alison A. McCormick[1], Tina Corbo[2], Jacqueline Bernales[2], John A. Lindbo[3], Kenneth E. Palmer[4], and Mark L. Smith[5]. (1) College of Pharmacy, Touro University, California, 1310 Johnson Lane, Mare Island, Vallejo, CA 94592, Fax: 707-638-5255, alison.mccormick@touro.edu, (2) Vaccine Development, Large Scale Biology Corporation, Vacaville, CA 95688, (3) Department of Plant Pathology, Ohio State University, Wooster, OH 44691, (4) Department of Pharmacology and Toxicology, University of Louisville, Kentucky, Louisville, KY 40202, (5) Genentech, South San Francisco, CA 94080

RNA virus vectors are attractive vaccine delivery agents capable of directing high-level gene expression, however, methods of encapsidation by animal cells is relatively inefficient. We generated an SFV expression vector that could be efficiently trans-encapsidated in vitro by purified Tobacco Mosaic Virus (TMV) coat protein (CP) by introducing a TMV-specific origin of assembly. RNA encapsidation was confirmed by EM, and disassembly, replication and bGalatosidase (bGal) reporter gene expression was demonstrated by cellular analysis. In vitro, TMV encapsidated RNA stimulates robust targeted immune cell uptake and activation, and in vivo, encapsidation significantly improved the humoral and cellular immune responses compared to naked RNA. Reassembly with recombinant TMV CP also facilitates conjugation of peptide epitopes onto the capsid surface to complement the immunoreactivity of the encapsidated RNA. The SFV vector/TMV CP system is safe, easy to manufacture and facilitates the generation of unique nucleic acid/protein antigen compositions that target effective antigen presentation.

WENDESDAY AFTERNOON

3:00 – 5:20 pm BCEC 107 C

Commercialization of Biologics: Characterization and Improvement of Platforms to Aid Commercialization of Biologics

S. Gadam and F. Olsen, Organizers Papers 220 – 225

BIOT 220 - Development and implementation of a robust chemically-defined and high-yielding GS-CHO platform mAb production process

Susan A. Casnocha, Bioprocess R&D, Pfizer Global Biologics, 700 Chesterfield Parkway West, MZ HH2, Chesterfield, MO 63017, Fax: 636-247-6098, susan.a.casnocha@pfizer.com

Yield of monoclonal antibody production from mammalian cell culture systems has been steadily on the rise over the past two decades. In addition to improvements in host cell/clone selection and vector construction, nutritional improvements in cell culture processes contribute significantly to this productivity enhancement. With metabolic knowledge of industrial relevant cell lines (e.g. NS0 and CHO) accumulated over decades of use, the reality of a wholly chemically defined process has been achieved. In many cases the chemically defined processes have demonstrated high productivity. The development of high-producing chemically defined processes will further enrich our knowledge of nutrient requirements, as well as regulation of cellular metabolism and protein production. This knowledge will drive a positive feedback loop between improving cell biology understanding and improving productivity through rational medium/nutrient feed design. The development and implementation of a chemically defined GS-CHO platform process for mAb production in Pfizer Global Biologics will be presented. The beneficial effects and some challenges will be discussed.

BIOT 221 - Use of mathematical modeling to enable effective implementation of platform aeration strategies in process scale-up and transfer

Gregg Nyberg[1], Franqui Jimenez[2], Frank Olsen[1], and Gustavo Grampp[1]. (1) Manufacturing Sciences and Technology, Amgen, Inc, 4000 Nelson Road, Longmont, CO 80503, Fax: 303-401-4401, gnyberg@amgen.com, (2) Manufacturing Sciences and Technology, Amgen Manufacturing, Limited, Juncos, PR 00777-4060

Achieving consistent aeration characteristics across scales is an important consideration in the technology transfer of stirred-tank

cell culture processes. It is necessary to balance oxygen supply and carbon dioxide removal in such a way as to achieve effective dissolved oxygen control, while also ensuring consistent dissolved carbon dioxide concentrations. We have developed a mass-transfer modeling tool, which we have used to guide implementation of our platform aeration strategies to achieve consistent dissolved carbon dioxide profiles across sites and scales. The mass transfer model is based upon mass balancing around the bioreactor using the well-known kLa mass transfer coefficient. Empirical measurement of mass transfer coefficients during equipment commissioning, combined with our mass transfer model, recently allowed us to scale two different processes up to 15,000L in new equipment without empirical iteration. By combining readily scaleable aeration strategies with effective mathematical modeling, we have been able to simplify process transfer and scale-up.

BIOT 222 - Robustness screening to enable cell culture process commercialization

Andrew Michael Rusiniak[1], Tongtong Wang[2], and Guillermo Miroquesada[2]. (1) Bioprocess Research & Development, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-276-5499, rusiniak_andrew_michael@lilly.com, (2) Bioprocess Research & Development, Eli Lilly & Company

We believe the delivery of a robust cell culture process for commercial manufacturing requires phase appropriate evaluations of the process concurrent to the product's development cycle. These evaluations begin with screening of multiple process parameters on multiple production clones prior to the selection of the commercial clone. Once the clone is selected and a baseline process is defined, the robustness of each unit operation within the cell culture process (flask expansion, bioreactor expansion, bioreactor production) is evaluated to ensure the process is commercially viable. After the robustness has been screened, further efforts to improve and characterize the process can be implemented. We will present some of the tools used for a CHO derived antibody production process. These tools include fishbone diagrams and statistical design of experiments (DOE) to evaluate how the common cause variability of process inputs influence the outputs (growth, yield, product quality) of the cell culture process.

BIOT 223 - Process improvement through media development

Wang Wenge, Bioreactor Process Development, Wyeth Biotech, 1 Burtt Road, Andover, MA 01810, WWang@wyeth.com
This presentation focuses on process improvement strategies for therapeutic protein production in mammalian cell culture through medium development. Two approaches will be presented with brief descriptions of relevant case studies. The first approach entails the evaluation of medium composition through additive screening with the intent of improving product quality endpoints. A case study for additive screening resulted in identification of a medium component that can decrease the percentage of high molecular weight forms of a monoclonal antibody expressed in CHO-cell culture. The second approach entails optimization of the relative quantities of components with the objective of increasing overall volumetric productivity. A separate case study will be presented that resulted in a well-balanced medium, free of serum and hydrolysates, that maintains high viability and high productivity for extended time in production.

BIOT 224 - Rapid development of mammalian cell production process of clinical material for proof of principle of novel therapeutic molecules

Jianwei Zhu[1], Yueqing Xie[1], Vinay V. Vyas[1], Beverly Keseling[1], John Roach[2], George Mitra[3], and Stephen P. Creekmore[4]. (1) Biopharmaceutical Development Program, SAIC Frederick, Frederick, MD 21702, jianweiz@ncifcrf.gov, (2) SAIC-Frederick, NCI-FCRDC, (3) Biopharmaceutical Development Program, SAIC-Frederick, NCI-FCRDC, Frederick, MD 21702, (4) Biological Resources Branch, DTP, DCTD, National Cancer Institute
The Biopharmaceutical Development Program (BDP) of the National Cancer Institute at Frederick and SAIC Frederick manufactures biopharmaceuticals, including monoclonal antibodies, recombinant therapeutic proteins, and other biologics through mammalian cell culture. The BDP produces “research grade” as well as “clinical grade” materials for the purpose of exploring “proof of principle” of novel therapeutic concepts and early clinical trials. Our goal is the rapid translation of innovative scientific discoveries into therapeutic products that hold the real hope for preventing and curing cancer, AIDS, and other diseases. In the presentation, we elucidate the procedure that leads to successful development of a variety of therapeutic biologics through case studies, as well as expose development challenges in bringing projects from laboratory bench to clinic. A few projects will be analyzed in detail in terms of cell culture process development, process scale up, and GMP manufacturing of therapeutic biologics through cell line development, medium study, process development and scale-up, and technology transfer to GMP production.

BIOT 225 - Rapid upstream commercial development of an Escherichia coli.-based process for producing therapeutic proteins in inclusion bodies

Henry Lin, Irene Liu, Hedieh Barkhordarian, Jonathan Lull, and Xiaoming Yang, Process Development, Amgen Inc, One Amgen Center Drive, 18S-1-A, Thousand Oaks, CA 91320

As numerous microbially-derived products mature in the pipeline, reducing timeline and cost are critical to successful commercialization. Manufacturing aspects beyond the clinical setting need be considered, which often fall outside the scope of a platform process. Relying on process improvement to unilaterally meet launch demand creates strain on development and heightens risks. Commercial development strategies that take into account quality, manufacturing fit, facility capacity, and process robustness early on help reduce cost, maintain focus and streamline development. In addition, these strategies mitigate the risks for meeting commercial demand. Such philosophy coupled with design of experiments for optimization was applied to the commercial development of a fermentation process that produced therapeutic proteins as inclusion bodies in E. coli. Results of a successful development that not only considers optimizing production, but also the commercial manufacturability will be discussed.

WEDNESDAY AFTERNOON

3:00 – 5:20 pm BCEC 108

Biophysical and Biomolecular Symposium: Protein Aggregation

C. J. Roberts and A. Grillo, Organizers, Papers 226 - 231

BIOT 226 - Biophysical characterization of the amylin-derived peptide pramlintide

Akihisa Nonoyama[1], Jennifer S. Laurence[2], Liza Garriques[3], Hong Qi[3], Thao Le[3], and C. Russell Middaugh[1]. (1) Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047, nonoyama@ku.edu, (2) Depts. of Pharmaceutical Chemistry and Chemical & Petroleum Engineering, The University of Kansas, Lawrence, KS 66047, (3) Amylin Pharmaceuticals, Inc, San Diego, CA 92121

AC137 (pramlintide) is a 37-residue peptide analogue of the hormone amylin. Pramlintide has been studied as an antihyperglycemic adjunct treatment for patients with type-2 or type-1 diabetes who use insulin. This study has taken an empirical phase diagram (EPD) -based approach to obtain structural stability information of this peptide by compiling thermal perturbation data acquired from multiple spectroscopic methods including high-resolution second-derivative UV absorbance spectroscopy, optical density, fluorescence and circular dichroism. The multi-pronged analysis allowed us to obtain information about the peptide's secondary and tertiary structures as well as its aggregation behavior, all as functions of temperature and pH. Although low concentrations of the peptide were shown to be stable across the entire pH range (4-8) examined, high concentrations produced a tendency for pramlintide to aggregate at certain pH values. This comprehensive characterization of the peptide's innate structural properties provides a basis for further work aimed at stabilizing the molecule.

BIOT 227 - Silicone oil contamination of therapeutic protein formulations: Surfactant and protein effects

John Gabrielson[1], Daniel G. Bates[1], Benjamin M. Williams[1], Jean-Bernard Hamel[2], John F. Carpenter[3], and Theodore W. Randolph[4]. (1) Department of Chemical and Biological Engineering, University of Colorado, Boulder, Boulder, CO 80309, (2) Pharmaceutical Systems, BD Medical, 38800 Le Pont de Claix, France, (3) Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262, (4) Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80309

Purpose: Silicone oil has been shown under certain conditions, even at low In addition to potential risk to∗concentrations, to induce protein aggregation. patients, silicone oil induced therapeutic protein aggregation is a major concern in the pharmaceutical industry, potentially leading to loss of product and increased manufacturing costs. This study explores the complex interactions between silicone oil, therapeutic monoclonal antibody, and non-ionic surfactant in oil/aqueous emulsions. Methods: Medical grade polydimethylsiloxane (PDMS, or silicone oil) is added (0.01 – 10% v/v) to two aqueous buffering systems (10 mM sodium phosphate, 130 mM NaCl, pH 7.2; 10 mM sodium acetate, pH 5.0). Resulting suspensions are passed once through a high pressure homogenizer (Avestin, Inc., Emulsiflex C5 Homogenizer). Polysorbate 20 solution (Tween 20®, 0.002% - 2%) and Herceptin® (trastuzumab, 1 mg/mL) are added to the emulsion by repeated pipeting until fully mixed. Ultraviolet-visible and fluorescence spectroscopy, light scattering particle sizing, and fluorescence activated particle scanning are used for sample analysis. Results: Emulsion half-life is inversely proportional to silicone oil concentration. At pharmaceutically relevant silicone oil concentrations (< 10 µL/mL), emulsions are stable for many hours. Addition of the non-ionic surfactant, Tween 20®, further stabilizes the emulsions, particularly when the surfactant is added prior to homogenization. In the absence of Tween 20®, monoclonal antibody adsorbs to the oil surface, and acting as a surfactant, it moderately stabilizes the emulsion. As the size of oil-antibody conjugate particles increases, the ratio of adsorbed antibody to oil remains constant. Conclusions: In pharmaceutical formulations containing a non-ionic surfactant, presence of the surfactant may aid in the suspension and stabilization of oil droplets in aqueous buffer. Small oil droplets (ca. 1 µm) with adsorbed antibody may agglomerate to form larger, "multidroplet" particles.

BIOT 228 - Self-interaction chromatography applied to the rapid development of formulation for therapeutic proteins

Henri Kornmann, Natacha Collet, and Gianni Baer, Biotechnology Process Development, EMD-Serono, CH-1809 Fenil-sur-Corsier, Switzerland, Henri.Kornmann@merckserono.net

The degradation of therapeutic proteins due to inappropriate process or storage conditions is a major issue in biopharmaceuticals manufacturing both in terms of costs and safety. Today, we classically test the stability of a protein by time- and product-consuming accelerated degradation studies. While this type of experiments usually takes weeks, information about the stability of a protein in terms of aggregation can be obtained in few minutes by directly measuring the protein-protein self-interactions in solution. The magnitude of these interactions is captured in a thermodynamic parameter termed the second osmotic virial coefficient (B22). In this study, Self-Interaction Chromatography (SIC) was used to rapidly measure the level of B22 of purification process intermediates and drug substance formulations. This work shows that monoclonal antibodies or Fc-fusion proteins are less prone to aggregation when the formulation conditions induce strong repulsive self-interactions (B22 > 1.0*10-4 [mol ml/g2]). Industrial applications and case studies of this method will be presented. Miniaturization and high throughput format of the method will also be discussed.

BIOT 229 - Stability of a heterogeneous IgG1 antibody with atypical glycosylation: A comparison between individual and mixtures of its glycoforms

Grace C. Chu[1], Sururat B. Coulibaly[2], and Hui Koon Khor[1]. (1) Department of Pharmaceutics, Amgen, Inc, 1 Amgen Center Drive, M/S 8-1-C, Thousand Oaks, CA 91320, gchu@amgen.com, (2) Department of Analytical Sciences, Amgen, Inc, Seattle, WA 98119

Heterogeneity is often observed in antibodies, and can result in multiple isoforms that differ in post-translational modifications such as glycosylation, disulfide linkages, or lysine variations. In this study, we have compared the stability of a heterogeneous IgG1, which is glycosylated in both variable and constant domains, to purified preparations of each of its glycoforms. We also tested the stability of mixtures of the different glycoforms. At least eight glycoforms were detected in the bulk material by cation exchange HPLC. Individual glycoforms, mixtures of different glycoforms, and the original bulk material were tested for stability as a function of pH, buffering agent, temperature, and time. Analyses by multiple HPLC and spectroscopic techniques indicated differences in protein stability between purified glycoforms and mixtures of isoforms, including the original bulk material.

BIOT 230 - Discrepancies in the phase behaviors of therapeutic antibodies

Branden Salinas[1], Sathish Hasige[1], Chris Allan[2], Steven Bishop[2], Ambarish Shah[2], John F. Carpenter[3], and Theodore W. Randolph[4]. (1) Department of Chemical and Biological Engineering, University of Colorado, Boulder, Boulder, CO 80309, Branden.Salinas@Colorado.EDU, (2) MedImmune, Inc, Gaithersburg, MD 20878, (3) Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262, (4) Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80309

Monoclonal antibodies (mAbs) have become popular drug candidates. Since antibodies produced in the same manner have high sequence identities it is often thought they will exhibit similar phase behavior. This study is concerned with two therapeutically relevant mAbs that provide an example to the contrary. There is a difference of an order of magnitude in the mAbs solubility's at their pIs.
A combination of static light scattering (SLS), membrane osmometry, differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) are utilized to analyze colloidal and structural non-idealities at wide range of protein concentrations. Along with the aforementioned solubility difference, the results have illuminated a reversible association in solution for one of the two mAbs. Furthermore, both events are buffer type and salt concentration dependent. This work reveals likely sources of these non-idealities and suggests that certain buffer types disrupt domain-domain interactions leading to intermolecular attraction and associations.

BIOT 231 - Chemometric approach toward protein aggregation propensity

Mark Cornell Manning[1], Cody M Van Pelt[1], Robert R. Meglen[2], and Fred J. Stevens[3]. (1) Legacy BioDesign LLC, 1826 Monarch Circle, Loveland, CO 80538, Fax: 970-663-6006, manning@legacybiodesign.com, (2) Latent Structures, LLC, Boulder, CO 80303, (3) Argonne National Laboratory, Argonne, IL 60439

Replacement of one amino acid for another within a protein sequence alters a number of physicochemical properties at one time (e.g., size, hydrophobicity, polarity). Therefore, a multivariate approach to analyzing the effects of mutation on aggregation propensity must be taken. We have used reduced properties of amino acids and used them to analyze protein sequences, in an effort not only to identity the critical residues, but also the specific physicochemical property at that site having the greatest impact on aggregation rate. The application of this approach to antibody light chains will be presented, along with results on A-beta peptide mutants. In addition, the methodology has been used to study the sequence dependence of conformational stability of the variable region of antibody light chains, as increased conformational stability is critical for retarding aggregation.

WEDNESDAY

5:30 – 7:30 pm HALL C

POSTER SESSION, Y. Tang, S. Ozturk, and S. Subramanian, Organizers, Papers 232 – 428

BIOT 232 - Role of astroglial fatty acid metabolism in the pathogenesis of Alzheimer's disease: A metabolic flux analysis (MFA) study

Sachin Patil, Department of Chemical Engineering, Michigan State University, East Lansing, MI 48824, patilsac@egr.msu.edu, and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by various pathophysiological (e.g. production of amyloid &beta protein and hyperphosphorylation of tau protein) and metabolic (e.g. glucose hypometabolism) abnormalities. Epidemiological studies suggest that high fat diet significantly increases the risk of AD and the degree of saturation of fatty acids is critical in determining the risk for AD. In this context, we have previously shown that saturated free fatty acids (FFAs), palmitic acid (PA) and stearic acid (SA), had no direct effect on primary rat cortical neurons, but through astroglial metabolism these fatty acids induced increased &beta-amyloidogenesis and tau hyperphosphorylation in rat cortical neurons. These data suggested the involvement of astrocytes in mediating the FFA-induced, AD-specific effects observed in neurons and thus, further warranted a more comprehensive analysis of astroglial FFA metabolism and their role in causing AD-specific changes in neurons. In this regard, we applied

metabolic flux analysis (MFA) as a tool here to gain a comprehensive insight into the metabolic profile of primary cortical astroglia in the presence of saturated FFAs. This analysis may help to identify i) the metabolic pathways involved in inducing the observed AD-specific phenotypes in the neurons and ii) potentially novel targets for therapeutic intervention in AD. Our preliminary results showed that glucose metabolism was significantly reduced in astroglia treated with pathological concentrations of PA as compared to untreated ones, thus suggesting involvement of FFAs in causing the metabolic changes in addition to the pathophysiological changes associated with AD. Study is underway to further investigate the effects of PA on other metabolic pathways in astroglia (e.g. abnormal lipid synthesis) that may contribute to development of the AD-related phenotypes. The pathways identified by the MFA are being further evaluated for their role in causing these AD-associated changes through various pharmacological and/or molecular biological means.

BIOT 233 - Suppression of PKR activity mediates the apoptosis induced by palmitic acid and TNF-α in HepG2 cells

Xuerui Yang and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, 2527 Engineering Building, East Lansing, MI 48824, yangxuer@egr.msu.edu

Saturated free fatty acid (FFA, e.g. palmitate) induced apoptosis is associated with the development of a variety of diseases. Palmitate has been shown to induce apoptosis in cardiac cells, pancreatic beta cells, breast cancer cells, hepatocytes, and many other cell types, while unsaturated FFAs (e.g. oleate) have been shown to protect cells from apoptosis induced by saturated FFAs. Palmitate induces apoptosis in liver cells by decreasing the protein level of Bcl-2, however, the intermediates that mediate this affect have not been identified. In the present study we determined that the double-stranded RNA-dependent protein kinase (PKR) exploited an anti-apoptotic role in human hepatocellular carcinoma cell (HepG2) by regulating the Bcl-2 protein. We showed for the first time that palmitate and tumor necrosis factor (TNF)-α suppressed the activity of PKR, and PKR is required to maintain the level of Bcl-2 in HepG2 cells. We propose that the transcription factor, NF-κB, is involved in mediating the effect of PKR on the protein level of Bcl-2. In addition, the phosphorylation of Bcl-2 at different amino acid residues, such as Ser70 and Ser87, is critical in determining the role of Bcl-2 in regulating apoptosis. We found that a decrease in the phosphorylation of Bcl-2 at Ser70 was mediated by PKR and JNK, while the phosphorylation of Bcl-2 at Ser87 was not affected by and PP2A, the mostαpalmitate and TNF-α. Furthermore, we showed that eIF-2 well-known substrates of PKR, are not involved in mediating the apoptosis induced by palmitate and TNF-α. In summary, we identified the signaling pathways that regulate the level and phosphorylation status of the Bcl-2 protein and thereby apoptosis in HepG2 cells upon exposure to palmitate are mediated by PKR.

BIOT 234 - Studies on novel optimization parameters for production of protease using response surface methodology

Asha Immanuel Raju Chaduvula[1], Kiran Kumar Nalla[1], Murali Yugandhar Nikku[1], Sarat Babu Imandi[1], Sita Kumari Karanam[2], and Israel Yedluri[3]. (1) Chemical Engineering Department, Andhra University, Room No: 109, Chemical Engineering Department, Andhra University, Visakahpatnam 530003, India, prof_chair@yahoo.com, (2) Department of Pharmaceutical Sciences, M. R. P. G. College, Vizianagaram 535002, India, (3) Department of Geophysics, Andhra University, Visakhapatnam 530003, India

ABSTRACT Protease production was maximized by Yarrowia lipolytica NCIM 3472 in submerged fermentation. Response surface methodology (RSM) involving Box-Wilson central composite design was adopted to evaluate the protease activity produced, by most important factors, such as carbon concentration, nitrogen concentration and salt solution concentration. The optimal set of conditions for maximum protease activity was as follows: carbon concentration 1.29925 % (v/v), nitrogen concentration 0.39977 % (v/v), and salt solution concentration 16.56754 % (v/v). Thus by using the central composite design, it is possible to know the accurate values of the kinetic parameters where the maximum protease activity occurs.

BIOT 235 - Evaluating biopharmaceutical economics and capacity with process modeling and simulation

Alexandros Koulouris, Intelligen Europe, 6th km Harilaou-Thermi, PO Box 328, Thermi, Thessaloniki 57001, Greece, akoulouris@intelligen.com, Charles Siletti, INTELLIGEN, INC, Mt. Laurel, NJ 08054, and Demetri Petrides, INTELLIGEN, INC, Scotch Plains, NJ 07076

The capital investment for new cell culture facilities is around $5,000 per liter of production bioreactor capacity. Moderate to large facilities can cost hundreds of millions, yet such investments are often made with uncertain information about process performance, media and materials prices, and market conditions. This paper presents a systematic way to evaluate the critical costs and capacity issues in biotech plants. Examples will be presented on determining the target product titer, optimizing bioreactor batch size and choosing where or whether to use disposable equipment. Because many process decisions must be based on partial or uncertain information, this presentation will also describe how to evaluate the risk associated with process and economic assumptions.

BIOT 236 - Monitoring the expression of biosynthetic pathway genes in *Catharanthus roseus* hairy root cultures under optimum elicitation conditions

Sheba Goklany, R. H. Loring, and Carolyn WT. Lee-Parsons, Department of Chemical Engineering, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, goklany.s@neu.edu

Plant cell cultures are being investigated as a source of alkaloids since alkaloid yields from whole plants are low and subject to weather conditions. The *Catharanthus roseus* plant produces two anti-cancer compounds, vincristine and vinblastine, in addition to anti-hypertensive and sedative compounds, ajmalicine and serpentine, respectively. In this study, *C. roseus* hairy root cultures were treated with methyl jasmonate (MeJA) or purified yeast extract (PYE) under optimum elicitation conditions. The transcript levels of three terpenoid indole alkaloid (TIA) pathway genes (*G10h*, *Tdc*, and *Str*) and alkaloid production were measured using quantitative Real Time-Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) and HPLC, respectively. MeJA and PYE treatment enhanced the expression of the three TIA pathway genes by 2 – 4 fold within the first 12 hours, followed by increases in alkaloid levels ranging from 40 – 300% by 3 days after elicitation.

BIOT 237 - Analysis of suspended and biofilm atrazine degrading cells

Nicole Kim Biglione, Chemical and Biochemical Engineering, The University of Iowa, 4133 Seamans Center, Iowa City, IA 52242, Fax: 319-335-1415, biglione@engineering.uiowa.edu, Victor GJ. Rodgers, Department of Bioengineering, University of California Riverside, Riverside, CA 92521, and **Tonya L. Peeples**, Department of Chemical and Biochemical Engineering, The University of Iowa, Iowa City, IA 52242, Fax: 319-335-1415, tonya-peeples@uiowa.edu

Increasing public concern over the safety of the s-triazine herbicide atrazine is fueling demands for methods to remove atrazine from water. In this research the ability of microbes in suspended and biofilm cultures to degrade atrazine is evaluated. Monod kinetic parameters, μ_{max} and K_s, of *Pseudomonas* sp. ADP (P. ADP) in suspension were determined to be 0.14(+/-0.01) h^{-1} and 1.86(+/-1.80) mg/L at 200 rpm and 27ºC and 0.22(+/-0.01) h^{-1} and 6.30(+/-1.44) mg/L at 200 rpm and 37ºC, respectively. Preliminary studies showed that P. ADP forms a biofilm. This work studies the relationship of this biofilm to atrazine consumption.

A spherical biofilm device was designed to allow for ease in modeling and experimental analysis of bioconversion and mass transfer. The mass transfer coefficient in this reactor was determined to 0.40 cm/sec at impeller speeds higher than 55 rpm. COMSOL 3.2 was used to model cell growth and atrazine consumption the biofilm reactor.

The main goal of this research is to determine the influence of suspended and biofilm cell populations on atrazine degradation. The degradation rate of atrazine and the cell growth rate in the suspended and biofilm cells will be presented. The rate-limiting step in the metabolic pathway as well as the transport limitations in each case will be discussed. Parameters developed from this research will be applied in the design of appropriate bioreactors for the application in industrial and environmental settings.

BIOT 238 - Biosynthesis and application of colorants from fungi

Farzaneh Alihosseini, College of Agricultural and Environmental Chemistry, University of California, Davis, Division of Textiles and Clothing, One Shields Avenue, Davis, CA 95616-8515, fhosseini@ucdavis.edu, and Gang Sun, Division of Textiles & Clothing, University of California, Davis, CA 95616

The successful use of microorganisms in dying the fabrics proved the bio-production of textile colorants by fungi and bacteria. Using bio-resource is believed as an alternative to use of non-renewable resources. We found that a new isolated marine bacterium similar to category of bacteria Vibrio gazogenes produces a bright red colorant. The preliminary dying test shows that this colorant can dye the synthetic fabric and has similar characteristic to disperse dyes. The UV-Visible spectra of methanol solution has absorption wavelength at λmax 530nm. The Electron Spray Ionization mass spectroscopy(ESI-MS)and Nuclear Magnetic Resonance(NMR)are used to identify the structure of this compound. The accurate mass result shows that the colorant has molecular mass of 323.19975. Further analyzing by mass fragmentation, H-NMR and COSY NMR are done and the structure of colorant has completely identified with formula of C20H25N3O. The light and heat stability of the compound under dyeing condition prove the quality of colorant as textile dye.

BIOT 239 - The evolution of a disposable stirred tank bioreactor at Centocor: From concept to GMP implementation

Wood Christian, Ravinder Bhatia, Nicole Richardson, and Sadettin Ozturk, BioProcess Development, Centocor R&D, PO Box 776, Welsh and McKean Roads, , PA, Spring House, PA 19477, CWood@CNTUS.JNJ.COM

Over the last few years, Centocor has been developing a disposable bioreactor system for use in the Development and GMP Pilot Plant Facilities. The project began internally at the 50 L scale, and through collaboration with Hyclone, evolved to a GMP compliant 1000 L bioreactor. Initially, a set of design requirements was established to ensure scalability, and adequate mass transfer and mixing to support cell growth. At each stage of the project, the disposable bioreactors were able to achieve growth and productivity comparable to stainless steel bioreactors. However, several issues and design limitations were encountered which required practical solutions to improve usability and safety, and meet GMP requirements. This presentation will summarize these development efforts, and highlight some of the challenges and solutions which resulted in a fully GMP compliant 1000 L disposable bioreactor system.

BIOT 240 - Biotransformation of steroids by *Exophiala jeanselmei* var. *lecanii corni*

Kayanne Patrice McCook, Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica, Fax: 876-977-1835, kayanne.mccook@uwimona.edu.jm, and Paul B. Reese,

Department of Chemistry, University of the West Indies, Mona, Kingston, Jamaica

The steroids dehydroepiandrosterone, pregnenolone, testosterone, progesterone, prednisone, cortisone, estrone and sarsasapogenin were fed to growing cultures of *Exophiala jeanselmei* var. *lecanii corni*. With 3B-hydroxy-Δ^5-steroids, dehydroepiandrosterone and pregnenolone there was hydroxylation at C-7. Alternately, these compounds were oxidized to the 3-ketone; the 5,6-double bond isomerised into conjugation; the olefin was then reduced and the 3-ketone was converted to the 3A-alcohol. For the Δ^4-3 keto steroids testosterone and progesterone both the double bond and carbonyl were reduced to the 3A-hydroxy analogue and position 5 was hydroxylated. Cortisone and prednisone underwent reduction of the C-20 ketone only to give the 17A,20*S*,21 triol, while estrone was simply hydroxylated at C-15. Sarsasapogenin remained untransformed.

BIOT 241 - Multiplex transcript analysis for identifying high-producing cell lines

Christina J Lee, Gargi Seth, Joni Tsukuda, and Robert W Hamilton, Early Stage Cell Culture, Genentech, Inc, 1 DNA Way, MS 32, San Francisco, CA 94080, Fax: 650-225-2006, leec@gene.com

Chinese hamster ovary cells are used as hosts for the production of therapeutic recombinant proteins. However, the current process of selecting high-producing clones is both time-consuming and labor-intensive. Improved methods of clone screening are required to meet increasing demands for efficient and streamlined production processes. In particular, we examined the potential of using antibody transcript levels as criteria for two aspects of clone screening: selecting for high-producing clones and identifying clones with questionable product quality. We used QuantiGene® Plex (Panomics, Inc., Fremont, CA), a high-throughput, high-sensitivity assay for measuring multiple transcripts from cell lysate, to enhance our current process of clone screening. Using the development of stable cell lines as examples, we investigated the relationship between transcript and protein levels through several rounds of screening. First, we observed that measured heavy chain expression levels are correlated with specific productivity, enabling the identification of high-producing clones from mRNA. Second, we observed that low ratios of light to heavy chain expression levels may be indicative of product aggregation levels, allowing us to rapidly identify and eliminate clones of questionable product quality. Therefore, an efficient process of identifying high-producing clones of desirable product quality is possible by using QuantiGene® Plex assay to measure antibody transcript levels.

BIOT 242 - ReBiT, a database for enzymatic pathway design

Collin H. Martin, Darcy Prather, and Kristala Jones Prather, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue Room 66-425, Cambridge, MA 02139, collin@mit.edu

Designing novel biochemical pathways is a highly subjective process. The current range of chemistries that enzymes catalyze is diverse enough that for most target molecules, there exist many possible combinations of enzymes that can theoretically synthesize the target compound. Popular enzyme databases like BRENDA, MetaCyc, and KEGG do not readily lend themselves to pathway design, because the information contained within these databases cannot be queried based on the chemical or functional group transformations that the enzymes themselves catalyze. That is, while these databases do allow users to query their contents based on static substructures, they do not allow querying for dynamic chemical changes that either create or destroy a given substructure. This type of query would be useful when identifying enzymes that create structures and compounds of interest. To address this issue, we have created ReBiT (Retro-Biosynthesis Tool), the database of enzymatic transformations, to assist synthetic biologists and metabolic engineers in designing novel pathways towards valuable target compounds.

ReBiT allows a user to query molecular structures against a database of enzyme-catalyzed chemical transformations. ReBiT returns hits (enzyme-catalyzed chemical transformations) in which the queried structure changes or reacts. Selecting a particular transformation lists all three-digit enzyme classification (E.C.) numbers that contain enzymes catalyzing that transformation as well as links to the ExPASy database for more detailed enzyme-specific information. ReBiT also displays graphical representations of all structures involved in each transformation, any cofactors or co-substrates used, and additional, useful commentary (usually specific four-digit E.C. numbers catalyzing each transformation).

BIOT 243 - Construction of ptb disrupted mutant for characterization of its function in Clostridium tyrobutyricum fermentation

Yali Zhang and Shang-Tian Yang, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 w 19th Ave., Columbus, OH 43210, zhang.387@osu.edu

Clostridium tyrobutyricum could produce acetate, butyrate and hydrogen directly from carbohydrates during anaerobic fermentation. The co-production of acetate and butyrate increases the separation cost and make the process uneconomical. Our attempt is to disrupt the ptb gene encoding phosphotransbutyrylase involved in butyrate formation pathway to shut down the butyrate production and to increase the selectivity. A ptb fragment is cloned by the degenerated primers and an erythromycin resistant gene Emr is inserted into the ptb fragment. The ptb gene in chromosome is knock out by homologous recombination after the disrupted ptb fragment is transformed into the wild type Clostridium tyrobutyricum. The mutant is characterized by southern blotting, enzyme assay and SDS-PAGE. The gene knock-out effect on the production is also evaluated.

BIOT 244 - Biotransformation of steroids by *Thielaviopsis paradoxa*

Floyd Alexander Russell, Department of Chemistry, The University of the West Indies, Mona, Kingston 7, Jamaica, Fax: 876-977-1835, floyd.russell@uwimona.edu.jm, and Paul B. Reese, Department of Chemistry, University of the West Indies, Mona, Kingston, Jamaica

The biotransformation potential of *Thielaviopsis paradoxa*, a pathogen of coconuts, was investigated using steroid substrates.

Incubation of the fungus with prednisone and cortisone resulted in the reduction of the C-20 ketone. Testosterone afforded two products of transformation, i.e. reduction of the 4,5-double bond and oxidation of the C-17 alcohol. With dehydroepiandrosterone, reduction of the C-17 ketone and hydroxylation at C-7 occurred. Biotransformation of progesterone resulted in degradation of the C-17 side chain and reduction of the 4,5-double bond. Pregnenolone underwent Baeyer-Villiger oxidation in ring D while the 17-ketone in estrone was reduced. All biotransformation reactions were carried out in a modified Salmink medium, shaking at 200 rpm for 10 days.

BIOT 245 - Continuous perfusion process in a disposable stirred-tank bioreactor: A case study

Barbara Chiang, Nicole Richardson, Hong Haddock, Christian Wood, Ravinder Bhatia, and Sadettin Ozturk, BioProcess Development, Centocor R&D, PO Box 776, Welsh and McKean Roads, Spring House, PA 19477, sozturk@cntus.jnj.com

Disposable bioreactor systems are making their way into production of therapeutic products. A 28-day perfusion run was performed in the disposable stirred-tank using an external cell retention device to retain cells in the bioreactor. Results will show that the disposable stirred-tank was able to sustain high cell densities (> 15x106 cells/ml) and productivity comparable to conventional stainless steel bioreactor. Also, in this case study we will present equipment design challenges to scale-up perfusion process in the disposable bioreactor system.

BIOT 246 - Screening of strains for bioconversion from DL-ATC to L-cysteine

Tao Dong[1], **Zhao Lin**[2], and Yu Huang[2]. (1) Department of Chemical Engineering,School of Chemical Engineering and Technology, Tianjin University, 92 Weijin Road, Nankai District, TianJin 300072, China, supermandt@126.com, (2) Department of Environmental Science, School of Environmental Technology and Science, Tianjin University, 92 Weijin Road ,Nankai District,Tianjin,China, Tianjin,300072, China, zhaolin@tju.edu.cn

Micrococcus P2 for bioconversion from DL-2-amino-Ä2-Thiazoline-4-Carboxylic acid (DL-ATC) to L-cysteine were isolated from sediment samples with DL-ATC used as the only nitrogen source. When mass concentration of DL-ATC was 2%, the productivity of L-cysteine reached to 11.2mg/mL with a molar yield of 92.4% after 6h bioconversion. The heredity for Micrococcus P2 was stable, and the relatively activity of the tenth generator was 96.3%.

BIOT 247 - Development of generic feed media for protein production in contract manufacturing

Guangli Wang, Laureate Pharma Inc, 201 College Road East, Princeton, NJ 08540, guangli.wang@laureatepharma.com

There have been many challenges in the upstream process development in contract manufacturing organizations because of extremely short timeline, limited resources and a diversity of client projects. Many approaches have been used for feed medium development to increase viable cell density and culture duration in upstream process development. We have been employing platform approaches for the generic feed medium development to improve the productivity of cell culture processes while preserving the product quality. This presentation will present case study results to show the efficiency and flexibility of using generic feeds in upstream process optimization to meet the respective expectations of the different clients.

BIOT 248 - Enhancement of mass transfer in a 250 L bioreactor by sparger design modification

Christian Wood, Hong Haddock, Brian Cottee, and Sadettin Ozturk, Bioprocess Development, Centocor R&D, PO Box 776, Welsh and McKean Roads, Spring House, PA 19477, Fax: 215-540-4840, sozturk@cntus.jnj.com

The transfer of oxygen and carbon dioxide gases to and from a cell culture broth are essential parameters to consider in the design and operation of a bioreactor. Sufficient oxygenation and regulation of carbon dioxide must be provided, while minimizing foaming and shear stress on the cells. Currently, the mass transfer rates in Centocor's GMP Pilot Plant 250 L bioreactors are adequate to support most processes in operation. However, new development processes with greater peak cell densities demand significant increases in mass transfer efficiency. In this work, we evaluated microspargers of varying surface area and pore size, and measured the corresponding mass transfer coefficients (kLa) as a function of gas flow rate and agitation speed for each microsparger. The resulting oxygen kLa values were not impacted by the microsparger pore sizes evaluated, however, significant increases were observed with greater surface area. The larger surface area microspargers effectively eliminated the saturation or “flooding” effect observed at high gas flow rates with the smaller microspargers. Based on these results, new microspargers with increased surface area were installed which provided oxygenation similar to the smaller microspargers at 2-4 times lower gas flow. The results of the mass transfer measurements and a subsequent bioreactor run will be discussed.

BIOT 249 - Fed-batch process development: A platform-based approach

Kristin Stoeber, Colette Ranucci, and Natalie Villani, Fermentation and Cell Culture Development, Merck & Company, WP17-201, West Point, PA 19486, kristin_stoeber@merck.com

In today's highly competitive process development environment, the objective is to rapidly bring forward early stage product candidates with minimal time and resource investments. Maximizing efficiency through establishment of a standardized approach to process development is vital to achieving this goal. Hence, we have instituted a platform based approach for cell culture-produced therapeutic protein products. This allows for expedited manufacture of early phase clinical material by eliminating lengthy process development timelines while still achieving process performance capable of supporting clinical programs.
Fundamental elements of the platform process developed at Merck include the cell line, medium and feed formulations, operational parameters, step definition, and timing for both the cell expansion

and production stages. The work described here demonstrates the utility of this kind of platform in quickly establishing robust and productive fed batch cell culture processes which can be readily implemented in a clinical manufacturing environment.

BIOT 250 - Fluid mixing practice for bioreactor scale-up and scale-down

Richard D. LaRoche, ANSYS, Inc, 10 Cavendish Ct, Lebanon, NH 03766, richard.laroche@ansys.com

This poster describes how industrial fluid mixing techniques, pilot-scale experiments and computational fluid dynamics (CFD) can be combined to insure optimal bioreactor scale-up and scale-down. The author will review industrial fluid mixing practice applied to bioreactor design along key validations for the use of CFD in modeling stirred tank reactors such as blend time, velocity fields and impeller power numbers. Turbulent energy dissipation rate will be discussed as an appropriate scale-up parameter for ensuring adequate mass transfer in bioreactors. The author will show how CFD can be used as a complementary tool with accepted industrial fluid mixing practice in the following areas: mixing equipment design outside standard mixing correlations, solid-liquid mixing, gas-liquid mixing, liquid-liquid mixing, probability statistics for turbulent energy dissipation and strain rate. The author offers his perspective on how CFD techniques can be used to complement the classic scale-up methods of industrial fluid mixing based on his experience and that of his colleagues while working for Cray Research, DuPont and Fluent.

BIOT 251 - Host system choice challenges bioprocessing of plant-derived therapeutics

Lisa R. Wilken, **Susan Woodard**, Georgia Barros, Steve White, and Zivko L. Nikolov, Biological & Agricultural Engineering, Texas A&M University, 201 Scoates Hall, MS 2117, College Station, TX 77843, lwilken@tamu.edu, swoodard@tamu.edu

The emergence of transgenic crops as alternative production hosts for therapeutic proteins presents unique challenges for downstream process development. Each plant host contains a unique mixture of impurities (plant protein, lipids, phenolics, alkaloids, etc.) that must be removed during purification of the target protein. The presence and quantity of these impurities in the extract could vary significantly with extraction conditions such as the solid-to-liquid ratio, pH, buffer ionic strength, and homogenization method. In some situations, specific impurities (e.g. proteases and phenolics) dictate the sequence of unit operations and operating conditions. Key tasks in downstream process development are to identify critical extract impurities, evaluate their impact on selected recovery and purification steps, and to rationally design an integrated process that exploits potential advantages of each host system. This presentation will address strategies that could be employed in the recovery of pharmaceutical proteins from seed and leafy plant tissues. The potentially detrimental effects of plant proteases and the identification and removal of interfering phenolics from Lemna minor, rice, and tobacco extracts will also be discussed.

BIOT 252 - Implementing an automated reactor sampling system for monitoring cell culture bioreactors

Lauren Speciner[1], George Barringer[2], Joseph Perez[1], Chris Grimaldi[1], Richard Reineke[1], and Adema Arroyo[1]. (1) Process Development Engineering, Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, speciner.lauren@gene.com, (2) Groton Technology, Inc, Acton, MA 01720

Monitoring of cell culture bioreactors is critical to process control and optimization. Currently, offline sampling is used in various cell culture operations. This process is timely and labor intensive. Automating the sampling process is highly desired but concerns about sterility, sample volume and cross contamination are common. This paper presents the evaluation of an automated sampling system by Groton Biosystems. This system allows sampling from up to eight bioreactors and connections to up to four analytical devices. It automates the sampling process from the reactor through the analytical device, reporting the results directly into a data historian. Several aspects of the evaluation will be discussed, including ease of use, robustness, sample viability, data reporting capabilities, and bioreactor contamination risk. The performance of the system was compared to the current manual sampling technique. Results showed that the two techniques are comparable. Bioreactor contamination was not observed. The system is easy to use and does not adversely affect the cell culture process.

BIOT 253 - Physiological characterization of Saccharopolyspora erythraea deletion strains: Global and pathway specific regulators

Isaac Adedeji Akinrinsola, DEPARTMENT OF BIOCHEMICAL ENGINEERING, UNIVERSITY COLLEGE LONDON, Torrington Place, London WC1E 7JE, United Kingdom, Fax: 02079163943, ucbeiaa@ucl.ac.uk

Recent advances in the understanding of polyketide enzyme complexes have enabled the rational design and synthesis of novel polyketide molecules. This will soon enable a targeted approach to increase the titres of polyketides both novel and existing. The publication of genome sequences from filamentous actinomycetes has shown that these strains contain a vast potential for production of secondary metabolites. This represents a potential that is mainly unexplored and so far attempts to tap into this potential have been met with limited success. Generic rules for tapping this potential will be needed for rapid exploration/exploitation of these novel compounds. Modification of transcriptional regulators is an obvious option. This study describes the effect of targeted deletions in both global and pathway specific regulators in Saccharopolyspora erythraea. Five deletions strains were constructed which contained a deletion in each of 3 global regulators and 2 pathway specific regulators in Saccharopolyspora erythraea.

BIOT 254 - Production of Galacto-oligosaccharides from whey lactose by using two-step Plug-Flow Reactor with immobilized enzyme β-Galactosidase from Aspergillus oryzae and Bacillus circulans and posterior chromatographic separation

Juan Ignacio Sanz Valero, Department of Chemical and Biomolecular Engineering, Ohio State University, 140 West 19th Avenue, Columbus, OH 43210, sanz-valero.1@osu.edu, and Shang-Tian Yang, Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210

In this project we developed a production process of galacto-oligosaccharides (GOS) and posterior chromatographic separation for separating and purifying GOS. GOS, also known as *Bifidus growth factor*, are produced from whey permeate or lactose. GOS and other non-digestible oligosaccharides are known to have many beneficial health effects, and are expected to have wide applications as prebiotic food ingredients and dietary supplements. The process for GOS production from whey lactose involves two sequential immobilized enzyme reactors and a chromatographic separation process for purification of GOS. Lactases, β-glycosidase enzymes, from *Aspergillus oryzae* (fungi) and *Bacillus circulans* (bacterium) were used to convert lactose to GOS by a transgalactosylation reaction. Products containing 28% of GOS (at 50% conversion) and 40% of GOS (at 60% lactose conversion) were obtained using lactase from *A. oryzae* and *B. circulans*, respectively. Two sequential plug-flow reactors with the immobilized β-galactosidases were used to convert lactose (400g/L) to GOS with a high productivity of 2000 g/L/h, which is about 100-fold higher than most other processes. To further increase the GOS content in the final product, a simulated moving bed (SMB) chromatographic technique was studied to separate GOS from lactose and monosaccharides. SMB offers a high efficiency due to the continuous operation and efficient use of the mobile and stationary phases, allowing high sample loading with improved productivity and saving 90% in solvent consumption as compared with conventional liquid chromatography methods.

BIOT 255 - Rapid enhancement of antibody production through DOE-based hydrolysate supplementation of an optimized base medium

Cindy Hunt, Kimesha Hammett, Tammy Hill, Toyin Oshunwusi, Stacy Holdread, **Yunling Bai**, and James W. Brooks, BD Advanced Bioprocessing, 54 Loveton Circle, Sparks, MD 21152

It has been well documented that different cell lines have unique nutritional requirements for maximum protein production. With current regulatory standards, undefined components, such as serum, have historically been eliminated from formulations. Additionally, process development timelines and production schedules do not always allow for a complete optimization of a chemically defined medium, feed strategy, and process. Therefore, the supplementation of a chemically defined medium with animal free hydrolysates has become a more prevalent and productive industry practice. Our unique media and process development procedures were utilized to achieve a significant increase in huIgG production. An initial productivity improvement was achieved through DOE-based chemically defined medium optimization. The production level was further increased by extending the work to include our DOE-based approach to peptone supplementation and process development. Feed media optimization and strategy development were further investigated in order to improve production in both shaker flask and bioreactor.

BIOT 256 - Simultaneous determination of multiple components in CHO cell culture using raman spectroscopy

Samir Varma, Bioprocess Development, Bristol-Myers Squibb Co, 6000 Thompson Road, Syracuse, NY 13057, samir.varma@bms.com, Sivakesava Sakhamuri, Process Development, Technical Operations, Bristol-Myers Squibb, Syracuse, NY 13057, Michael Hausladen, Bristol-Myers Squibb Company, Syracuse, NY 13057, Zhengjian Li, Bioprocess Development, Bristol-Myers Squibb Company, Syracuse, NY 13057, and Steven S. Lee, Biotechnology Development, Bristol-Myers Squibb Company, Syracuse, NY 13221-5050

To aid in the development of a more efficient CHO cell culture process and to monitor production, rapid feed back on the state of cell culture process is crucial. There is a need for on-line methods for the characterization of bioprocesses that will enhance process analysis and control. Monitoring of bioprocesses requires sensors providing real-time measurement of several variables to analyze, model, and control optimally the time course of these processes in bioreactors. Raman spectroscopy is a promising tool for the rapid, non-invasive, and multi-parameter analysis of aqueous biological systems. Additionally it may be considered as an essential element for PAT strategy.

The potential of non-invasive and at-line determination of glucose, lactate, glutamine, ammonia, total cells, viable cells and protein titer in a CHO cell culture was investigated using Raman spectroscopy. Samples obtained from a fed batch bioreactor were analyzed with traditional methods and Raman spectroscopy. The Raman spectra were interpreted by using suitable spectra wavenumber regions through multivariate statistical techniques such as partial least square (PLS) and principal component regression (PCR). The correlation coefficient (R2) values for the PLS-1 calibration models for glucose, lactate, glutamine, ammonia, viable cells and total cells were greater than 0.9, where as, protein titer had a correlation coefficient of 0.84. Results indicated that Raman spectroscopy could be used for rapid detection of glucose, lactate, glutamine, ammonia, total cells, viable cells and protein titer in a CHO cell culture.

BIOT 257 - Strategies for implementation of a new bioprocess container in commercial biologics manufacturing

Rachael Marcklinger[1], Carolyn Williamson[1], Joel Gates[1], Abhinav A Shukla[1], and Steven Lee[2]. (1) Bioprocess Engineering, Bristol-Myers Squibb, PO BOX 4755, Syracuse, NY 13221, rachael.marcklinger@bms.com, (2) Technical Operations, Bristol-Myers Squibb, East Syracuse, NY 13057

There are numerous important aspects that must be considered when implementing a new bioprocess container into a commercial

manufacturing process. These include vendor sourcing, design, review of vendor data, compatibility with process streams, and container closure integrity. Strategies to streamline implementation will be presented. A grouping rationale will be presented to minimize the testing required. Finally, several case studies will be used to highlight key aspects of this strategy.

BIOT 258 - Toward reproducible and optimal operation of mammalian cell cultures: A model based approach for fault detection and isolation

Srinivas Karra and M. Nazmul Karim, Department of Chemical Engineering, Texas Tech University, MS 43121, Lubbock, TX 79409-3121, Fax: 806-742-3552, srini.karra@ttu.edu

The reproducibility and optimal operation of mammalian cell cultures depends on various environmental and physiological factors. High variability in the mammalian cell metabolism towards the growth medium, biological oscillations, metabolism shifts in case of thermal, mechanical or chemical shocks makes these processes worse in control prospective as identification of these phenomena itself offers a great challenge. In this work, those predominant physiological phenomena that determine the intrinsic state of mammalian cells are modeled in an effort to unify them to estimate the onset of metabolism shift from the available on-line and off-line measurements which may be noisy. A hybrid (structured-unstructured) single cell model is developed to quantify cell growth, death, lysis, nutrient uptake, metabolite and protein production, protein deactivation, and their dependency on various environmental and physiological factors. This model is validated against the experimental data obtained in the culture of genetically modified Chinese hamster ovary (CHO) cells producing recombinant tissue type plasminogen activator (r-tPA). Experimental design based on parametric sensitivity is performed to capture accurate information on cell metabolism dynamics. Finally, a novel online model based fault detection and isolation procedure is developed for mammalian cell culture monitoring. Model parametric variations are identified using a probabilistic optimization algorithm online. Onset of special events related to cell metabolism is detected using multi-variate statistical analysis on these model parameters. This information can be used in closed loop strategy to drive the cell metabolism towards specified objectives (e.g., higher yield of therapeutic protein).

BIOT 259 - Using CFD to model gas transfer in bioreactors

William J. Kelly and Joseph Anderson, Chemical Engineering Department, Villanova University, 800 Lancaster Avenue, 318 White Hall, Villanova, PA 19085-1681, Fax: 610-519-7354, william.j.kelly@villanova.edu

The power of pc clusters allows for utilization of Computational Fluid Dynamics (CFD) models that can track the local size and concentration of sparged gas bubbles in a complex 3D geometry using multiphase models. In this study, a CFD model was developed that predicts the steady state concentrations of dissolved O2 and CO2 in a pilot scale perfusion bioreactor by considering the transfer of O2 and CO2 between the phases and the reaction of CO2 with the bicarbonate buffer in the liquid media. The results indicate how the accuracy and computational time are affected by the choice of multiphase modeling approaches and operating conditions.

BIOT 260 - Improvement of the bleachability and brightness of wheat straw chemomechanical pulp with a pretreatment of xylanase from Trichoderma reesei

Feng Hong, Institute of Biological Sciences and Biotechnology, Donghua University, Ren Min North Road No.2999, Shanghai 201620, China, fhong@dhu.edu.cn

Pretreatment of pulp with xylanase before bleaching has received considerable attention in recent years, however, almost all studies focused on chemical pulp such as kraft pulp. In this study, xylanase produced by T. reesei Rut C-30 with coarse corncob meal as the carbon source was used to improve the bleachability and brightness of wheat straw chemomechanical pulp (CMP). The results indicated that the bleachability of the pulp was improved considerably. The treatment also enhanced the effect of the hydrogen peroxide bleaching, decreased the consumption of the bleach agent and increased the brightness effectively. The investigation showed that 50 % hydrogen peroxide was saved after enzymic pretreatment when wheat straw CMP was bleached to the same brightness with only one-stage conventional H2O2 treatment. If wheat straw CMP was bleached with high consistence two-stage hydrogen peroxide and XP3P3 bleaching sequence after enzymatic pretreatment, the brightness could reach 60.31 % ISO. This study of xylanase pretreatment of wheat straw CMP led to satisfactory results, which may facilitate the utilization of wheat straw for pulping.

BIOT 261 - Using MAb enhancing compounds to investigate the MAb production pathway

Tiffany D. Rau, Department of Chemical Engineering, Vanderbilt University, VU Station B, Box 351604, Nashville, TN 37235, tiffanyrau@aol.com, and R. Robert Balcarcel, Cell and Tissue Technologies, BD Bioscience, San Jose, CA 95131-1807

There are more than 10 reported MAb enhancing compounds but there is no unifying feature of the group except that they increase MAb production. This work will focus on a method for identifying MAb enhancing agents and then using them to probe the MAb production pathway in an effort to identify the limiting step(s) in the pathway. The investigation is divided into two main branches, the general MAb productivity pathway and the compound specific pathway, which allows us flexibility in choosing our compounds to investigate. At the same time the method allows the unknown mechanisms behind MAb production enhancement to be investigated. We will use rapamycin and sodium butyrate to investigate changes in the general pathway, specifically transcription and translation. The second branch of our investigation will examine whether the specific targets of rapamycin and sodium butyrate, mTOR and HDAC, respectively, are inhibited when MAb production is increased.

BIOT 262 - Analysis of gene dosage effects for metabolic engineering in Escherichia coli

Neidi Negron Rodriguez, Kathryn Schumacher, and Kristala Jones Prather, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room 66-425, Cambridge, MA 02139, neidi@mit.edu

In many biopharmaceutical processes the use of high copy plasmids for the production of foreign proteins in host organisms is preferred in order to maximize product yields. However, the introduction of foreign genetic material directly interferes with the physiology of the host cell, altering the cell's metabolic activities. This effect, generally known as metabolic burden, has the potential to reduce the productive capability of processes that require certain metabolites as substrates for small molecule production. In these cases, the expression level must be optimized to ensure maximum productivity. This work aims to explore the effects of changes in expression level by varying the gene dosage in a system for the production of polyphosphates (polyP) in E.coli. PolyP molecules are produced in a one-step reaction in which a terminal phosphate from an ATP molecule is transferred to a growing polyP chain by the action of the enzyme polyphosphate kinase (PPK). PolyP is ubiquitous in nature, but its production can be enhanced by the introduction and induction of additional copies of the ppk gene. Three different plasmids are used for this study: a low-copy mini-F plasmid; a medium-copy p15A type plasmid; and a high-copy pMB1 type plasmid. Measurements of copy number, mRNA levels, specific polyP content and PPK activity as a function of copy number are made during a 24-hour period. The time course experiments indicate the relationship between copy number and final product concentration and allow us to establish the optimal gene dosage for the system. The ultimate goal of this research is to derive some fundamental relationships that will allow the construction of optimal recombinant microorganisms for metabolic engineering purposes.

BIOT 263 - Analysis of substrate metabolism for an immobilized CYP2C9 construct: A potential bioreactor application

Lance Wollenberg[1], Jarod L. Kabulski[1], Timothy S. Tracy[2], David Lederman[3], and Peter M. Gannett[1]. (1) Department of Pharmaceutical and Pharmacological Sciences, West Virginia University, 1 Medical Center Drive, Morgantown, WV 26505, Fax: 304-293-2576, lwollenb@mix.wvu.edu, (2) Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN 55455, (3) Department of Physics, West Virginia University, Morgantown, WV 26506

Essential in the metabolism of xenobiotics are the heme-containing enzymes, the cytochrome P450s (CYP). We have demonstrated a method to immobilize CYP2C9 on gold. The metabolic activity of this construct was verified using Δ-9-tetrahydrocannibinol (THC), a high turnover CYP2C9 substrate. Here we report on the metabolic competency of immobilized CYP2C9 using 1) model CYP2C9 substrates, including non-steroidal anti-inflammatory drugs, which often show lower substrate turnover rates in comparison to THC, and 2) the effect of various effector molecules known to accelerate model substrate metabolism in vitro in our immobilized CYP2C9 constructs. The results for dapsone (effector) on flurbiprofen (substrate) metabolism parallel solution data. Additional examples will be discussed. Our results show that the immobilized enzyme activity parallels the in vitro activity. Therefore this system could be used in bioreactors for metabolite production, creating a practical tool for drug development. (Supported by WVEPSCoR STEM program and NIH GM063215 and GM069753.)

BIOT 264 - Isolation and identification of acetic acid bacteria from Shanxi superior mature vinegar in China

Ning Yang and Lin Hao, Department of Bioengineering, College of Food Science and Engineering, Shanxi Agricultural University, Shanxi, Taigu P.R. China, Taigu, Shanxi, AL 030801, China, yanglvning80@163.com

Shanxi Superior Mature Vinegar is ranked the first position among Chinese four famous vinegar as its "special flavor and good quality" and its good color, savory and taste. Shanxi Superior Mature Vinegar collected a large amount of acetic acid bacteria over the course of many years' production process. The experimental sample came from the Shanxi Mature Vinegar Factory's solid-fermenter. By using the changing color circle method to separate Acetobacter spp., then by conducting Gram dyeing and defining experiments which produce acetic acid, the study finally isolated 176 strains of Acetobacter spp.. On the basis of their shape, cultivated traits, physiological and biochemical properties, and the determination of G+C mol% of DNA (GC ratio), the isolated strains were determined to belong to the following five species of Acetobacter, respectively: A. aceti (G+C mol%=64.1%), A. pasteurianus (G+C mol%=61.2%), A. hansenii (G+C mol%=59.2%), A. rancens (G+C mol%=57.5%) and A. liquefaciens (G+C mol%=55.4%).

BIOT 265 - Effect of β-Mercaptoethanol on the fed-batch performance of GS-NS0 cells

Zubia Naji, Serena Fries, Michel Chartrain, and Craig Seamans, Fermentation & Cell Culture, Merck & Co., Inc, Rahway, NJ 07065, *zubia_naji@merck.com*

β-Mercaptoethanol (BME) is a reducing agent and an important component of culture media. It has been shown to contribute to survival and growth for in vitro cultivation of lymphocytes, fetal mouse brain neurons, as well as proliferation and differentiation of human osteoprogenitor cells. Though the complete role of BME in cell culture is not well understood, it is thought to protect cells from reactive oxidative metabolites produced during cultivation. In the development of a cell culture process using GS-NS0 cells to produce a therapeutic protein, it was observed that cultures lacking in BME did not support optimal cell growth but maintained volumetric productivity. Sequential titrations of BME in the feed and medium showed that BME had an overall impact on cellular specific productivity. Investigation was pursued by determining the overall effect of BME on cell culture at both the cellular and molecular level.

BIOT 266 - Engineering of an L-arabinose metabolic pathway in Corynebacterium glutamicum

Miho Sasaki, Hideo Kawaguchi, Shohei Okino, Toru Jyojima, Masayuki Inui, and Hideaki Yukawa, Microbiology Research Group, Research Institute of Innovative Technology for the Earth (RITE), 9-2 Kizugawadai, Kizu-shi, Kyoto 619-0292, Japan, Fax: +81-774-75-2321, *mmg-lab@rite.or.jp*

Lignocellulosic biomass from agricultural wastes represents abundant and cost-effective renewable energy source that are to date underutilized for production of ethanol and most chemicals. Lignocellulose is mainly composed of cellulose, and contains a significant amount of pentose-derived compounds, D-xylose and L-arabinose. To expand the catabolic properties of industrial corynebacteria with the objective to make possible the conversion of pentoses to a variety of compounds, we previously developed genetically modified strains of Corynebacterium glutamicum capable of utilizing D-xylose. In this study, C. glutamicum was metabolically engineered to broaden its substrate utilization range to include another pentose, L-arabinose. The resulting transformant exerted simultaneous utilization of glucose and L-arabinose.

This study was partially supported by a grant from New Energy and Industrial Technology Development Organization (NEDO).

BIOT 267 - Novel large-scale production of genetically engineered elastin-like-biopolymer and endotoxin-binding peptide fusion for neutralization of endotoxin

Wenzhao Jia and **Yu Lei**, Department of Chemical, Materials and Biomolecular Engineering, University of Connecticut, 191 Auditorium Road, U3222, Storrs, CT 06269, Fax: 860-486-2569, wenzhao.jia@engr.uconn.edu, ylei@engr.uconn.edu

Endotoxins (or lipopolysaccharide complexes) are fragments of outer wall of Gram-negative bacteria which are released during normal cell metabolism and the death of Gram-negative cells. Endotoxin infection can lead to a simple fever up to multi-organs failure associated with sepsis. It has been reported that sepsis has caused 10-15% of children mortality rate and up to 40% in adults. Sterilization, such as autoclaving or sterile filtration which is normally practiced in pharmaceutical and medical industries, barely has effect on removing endotoxin. Therefore, simple, cost-effective and fast removal of endotoxin still persists as a problem in food, water resources, medical, pharmaceutical and microelectronics industries. The Sushi3 domain (a peptide consists of 34 amino acids), isolated from the horseshoe crab, exhibits exceptionally high binding affinity with endotoxin. Even though the Sushi 3 has been expressed in E. coli, but the purification steps are very complicated, costly and time-consuming, which is not suitable for mass-production and large-scale removal/neutralization of endotoxin. A peptide can also be synthesized by chemical method, but it is very expensive to synthesize 34-amino acid Sushi3 peptide. So there is an urgent need to develop a new, simple, fast and cost-effective method for mass-production of such peptide in order to achieve large-scale neutralization/removal of endotoxin. In this project, we will use DNA recombinant technology to construct the gene coding for the polypeptide fusion of elastin like polymer-Sushi3 and perform the thermally-triggered purification, and achieve the simple and cost-effective mass-production of Sushi 3. Large-scale neutralization/removal of endotoxin will also be investigated in this project.

BIOT 268 - Establishment of a WaveTM bioreactor scale-down model for process development and characterization studies

Sarwat F. Khattak[1], Sivakesava Sakhamuri[1], Zhengjian Li[1], and Steven Lee[2]. (1) Process Development, Technical Operations, Bristol-Myers Squibb, 6000 Thompson Rd, L-2, Syracuse, NY 13057, sarwat.khattak@bms.com, (2) Biotechnology Development & Operations, Bristol-Myers Squibb Co, Syracuse, NY 13221-4755

Process characterization and development studies require the use of scale-down bioreactor models to mimic large-scale cell culture processes. The Wave^{TM} bioreactor was evaluated as an alternative to the stirred-tank bioreactor as a scale-down fed-batch model. The Wave^{TM} bioreactor system is simple to use, low in cost, and is in compliance with cGMP guidelines. Additional advantages of a Wave^{TM} bioreactor over the traditional stirred-tank bioreactor are minimal preparation and cleaning cycles. A Wave^{TM} bioreactor scale-down model could serve as an alternative method to evaluate cell lines and their productivity.

Rocking speed, rocking angle, CO_2 delivery, air flow rate and temperature were identified as important process parameters. Process parameters such as pH, pCO_2 and air saturation were compared to those of a stirred-tank bioreactor. Cell line performance was evaluated by metabolite analysis, cell growth, and protein characterization. Process parameters and media were optimized to improve productivity and product quality. The results were compared with those of a stirred-tank bioreactor. We present here a case study of the Wave^{TM} bioreactor as a scale-down model which has similar performance in terms of productivity and product quality attributes to those of a stirred-tank bioreactor.

BIOT 269 - Expression of recombinant human erythropoietin having more complex N-glycans in Drosophila S2 cells through hexosaminidase inhibition

Yeon Kyu Kim[1], Kyoung Ro Kim[1], Young Hwan Kim[2], and Hyung Joon Cha[1]. (1) Department of Chemical Engineering, POSTECH (Pohang University of Science and Technology), Pohang 790-784, South Korea, livest@postech.ac.kr, (2) Proteomics Team, Korea Basic Science Institute, Daejon 305-333, South Korea

Human proteins often require post-translational modifications for their biological activities. Insect Drosophila melanogaster S2 cell system is a non-lytic expression system for eukaryotic foreign protein production and has a high productivity. We performed successful secreted production of human erythropoietin (hEPO) in stably transfected S2 cell system. However, this system has different N-glycosylation pattern with mammalian cell system. Thus, we tried the modification of their N-glycosylation pathway.

A chemical inhibitor for hexosaminidase was applied to the culture because we guessed this enzyme is a main factor of simple N-glycosylation in Drosophila S2 cells. According to MALDI-TOF mass spectrometry or Western blot, purified S2-cells derived proteins hEPO, had a smaller molecular weight than mammalian derived native proteins. These data suggested that S2-cells derived proteins were incompletely glycosylated, so their biological activities in human body could be abnormal. We also analyzed the N-glycan itself with 2D- HPLC. From these data, we found that the proteins produced in Drosophila S2 cells have N-glycans of simple core structures. When 2-ADN was added, the culture profile was not changed and hEPO had a slightly bigger molecular weight than an original one. Its N-glycans were also analyzed by MALDI-TOF MS and the more complex structure that had a terminal N-acetylglucosamine was clearly detected.

BIOT 270 - Inhibiting the apoptosis pathway using MDM2 in mammalian cell cultures

Nilou Arden, **Brian S. Majors**, Shin-hyung Ahn, George A. Oyler, and Michael J. Betenbaugh, Department of Chemical and Biomolecular Engineering, Johns Hopkins University, 3400 North Charles Street, Maryland Hall, Room 221, Baltimore, MD 21218, bmajors1@jhu.edu

Genetic modification of mammalian cells using antiapoptotic genes is one method that may be used to improve cellular performance in commercial biotherapeutic production processes. This study investigates a method to inhibit upstream apoptosis pathways through the overexpression of MDM2, an E3 ubiquitin ligase for p53. Both 293 and CHO cells expressing MDM2 were examined under both batch and spent media conditions. MDM2 overexpression increased viable cell densities and viabilities over control cells with the largest enhancements observed in CHO cells. When CHO cells were passaged without medium exchange, cells expressing MDM2 reached a viable cell density that was nearly double the control and survived for an extra day in culture. Analysis of apoptotic DNA fragmentation confirmed that CHO-MDM2 cells exhibited DNA degradation at a much lower rate than controls. These results suggest that the overexpression of heterologous MDM2 represents a promising method to delay apoptosis in mammalian cell cultures.

BIOT 271 - Monitoring of glucose in cell culture by glucose binding protein (GBP) combined with ultraslow microdialysis

Xudong Ge[1], Michael A. Hanson[2], Govind Rao[3], and Leah Tolosa[3]. (1) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, 5200 Westland Blvd., Baltimore, MD 21227, Fax: 410-455-6500, xge1@umbc.edu, (2) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, Baltimore, MD 21250, (3) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland at Baltimore County, Baltimore, MD 21250

Glucose is the major carbon and energy source in cellular metabolism. The lack of glucose in the medium will limit cell growth and product yield in bioprocesses while excessive glucose can also be detrimental leading to lactate formation via the glycolytic pathway. Here we describe a GBP-based assay combined with ultraslow microdialysis for glucose monitoring in cell culture. GBP is one of the soluble binding proteins found in the periplasmic space of gram-negative bacteria. It can be used for glucose monitoring by attaching a polarity sensitive probe to a site on the protein that is allosterically responsive to glucose binding. The microinjector used for sampling is connected through plastic tubing to a dialysis probe immersed in the medium. The glucose concentration in the dialysate depends on the glucose concentration in the medium, the flowrate of the perfusion buffer, and temperature. Depending on the desired dialysis efficiency, the flowrate can be adjusted stepwisely from 0.1 μL/min to 100 μL/min to accommodate the sensitivity of the glucose assay, which is in micromolar range.

BIOT 272 - The impact of residual air in bioreactor sterilization

William J. Kelly[1], Stephen Hannon[1], Monica Paddock[1], and T. Bruce Vickroy[2]. (1) Chemical Engineering Department, Villanova University, 800 Lancaster Avenue, 318 White Hall, Villanova, PA 19085-1681, Fax: 610-519-7354, william.j.kelly@villanova.edu, (2) Biopharmaceutical Development, GlaxoSmithKline, King of Prussia, PA 19406-0939

The presence of air pockets in a bioreactor during a steam sterilization process could result in a less effective sterilization. Experimental results from this study support previously published data indicating that the concentration of air (or humidity) , in addition to temperature and time, can effect the effectiveness of a steam sterilization process. The death rate of bacillus spores was found to decrease as the moisture levels decreased from 100% to 80%. The results of a Computational Fluid Dynamic (CFD) simulation indicates that the % moisture in peripheral ports (addition etc..) at the end of a 30 minute sterilization was not always 100%, and varied depending on the dimensions and location of the port.

BIOT 273 - Optimization of monoclonal antibody production using process simulation and scheduling tools

Victor Papavasileiou, Intelligen Europe, Rijnsburgerweg 119, Leiden 2334 BL, Netherlands, vp@intelligen.com, Demetri Petrides, INTELLIGEN, INC, Scotch Plains, NJ 07076, and Charles Siletti, INTELLIGEN, INC, Mt. Laurel, NJ 08054

The new generation of monoclonal antibodies that are required in large quantities (hundreds of kilo-grams per year) present a number of manufacturing challenges to the biotech industry. Such products typically require facilities with multiple upstream and downstream suites. Furthermore, such biopharmaceuticals are often produced in multi-product manufacturing facilities. In such environments, the multiple production lines interact with each other through sharing of labor, utilities (e.g., steam, purified water), auxiliary equipment (e.g., CIP skids), buffer preparation tanks, etc. Occasionally, they also share rooms and other work areas. Sharing of resources, however, leads to scheduling bottlenecks that limit the throughput of the entire facility. Our experience in using process modeling and scheduling tools to

improve the design of new biopharmaceutical facilities and the operation of existing ones will be presented using an industrial case study.

BIOT 274 - Proteomic investigation of elicited *Eschscholzia californica* cultures producing enhanced levels of benzophenanthridine alkaloids

John T. Oldham[1], Marina Hincapie[2], Tomas Rejtar[2], Jennifer G. Dy[3], and Carolyn WT. Lee-Parsons[1]. (1) Department of Chemical Engineering, Northeastern University, 360 Huntington Avenue, 342 Snell Engineering Center, Boston, MA 02115, (2) Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA 02115, (3) Department of Electrical and Computer Engineering, Northeastern University, Boston, MA 02115

Recent developments in proteomics have enabled the global protein profiling of organisms with unsequenced genomes, including many medicinal plants. *Eschscholzia californica* produces biologically active benzophenanthridine alkaloids (BPAs) and was used as a model system to investigate the complex metabolic mechanisms leading to alkaloid production using proteomics. Cells grown without the hormone 2,4-dichlorophenoxy acetic acid were elicited with purified yeast extract leading to an 18-fold increase in BPAs compared to untreated cultures. Protein extracts of elicited and untreated cultures were separated by HPLC, which revealed differences in protein abundance. HPLC fractions were further separated by gel electrophoresis followed by in-gel digestion of individual bands. Peptide digests were analyzed using MALDI-TOF tandem mass spectrometry and *de novo* sequencing. Proteins were identified by homology searching against the plant database. Identification of differentially expressed proteins will provide insight for increasing secondary metabolism in plant cell culture.

BIOT 275 - Rational design of main-chain elongated substrates acceptable by the E.coli ribosome

Keigo Mizusawa, Shinsuke Sando, and Yasuhiro Aoyama, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyoku, Kyoto 615-8510, Japan, Fax: +81-75-383-2767, keigo8558523@yahoo.co.jp

The ribosome is a highly sophisticated RNA machine for the mRNA-templated amino acid oligo/polymerization to yield their size-, shape-, and sequence-defined peptides/proteins. Following the early demonstration of adaptor hypothesis, there has been much current challenge on applying the ribosomal decoding system for the synthesis of peptidomimetic libraries. However, the ribosome has strict limitations on substrates with elongated backbones. We show an unexpected loophole in the E.coli translation system. Nonsense suppression method revealed that the prokaryotic ribosomal system can accept main-chain elongated substrates if the pKa of the substrate was appropriately chosen. The factors governing the incorporation of main-chain elongated substrates will also be discussed.

BIOT 276 - Using a global model of protein production with substrate competition to explain the complex mRNA vs. protein relationship arising from genetic perturbations

Robert S. Kuczenski and Kelvin H. Lee, School of Chemical and Biomolecular Engineering, Cornell University, 120 Olin Hall, Ithaca, NY 14853-5201, rsk23@cornell.edu

With the exception of the most highly expressed genes, changes in mRNA expression are found to have a complex and nonlinear relationship to the resulting changes in protein expression. Thus, the predictable tuning of protein expression in genetic circuits, such as those found in synthetic biology, would benefit from an improved predictive capability. Here we present a cell-wide mathematical model of protein production which incorporates translation elongation rates, translation substrate competition (*e.g.* ribosomes), and mRNA degradation. We verify the model results by comparing the average translation elongation rate, protein production burst sizes, and mRNA decay rates to experimentally observed values. Finally, this model is tested to both qualitatively and quantitatively describe the experimentally observed relationship between changes in mRNA and protein expression in *Escherichia coli*.

BIOT 277 - Recombinant baculovirus-based multiple protein expression platform for Drosophila S2 cell culture

Kyoung Ro Kim[1], Yeon Kyu Kim[1], and Hyung Joon Cha[2]. (1) Department of Chemical Engineering, POSTECH (Pohang University of Science and Technology), Pohang 790-784, South Korea, icejijon@postech.ac.kr, (2) Department of Chemical Engineering & Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, South Korea

We developed a platform for selective and controllable expression of multiple foreign protein types in insect cell culture. Based on the fact that baculovirus cannot replicate in nonpermissive Drosophila melanogaster Schneider line 2 (S2) cells, S2 cells that stably express human erythropoietin (hEPO) under the control of the S2-derived inducible metallothionein (MT) promoter were infected with three types of recombinant baculoviruses, each of which expressed a fluorescent protein (enhanced green fluorescent protein [EGFP], enhanced cyan fluorescent protein [ECFP], or enhanced yellow fluorescent protein [EYFP]) gene under the control of MT promoter. Addition of copper sulfate as an inducer to infected, stably transfected S2 cells resulted in simultaneous expression of hEPO and three fluorescent proteins. Expression profiles and levels of the three induced fluorescent proteins were similar in all single infected cells. Importantly, expression profiles and levels of hEPO were similar in both non-infected and infected cells, indicating that baculovirus infection did not affect the expression of stably introduced foreign genes. Expression of the three fluorescent proteins was able to be selectively regulated by altering combination ratios of the three types of recombinant baculoviruses. Collectively, these data indicate that the baculovirus/stably transfected S2 cell system can be successfully used to express multiple foreign proteins in a controlled and selective manner without the burden of additional selection

markers. Such a system would be expected to be attractive as a multiple protein expression platform for engineering metabolic or glycosylation pathways.

BIOT 278 - Systematic approach to troubleshooting in support of a late stage antibody manufacturing campaign

Le Ly, Michelle Wyckoff, Ching Lee, Valerie Tsang, and Helena Yusuf-Makagiansar, Cell Culture Development, Biogen IDEC, 5200 Research Place, San Diego, CA 92122, le.ly@biogenidec.com

A case study is presented in which Process Development and Manufacturing groups at Biogen Idec worked closely to identify the root cause and provide a solution for a critical problem in a recent late stage antibody manufacturing campaign. In addition to Cell Culture Development's (CCD) primary role of developing and transferring cell culture processes to Manufacturing, CCD also continues to support Manufacturing throughout the campaigns to ensure success, predominantly by helping to troubleshoot issues that may arise. These activities involve working with Manufacturing to identify the source of a problem, performing laboratory studies to evaluate solutions to the problem, and offering recommendations as a path forward for the campaign. Recently, this type of support was demonstrated in a late state antibody manufacturing campaign in which the cells from the Working Cell Bank (WCB) vials failed to recover from thaw. Since the manufacture of the drug depends upon the successful growth and productivity of these cells, resolving this issue quickly was crucial to avoid campaign delays or cancellation. In order to determine the possible cause of thaw failure, Cell Culture Development launched an investigation to systematically identify the root of the problem, determined the best path forward for the campaign, and made provisions for future campaigns.

BIOT 279 - Reduction in high mannose glycoforms in mammalian cell culture through media optimization

Nicole Le, Cell Science and Technology, Amgen Inc, One Amgen Center Drive, Mailstop 18s-1-a, thousand oaks, CA 91320, nle@amgen.com, and Bob Pendleton, Cell Science & Technology, Amgen, Inc, thousand oaks, CA 91320

The culture environment is a highly critical factor to maximize cell growth and product titer. Over the years, product quality and the ability to modulate it was of high interest to Amgen. We could demonstrate that various culture methods, process setpoints and some nutrient concentrations can have a profound effect on the oligosaccharide structures of glycoproteins. This presentation will discuss how the percentage of high mannose species of an antibody can be significantly reduced, even modulated utilizing medium optimization strategies without compromising the overall product titer.

BIOT 280 - Development of novel sourdoughs with the use of different cereal flours

Humberto Hernandez-Sanchez and Ixchel Nava-Arenas, Dept. Graduados e Investigacion en Alimentos, Escuela Nacional de Ciencias Biologicas - Instituto Politecnico Nacional, Carpio y Plan de Ayala, Col. Sto. Tomas, Mexico City, DF, Mexico, hhernan@encb.ipn.mx

Sourdough is an acid tasting mixture of wheat or rye flour and water used for making a bread with characteristics given by the special interactions between lactic acid bacteria and yeasts. The purpose of this work was to study the effect of oats, barley and corn flours on the sourdough fermentation. The starter culture contained a mixture of *Lactobacillus sp.* C1 and *Saccharomyces cerevisiae* and was mixed with water and the selected flour and incubated at 27ºC for 8 h to form the sourdough which was used to elaborate bread by the sponge and dough method. The final pH of the sourdoughs ranged from 4.46 (wheat) to 4.91 (whole wheat). The breads of all the non-wheat flours were heavy and compact. Densities ranged from 0.65 (wheat) to 0.92 g/cm^3 (oats) and pH ranged from 4.46 (wheat) to 5.2 (whole wheat). The breads prepared from oats and corn were sensorially inferior.

BIOT 281 - Production of 14-oxo-*cis*-11-eicosenoic acid from lesquerolic acid by genetically variable *Sphingobacterium multivorum* strains

Tsung Min Kuo[1], Alejandro Rooney[1], and Terry A. Isbell[2]. (1) Microbial Genomics & Bioprocessing Research Unit, USDA-ARS-NCAUR, 1815 N. University Street, Peoria, IL 61604, Fax: 309-681-6672, kuotm@ncaur.usda.gov, (2) New Crops Processing & Technology Research Unit, USDA-ARS-NCAUR, Peoria, IL 61604

The objective of this study was to explore the extent of microbial conversion of lesquerolic acid (LQA; 14-hydroxy-*cis*-11-eicosenoic acid) by whole cell catalysis and to identify the newly converted product. Among 17 environmental isolates selected from compost amended with soybean oil and unsaturated fatty acids including such species as *Sphingobacterium*, *Acinetobacter*, *Enterobacter*, *Escherichia*, *Pseudomonas*, and *Serratia*, *Sphingobacterium multivorum* identified by 16S rRNA gene sequence analysis was the only microbial species that exhibited the ability of LQA conversion. In small shake flask experiments, strains of *S. multivorum* displayed the activity for converting LQA to a major new product with yields ranging from about 45% for NRRL B-23213 to none for NRRL B-14797. For structural analysis, 6.88 g of the new compound representing a yield of more than 62% in 72 h was produced by strain NRRL B-23213 in Fernbach flasks using a culture medium that also contained EDTA and used glycerol in lieu of glucose as carbon source. Displaying a similar retention time as LQA on GC, the new compound was determined as 14-oxo-*cis*-11-eicosenoic acid by GC-MS and NMR analyses. Therefore, strains of *S. multivorum* have possessed specific enzymatic activity, presumably a secondary alcohol dehydrogenase, for converting LQA to produce 14-oxo-*cis*-11-eicosenoic acid, a first report that demonstrates the functional modification of LQA by whole cell catalysis.

BIOT 282 - Production of D-lactic acid by the RITE bioprocess using genetically engineered *Corynebacterium glutamicum*

Shohei Okino[1], Masako Suda[1], Masayuki Inui[1], Hideaki Yukawa[1], and Yoshiki Tsuchida[2]. (1) Microbiology Research Group,

Research Institute of Innovative Technology for the Earth (RITE), 9-2 Kizugawadai, Kizu-shi, Kyoto 619-0292, Japan, Fax: +81-774-75-2321, (2) Honda R&D Co., Ltd, Saitama 351-0193, Japan

The biodegradable polymer PLLA (poly L-lactic acid) can be produced from renewable resources. Its use, limited by its low heat stability, can be enhanced by blending with D-lactic acid derived PDLA (poly D-lactic acid). D-lactic acid production bioprocess need therefore be established for better utilization of PLLA. The *Corynebacterium glutamicum*-dependent RITE bioprocess has been demonstrated to achieve high volumetric productivities of various organic compounds under growth-arrested conditions. In this study, a genetically engineered *C. glutamicum* producing D-lactic acid was constructed, for RITE bioprocess application for D-lactic production. This study was partially supported by a grant from New Energy and Industrial Technology Development Organization (NEDO).

BIOT 283 - Effects of redox buffers on the folding of disulfide containing proteins

Watson J. Lees, Minakshi Gurbhele, and Farhana Kibria, Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th Street CP 314, Miami, FL 33199, Fax: 305-348-3772, leeswj@fiu.edu

Almost all therapeutic proteins and most extracellular proteins contain disulfide bonds. The production of these proteins in bacteria or in vitro is challenging due to the need to form the correctly matched disulfide bonds during protein folding. One important parameter for efficient in vitro folding is the composition of the redox buffer, a mixture of a small molecule thiol and small molecule disulfide. The effects of different redox buffers on the folding of proteins, however, have received limited attention. The oxidative folding of a several denatured reduced proteins was followed in the presence of redox buffers containing varying concentrations of different aromatic thiols or the traditional aliphatic thiol glutathione (GSH). Under each set of conditions the optimal or best concentration of each thiol was elucidated. Specific and general trends linking the composition and properties of the redox buffer to protein folding were identified.

BIOT 284 - Bridging timescales between atomistic simulation and experiments with master equation models of protein folding and dynamics

John D. Chodera[1], Nina Singhal[2], Imran Haque[2], William C. Swope[3], Jed W. Pitera[4], Ken A. Dill[5], and Vijay S. Pande[6]. (1) Department of Chemistry, Stanford University, Clark Center, MC 5447, Stanford, CA 94305-5447, jchodera@gmail.com, (2) Department of Computer Science, Stanford University, Stanford, CA 94305-5447, (3) IBM Almaden Research Center, IBM, San Jose, CA 95120, (4) IBM Almaden Research Center, San Jose, CA 95120, (5) University of California San Francisco, San Francisco, CA 94158-2517, (6) Departments of Chemistry and Structural Biology, Stanford University, Stanford, CA 94035

While atomistic simulations allow for the study of protein folding in atomic detail, it has been difficult to connect simulations with experimental measurements due to the fact that simulations typically consider the dynamics of a single molecule over short timescales (nanoseconds) while high-resolution experiments such as laser T-jump monitor the time evolution of an ensemble of molecules over long timescales (microseconds). To bridge this gap, as well as extract insight about statistical macromolecular dynamics over timescales much longer than are computationally accessible, we and others have proposed the construction of discrete-state master equation or Markov models from many short trajectories which would describe statistical dynamics over long timescales. Constructing these models requires both a method of identifying kinetically metastable states, a process tantamount to identifying the slow degrees of freedom, and an efficient method for computing transition probabilities or rates between the states. We present our recent progress toward the automatic construction of these models, validation that they accurately reproduce the kinetic behavior of the simulations from which they were constructed, and direct comparison with laser T-jump experiments.

BIOT 285 - A simple and generic immunoplatform for detecting histidine tagged proteins based on biofabrication

Hsuan-Chen Wu, Xiaowen Shi, Angela T. Lewandowski, Chen-Yu Tsao, Rohan Fernandes, Chi-Wei Hung, Philip DeShong, Eiry Kobatake, Gregory F. Payne, and William E. Bentley, 5115 Plant Sciences Bldg, University of Maryland, College Park, College Park, MD 20742, Fax: 301-405-4255, hcwu@umd.edu

We have developed a generic strategy for the covalent assembly of proteins onto patterned surfaces, including sensor surfaces, by incorporating a tyrosine rich “pro-tag” at the C- or N-terminus of a protein of interest (Lewandowski et al., 2006). The tyrosyl residues of the pro-tag are enzymatically activated by tyrosinase to quinones, which then covalently couple to the primary amines of pH-stimuli responsive polysaccharide chitosan. The resulting protein-polysaccharide conjugate retains the pH-dependent solubility of chitosan, which enables its electrodeposition from aqueous solutions directly onto conductive polymers and electrode surfaces. Protein G has a specific binding affinity to the heavy chain constant (Fc) region of immunoglobulin G (IgG). Taking advantage of this feature, we fused a pentatyrosine pro-tag to the C-terminus of protein G, and then conjugated the fusion protein onto electrodeposited aminopolysaccharide chitosan. The assembled complex then retains affinity for IgG, including an anti-hexahistidine IgG. This forms the basis for a generic sensor for any recombinant protein product that contains the histidine-rich purification tag. Western blot and ELISA results confirm the expression and purification of proteinG-tyr and that the fusion protein retains its native activity in IgG binding. Spatially-directed immobilization of antibodies and his tag proteins on electrodeposited chitosan chips will be described.

BIOT 286 - Simulation of an industrial ion exchange step: The use of simulation for robustness analysis

Thomas B. Hansen, Lars Sejergaard, Ernst Hansen, and Steffen Kidal, Protein Separation, CMC API Production, Novo Nordisk A/S, Hagedornsvej 1, DK-2820 Gentofte, Denmark, wilfred@engr.ucr.edu

In the production of peptides and proteins it is of great advantage to have the ability of predicting the behavior of purification steps i.e. retention of the product and various closely related impurities

as a function of variables like salt concentration, gradient and loading conditions. The ability to simulate the chromatographic process facilitates fast development and optimization, and it assists robustness analysis of the purification step.

The present work demonstrates how simulation, using the SMA isotherm and a general mass balance, can be applied during robustness analysis of a process step. An example of robustness analysis of an ion exchange step will be presented and it will be discussed which parameters are of the highest importance regarding purity and yield. The simulated robustness analysis will be compared to a robustness analysis run in the laboratory.

BIOT 287 - Simulation of an industrial ion exchange step: The use of simulation to yield a constant output with a variable input to a process step

Steffen Kidal, Ernst Hansen, Lars Sejergaard, and Thomas B. Hansen, Protein Separation, CMC API Production, Novo Nordisk A/S, Hagedornsvej 1, DK-2820 Gentofte, Denmark, wilfred@engr.ucr.edu

US Food and Drug Administration (FDA) recently published a guideline (PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance) in which the process understanding is emphasized. It is stated that "the ability to predict reflects a high degree of process understanding". With this initiative it is becoming even more important to demonstrate process understanding - and simulation fits this purpose.

The presented work demonstrates how to apply simulation to obtain a constant output from the process when the input is varying. When the input to a purification step is changing it can be helpful to have the ability to adjust the purification scheme in order to get a constant and comparable output from the process. In this case simulation has proven to be very useful as it was possible to predict chromatographic performance with different starting material.

BIOT 288 - Strategies for integrating spectral data from raw materials to control biopharmaceutical manufacture

Maureen Lanan, Analytical Development, Biogen Idec, 14 Cambridge Center, B8-4, Cambridge, MA 02142, Fax: 6176793476

Abstract text not available.

BIOT 289 - Characterization of peak broadening during size-exclusion chromatography of IgG2 antibodies

Alexis Leuras, Eugene Babcock, Tiansheng Li, and Rahul Rajan, Pharmaceutics Department, Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, aleuras@amgen.com

During the commercial formulation development of an IgG2 antibody, a systematic peak broadening was noticed during size-exclusion chromatographic analysis for samples at pH 4–6 and accelerated temperatures (29 – 45oC). At a given temperature, the monomer peak broadening was most pronounced at pH 4, and decreased with increasing pH, and was undetectable at pH 7 and above. The peak broadening also increased with temperature and time for a given pH. A series of investigative efforts were initiated to understand the underlying reason behind the low-pH peak broadening in the monomer peak for this IgG2 in SEC. Different analyses showed that this phenomenon was not due to column performance or antibody fragmentation. Consistent with this observation, there was no significant loss in bioactivity at lower pH values. Dynamic light scattering measurements for samples incubated at 4oC or 45oC showed no significant difference in hydrodynamic radius (Rh) in the pH 4–7 range. Interestingly, ANS binding experiments revealed a temperature dependent exposure of hydrophobic surfaces that correlated with increase in peak broadening. Further, changing the salt in the mobile phase from sodium chloride to sodium perchlorate dramatically resulted in complete disappearance of the main peak broadening, and yet the ANS binding did not change in the different mobile phases. These observations suggest that aberrant behavior in SEC, which may be expected for hydrophobic IgG2 antibodies at low pH formulations, was due to a pH- and temperature-dependent perturbation of the tertiary structure that resulted in an altered interaction with the column's stationary phase, giving rise to peak broadening. This study has important implications for formulation development, as similar peak broadening effects were also later observed for a number of different IgG2's and therefore not limited to the antibody characterized in this study.

BIOT 290 - Cell culture conditioning to improve harvest performance and impurity removal

Matthew Westoby, Andy Koswara, and Ryan Haverstock, Biopharmaceutical Development, Biogen Idec, 5200 Research Place, San Diego, CA 92122, Fax: 858-431-2714, matthew.westoby@biogenidec.com

Increasing cell culture densities and productivities are placing a larger burden on downstream clarification and purification operations due to increased solids mass and other impurities. To accommodate these challenges, innovative process techniques are needed that can facilitate the removal of process impurities and improve clarification performance. A method was developed that examines the effects of cell culture pH conditioning and predicts optimal conditions for cell culture clarification by microfiltration or centrifugation. Implementation of this method has led to improved impurity removal and increased clarification performance.

BIOT 291 - Column simulations of chemically selective displacement systems

Alexander S. Freed, Christopher J. Morrison, and Steven M. Cramer, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, freeda@rpi.edu

The design of chemically selective displacement separations requires an appropriate understanding of the interplay of displacer protein interactions as well as the multicomponent equilibria in these systems. Accordingly, a model was developed to describe the behavior of these complex chemically selective displacement

systems. This model takes into account the interaction between displacer and protein and the resulting complex's binding to the stationary phase. All parameters for the model were estimated using batch multicomponent isotherms. Chromatographic simulations based on this model were shown to accurately predict column experiments under a range of conditions. Examination of the parameters in the model also helped to elucidate the necessary protein-displacer affinity required for selectivity for different classes of separation problems. The presented approach for parameter estimation and column modeling will aid in the development of efficient chemically selective displacement separations for important bioanalytical and bioprocessing applications.

BIOT 292 - Interrelating protein precipitation and hydrophobic interaction chromatography

Beckley K. Nfor1, Koenraad Wiedhaup2, Tangir Ahamed1, Luuk A. M. van der Wielen1, Gijs W. K. van Dedem1, Emile J. A. X. van de Sandt3, Michel H. M. Eppink[4], *and Marcel Ottens*[1]. *(1) Department of Biotechnology, Delft University of Technology, Julianalaan 67, Delft 2628 BC, Netherlands, Fax: 0031 (0)15 27 82355, b.kungahnfor@tudelft.nl, m.ottens@tudelft.nl, (2) Biotechnology, Delft University of Technology, Delft 2628 BC, Netherlands, (3) DSM Anti-infectives B.V, Delft 2600 AK, Netherlands, (4) Biotechnology Operations, N.V. Organon, Oss 5340 BH, Netherlands*

Protein purification generally involves a series of processing steps intended to isolate a target protein from a fermentation broth and finally separate the desired protein from a complex mixture of other proteins. The latter step is the most challenging, typically involving unit operations such as protein precipitation and various types of chromatography. A potential way to speed-up protein purification process design is by interrelating the critical thermodynamic parameters required for designing the different unit operations. In this way, fewer experiments have to be conducted to rationally design a purification cascade. The interrelation of purification methods can lead to significant savings in time and cost especially in Pharma and Biotech industries where high product purity is mandatory and a short time to market is paramount for the commercial success of the biopharmaceutical product. An interrelation model between Hydrophobic Interaction Chromatography (HIC) and protein precipitation was developed based on the cavity theory of Melander and Horváth and the preferential interaction theory. In the HCA-dipole interrelation, the salting-out constant in protein precipitation is calculated from the hydrophobic contact area in HIC and the dipole moment of a protein. The HCA is predicted from HIC retention data using the preferential interaction model. The HCA-dipole interrelation model was investigated for different proteins and the predicted salting out constants were in good agreement with the experimental values. For a given salt type and ligand, this model can be used to interrelate HIC and protein precipitation for the purpose of speeding-up bioseparations process design.

Acknowledgement: This project is financialy supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public private NWO-ACTS program.

BIOT 293 - Purification of recombinant collagen from transgenic corn seed

Cheng Zhang1, Julio Baez2, and Charles E. Glatz1. (1) Department of Chemical Engineering, Iowa State University, 2114 Sweeney Hall, Ames, IA 50010, Fax: 515-294-2689, czhang@iastate.edu, (2) FibroGen, Inc, South San Francisco, CA 94080

Gelatin derived from collagen is widely used as a biomaterial in pharmaceutical capsules and other medical applications. Currently, collagen is produced from animal sources leading to concerns related with the presence of infectious agents and lot-to-lot variability resulting in lack of uniformity in composition and size. We are investigating the possibility of using genetically modified corn to develop a more consistent and animal-contaminant free collagen to be used for gelatin production.
A pure protein sample is needed to determine the composition of the collagen protein expressed in corn. The low expression level (3 mg of protein/kg of seed or 3 ppm) obtained in the first generation corn seeds posed a significant challenge for the development of an effective purification process. As no effective affinity chromatography system was available, a purification process based on ultrafiltration and a three-step chromatography was developed to achieve a recombinant collagen purity of about 80-90% with a purification factor of more than 3000. Ultrafiltration was a useful early step that took advantage of the size difference between the recombinant collagen and contaminating corn seed proteins. This step not only greatly reduced the process volume for later chromatographic steps, but also achieved a purification factor of 5-10. Chromatographic resins and elution conditions (pH, ionic strength, gradient) were identified to successfully obtain samples suitable for the characterization of collagen from a low purity transgenic source.

BIOT 294 - Activation of the IRF-3 transcription factor: The mechanisms of phosphorylation-induced IRF-3 dimerization and DNA binding

Anatoly I. Dragan, Victoria Hargreaves, Elena N. Makeyeva, and Peter L. Privalov, Department of Biology, Johns Hopkins University, Baltimore, MD 21218, Fax: 410-261-1064, **dragan@jhu.edu**

The IRF-3 transcription factor plays a central role in activation of the interferon-β gene upon virus infection. IRF-3 activates by phosphorylation of certain Ser/Thr residues clustered in the 20 a.a.r. loop at the C-terminal domain (CTD). Phosphorylated IRF-3 dimerizes, translocates to the nucleus, binds specific DNA sequences and activates the target gene. To understand why phosphorylation of certain residues of CTD results in dimerization of IRF and induces association of its N-terminal domain (NTD) with the DNA, we investigate the dimerization and the DNA-binding abilities of the IRF-3 and its phosphomimic mutants, 2D (S396D, S398D) and 5D (S396D, S398D, S402D, T404, S405); particularly was studied association of the wild type and mutant IRF-3 with the DNA containing one PRDI binding site of IRF and DNA containing PRDI and PRDII binding sites. It was found that: the mutations in the CTD do not affect the state of the NTD; in the 5D mutant the local increase of negative charge in the CTD induces its partial unfolding, probably deflecting the loop

containing 383-410 residues, which might result in exposure of about 586 A^2 of polar and 1060 A^2 of apolar surface area; partial unfolding of the CTD results in dimerization of IRF-3 with the Gibbs energy of –45 kJ/mol; the dimerization of IRF3-5D is the only reason of its strong binding to the DNA containing two binding sites (ΔG^a=–46 kJ/mol) since its binding to the DNA containing only one binding site is almost the same as of the not activated IRF3 (ΔG^a=–33 kJ/mol). Analysis of the binding characteristics and possible reduction of the translational entropy upon dimerization of IRF leads to conclusion that binding of the dimeric IRF3 with the DNA containing two binding sites, which are located on its opposite faces, requires considerable work for the DNA bending.

BIOT 295 - Chromatographic HTS and simulation for improved process development and optimization

Matthias Bensch[1], Jacob Nielsen[2], Janus Krarup[2], Thomas Budde Hansen[3], Steffen Kidal[4], and Lars Sejergaard[5]. (1) Institute of Biotechnology 2, Research Center Jülich, Jülich 52425, Julich, Germany, (2) Protein Separation, Novo Nordisk A/S, Gentofte, Denmark, (3) Department of Protein Separation, Novo Nordisk A/S, Gentofte DK-2820, Denmark, (4) HAD1.175, Ge, Novo Nordisk A/S, DK-2820 Gentofte, Denmark, (5) Novo Nordisk, Denmark

Abstract text not available.

BIOT 296 - Impact of process parameters on the performance of Normal Flow Parvovirus (NFP) filters in an antibody purification process

Jerrod Einerwold, Jeff Jorgensen, Brin Petersen, and Amitava Kundu, Purification Process Engineering, PDL BioPharma, 9450 Winnetka Ave N, Brooklyn Park, MN 55445, Fax: 763-383-7058, *jerrod.einerwold@pdl.com*

Current regulatory expectations necessitate the use of small pore virus filters such as Normal Flow Parvovirus (NFP) filters in an antibody downstream process to remove both small and large viruses. Due to the small size of the pores, these filters are prone to fouling by the antibody and other process contaminants. Mobile phase conditions such as pH and conductivity could also impact the process performance of these filters. Process performance parameters include volumetric (and mass) capacity and volumetric flux. The loss of volumetric flux as a function of increasing capacity is a critical process performance parameter as this is related to the clearance of small model viruses (such as Minute Mouse Virus), across this filtration step. Minimizing flux decay is thus critical to maximizing the log reduction value across these NFP filters. Thus, to ensure maximal clearance, it is important to determine the factors that have an impact on the volumetric flux. A case study will be presented to show how the performance of this filtration step is sensitive to mobile phase conditions such as pH and conductivity. Using this understanding, a NFP filtration step was moved further upstream in the purification process to enhance volumetric capacity and minimize flux decay contrary to expectations. Furthermore, strategies of how to mitigate the impact of the processing conditions on volumetric flux will also be presented. Case studies will be presented to show how viral clearance is impacted by the decay in the volumetric flux, further underlining the importance of obtaining a good understanding of the factors that impact volumetric flux during Viral filtration with small pore NFP filters.

BIOT 297 - Stability of the bZIP homo- and heterodimers and energetics of their interactions with DNA

Ralf Carrillo, **Anatoly I. Dragan**, and Peter L. Privalov, Department of Biology, Johns Hopkins University, Baltimore, MD 21218, Fax: 410-261-1064, dragan@jhu.edu

Distinguishing specificity of the bZIP transcription factors is that they are dimers assembled form two polypeptide chains held together by the leucine-zipper coiled-coil dimerization domain. Their basic unfolded segments recognize the tandem binding sites on DNA and, being neutralized by its phosphates, enter into its major groove in helical conformation. The advantage of such scissor-grip motif of DNA recognition is that several different polypeptides can form large number of hetero-dimers recognizing different combinations of the tandem binding sites. Our studies of the homo- and hetero-dimmers of GCN4, ATF2 and cJun proteins and their interaction with DNA duplexes containing various tandem binding sites (AP-1, PRD-IV, CREB) by optical (CD, fluorescence spectroscopy, FRET) and calorimetric (DSC, ITC) methods has shown that: (a) In all cases the leucine zipper domain unfolds upon heating in two cooperative stages the thermodynamic characteristics of which are different for different combinations of the polypeptide chains. (b) The basic segments of all studied proteins are partly unfolded even at 0oC and unfold completely upon heating to 40oC. (c) Association of the homo and hetero-dimers with the DNA duplex containing their cognate tandem binding sites proceeds with increase of helicity, i.e. with folding of the basic segments, and upon heating such complex unfolds cooperatively in a single stage. (d) Association of the bZIPs with the non-cognate DNA results in decrease of helicity and melting of such complex proceeds non-cooperatively; it appears as in that cases the basic segments do not enter into the major groove of DNA but stick to its surface. (e) Analysis of the bZIP/DNA interaction in the presence of various concentration of salt shows that almost half of the Gibbs energy of binding is entropic, i.e. results from release of the counter ions of phosphates upon their neutralization by the basic groups of protein.

BIOT 298 - Strategies for optimizing upstream clarification of cell culture fluids

Bala Raghunath, Millipore Corporation, Billerica, MA 01821, Bala_raghunath@millipore.com, Benjamin Cacace, Millipore Corporation, Bedford, MA 01730, and David Yavorsky, R&D, Millipore Corporation, Bedford, MA 01730

Successful clarification of mammalian cell culture fluid containing a therapeutic protein is a key first step in preparing the protein for downstream chromatography purification. The challenges encountered in cell culture clarification are underscored by the inherent variability and complexity of the fluid particularly with respect to cell densities, viability, colloid concentration, and

presence of soluble entities such as media components, antifoam agents, cell stabilizers etc.

Clarification involves multiple steps and typically involves a systematic combination of unit operations such as centrifugation, tangential flow filtration, depth filtration and sterile filtration. While some components of the cell culture fluid are completely removed by a unit operation, others are only partially removed depending on the process operating parameters. Therefore, understanding process inter-dependencies is a key factor in establishing an optimized, robust clarification scheme.
The current presentation examines optimized strategies for clarification of cell culture fluids based on process economics for a variety of commonly encountered situations taking into consideration factors such as process batch volumes, utilization, ease of use, equipment constraints etc.

BIOT 299 - Accelerated physico-chemical characterization of complex protein mixtures for optimized ion-exchange chromatographic separation

Alex Berrill[1], Sa Ho[1], Nigel Titchener-Hooker[2], and Daniel G. Bracewell[2]. (1) R&D Global Biologics, Pfizer, 700 Chesterfield Parkway, Chesterfield, MO 63017, Alex.Berrill@pfizer.com, (2) Department of Biochemical Engineering, University College London, London WC1E 6JW, United Kingdom

Development of chromatographic separation for complex protein mixtures such as crude extract or lysate is typically very labor intensive and time consuming. Selection of chromatography sorbents and buffer conditions to use with them are traditionally made based on knowledge from similar processes and a large number of small-scale chromatography runs. This could require a significant quantity of feed material and extensive analytical support, which at early development stage are likely in limited supply or not available. This study outlines the use of Surface Enhanced Laser Desorption Ionisation-Time of Flight-Mass Spectrometry (SELDI-TOF-MS) technology to obtain physico-chemical properties of proteins in complex mixtures, which can then be utilized to determine the optimized chromatographic conditions for purification of a target protein. The key benefits of this approach over the traditional methods are the speed and ease of ProteinChip preparation and data acquisition to generate a large amount of quality data within a few days with only microliters of the complex protein solution. Data from the SELDI are processed to provide detailed knowledge of the bind/release properties of each protein in the solution under various buffer conditions. This knowledge will eventually enable to the selection of sorbents and buffer conditions that exploit the greatest difference in adsorption/desorption characteristics between the protein of interest and impurities in order to find the most effective chromatographic separation step.

BIOT 300 - High throughput synthesis and screening for membrane filtration

Mingyan Zhou, Department of Civil and Environmental Engineering, Rensselaer Polytechnic Insitute, 110 8th St, Troy, NY 12180-3590, zhoumy@rpi.edu, James E. Kilduff, Department of Civil and Environmental Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, Daniel G. Anderson, Center for Cancer Research, Massachusetts Institute of Technology, Boston, MA 02139, Robert S. Langer, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, and Georges Belfort, Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180-3590, Fax: 518-276-4030

The current most widely used filtration membrane, poly(ether sulfone) (PES), exhibits high non-specific protein fouling and needs to be replaced. Also, recently commercialized regenerated cellulose (RC) composite membranes are sub-optimal with low permeation fluxes, negative influence on protein secondary structure and susceptibility to large pH changes. However, developing new polymeric materials with appropriate surface or functional characteristics for different membrane filtration applications involves great effort and expense and often takes many years. More importantly, surface science has not yet developed to the point that allows prediction of the surface or functional characteristics needed to reduce such protein fouling. As a result, over the past 30 years, few new polymers have been used for membrane production. What is urgently needed is a fast, efficient and reproducible process to allow quick selection of the best polymer, and subsequent analysis of its mechanism of action to gain understanding for future design of surfaces for membrane and other separations. We offer such a method, by adapting, for the first time, high throughput platform (HTP) approaches successfully used in chemistry and biology to the facile modification of commercial PES membranes, using a HTP together with our patented photo-induced graft polymerization (PGP) method. We call this method HTP-PGP. The novel method proffers an inexpensive, fast, simple, reproducible and scalable modification procedure for modifying poly(aryl sulfone) membranes, which have excellent physical and transport characteristics but poor surface chemistry. Using the HTP-PGP process we first identify the best-grafted monomers from a large library of candidates, optimize the grafting and filtration conditions, and then focus on mechanistic studies.

BIOT 301 - Investigation of chemically selective displacers using robotic high throughput screening, SPR, NMR and MD simulations

Christopher J. Morrison[1], Scott A. McCallum[2], Rahul Godawat[1], JA. Moore[3], Shekhar Garde[1], and Steven M. Cramer[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Ricketts Building, 110 8th St, Troy, NY 12180, morric2@rpi.edu, (2) Director, NMR Core Facility, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, (3) New York State Center for Polymer Synthesis, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180

High throughput screening was employed in concert with several analytical techniques to identify and evaluate the behavior of chemically selective displacers for protein purification in ion exchange systems. A robotic liquid handling system was adapted to efficiently carry out this parallel batch screen of selective displacers on multiple protein pairs. The results identified potential selective displacers and important functional group chemistries.

The screen also indicated that this selectivity was due primarily to the specific binding between the displacer and targeted proteins. Nuclear Magnetic Resonance was then conducted on several protein/displacer mixtures verifying the binding of the selective displacers to targeted proteins and the location of the binding event. Surface plasmon resonance experiments and molecular dynamic simulations were also carried out to corroborate the NMR results. This proof of concept study shows that more specific selectivities may also be possible by utilizing affinity based selective displacers for explicit protein systems.

BIOT 302 - Platform technology for developing purification processes

Michel H. M. Eppink1, Rick Schreurs II2, Anke Gijsen2, Bram Kamps[2], and Kees Verhoeven[2]. (1) Biotechnology Operations, N.V. Organon, Molenweg 50, Oss 5340 BH, Netherlands, Fax: +31 412662520, michel.eppink@organon.com, (2) Biotechnology Operations, NV Organon, Oss 5340 BH, Netherlands

Biotechnology Operations, a division of N.V. Organon, is a globally operating manufacturer of active pharmaceutical ingredients, specialised in biopharmaceutical preparations of therapeutic proteins. “Time to market” is for the biotechnology industry an important milestone to improve overall efficiency and reduce costprice. A crucial part of the biotechnology processes concerns the development of the downstream process (DSP) for therapeutic proteins (biologicals). In this way new and fast methods such as high throughput screening techniques are needed to speed up DSP processes. The platform technology as will be discussed in this presentation includes a structural approach of different sophisticated techniques. At first, ligand screening/selection on chip is carried out with the SELDI-TOF (Surface Enhanced Laser Desorption Ionization-Time of Flight) system. Secondly, resin screening/selection of different suppliers occurs with the pipetting robot followed by scouting studies with purification systems to select the most ideal resin. Finally, optimization studies occur with statistical design approaches by means of design of experiments. A few examples will be presented to explain the platform approach for purification development.

BIOT 303 - Rapid optimization of Protein A chromatography using a high-throughput format

Anna Gronberg, Frida Lindstrom, Tomas Bjorkman, and Gustav Rodrigo, Life Sciences, R&D, GE Healthcare, Bjorkgatan 30, Uppsala 751 84, Sweden, anna.gronberg@ge.com

Protein A affinity chromatography is the foundation in most monoclonal antibody purification platform processes. Capture with Protein A is highly generic, selective and results in a large purification and volume reduction factor in one single step. The use of Protein A-based purification platforms can minimize development times for biopharmaceutical manufacturing by several months. Further optimization of such platforms can generate significant economical benefits such as increased product recovery and purity.
Here, the effect of different loading, wash and elution conditions on Protein A resin will be explored with respect to capacity, product purity and recovery. Parallel screening and optimization of conditions in a 96-well format enables fast evaluation of the chromatography step. In this context, high throughput analytical methods for impurities and target protein will also be discussed. Confirming the 96-well format results with traditional column chromatography demonstrates excellent correlation between the different methodologies.

BIOT 304 - Streamline hydrophobic interaction chromatography resin screening for protein purification process development

Junfen Ma, Paul Jorjorian, Colleen Mason, and Ronald Bates, Bioprocess Development, Bristol-Myers Squibb Company, 6000 Thompson Road, East Syracuse, NY 13057, junfen.ma@bms.com

Chromatography resin manufacturers use retention times for model proteins, such as myoglobin or lysozyme, under linear conditions as their product release specifications for hydrophobic interaction chromatography (HIC) resins. The retention time of a model protein can be indicative of binding capacity and resolution capability. In preparative chromatography process development, HIC resin selection is based on a trade-off between binding capacity, step yield and resolution of product and impurities. This presentation will identify an approach to bridge the manufacturers' release specifications and preparative chromatography process development. A correlation between the binding capacity / resolution strength and the retention times of model proteins will be presented. Recommendations will be made regarding whether the retention times are useful or reliable parameters or tools for preparative chromatography resin screening and process development.

BIOT 305 - Bench scale cleaning characterization of drug products in a fill-finish facility

Nitin Rathore1, Nishant Bhasin2, Parag Kolhe2, Rich Law2, Ahmad Abdul-Fattah2, Linda Li2, Wenchang Ji2, and Keith Murphy2. (1) Drug Product and Device Development, Amgen, Inc, One Amgen Center Dr, Thousand Oaks, NC 91320, nrathore@amgen.com, (2) Global Drug Product & Device Development, Amgen, Thousand Oaks, CA 9*1320*

Characterization and validation of equipment cleanliness is a key requirement for a bio-pharmaceutical facility. Manufacturing scale cleaning cycle has to be developed and validated for its ability to clean all the equipment parts for a given soil. Cleaning validation in a multi-product fill-finish facility could benefit from using a worst-case based approach which involves cleaning and validating the most difficult to clean product. Such an approach will minimize the number of required validation runs. Scaled down cleaning evaluation can prove helpful in determining the worst case product. Our current study will present the bench scale characterization of the cleaning process for several drug products. The presentation will cover the effect of several process parameters including cleaning conditions on the cleanabiltiy and establish a bench scale process to be used as a platform for comparing the relative cleanabiltiy of different drug products.

BIOT 306 - Regulatory strategy for changing a chromatography resin in a licensed process by the use of a comparability protocol

Michael J. Rubino, Regulatory Affairs, Eli Lilly and Company, 1 Lilly Corporate Center, Indianapolis, IN 46285, and Philip R. DeVoe, Manufacturing Science & Technology, Eli Lilly and Company, Indianapolis, IN 46285

The development and implementation of a process change of a chromatography resin in an approved process using a comparability protocol is described. Through the use of multivariate, statistically designed experiments to define the design space, an alternate chromatography resin was first tested then incorporated into the bulk manufacturing process. The new process was then tested at manufacturing scale to confirm the small-scale design space and then validated in the commercial production facility. The new process was shown to be robust over the ranges tested during validation. Although the process change was considered to be a Prior Approval Submission, the use of a comparability protocol allowed for early discussions with the FDA and a reduction of the reporting category for the subsequent submission to a 30 day approval process, reducing the approval time by up to five months.

BIOT 307 - Chromatographic HTS and simulation for improved process development and optimization

Matthias Bensch1, Jacob Nielsen2, Janus Krarup2, Thomas Budde Hansen3, Steffen Kidal[4], Arne Stabe[2], Jürgen Hubbuch[1], Ernst Hansen[5], and Lars Sejergaard[6]. (1) Institute of Biotechnology 2, Research Center Jülich, Jülich 52425, Julich, Germany, (2) Protein Separation, Novo Nordisk A/S, Gentofte, Denmark, (3) Department of Protein Separation, Novo Nordisk A/S, Gentofte DK-2820, Denmark, (4) HAD1.175, Ge, Novo Nordisk A/S, DK-2820 Gentofte, Denmark, (5) Protein Separation, CMC API Production, Novo Nordisk A/S, DK-2820 Gentofte, Denmark, (6) Novo Nordisk, Denmark

Increased demand of material for clinical trials and handling of an increasing number of projects in the biopharmaceutical industry are calling for different ways of performing process development. This paper will present true examples of implementation of high-throughput screening techniques as stand alone tool and in combination with mathematical modelling for separation development, trouble shooting, and batch release for chromatographic purification steps. Further, these techniques will be discussed in relation to scale-up, general accuracy of results, and PAT.

BIOT 308 - Engineering the adsorption of plasmid DNA

William J. Kelly and Paul Butts, Chemical Engineering Department, Villanova University, 800 Lancaster Avenue, 318 White Hall, Villanova, PA 19085-1681, Fax: 610-519-7354, william.j.kelly@villanova.edu

The production of plasmid DNA (pDNA) for use in DNA vaccines and gene therapy is limited by the high costs of the process, especially the isolation and purification steps. The separation of the pDNA from the genomic DNA (gDNA) is a significant challenge, because of the chemical similarity of the molecules. In this study, the feasibility of engineering pDNA to contain a triplex forming region that allows for selective binding to some relatively inexpensive hydrophobic adsorbents is evaluated. Preliminary results have identified conditions (pH and ionic strength) that encourage binding of the pDNA while discouraging binding of gDNA.

BIOT 309 - AKTA Crossflow for characterization of an ultrafiltration and diafiltration step

Carl W. Richey Jr., Process and Clinical Operations, Amgen Colorado, 4765 E. Walnut, Boulder, CO 80301, carlr@amgen.com

Following inclusion body solubilization and refolding, a recombinant protein is purified through 3 chromatographic steps and then processed though an ultrafiltration and diafiltration step (UFDF 2) to produce the drug product. An ÄKTA Crossflow (GE Healthcare) automated UFDF system was first qualified as a scale-down model of the UFDF 2 process step, then used for characterization of the step. Failure Mode and Effects Analysis selected the operational parameters of load rate, temperature, feed flow rate, transmembrane pressure, concentration volume target and diafiltration factor for experimentation. Testing at 2-3X the normal operating ranges showed no significant effects on product purity, step yield or exchange of buffer components. Low transmembrane pressure significantly increased process time. Overall, the UFDF 2 process step was found to be very robust. The automated processing and data collection enable simultaneous performance of additional tasks with excellent comparative analysis.

BIOT 310 - Development of lab scale systems to evaluate micromixing in biological separations

Jennifer Girard, BioProcess R&D, Merck and Co, Sumney town Pike, WestPoint, PA 19486, Fax: 215-993-3348, jennifer_girard@merck.com, and Matthew P Watson, BioProcess R&D, Merck &Co., Inc, West Point, PA 19486

Poor mixing coupled with the harsh physical environments and reagent conditions often found in biopurification processes can result in irreversible damage to sensitive biological products. It is essential to reduce scale dependant variability in mixing to avoid unforeseen reductions in product yields and purity, during pilot and manufacturing processing. Lab scale batch and continuous mixing systems were developed along with a scale independent model of micromixing using a competitive reaction system, which utilizes acid/base instantaneous reactions and iodide/iodate fast reactions. The model was used to compare the differences in micromixing in current lab, pilot, and manufacturing scale equipment. Applications of this model as well as three common industrial mixing parameters (power, tip speed and turn over), will be discussed with respect to biological separations.

BIOT 311 - ELISA based selection of elution buffer for IgG-affinity chromatography

Rajaramesh Varakala[1], Robert R. Beitle[1], Kevin Clark[2], and Tom W Smith[2]. (1) Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, AR 72701, Fax: 479-575-7926, rvaraka@uark.edu, (2) Immunovision, Springdale, AR 72764

Patients with tissue diseases produce autoantibodies to diverse cellular components. The presence of autoantibodies may be diagnosed using corresponding antigens. Anti-(Ro) SS-A autoantibodies were described originally as precipitating autoantibodies in sera of Sjogren's Syndrome and Systemic Lupus Erythematosus patients. The (Ro) SS-A antigen is comprised or an acidic 60 kD protein that may also be associated with a RNA ranging in size from 80 to 112 bases **(1)**. Elevated levels of Anti-(Ro) SS-A have been detected in as high as 96% of Sjogren's Syndrome patients **(2)**, 49% of patients with Systemic Lupus Erythematosus **(2)**, 83% of mothers of infants with isolated complete congenital heartblock or Neonatal Lupus Dermatitis, and over 75% of patients with Subacute Cutaneous Lupus. In addition, it has been shown that 70% of patients with (Ro) SS-A precipitating antibodies also have rheumatoid factor

Extraction of Ro-SSA from bovine spleen has several steps including Immunoaffinity chromatography. Optimization of variables, such as the elution condition, is often necessary within this method. Affinity chromatography has a purification factor of 2,000- to 20,000 due to its specificity, it has limitation of dissociation of bond between biomolecules and their ligand at the cost of biological acitivity and yield. Various buffer systems utilizing different conditions such as extreme pH, ionic strength, chaotropic agents and commercially available elution buffers were investigated for high yield. Capture ELISA (Enzyme-linked Immunosorbent Assay) was preformed to screen the optimum buffer for biological activity of Ro-SSA.

References: **1**.Mamula MJ, et al. "The (Ro) SS-A Autoantigen as an Immunogen Some Anti-(Ro) SSA Antibody Binds IgG." J of Exp Med Vol 86: pg 1889-1901. **2**.Rader MD, et al. "Heterogeneity of the (Ro) SSA Antigen, Different Molecular Forms in Lymphocytes and Red Blood Cells." J. Clin. Invest Vol 83: 1989, pg 1293-1298.

BIOT 312 - Enantioselective hydrolysis of epoxides in organic solvents by Beauveria bassiana

Emmanuel G. Ankudey and Tonya L. Peeples, Department of Chemical and Biochemical Engineering, The University of Iowa, Iowa City, IA 52242, eankudey@engineering.uiowa.edu

The enzymatic enantioselective hydrolysis of epoxides is usually carried out in aqueous reaction media with whole microbial cells. The drawbacks of this method include the low solubility of these epoxides in the aqueous buffer systems and the spontaneous hydrolysis of the epoxide leading to the undesired racemic epoxides and diols. These problems can be overcome by using organic solvents as the reaction medium. However, many common organic solvents are toxic to the enzyme and the enzyme activity is reduced.

In this study, a crude enzyme preparation from B. bassiana was immobilized in sol gels and is being applied in the kinetic resolution of styrene oxide and indene oxide. Several organic solvents are being examined at different conditions for optimal yield and selectivity. The initial results indicate a water content of 2% to optimize the yield and enantiomeric excess of the residual epoxide.

BIOT 313 - Risk mitigation and optimization strategies for viral filtration

Lisa Flansburg[1], Renyo Forng[1], Steven Lee[2], and Abhinav A Shukla[1]. (1) Bioprocess Engineering, Bristol-Myers Squibb, PO Box 4755, Syracuse, NY 13221, lisa.flansburg@bms.com, (2) Technical Operations, Bristol-Myers Squibb, East Syracuse, NY 13057

Therapeutic recombinant proteins expressed by mammalian cell lines are susceptible to contamination by retrovirus-like particles. Clearance of viral particles from product streams must be demonstrated in accordance with regulatory requirements for viral safety. Adequate viral safety in manufacturing processes for therapeutic recombinant proteins and plasma-derived biologics can be achieved by the viral filtration step because of its robustness and ease of validation. Viral filters are single use devices employing membranes with tight and narrowly controlled pore structures. Consequently, viral filtration is a costly but necessary unit operation that is commonly employed in many downstream bioprocesses.

Risk mitigation strategies for a viral filter in a commercial bioprocess are identified using an FMEA analysis. A key risk identified was the single sourcing of viral filters. The identification of an alternative viral removal filter for the current bioprocess would mitigate the risk of potential interruptions due to the availability of the single sourced filter used. A strategy using a combination of functional testing studies, economic analysis and viral clearance validation is described for the insertion of a second source of viral filters into a licensed process.

BIOT 314 - Robust microscale method for yeast cell disruption in the purification of intracellular proteins

Marc D. Wenger, Peter DePhillips, and Daniel G. Bracewell, Bioanalytical Development, Merck & Co, West Point, PA 19486, marc_wenger@merck.com

Microscale bioprocessing techniques hold the promise of accelerating process development. For intracellular proteins expressed in yeast, small-scale cell breakage methods are needed that match the protein release and contaminant profile of full-scale methods like homogenization, enabling representative studies of subsequent downstream operations to be performed. In this study, a non-contact method known as adaptive focused acoustics (AFA) was used for the disruption of milligram quantities of yeast in the purification of a recombinant protein. AFA operates at higher frequencies than that of conventional ultrasonication, delivering highly focused acoustic radiation without appreciable sample heating. The product and soluble protein release of the method was equivalent to laboratory-scale homogenization, although the addition of a lytic enzyme decreases processing time. In addition, no adverse effects were observed on the downstream chromatography. This disruption technique in combination with

microscale chromatography supports a strategy for a completely microscale purification of intracellular proteins expressed in yeast.

BIOT 315 - Anomalous peak splitting of a human IgG2 mAb on HPLCSEC caused by charge variants

Joseph Martin, Michael Dupuis, Joan Kwong, and Gregory Waszak, Pfizer Global Research and Development, St. Louis, MO 63017

A human IgG2 shows a single peak on the Phenomenex Biosep SEC 3000 but multiple peaks when run on the silica-based SEC columns of Toso-Haas or BioRad. Interactions with the silica-based matrices would explain the elution profiles. Thus: The Kav on the BioSil column showed increased retention in more lyotropic mobile phases. Although the standard proteins on the BioSil column conform to the expected relationships of Kav to the Stokes radius, the mAb does not and the mAb peaks' radii varied by more than 2x. However, the Stokes radii of the peaks were identical when evaluated by analytical ultracentrifugation (AUC). AUC and mass spectrometry further established that the mAb was intact and contained no half molecule or free light chain, ruling out dissociation as an explanation for the anomalous retention on HPLC. Separation of the peaks by SP Sepharose verified that the peaks were actually charge variants.

BIOT 316 - Buffer distribution capacity analysis at the pilot scale

Amy Caparoni, Jason B. Fletcher, and Jack Sinclair, Bioprocess Clinical Manufacturing & Technology, Merck & Co., Inc, West Point, PA 19486, amy_caparoni@merck.com

Clinical supplies for several therapeutic areas in Merck & Co., Inc.'s growing pipeline are currently being supported in the Biologics Pilot Plant (BPP) in West Point, PA. The BPP is a state-of-the-art, multi-product cGMP facility, designed to simultaneously process vaccines and therapeutic proteins up to 2000L in scale. While processes are being optimized to increase yields and platforms are being developed to accelerate clinical material timelines, more demands are being placed on the facility. As a consequence, the buffer requirements for downstream processing are projected to surpass the current storage and distribution capacity of the facility. This poster outlines the assumptions used for a clinical capacity analysis and summarizes the strategy developed to meet the forecast needs. The poster will also delineate how simple material handling solutions can be implemented to defer $3MM in capital spending and result in anticipated labor savings of $500,000 per year as well as a 10X reduction in required storage area.

BIOT 317 - Characterization of a new IgG binder adsorbent

Patrik Adielsson, Stefan Eriksson, Faramarz Alamravesh, Hanna Wlad, and Johan Färenmark, R&D, Custom Design Media, GE Healthcare Biosciences AB, Björkgatan 30, 75184 Uppsala 751 84, Sweden, Patrik.Adielsson@ge.com

A new affinity chromatography resin for the purification of all subclasses of human IgG is under development. The prototype resin is constructed by immobilizing the caustic stable human IgG capture ligand developed by BAC B.V., through multi point attachment, to a rigid agarose base matrix. Physical and functional characteristics have been determined and will be presented. The dynamic binding capacity QB10% for this product is over 17 mg IgG/ml gel. The CIP cycle study shows that the resin has a good stability to alkaline conditions which extends functional life-time and reduces overall production costs. The selectivity of the media was checked by loading human serum to the column. Distribution of all IgG subclasses was determined by ELISA and the purity of the eluted sample was checked by SDS PAGE. The results indicate the identical subclass distribution in the eluted material as in the loaded serum. This is a product with high potential within plasma industry and other companies with interest in purification of human IgG from different sources.

BIOT 318 - Characterization of cation exchange adsorbents for monoclonal antibody purification

Daniel Roth, Chris Daniels, and Brian To, Biopurification Development, Merck & Co., Inc, West Point, PA 19486, **brian_to@merck.com**

Cation Exchange Chromatograhy (CEX) is a powerful tool to purify protein such as monoclonal antibodies. The separation efficiency of a CEX adsorbent depends on its affinity to the target proteins and pore structure. In addition, the protein adsorption capacity is another important factor to consider when choosing the appropriate adsorbent for a purification process. In this study, the pore structures of 8 strong CEX resins were measured by inverse size exclusion chromatography. In addition, their binding capacities and adsorption affinities to a monoclonal antibody were determined from their adsorption isotherms. The results show that only a small fraction of the pore volume in each adsorbent is accessible to monoclonal antibody. In addition, the pore structures, affinities to antibody and binding capacities of these adsorbents are different.

BIOT 319 - Coimmobilization of pyranose 2-oxidase, laccase, and catalase on solid supports for enhanced production of 2-keto-aldoses

Roland Ludwig[1], Prakit Sukyai[2], Tonci Resic[2], and Dietmar Haltrich[2]. (1) Department of Food Sciences and Technology, Research Centre Applied Biocatalysis, Muthgasse 18/2/71, 1190 Wien, Austria, Fax: +43-1-36006-6251, roland.ludwig@a-b.at, (2) Department of Food Sciences and Technology, University of Natural Resources and Applied Life Sciences, Vienna, 1190 Vienna, Austria

We describe the coimmobilization of pyranose 2-oxidase (P2O) which is the key catalyst for the production of 2-keto-glucose, a glucose intermediate of growing industrial interest and two supporting enzymes, laccase and catalase. During C2-oxidation of glucose, P2O concomitantly forms hydrogen peroxide, which has a deleterious effect on the biocatalyst, when using molecular oxygen as electron acceptor. Two strategies for reducing hydrogen peroxide concentrations were tested: the common one removes hydrogen peroxide by an excess of catalase activity, the novel

approach uses a laccase/mediator regeneration system to circumvent the formation of highly reactive oxygen species by using an electron acceptor (benzoquinone) other than oxygen. In addition to the protection against hazardous by-products the immobilized enzymes were not only more resistant to thermal denaturation than the free enzymes, but also more stable under acidic conditions resulting in higher total turnover numbers.

BIOT 320 - Development of a high throughput purification method for analysis of cell culture samples

Sarah Koob1, Nadine Barron1, Judy H. Chou2, and Martin Sinacore1. (1) Cell and Molecular Sciences, Wyeth Biotech, One Burtt Road, Andover, MA 01810, Fax: 978 247-2600, skoob@wyeth.com, (2) Genentech, Oceanside, CA 92056

The proliferation of monoclonal antibodies as potential therapeutic agents provides opportunities to "platformize" process development approaches, adding speed and reducing the cost of early-stage process development. We have exploited the properties of antibodies (and other Fc-containing proteins) to develop a high throughput purification scheme to enable qualitative protein analysis on large numbers of samples, for example as part of a cell culture screening process. The method provides rapid, high throughput Protein A cleanup of conditioned medium samples for antibodies and other Fc-containing proteins suitable for downstream analytical techniques such as size exclusion HPLC, cation exchange HPLC, capillary electrophoresis, hydrophobic interaction chromatography, and carbohydrate (e.g., sialic acid) analyses. Using a Beckman Biomek Fx robotic liquid handling station equipped with a solid phase extraction apparatus (SPE), 96 samples can be automatically purified in approximately 45 minutes. Alternatively, the method can be performed manually, using a centrifuge. The method has been fine tuned to deliver a single high concentration sample elution in a matrix that is compatible with all downstream assays. Application of the method allowed us to compare product quality attributes among a cohort of cell lines for each of seven development projects, and to check for effects of changes in cell culture processes on product quality. The results of this method are comparable to existing low-throughput technologies such as proA drip columns or large-scale proA purification.

BIOT 321 - Development of a high-capacity downstream process for an NS0-derived human IgG2 based on 11-12 day fed batch fermentations with defined feed media

Gregory Waszak, Erwin Yu, Michael Dupuis, and **Joseph Martin**, Pfizer Global Research and Development, St. Louis, MO 63017

The current version of the incipient downstream process for this antibody is patterned after the successful platform developed at Pfizer. This process can handle product titers up to 2.2 g/L at production scale. As upstream process developments increase mAb titers to 4 g/L, processes that could manufacture 40+ Kg/cycle in the same manufacturing footprint are desired. In developing a higher titer purification process, the following changes were evaluated: 1) Removal of one or two of the ultrafiltration/diafiltration steps. 2) Substitution of rProtein A resin with other affinity resins while increasing the bed height, and the flow rate. 3) Using alternate ion exchange resins that increase column capacity. 4) Increasing the load on the ion exchange column. In a scaled-down processing model, a number of high capacity downstream process variants yielded mAb of comparable quality when evaluated by a panel of quality-indicating assays.

BIOT 322 - Development of Biacore application for influenza virus titer determination

Camilla Estmer Nilsson, Mia Bängtsson, Anita Larsson, and Raf Lemmens, R&D, Life Sciences, GE Healthcare, Bjorkgatan 30, 75184 Uppsala, Sweden, Fax: +46-18-6121844, **camilla.estmernilsson@ge.com**

The challenge in developing and optimizing a vaccine purification process is to reliably measure the yield and recovery of the virus over each step of the process. This put strains on the quality, timeline and simplicity of the methods as process development typically generates a high number of samples. The influenza virus hemagglutinin (HA) titer is commonly determined by single radial immuno-diffusion assay (SRID) as recommended by the European Pharmacopoeia and WHO. However, the SRID assay has a number of disadvantages as it is laborious and time consuming, has a low detection range and is mainly suited for relatively pure vaccine products as precision and accuracy is affected by the sample matrix. Therefore, SRID is not an optimal method for the analysis during process development. Surface Plasmon Resonance technology monitors the interactions on the surface of a chip and has the valuable advantage of significantly lowering time required for analysis during process development. In this study an analytical method using Biacore™ was developed with the goal of receiving a robust and accurate assay for three influenza virus types (A/H1N1, A/H3N2 and B) with lower detection limit than SRID (<5µg HA/ml). The robustness, accuracy, precision and detection range of the three influenza virus assays using Biacore were investigated and compared to SRID analysis.

BIOT 323 - Monte-Carlo simulations of dye-labeled proteins diffusing in nanopores

Johannes Hohlbein[1], Martin Steinhart[2], Erik Hinze[3], Cordelia Schiene-Fischer[3], Christian G. Hübner[4], and Ulrich Gösele[2]. (1) Max Planck Institute of Microstructure Physics, Weinberg 2, 06120, Halle, Germany, Fax: +49-345-5511223, hohlbein@mpi-halle.de, (2) Max Planck Institute of Microstructure Physics, Halle D-06120, Germany, (3) Max Planck Research Group for Enzmology of Protein Folding, 06120, Halle, Germany, (4) Department of Physics, University of Lübeck, 23538, Lübeck, Germany

The investigation of fluorescence resonance energy transfer (FRET) in donor-acceptor labeled proteins allows monitoring their internal dynamics. Probe molecules confined to nanopores having their pore axes oriented parallel with the long axis of the focal volume of a confocal microscope show apparent one-dimensional diffusion. Thereby, their dwell time in the focal volume is more than one order of magnitude longer than in free solution. Simulations revealed that conformational changes of doubly labeled proteins can thus be monitored with significantly higher

accuracy on an extended timescale. Moreover, alternating laser excitation allows the separation of FRET signals from signals of proteins bearing only one chromophor. Single molecule fluorescence detection with dually labeled protein probes confined to properly oriented nanopores should be a viable and robust strategy potentially superior to measurements in free solution.

BIOT 324 - ELISA for Quantifying trace amount of an alkaline stabilized protein A in high concentration of mAbs

Tomas Bjorkman, Life Sciences, R&D, GE Healthcare, Bjorkgatan 30, Uppsala 751 84, Sweden, Tomas.Bjorkman@ge.com

Immobilized Protein A is the standard for the first chromatography step in the purification of monoclonal antibodies and Fc fusion proteins. This is normally achieved by applying clarified cell culture supernatant to a packed bed of a protein A resin followed by an acidic elution of the product. This gives excellent clearance of contaminants such as host cell proteins and host cell DNA. However, variable amounts of the immobilized protein A leach and the clearance of the leached ligand must be carefully monitored throughout the process. For the protein A variants currently available both commercial and in-house developed ELISA are used for quantifying the leached ligand. Since two years ago a new Protein A B-domain derived alkali stabilized protein A is available and in this paper we have studied the ability of one commercial kit to detect and quantify the new Protein a ligand. Data on e.g. precision and specificity will be shown.

BIOT 325 - High resolution anion exchange chromatography purification of oligonucleotides

J. Kevin O'Donnell, Eric Gershenow, Bruce Kempf, and Shigeru Nakatani, Tosoh Bioscience, 156 Keystone Drive, Montgomeryville, PA 18936, Fax: 215-283-5035, **kevin.odonnell@tosohbioscience.com**

The use of oligonucleotides as therapeutics continues to grow each year. These include both RNA and DNA based oligos. Using high resolution anion exchange chromatography resins, a crude oligonucleotide was purified to greater than 98%. Initial experiments indicated that at a higher pH better selectivity was obtained, thus requiring a strong anion exchange resin. For TSKgel SuperQ-5PW resins (both 20 and 30 microns nominal particle size), pH 9.0 gave optimal results. Not surprisingly, a longer column (15 vs. 7.5cm) gave better resolution between the main peak and the N-1 (and smaller) moieties. The 1000Å pore size of this resin offered excellent binding kinetics and almost quantitative recoveries. The TSKgel SuperQ-5PW capacities were excellent and resolution was superior to competitive resins even under saturation conditions. This resin is ideal for use in oligonucleotide purifications of all process scales.

BIOT 326 - Enhancing the thermostability of β-mannanase by addition of sugars, polylols and their combinations

Weina Wu, Zhaohui Liu, **Wei Qi**, and Zhimin He, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China, qiwei@tju.edu.cn

In this study, various sugars (glucose, mannose, fructose, sucrose and chitosan) and polylols (glycol, glycerol and sorbitol) as well as their combinations were used to improve the thermostability of an important industry enzyme: β-mannanase. Through single-factor experiments, sucrose, chitosan and sorbitol were found to inhibit inactivation of β-mannanase dramatically and increase enzyme activity greatly. When sucrose, chitosan and sorbitol were added with the constant concentration (2.0 g/l), the activity of β-mannanase at 50 ^{o}C were increased 201.3%, 198.7% and 222.8%, respectively. Furthermore, in order to enhance the enzyme thermostability, combinations of these three additives with different ratios were screened. The optimal ingredient was obtained as follows: sorbitol of 2.0 g/l, chitosan of 2.0 g/l and sucrose of 1.0 g/l, and the β-mannanase activity was increased 250.5% using this composition. Moreover, the reaction temperature profiles of β-mannanase suggested that the optimal reaction temperature was shifted from 50 ^{o}C to 60 ^{o}C by addition of sucrose, chitosan, sorbitol or their combinations, which verified the protective effect of the additives.

BIOT 327 - Evaluation of protein A chromatography media for capture of monoclonal antibodies

Bjarne R. Nielsen and John S. Nielsen, Protein Separation, Novo Nordisk A/S, Hagedornsvej 1, DK-2820 Gentofte, Denmark, Fax: +45 4443 8400, brni@novonordisk.com

Affinity chromatography using Protein A as ligand is state-of-the art for capture of mAbs. To ensure high productivity at lowest possible cost, the choice of Protein A resin is dependent on parameters such as binding capacity, yield and process time as well as ligand stability during processing and CIP. Binding and elution properties of the resin are also important to facilitate a generic platform approach. Three commercially available Protein A media including macro-porous basis resins and mutated protein A domains were evaluated in this study with focus on dynamic binding capacity, elution conditions, HCP removal, Protein A leakage and product stability.

BIOT 328 - Extraction of intracellular oxalate decarboxylase from the white-rot wood decay fungus Trametes versicolor

Cui Xia Zhu and **Feng Hong**, Institute of Biological Sciences and Biotechnology, Donghua University, Ren Min North Road No.2999, Shanghai 201620, China, fhong@dhu.edu.cn

Oxalate decarboxylase (OxDc, EC 4.1.1.2) catalyses the conversion of oxalate to formate and carbon dioxide and utilizes dioxygen as a cofactor. In this study, an intracellular OxDc was induced by oxalic acid and was extracted from the pestle-milled

mycelia of Trametes versicolor with an acetate buffer (200 mmol/L, pH3.7) at room temperature. More than 30% of total OxDc activity was found in the first time extracted enzyme solution, but the activity was hardly detected in the second and third time extracted acetate solutions. Approximately 60% of total OxDc activity was detected in the residual mycelia debris after the three times extraction using acetate buffer. The result suggested that some OxDc probably bond with the mycelia debris, and also implied that the bond/adhered OxDc might have higher catalytic activity. Non-ionic surfactants will be added in the extracting buffer to investigate if it is possible to improve the efficiency of enzyme extraction and whether more free enzymes could be released. The effects of buffer pH, types and concentrations of buffers, and extraction temperature on the enzyme harvest yield will be studied.

BIOT 329 - Impact of lot-to-lot variability of cation exchange resin on process performance

Jane Wahome1, Aberash Berhe1, Jeff Jorgensen1, Jerrod Einerwold1, Weichang Zhou2, and Amitava Kundu[1]. (1) Purification Process Engineering, PDL BioPharma, 9450 Winnetka Ave N, Brooklyn Park, MN 55445, Fax: 763-383-7058, jane.wahome@pdl.com, (2) Process Sciences Engineering, PDL BioPharma, Fremont, CA 94555

Cation Exchange chromatography is commonly used as a polishing step in the purification of monoclonal antibodies. This process step is typically designed to remove product related impurities such as aggregates, and process related impurities such as DNA and host cell proteins. Additionally, the cation exchange step has the potential to alter the distribution profile of product related substances such as charged isoforms of the antibody. Cation exchange resins that are used in the manufacturing process typically have some lot-to-lot variability. This variability may result in a visually different elution profile depending on the conditions used in this chromatography step. Depending on the product collection criteria, product elution volumes may also be significantly different. Despite these differences, there may or may not be any significant differences in the clearance of impurities. However, differences in product volume could lead to differences in the pH and conductivity of the product that could have an impact on the subsequent unit operation. This poster will present a case study that shows how the lot-to-lot variability of a cation exchange resin impacts its process performance and the performance of the subsequent process step. Possible correlations between the physical attributes of the resin to the process outputs will also be presented.

BIOT 330 - Impact of process parameters on the performance of Normal Flow Parvovirus (NFP) filters in an antibody purification process

Jerrod Einerwold, Jeff Jorgensen, Brin Petersen, and Amitava Kundu, Purification Process Engineering, PDL BioPharma, 9450 Winnetka Ave N, Brooklyn Park, MN 55445, Fax: 763-383-7058, **jerrod.einerwold@pdl.com**

Current regulatory expectations necessitate the use of small pore virus filters such as Normal Flow Parvovirus (NFP) filters in an antibody downstream process to remove both small and large viruses. Due to the small size of the pores, these filters are prone to fouling by the antibody and other process contaminants. Mobile phase conditions such as pH and conductivity could also impact the process performance of these filters. Process performance parameters include volumetric (and mass) capacity and volumetric flux. The loss of volumetric flux as a function of increasing capacity is a critical process performance parameter as this is related to the clearance of small model viruses (such as Minute Mouse Virus), across this filtration step. Minimizing flux decay is thus critical to maximizing the log reduction value across these NFP filters. Thus, to ensure maximal clearance, it is important to determine the factors that have an impact on the volumetric flux. A case study will be presented to show how the performance of this filtration step is sensitive to mobile phase conditions such as pH and conductivity. Using this understanding, a NFP filtration step was moved further upstream in the purification process to enhance volumetric capacity and minimize flux decay contrary to expectations. Furthermore, strategies of how to mitigate the impact of the processing conditions on volumetric flux will also be presented. Case studies will be presented to show how viral clearance is impacted by the decay in the volumetric flux, further underlining the importance of obtaining a good understanding of the factors that impact volumetric flux during Viral filtration with small pore NFP filters.

BIOT 331 - Isolation, identification, and characterization of heavy chain clips and an expressed intron in a CHO-produced mAb

Michael Dupuis, John Finnessy, Joseph Leone, Tara Carter, and Joseph Martin, Pfizer Glob*al Research and Development, St. Louis, MO 63017*

During process development for a human IgG2 mAb we noticed extra bands on SDS-PAGE gels and a “shoulder” on analytical HPLC-SEC. Separation and enrichment of these bands during purification showed that they were distinct molecular entities and not method-related artifacts. After preparation of sufficient material, N-terminal sequencing, tryptic digests, and mass spectrometry were used to identify the bands. We identified a clip between two heavy chain residues which were also responsible for part of the “shoulder” seen on analytical HPLC-SEC. An expressed intron within the heavy chain was also identified. The expressed intron was 49 amino acids in length and was between the variable and constant domains. At least one other documented case of intron expression in CHO has been noted in the literature.

BIOT 332 - Kinetics and thermodynamics of thermal inactivation and the thermostable mechanism of protective additives for beta-mannanase

Zhaohui Liu, Weina Wu, Wei Qi, and **Zhimin He**, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China, enzyme@tju.edu.cn

The inactivation experiments of an important industry enzyme: beta-mannanase with and without protective additives (sucrose, chitosan, sorbitol and their combinations) were carried out at high temperature (65 ^{o}C). The kinetic and thermodynamic parameters,

kinac and ΔG, were calculated from the curves of enzyme inactivation given that these inactivation reactions were the first order. The computed results showed that the addition of protective additives might obviously prolong half life of reaction, but slightly decrease kinetic constant and increase free energy of inactivation reaction. The protective mechanism of the additives was elucidated as follows: the additives could increase the surface tension of water, which govern the interaction of enzyme with solvent components in aqueous; and the formation of intra- and inter-crosslink hydrogen bonds between additives and enzyme might reduce the stretch activity of polypeptide residues and the enzyme-enzyme interaction; and then the themostability of enzyme was largely enhanced. In view of the slight change of ΔG, however, the use of additives may not alter the intrinsic conformational stability of beta-mannanase.

BIOT 333 - Libraries of multimodal chromatographic ion exchanger for the discovery of new selectivities and as a tool for HTPD

Ingela Blomqvist[1], Patrik Adielsson[2], Johan Färenmark[2], and Jean-Luc Maloisel[3]. (1) R&D Custom Design Media, GE Healthcare Biosciences AB, Björkgatan 30, 751 84 Uppsala, Sweden, Fax: + 46 18 6121191, ingela.blomqvist@ge.com, (2) R&D, Custom Design Media, GE Healthcare Biosciences AB, 75184 Uppsala 751 84, Sweden, (3) R&D Chemical synthesis, GE Healthcare Biosciences AB, Uppsala SE-751 84, Sweden

Time to market and production cost are getting more and more critical for biopharmaceutical companies. This leads to an increasing demand for extremely robust and generic chromatographic media, and also for technological platforms that allows fast generation and screening of new media. The development of multimodal chromatographic media, with the introduction of new combinations of interactions, allows an enhanced chromatographic performance as compared to more traditional media. This approach is particularly effective in the preparation of media with new specific selectivities. It has led to the development of Capto ™ MMC, a salt tolerant ion exchanger, and of Capto ™ Adhere, which is a scavenger media designed for the second step of a MAb purification process. This approach has been further extended to generate libraries containing custom designed media based on modifications of traditional media as well as highly diverse multimodal media based on a computer assisted design. The utilization of these diverse libraries in a ready-to-screen format and as a new HTPD tool will be introduced and further discussed.

BIOT 334 - Mechanisms of protein A leaching

Corazon Victa[1], Jayme N. Franklin[1], Paul J McDonald[2], and Robert Fahrner[2]. (1) Early Stage Purification, Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, (2) Bioprocess Development, Genentech, Inc, South San Francisco, CA 94080

Protein A affinity chromatography is a common method for process scale purification of monoclonal antibodies. During protein A affinity chromatography, protein A ligand co-elutes with the antibody (commonly called leaching), which is a potential disadvantage since the leached protein A may need to be cleared for pharmaceutical antibodies. To determine the mechanism of protein A leaching and characterize the leached protein A, we fluorescently labeled the protein A ligand in situ on protein A affinity chromatography media. We found two distinct mechanisms of protein A leaching and two classes of leached protein A. The first mechanism leaches intact whole protein A and occurs when using either purified antibody or unpurified antibody in harvested cell culture fluid (HCCF). The second mechanism leaches fragments of protein A when using HCCF. The leaching of protein A fragments can be inhibited by EDTA, suggesting that proteinases contribute to the generation of protein A fragments. We found that protein A fragments can be measured by ELISA, and that they can be more difficult to clear than whole protein A by cation exchange chromatography.

BIOT 335 - Novel denitrification process of electrochemical biocatalyst and its stability

Jeong Soo Cho1, Jae Yeon Park[1], Young Tae Park[2], and Young Je Yoo[1]. (1) School of Chemical and Biological Engineering, Seoul National University, San 56-1, Shillim-dong, Gwanak-gu, Seoul 151-744, South Korea, Fax: 82-2-887-1659, cloud882@snu.ac.kr, (2) Interdisciplinary Program for Biochemical Engineering and Biotechnology, Seoul National University, Seoul 151-744, South Korea

Biological denitrification among various denitrification methods is the most widely accepted process because of economical and environmental advantages. However, the feeding of carbon source is required to maintain biological activity. Novel bioelectrochemical method which did not need carbon source was developed. Denitrification was carried out by permeabilized Ochrobactrum anthropi SY509 containing denitrifying enzymes; nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase. The mediator, neutral red was selected to transfer electrons from electrode to biocatalyst. Carbon nanoparticle was chosen as a support material for immobilization to enhance the electron transfer and to increase the immobilization efficiency. When the permeabilized cell and the immobilized mediator were entrapped on the carbon felt electrode, high denitrification efficiency was obtained. The environmental effects such as pH, temperature, copper, and benzene on the reaction efficiency were studied and the permeabilized cell showed 90 days of half-life in activity.

BIOT 336 - Preparation and characterization of alcohol dehydrogenase-containing liposomes for improving its quaternary structure

Makoto Yoshimoto[1], Mami Sato[1], and Katsumi Nakao[2]. (1) Department of Applied Molecular Bioscience, Yamaguchi University, 2-16-1 Tokiwadai, Ube 755-8611, Japan, Fax: +81-836-85-9201, yosimoto@yamaguchi-u.ac.jp, (2) Department of Applied Chemistry and Chemical Engineering, Yamaguchi University, Ube 755-8611, Japan

Alcohol dehydrogenase (ADH) catalyzes the oxidation of alcohol in the presence of cofactor NAD^+. An appropriate reaction system is required for stabilization of the tetrameric ADH as well as effective ADH-NAD^+ interaction. In this work, the liposomes encapsulating both ADH and NAD^+ were prepared and characterized. The liposomal enzyme system showed almost no

reactivity to ethanol added to the liquid bulk at 25 °C because of negligible permeation of the substrate through the lipid membrane. At 40 °C, the ethanol oxidation occurred in the liposomal aqueous phase due to the increased substrate permeability by increasing temperature. In addition, the liposomal ADH showed a higher thermal stability at 40 °C than free ADH because its conformational change was depressed by the liposomal environment. The results obtained indicated that the present liposomal ADH/NAD^+ system was advantageous for obtaining the intact enzyme structure, effective ADH-NAD^+ interaction and practical regulation of the enzyme activity.

BIOT 337 - Process-specific range finding of chromatography resin lots used in the purification process of a recombinant product

Lillian Lee[1], Jeffrey Suchecki[2], Kristie Apgar[2], and Dicky Abraham[2]. (1) Biologics Development and Engineering, Merck Research Laboratories, 770 Sumneytown Pike, West Point, PA 19486, lillian_lee@merck.com, (2) Vaccine Technology and Engineering, Merck & Co, West Point, PA 19486

A challenge to large-scale, high-throughput biopharmaceutical manufacture is the consistency of incoming raw materials, particularly chromatography resins used for primary purification. Resin re-use mitigates these risks. However, the selection of an appropriate resin lot is still important to ensure product consistency and quality. In this case-study, variable elution times were identified for a product at full-scale, which resulted in variable yield and purity for this step. Resin specifications were compiled, and the observed shift correlated with the vendor's dynamic capacity. While the capacity for these lots met the vendor specifications, they were not optimal for the manufacture process. To establish the appropriate process specification for the resin, lab-scale studies were performed to confirm the link between step performance and resin dynamic capacity. These results matched the full-scale trend and, hence, will be used be used to establish a product-specific range for acceptance, e.g. lot selection. This process input will enable more consistent performance and increased control of the process. In addition to optimization of the manufacturing process, this work seeks to increase understanding of the resin variability and the dependence of the elution trend for variants of the protein. Multiple lots of resins were characterized to detect biophysical hints to the interaction of the resin with specific variants of the recombinant protein product.

BIOT 338 - Production of biodiesel by transesterification with new supported solid state alkali catalyst CaO/NaY

Haitang Wu1, Yujie Zhou1, Jianan Zhang1, and Jian Liu2. (1) Division of Green Chemistry and Technology, Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, China, Fax: 86-10-69771*464, zhangja@tsinghua.edu.cn, (2) School of Environmental Science and Engineering, Chang'an University, Xi'an 710064, China*

Biodiesel, a kind of environmental friendly renewable green energy, is produced by transesterification with vegetable oil under catalyst. Using molecular sieve NaY and CaO as supported substrate and raw material respectively, a new supported solid alkali catalyst CaO/NaY was prepared by microwave irradiation. With CaO/NaY as catalyst, production of biodiesel by transesterification reaction of soybean oil with methanol was studied. The effects of operation parameters on yield of biodiesel were explored. The results showed that the optimal reaction conditions were that the mass ratio of catalyst to soybean oil was 3%, the molar ratio of methanol to soybean oil was 9:1, reaction temperature was 65°C and reaction time was 3h. Additionally, CaO/NaY was characterized by XRD analysis. The results made it clear that the catalyst activity of CaO/NaY was improved greatly and the surface area of CaO/NaY was higher, which were benefit for production of biodiesel.

BIOT 339 - Successful scale-up of pH elution on cation-exchange column for the purification of monoclonal antibodies

Ferhana Zaman, Judy Glynn, Natrajan Ramasubramanyan, and Paul Mensah, Bioprocess Research & Development, Global Biologics, Pfizer Inc, 700 Chesterfield Parkway West, HH2, Chesterfield, MO 63017, Fax: 636-247-6098, **ferhana.zaman@pfizer.com**

Successful process scale-up of pH elution on a cation-exchange column was demonstrated during the downstream purification of a monoclonal antibody. This elution process was developed in the laboratory and successfully scaled to the pilot and manufacturing scales generating similar contaminant profiles. Typically, the higher conductivity salt elution buffers used in cation-exchange columns necessitates the use of buffer exchange prior to further processing, however with the use of pH elution concept, the buffer exchange step was able to be eliminated. This resulted in reduction of buffer consumption at manufacturing scale and reduction in process cycle time. A second monoclonal antibody was also successfully purified by this process at pilot scale with the same benefits of reduction in buffer consumption and process cycle time.

BIOT 340 - Use of analytical ultracentrifugation with a fluorescence detector to characterize high protein concentration formulations

Zephania W Kwong[1], Jamie Moore[2], Jun Liu[2], Ye Shen[3], Qing Zhu[1], Tim Kamerzell[4], Thomas M. Laue[5], and Steven J. Shire[6]. (1) Late Stage Pharmaceutical Device and Development, Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, wzkwong@gene.com, (2) Pharmaceutical R&D, Genentech, Inc, South San Francisco, CA 94080-4918, (3) Early Stage Pharmaceutical Development, Genentech, Inc, South San Francisco, CA 94080, (4) University of Kansas, (5) Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824-2617, (6) Late Stage Pharmaceutical and Device Development, Genentech, Inc, South San Francisco, CA 94080-4918

One of the greatest challenges of formulation development of high protein concentration formulations (>100 mg/mL) is the concentration dependent degradation pathway leading to aggregation. In addition to non-native protein aggregation leading to precipitation and particulate formation, there may be aggregates derived from reversible self-association. These dissociable aggregates at high protein concentration are difficult to quantitate

by most electrophoretic and chromatographic techniques since sample preparation, separation and detection require substantial dilutions. To overcome these limitations, we have evaluated the use of analytical ultracentrifugation with a novel fluorescence detection system (AUC-FDS) to measure the biophysical properties of proteins at high concentrations. The results from this study identified optimal fluorescent labeling conditions and demonstrated that the fluorescent label does not significantly alter the conformation or biophysical properties of monoclonal antibodies. In addition we demonstrate that AUC-FDS can be used to monitor the biophysical properties of proteins at high concentrations.

BIOT 341 - A high-throughput genetic selection for protein folding and solubility in the bacterial periplasm

Thomas Mansell, Chemical and Biomolecular Engineering, Cornell University, 304 Olin Hall, Ithaca, NY 14853, Fax: 607-255-9166, tjm49@cornell.edu, and Matthew P. DeLisa, School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853-5201

Given the importance of protein folding to myriad biological applications, it is not surprising that several methods of monitoring protein folding and solubility have been engineered. However, while many reporters have been shown to be effective indicators of folding behavior (e.g., solubility, aggregation, inclusion body formation), all report folding that occurs in the cytoplasm of *E. coli*. At present, there are no reports of cell-based assays for monitoring protein folding in extracytoplasmic compartments such as the periplasmic space. Accordingly, we report the development of a genetic selection for periplasmic protein solubility and folding *in vivo* using the signal recognition particle (SRP)-dependent pathway. The advantage conferred by use of the SRP-dependent pathway is that target proteins are extruded directly into the periplasm without residence time in the cytoplasmic folding environment. Using this system, we have characterized the *in vivo* periplasmic folding and solubility of several classes of proteins including amyloid-beta peptides and single-chain Fv antibody fragments. In addition, we have demonstrated the potential of our technique for the directed evolution of proteins in the periplasm by creating libraries to screen for improved solubility of target proteins. This system can also be used to analyze the effectiveness of factors that influence protein folding in trans, such as association with chaperones. Finally, by coupling this SRP-dependent folding assay with a previously developed cytoplasmic folding assay based on the Tat-dependent pathway, it is possible to elucidate, in an unbiased manner, the difference in the folding environments of the cytoplasmic and periplasmic cellular compartments. The application of this system is geared towards unbiased whole-cell screening of combinatorial libraries to determine the optimal folding environment for heterologously expressed proteins.

BIOT 342 - Creation of a fluorescent protein biosensor

Eric Shusta and Tej Pavoor, Department of Chemical and Biological Engineering, University of Wisconsin Madison, 3631 Engineering Hall, 1415 Engineering Drive, Madison, WI 53706, shusta@engr.wisc.edu, **tvpavoor@wisc.edu**

Generating a fluorescent protein sensor capable of binding to a specific target is of considerable interest. Such a sensor has wide application in immunochemistry, cell biology, and proteomics. This has been previously attempted by grafting multiple binding loops onto GFP, but with limited success. In our approach, we sought to graft two loops onto GFP to create a binding interface. Starting with yEGFP, we have studied its ability to accommodate 13 and 12 amino acid insertions in the loop regions D102-D103 and E172-D173, respectively. Initially, the double loop insertion yEGFP was not produced by yeast. Using yeast surface display, we performed multiple rounds of mutagenesis and shuffling to make a well-expressed, fluorescent loop-grafted yEGFP scaffold with four mutations of which three are proximal to the chromophore region. Further, we have randomized both loops to produce a library of GFP-based biosensors that can be screened against any target of interest.

BIOT 343 - Designing AraC effector specificity through modeling and dual selection

Patrick C. Cirino, Shuang-Yan Tang, Hossein Fazelinia, and Costas D. Maranas, Department of Chemical Engineering, The Pennsylvania State University, 226A Fenske Laboratory, University Park, PA 16802, Fax: 814-865-7846, **cirino@engr.psu.edu**

Engineered regulatory proteins enable customized genetic selections and permit targeted gene transcription for applications in metabolic engineering and biosensing. Use of regulatory proteins for reporting or controlling metabolic pathways in response to specific metabolites requires a highly selective protein-effector interaction that is not influenced by similar compounds. We have developed dual selection strains and FACS-based screens to engineer the effector specificity of AraC regulatory protein, which naturally regulates the ara operon in response to L-arabinose in E. coli. L-Arabinose-producing enzymes and enzyme libraries were tested to validate the use of this system for reporting in vivo metabolite production and identifying strains with improved activity. A computational framework was also developed to direct the redesign of the AraC binding pocket. AraC was subsequently evolved to respond to a variety of non-native molecules. This presentation will highlight the construction and evaluation of our selection and screening systems, computational results, and experimental progress toward the design of AraC variants with novel effector specificity. Our results address the difficult yet critical requirement for binding selectivity and have allowed for comparisons between the use of selection versus FACS and computationally-guided versus random or structure-based protein design.

BIOT 344 - Gankyrin: A general model for Ankyrin Repeat (AR) protein studies

Anjali Mahajan[1], Yi Guo[2], Ming-Daw Tsai[1], and Junan Li[1]. (1) Department of Chemistry, The Ohio State University, columbus, OH 43210, mahajan.9@osu.edu, (2) Ohio State Biochemistry Program, The Ohio State University, columbus, OH 43210

Ankyrin repeat proteins (ARs) consist of linear arrays of small repeating units and play critical roles in almost every life process via protein-protein interactions. Gankyrin, an oncoprotein overexpressed in hepatocellular carcinomas, is composed of seven ankyrin repeats. Although, P16 and gankyrin both bind to CDK4, only P16 inhibits the kinase activity of CDK4 and hence acts a tumor suppressor. In this study we engineered a double mutant of gankyrin, L62H/I79D, that inhibits the kinase activity of CDK4 and also possess similar biophysical properties to P16. The difference between tumor suppressing and oncogenic functions of P16 and gankyrin, respectively, has thus been shown to reside in a single residue, and we propose that gankyrin has a potential to adjust its structure for performing such novel functions. In this study, we also investigated the conformational stability of gankyrin in comparison with other AR proteins, under different denaturant conditions, pH, GdnHcl, and temperatures, and our results showed that gankyrin follows a two-state transition in chemical- and heat-induced unfolding and has a high structural tolerance. Taken together, our studies provide insights into understanding the biophysical uniqueness of gankyrin (or AR proteins in general).

BIOT 345 - High yields of complex proteins with site-specific posttranslational modification using cell-free protein synthesis

Aaron R. Goerke, Jessica J Wuu, Wataru Ebina, and James R. Swartz, Department of Chemical Engineering, Stanford University, Stauffer III, Room 113, Stanford, CA 94305-5025, **argoerke@stanford.edu**

Expanding the set of 20 natural amino acids used for protein synthesis will enable site-specific protein modification for a variety of purposes. Our objective was to develop a cell-free production platform for producing complex disulfide-bonded proteins with site-specific unnatural amino acid incorporation. This was demonstrated with soluble bacterial proteins, therapeutic mammalian proteins, and membrane proteins. Bioactivity was verified by colorimetric, cell proliferation, and transport assays. All proteins express well with the best yield at 590 µg/mL, with 100% active protein. Targeted post-translational modification of p-azido-L-phenylalanine used new probes and linkers. With these advances we could rapidly produce functionalized membrane proteins and homogeneous post-translationally modified therapeutics and vaccines.

BIOT 346 - Mathematical model of protein secretion incorporating pore complex sequestration by aggregated protein

Robert S. Kuczenski, Prateek Gupta, and Kelvin H. Lee, School of Chemical and Biomolecular Engineering, Cornell University, 120 Olin Hall, Ithaca, NY 14853-5201, rsk23@cornell.edu

While secretion of heterologous protein from *Escherichia coli* may reduce downstream processing costs, low yields have made secretion uncompetitive. Recent Type 1 secretion results by our group and others have illustrated an inverse relationship between secretion and aggregation, possibly suggesting inhibition via secretion machinery sequestration by non-transportable aggregates. To study this phenomenon, we created a mathematical model incorporating protein translation, aggregate formation, and competition between aggregated and non-aggregated product for secretion. Using this model we explore the optimal balance between total protein production and the cell's secretion capacity. Specifically, we investigate the ability of changes to mRNA concentration or translation rate to maintain this optimal protein production rate. Finally, changes to the secretion capacity and other alternative methods of enhancing total secretion are investigated. This work is part of our group's effort to create a predictable *E. coli* secretion platform with an understanding of the significant protein-specific design variables.

BIOT 347 - New design strategies for FRET-based sensor proteins

Maarten Merkx[1], Elisabeth M. W. M. van Dongen[1], Toon H. Evers[1], Jan L. Vinkenborg[1], Leo W. J. Klomp[2], and E. W. Meijer[1]. (1) Department of Biomedical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, Netherlands, m.merkx@tue.nl, (2) University Medical Centre Utrecht

Sensor proteins based on FRET between two fluorescent proteins allow real-time imaging of molecular events in living cells. Here we report new FRET-based sensor approaches for the detection of Zn(II) and protease activity. Zn(II) sensors with a very high and tunable affinity (Kd = 30 fM -1.4 pM) have been created by connecting two fluorescently labeled metal binding domains using a series of flexible peptide linkers. Modelling the conformational distribution of the linker allows a quantitative understanding of the ratiometric changes and the Zn(II) affinity. In a different sensor approach, de novo Zn(II) binding sites are introduced directly on the surface of both fluorescent proteins. This sensor displays an impressive 9-fold increase in emission ratio and allows detection of Zn(II) from 10 nM to 1 mM. Finally, we present a new concept for FRET-based protease sensors that is based on intramolecular complex formation between donor and acceptor fluorescent domains.

BIOT 348 - Protein Engineering Call for Papers

Rahul S. Rajan, Department of Pharmaceutics, Amgen, Inc, One Amgen Center Drive 2-1-A, Thousand Oaks, CA 91320, Fax: (805)-498-8674, rrajan@amgen.com, and George Makhatadze, Department of Biochemistry and Molecular Biology, Penn State University, College of Medicine, 500 University Drive, Harrisburg, PA 17033, Fax: 717-5317072, makhatadze@psu.edu

Protein Engineering Call For Papers. Engineering and design of proteins with enhanced properties such as stability, solubility, novel structures and functions is one of the most dynamic and rapidly developing areas of protein biochemistry with broad industrial applications. Papers are welcome in several areas related to protein engineering. This session will feature presentations that discuss various strategies for protein engineering, including the most recent innovations in experimental methods (protein modification, in-vitro directed evolution, high through screen) as well as computational algorithms for rational design. Advances in fusion protein technology, peptide-protein conjugation (such as peptibodies) and polymer-protein conjugation (such as PEGylation) will also be considered.

BIOT 349 - Protein engineering of gene switches

Huimin Zhao and Karuppiah Chockalingam, Department of Chemical and Biomolecular Engineering, University of Illinois, 215 RAL, Box C3, 600 S. Mathews Ave., Urbana, IL 61801, Fax: 217-333-5052, zhao5@uiuc.edu

Abstract text not available.

BIOT 350 - Protein engineering of gene switches

Huimin Zhao[1], Karuppiah Chockalingam[1], and Michael McLachlan[2]. (1) Department of Chemical and Biomolecular Engineering, University of Illinois, 215 RAL, Box C3, 600 S. Mathews Ave., Urbana, IL 61801, Fax: 217-333-5052, zhao5@uiuc.edu, (2) Center for Biophysics and Computational Biology, University of Illinois, Urbana, IL 61801

Abstract text not available.

BIOT 351
Site-specific PEGylation of interferon–beta by Cu(I)–catalyzed cycloaddition

Natalie Winblade Nairn, Thomas J Graddis, Aijun Wang, Kurt Shanebeck, and Kenneth Grabstein, Allozyne, 1616 Eastlake Ave E, Suite 202, Seattle, WA 98102, Fax: 206-957-7399, nnairn@allozyne.com

PEGylation of protein therapeutics has been established as a means to improve properties such as pharmacokinetics, resistance to proteases, and solubility. Methods to conjugate PEG to the 20 natural amino acids provide little control over the site of PEGylation. However, selection of an appropriate conjugation site is critical to retain bioactivity of the resultant molecule. To address these limitations, we have incorporated a nonnatural amino acid, azidohomoalanine, into a single site in interferon-beta, followed by conjugation to an alkyne–PEG using Cu(I)–catalyzed ″click″ chemistry. Optimized conditions lead to routine yields of >90 percent PEGylation of the molecule at a single site. The PEGylation has been performed at scales of up to 12 mg protein with retention of up to 100 percent bioactivity in vitro.

BIOT 352 - Stability of the bZIP homo- and heterodimers and energetics of their interactions with DNA

Anatoly I. Dragan, Department of Biology, Johns Hopkins University, Baltimore, MD 21218, Fax: 410-261-1064, dragan@jhu.edu

Abstract text not available.

BIOT 353 - Structural stability and reactivity of bovine liver catalase modulated with liposomes

Makoto Yoshimoto[1], Hideyuki Sakamoto[1], and Katsumi Nakao[2]. (1) Department of Applied Molecular Bioscience, Yamaguchi University, 2-16-1 Tokiwadai, Ube 755-8611, Japan, Fax: +81-836-85-9201, yosimoto@yamaguchi-u.ac.jp, (2) Department of Applied Chemistry and Chemical Engineering, Yamaguchi University, Ube 755-8611, Japan

Catalase is industrially useful for H_2O_2 decomposition. Catalase is deactivated due to the substrate inhibition and dissociation into subunits at low concentrations. In this work, the catalase-containing liposomes (CAL) and the catalase-bound liposomes (CABL) were prepared and characterized. The CAL activity was stable at 55 °C because the quaternary structure of catalase was maintained by its interaction with liposome membrane and high concentration in the liposome. The CAL activity in catalyzing H_2O_2 decomposition was also stabilized because the limited H_2O_2 permeation through the liposome membrane depressed the catalase-H_2O_2 interaction and the enzyme deactivation. Catalase in the CABL showed a comparable reactivity to free enzyme because catalase in the CABL was covalently immobilized on the outer surface of liposome. The CABL stability depended on the number of bonding sites between catalase and liposome. Mechanism of the liposomal modulation on the stability of catalase was discussed based on the results obtained.

BIOT 354 - Resolution of disulfide “scrambling” through engineering of a modified form of the Nogo Receptor (NgR1) Fc fusion protein

Paul H Weinreb, Protein Biochemistry, Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142, Fax: 617.679.3148, paul.weinreb@biogenidec.com

In adult mammalian brain, axonal regeneration is limited by a group of myelin associated inhibitory factors (MAIFs). The activity of these factors, which include the structurally unrelated proteins Nogo-A, OMgp, and MAG, is mediated at least in part by Nogo-66 receptor 1 (NgR1), a receptor for all three MAIFs and forms part of a larger signaling complex. Treatment with soluble recombinant NgR1-Fc fusion proteins in animal models of spinal cord injury, stroke recovery, and Alzheimer's Disease promotes axon regeneration and reduces amyloid-beta plaque load. NgR1 is a 473 amino acid leucine-rich repeat (LRR) protein that contains eight LRRs flanked by N- and C-terminal disulfide-containing “cap” domains (LRRNT and LRRCT, respectively), a C-terminal stalk region, and a GPI anchor site. We recently showed that the disulfide pattern of the LRRCT in full-length NgR1 includes links to Cys335 and Cys336, in contrast with earlier reports using a truncated recombinant NgR1(27-310), in which a non-native disulfide bridge between Cys266 and Cys309 was observed. In this work we show that, while functionally active, the NgR1(27-310)-Fc fusion protein contains mislinked and heterogeneous disulfide patterns, and we report the generation of a series of variant molecules specifically designed to prevent this disulfide “scrambling”. One of these variants was shown to completely eliminate disulfide scrambling, while maintaining functional in vitro and in vivo efficacy. This modified NgR1-Fc molecule represents a suitable candidate for pharmaceutical development.

BIOT 355 - Dendritic molecular transporters as highly targeted, controlled intracellullar delivery vectors

Billie L. Evans III[1], Kui Huang[1], Mine Ikizler[2], Peter F. Wright[2], and **Eva Harth**[3]. (1) Department of Chemistry, Vanderbilt University, 7619 Stevenson Center, Nashville, TN 37235, (2)

Department of Pediatrics, Vanderbilt University Medical Center, Nashville 37232, (3) Department of Chemistry, Vanderbilt University, 7619 Stevenson Center, Nashville, TN 37235-1822, Fax: 615-343-1234, eva.harth@vanderbilt.edu

We present the utilization of novel dendritic molecular transporter molecules to deliver proteins, such as aprotinin, a serine protease inhibitor into the cytoplasm of human adenoid epithelial cells (HAEC). In this approach we modified the dendritic molecular transporter at the focal point of the macromolecule to add a PEG linker, endfunctionalized with a hydrazide functionality. In order to establish a conjugation to selected sites of the proteins we implemented a carbonyl group from tyrosines in a Mannich-type reaction. The coupled bioconjugation product is investigated in their uptake behavior towards HAEC cells and we observed the rapid and efficient delivery of the transporter-protein conjugate. Further studies evaluating the potential of the transporter molecules involving the modification of other proteins and peptides will be presented.

BIOT 356 - Directed evolution of targeted cell penetrating peptides for trans-BBB delivery

Shan Gao[1], Melissa Simon[2], Barclay Morrison III[2], and **Scott Banta**[1]. (1) Chemical Engineering, Columbia University, 500 W 120th St, New York, NY 10027, sg2323@columbia.edu, sbanta@cheme.columbia.edu, (2) Biomedical Engineering, Columbia University, New York, NY 10027

The delivery of therapeutic materials to the brain is limited by the blood brain barrier (BBB). Cell Penetrating Peptides (CPPs) can traverse membranes and deliver cargos to most cell types, including the brain, but they lack specificity. Homing Peptides (HPs) can target specific cells following systemic administration, but they generally do not penetrate cells. We are attempting to create novel peptides that exhibit both HP and CPP abilities (Specific Cell Penetrating Peptides, SCPPs) through an innovative Directed Evolution approach based on the delivery of a fluorescent transgene using plasmid display. The delivery of the plasmid display complex has been inefficient, and we are identifying probable bottlenecks. The TAT CPP can deliver the GFP protein to PC-12 cells, and we have systematically incorporated features of plasmid display to this system. The insights gained through these efforts are enabling us to redesign our system for the Directed Evolution of novel trans-BBB SCPPs.

BIOT 357 - Hemoglobin-drug conjugates for targeted delivery of nucleoside drugs

Gord Adamson and David N. Bell, Drug Development, Hemosol Corp, 2585 Meadowpine Blvd., Mississauga, ON L5N 8H9, Canada, gadamson@hemosol.com

Hemoglobin-drug conjugates (HDCs) may improve the safety and efficacy of the attached drugs for treatment of liver viral infection and cancer. HDCs target drugs to liver cells through the endogenous pathway for hemoglobin catabolism through the liver. Several nucleoside drugs including ribavirin (an essential co-therapy for hepatitis C virus infection with significant dose-limiting toxicity) were conjugated to hemoglobin as 5'-monophosphoramidates, allowing for intracellular drug release. HDCs with molar drug ratios up to 15 retained the ability to bind haptoglobin (the natural ligand for acellular hemoglobin) and to be taken up by cells expressing the hemoglobin-haptoglobin receptor, CD163. In a viral hepatitis mouse model, ribavirin-HDC prolonged survival, improved behavior, and reduced histological signs of disease. Viral replication and inflammatory cytokine production were inhibited in vitro. HDCs of the anti-cancer nucleoside floxuridine showed increased cytotoxicity against lymphoma cells expressing CD163. Further investigation of HDCs for targeted therapy of hepatic diseases is warranted.

BIOT 358 - Ketalized polyethylenimine for tunable delivery of plasmid DNA and siRNA to intracellular targets

Min Suk Shim, Department of Biomedical Engineering, Case Western Reserve University, 10900 Eulicd Ave, Cleveland 44106, min.shim@case.edu, and **Young Jik Kwon**, Department of Biomedical Engineering, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106, young.kwon@case.edu

Polyethylenimine (PEI) is one of the most widely studied polymeric gene carrier due to its superior transfection efficiency. However, its uses in vivo are limited due to high cytotoxicity and non-degradability. In this study, we ketalized PEI to achieve 1) enhanced intracellular release of nucleic acids, 2) efficient dissociation of nucleic acids from cationic polymeric backbone, and 3) lowered cytotoxicity by less attractive interactions between nucleic acids and hydrolyzed PEI. Ex vivo study showed remarkably reduced cytotoxicity of ketalized PEI and, more interestingly, transfection efficiency was found inversely proportional to molecular weight of ketalized PEI while RNA interference was observed in an opposite way. The results demonstrated that targeted delivery of plasmid DNA and siRNA to the nucleus and cytoplasm, respectively, can be achieved by appropriately modulating PEI. Characterization of the polymers, biological studies explained by a theoretical model, and implications in clinical gene therapy will be presented.

BIOT 359 - Polyelectrolyte multilayer films as platforms for efficient siRNA delivery

Srivatsan Kidambi, Sumit Mehrotra, Joseph A. Gredell, S. Patrick Walton, Ilsoon Lee, and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, 2527 Engineering Building, East Lansing, MI 48824, srivatsan.kidambi@gmail.com

For diseases that develop from the undesired production of a particular protein, RNA interference (RNAi) initiated by small interfering RNAs (siRNAs) is a highly promising therapeutic approach. Typically, chemical and physical techniques (e.g., lipofection or electroporation) are used for in vitro delivery of siRNAs, but targeted delivery of active siRNAs in vivo remains a significant challenge. Solution-phase and solid-phase delivery systems for in vivo applications are the focus of intense investigation by industrial and academic researchers. We propose using highly customizable polyelectrolyte multilayer (PEM) thin films to engineer surfaces for targeted siRNA delivery. We have engineered a PEM-based siRNA delivery system by capitalizing upon electrostatic interaction between polycations and the

negatively-charged siRNA. The construction of multilayer films by alternate self-assembly of poly-L-lysine (PLL) and siRNA was monitored using UV-Vis spectrometry and ellipsometry. These films are then deconstructed under physiological conditions ([NaCl] = 0.25 M), thus releasing the siRNA to the cells. Our preliminary cell uptake studies indicate that FITC-labeled siRNA were taken up by fibroblasts and primary hepatocytes providing confidence that this effect may be extended to other siRNA molecules attached on top of PEMs. To our knowledge, our study represents the first report of siRNA delivery from PEM films. A comparative study with several cell types, such as cell lines and primary cells, is underway to determine the delivery efficiency of the siRNA molecules from the PEMs to these different cell types.

BIOT 360 - Targeting mRNAs with a radiolabeled herceptin/tat/antisense MORF streptavidin nanoparticle

Xinrong Liu[1], Yi Wang[1], Kayoko Nakamura[2], Atsushi Kubo[2], and Donald J Hnatowich[1]. (1) Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, Fax: 508-856-4572, xinrong.liu@umassmed.edu, (2) Department of Radiology, Keio University School of Medicine, Tokyo, Japan

This nanoparticle consisted of the herceptin antibody to provide specific targeting of the Her2+ SUM190 breast cancer cells, the tat peptide for increased membrane transport and the antiRIalpha MORF antisense oligomer radiolabeled with Tc99m for imaging, all biotinylated and linked via streptavidin. Within the nanoparticle, the antibody was successfully targeting its antigen as shown by the 5-fold significantly higher accumulations in the 190 vs the Her2- SUM149 control cells, and the 30-fold significantly higher accumulations of the herceptin/tat/MORF nanoparticle compared to tat/MORF and naked MORF controls, the antisense MORF was successfully targeting its mRNA as shown by the significantly higher accumulations of the herceptin/tat MORF and the tat preserved its carrier function as shown by the 2-fold significantly higher accumulations of the tat/MORF nanoarticle compared to naked MORF. These results add to earlier evidence in tumored mice that combining two and three components with streptavidin may led to a simple solution to delivery for antisense, gene therapy and RNAi applications.

BIOT 361 - The influence of different antitumor antibodies on anticancer effector accumulation in solid tumors by MORF pretargeting

Guozheng Liu, Shuping Dou, Mary Rusckowski, and Donald J Hnatowich, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, Fax: 508-856-4572, guozheng.liu@umassmed.edu

By pretargeting, the antitumor antibody and the radiolabeled effector are separately administered but with mutual affinity. We are developing a novel pretargeting approach using a pair of phosphorodiamidate morpholino oligomers (MORF/cMORF) for recognition. We earlier described a semiempirical model capable of accurately predicting the behavior of a radiolabeled cMORF effector in pretargeted tumored mice with variations in dosages and timing. We have now extended the model to predict the effector behavior, in particular the maximum percent tumor accumulation, in tumored mice pretargeted with MORF conjugated MN14, B72.3 or CC49 antibodies. By comparing the results in mice pretargeted by these different antibodies with predictions, we confirmed that the maximum accumulation in the LS174T tumor of the radiolabeled effector is independent of the antibody. While it is possible to improve tumor-to-normal tissue ratios by the use of different antibodies, the development of novel antibodies in an attempt to improve the maximum percent tumor accumulation of anticancer effectors by pretargeting is therefore unproductive and unnecessary.

BIOT 362 - Two novel anti-TAG-72 cancer biomarkers identified by phage display

Ling Chen, Mary Rusckowski, and Donald J. Hnatowich, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, ling.chen@umassmed.edu

The TAG-72 glycoprotein, over expressed in a number of cancers, was subjected to selection using the f88-4/cys6 phage library expressing randomized 16 mer peptides with a 6 amino acid constrained disulfide loop. Of four consensus peptides from 25 clones, two highest binders were VHHSCTKLTHCCQNWH and GGVSCMQTSPVCENNL, present in 44% and 24% of clones in round 2 and 52% and 24% in round 3. These two novel peptides are distinctly more hydrophobic than those identified by us earlier. The phage-bound peptides showed high binding to the TAG-72 positive cells LS-174T by FACS with signal shifts of >50% and that were reduced upon competition with unlabeled material. Similar high binding was observed to cells when the phage-peptides were radiolabeled with ^{99m}Tc. Binding to the cell membrane was confirmed for both by fluorescence microscopy after labeling with Alexa 633. These phage-bound peptides are intended for use in tumor detection.

BIOT 363 - Characterizing protein structure in amorphous solids using hydrogen/deuterium exchange with mass spectrometry

Yunsong Li[1], Todd D. Williams[2], Richard L. Schowen[1], and Elizabeth M. Topp[1]. (1) Department of Pharmaceutical Chemistry, University of Kansas, 2095 Constant Ave., Lawrence, KS 66047, (2) Department of Chemistry, University of Kansas, Lawrence, KS 66045

Purpose. To develop a new technique for characterizing protein structure and protein-excipient interactions in the solid state for use in rational design of stable lyophilized protein formulations. Method. We applied hydrogen/deuterium exchange with electrospray ionization mass spectrometry (+ESI/MS), a method long used to characterize protein conformation in solution, to lyophilized formulations of a model protein (Calmodulin, CaM, 17 kDa). CaM was lyophilized in the presence or absence of trehalose and/or calcium chloride, and then exposed to D2O vapor at controlled relative humidity (RH) and temperature. Samples were quickly reconstituted by chilled low pH buffer and analyzed by tandem liquid chromatography (LC) and +ESI/MS, or subjected to peptic digestion to assess local differences in H/D exchange prior to analysis. Results. The results demonstrate that H/D exchange for CaM in amorphous solids is influenced by RH and by the inclusion of calcium chloride and/or trehalose in the solid. The effects are

not exhibited uniformly along the protein sequence, but occur in a site-specific manner, with calcium primarily influencing the calcium binding loops and trehalose primarily influencing the á-helices. Additional studies with a broader range of carbohydrate excipients show that these materials as a class primarily influence H/D exchange in á-helices for proteins in lyophilized solids. Conclusions. The method can provide quantitative and site specific structural information on proteins in amorphous solids, as well as on changes in structure induced by protein cofactors and formulation excipients. Such information is not readily available with other widely used techniques used to characterize protein structure in the solid state, such as FTIR, Raman and near infrared spectroscopy.

BIOT 364 - A highly efficient selection strategy for intracellular protein-protein interactions mediated by a unique bacterial hitchhiker transport mechanism

Dujduan Waraho, Chemical and Biomolecular Engineering, Cornell University, 304 Olin Hall, Ithaca, NY 14853, Fax: 607-255-9166, dw95@cornell.edu, and **Matthew P. DeLisa**, School of Chemical and Biomolecular Engineering, Cornell University, 254 Olin Hall, Ithaca, NY 14853-5201, Fax: 607-255-9166, md255@cornell.edu

We have developed a reliable genetic selection strategy based on the inherent 'hitchhiker' mechanism of the *Escherichia coli* twin-arginine translocation (Tat) pathway for the isolation of interacting proteins. This method, termed FLI-TRAP (Functional Ligand-binding Identification by Tat-based Recognition of Associating Proteins), is based upon the observation that non-covalent complexes of two folded polypeptides, where one polypeptide carries an N-terminal Tat signal peptide (ssTorA) fused to the protein to be screened for interactions and the second is a fusion between the cognate partner protein and the TEM-1 β-lactamase protein (Bla), are efficiently exported by the Escherichia coli Tat pathway. Using a series of leucine zipper motifs of known affinities derived from the c-Jun and c-Fos proteins, we observed that only those chimeras which were stably co-expressed and interacted strongly in the cytoplasm formed a physical linkage between ssTorA and Bla that was sufficient to co-translocate Bla into the periplasm and confer β-lactam antibiotic resistance to cells. We also demonstrate that intracellular binding between an anti-GCN4 single-chain Fv fragment (scFv) and GCN4 leucine zipper resulted in efficient delivery of Bla to the periplasm and growth of ~10 times more colonies than non-specific interactions, indicating that the assay can be used for the routine selection of high-affinity antigen/antibody interactions without the need for purification or immobilization of the antigen. Since the Tat mechanism requires its substrates to be correctly folded prior to transport, this system should naturally favor the identification of soluble, non-aggregating, protease resistant protein pairs.

BIOT 365 - Effects of water-miscible ionic liquids on the catalysis of horseradish peroxidase

Keungarp Ryu and Eunsik Hong, Department of Chemical Engineering and Bioengineering, University of Ulsan, Nam-gu, Moogeo-dong, Ulsan 680-749, South Korea, Fax: 82-52-259-1689, kgryu@mail.ulsan.ac.kr

Ionic liquids are nonvolatile and nonflammable; thus, possess great potential as environment-friendly green solvents. Peroxidases catalyze the oxidation of various aromatic compounds with peroxides. Peroxidases have been exploited for the environmental purposes to remove recalcitrant phenols and to develop benign alternative processes to produce phenolic polymers to replace phenol-formaldehyde resins. In this study, the effects of water-miscible ionic liquids on the horseradish peroxidase (HRP)-catalyzed oxidation of guaiacol were investigated. HRP maintains high activity in the aqueous mixtures containing various concentrations of the ionic liquids to 90% (v/v) ionic liquids. In order to elucidate the effects of ionic liquids on the catalytic mechanism of HRP, kinetic studies were performed in water-ionic liquid ([BMIM][BF4]) mixtures containing 25% (v/v) [BMIM][BF4] at maximum. The effects of the ionic liquid to stabilize the substrate's free energy were accounted for to explore the true catalytic properties of HRP in water-ionic liquid mixtures.

BIOT 366 - Formulation and characterization of components in vaccines

James D Chesko[1], Jina Kazzaz[2], Padma Malyala[1], Derek O'Hagan[1], Kathryn Patton[1], and Manmohan Singh[1]. (1) Vaccines and Diagnostics, Novartis, 4560 Horton Street, M/S 4.3, Emeryville, CA 94608, Fax: 510-923-2586, James.Chesko@novartis.com, (2) Novartis, Emeryville, CA 94709

Effective formulation and delivery of bioactive antigens such proteins, polysaccharides, oligonucleotides and conjugates benefits from methods of characterizing properties beyond composition such as adsorption and release of antigens, size and charge of particulate carrier polymers, interactions that may alter the molecular form, disposition and stability of the biomolecules and phases present. Vibrational and electronic spectroscopies were performed in addition to analytical chromatography and biophysical measurements to characterize and assess properties of antigens used in vaccines for HIV, anthrax, Meningitis B, influenza in the molecular context of various delivery systems including aluminum salts, MF59 emulsions and poly(lactide-co-glycolide) micro and nanoparticles. The potency of such vaccine formulations are enhanced by biophysical properties that result in more effective uptake and presentation of immunogenic epitopes that are recognized by cells of the immune system. The ability to measure and characterize relevant biomolecular properties and correlate them to an appropriate immune response will be emphasized.

BIOT 367 - Amplification bias in competitive templates is offset through the use of proofreading DNA polymerases: Application to the amplification of SAGE ditags

Austin P. So and C. A. Haynes, Michael Smith Laboratories, University of British Columbia, Rm 273, 2185 East Mall, Vancouver, BC V6T 1Z4, Canada, Fax: 604-822-2114, aphs@interchange.ubc.ca

Serial analysis of gene expression is a powerful technology that provides a portrait of gene expression on a global scale. Utilizing a sequencing-based approach, the abundance of each transcript is

inferred through short sequence representations (SAGE tags) presented in a form amenable to interrogation via sequencing. Numerous steps, however, are involved in preparing a sample for analysis via SAGE, requiring the extensive use of PCR to offset these losses. Through the application of Taqman assays, we have observed that the amplification of SAGE ditags is subject to an abundance dependent amplification bias that can affect the fidelity of SAGE analysis. This bias arises from the extensive homology and common priming sites shared between any two ditags, leading to the formation of intrastrand products during the annealing phase of the PCR, selectively biasing the amplification reaction against low abundance ditags. By applying Taqman-like assays on proofreading DNA polymerases, we are able to define conditions that offset this effect under conditions that enhance exonuclease activity. However, as this increase in performance occurs to the detriment of product yields, a streamlined concatenation protocol is developed for SAGE library construction, which permits the capture of ~4-orders of magnitude in ditag abundances. These results also provide a general application of proofreading DNA polymerases in the detection of competing templates that vary over 5-orders of magnitude in abundance, representing a significant increase in the sensitivity of these assays.

BIOT 368 - Dual fluorescence reporter system for studying +1 programmed ribosomal frameshifting in *Escherichia coli*

Pei-Yu Liao and Kelvin H. Lee, School of Chemical and Biomolecular Engineering, Cornell University, 120 Olin Hall, Ithaca, NY 14853-5201, Fax: 607-255-9166, pl248@cornell.edu

Programmed ribosomal frameshifting (PRF) is the process by which ribosomes produce two different polypeptides from the same mRNA. A new *in vivo* dual fluorescence reporter system has been developed to study +1 PRF in *Escherichia coli*. Frameshifting sites are inserted between two fluorescence reporter genes, DsRed and EGFP, contained in an *E. coli* expression vector. The red and green fluorescence for different strains are directly measured by 96-well plate reader. The system allows the efficient comparison of frameshifting efficiency for different recoding sites with the normalized fluorescence ratio. The system has been used for studying the effect of E-site on +1 PRF in terms of the codon:anticodon interaction and the stimulatory signals (Shine-Dalgarno-like sequence). The dual-fluorescence reporter system provides a high-throughput and non-invasive *in vivo* assay for +1 PRF studies.

BIOT 369 - Stabilizing protein conformations in solution with osmolytes and cosolvents

Catherine H Schein, Sealy Center for Structural Biology, Biochemistry and Molecular Biology, UTMB Galveston, 301 University Blvd., Galveston, TX 77555-0857, Fax: 409-7476850, chschein@utmb.edu

Proteins in solution typically require co-solvents to maintain activity and solubility. NMR data for VPg from poliovirus (Peptides, 2006, 63:719-26) indicates the natural osmolyte, trimethyl-amine n-oxide (TMAO), works in several ways to stabilize the peptide structure. The first is to lower the rate of proton exchange with solvent. A second is to specifically stabilize the interactions of side chains, and by increasing viscosity, to favor a more compact structure and reduce the mobility of large side chains. Any equation designed to summarize all these effects, i.e., an a priori computational method for predicting the ideal solvent conditions for any given protein, would require a heuristic solution. Molecular dynamics show some promise for simulating effects when a protein structure is known. Recognizing the physico-chemical properties of available co-solvents yields a matrix of solvent conditions to test when beginning the isolation of a novel protein of unknown structure.

BIOT 370 - Highly active enzyme formulations for use in nonaqueous media

Anne L. Serdakowski, Department of Biochemistry and Biophysics, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, serdaa@rpi.edu, John P. Lindsay, Metabolism and Biotransformations, Albany Molecular Research Incorporated, Albany, NY 12203, and Jonathan S. Dordick, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

The mechanism of enzyme activation in nonaqueous media is far from being elucidated. Despite the high degree of activation by lyophilizing an enzyme in the presence of simple salts, very little is known about the structural features on enzymes in the salt preparation in nonaqueous media. For example, it is not known whether salt activation results in an enzyme preparation that is structurally similar to a preparation generated by lyophilizing in the presence of trehalose, a lyoprotectant known to promote retention of native-like secondary structure. This study examines the properties of the enzymatic transition state of salt formulated enzymes through kinetic and structural analysis by focusing on the effect lyophilization has on the secondary structure of an enzyme and how that effect translates into a change in activity. A better understanding of this phenomenon will allow for the direct engineering and formulation of highly active biological catalysts for nonaqueous reactions.

BIOT 371 - Bioactive protein-based hydrogels for functional bioelectrode construction

Ian Wheeldon[1], Scott Calabrese Barton[2], and **Scott Banta**[1]. (1) Chemical Engineering, Columbia University, 500 W 120th St, New York, NY 10027, irw2101@columbia.edu, sbanta@cheme.columbia.edu, (2) Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI

The appropriate design of the enzyme-electrode interface is a key consideration for the creation of new bioelectrochemical systems such as biofuel cells and biosensors. We have created self-assembling bioactive hydrogels through the insertion of functional proteins into previously designed hydrogel-forming peptide systems. Hydrogels containing the green fluorescent protein (GFP) are stronger and more stable the originally designed hydrogels as demonstrated by rheology and timed release experiments. By combining these monomer units in mixtures with the original unmodified peptides, we are able to precisely control the loading of the proteins in the hydrogels. Confocal microscopy images and Forster Resonance Energy Transfer (FRET) experiments show that

these gels are homogenous. We have recently incorporated redox enzymes into these systems as well as redox mediators. This protein-engineering based approach may dramatically simplify the fabrication, characterization, and reproducibility of the bioelectrocatalytic interface employed in future devices.

BIOT 372 - Determining antibody stability: Creation of defined interfacial effects within a high shear environment

James G. Biddlecombe[1], Daniel G. Bracewell[1], Brendan C. Fish[2], and Shahid Uddin[2]. (1) Department of Biochemical Engineering, University College London, Advanced Centre of Biochemical Engineering, Torrington Place, London WC1E 7JE, United Kingdom, j.biddlecombe@ucl.ac.uk, (2) Cambridge Antibody Technology, Cambridge CB21 6GH, United Kingdom

Exposure of antibody to high shear at a solid liquid interface is shown to lead to loss of monomer and aggregation. The purpose of this study was to assess the stability of a monoclonal IgG4, using a rotating disk device designed to generate defined, quantifiable levels of shear in the presence of a solid-liquid interface and to exclude air-liquid interfaces at a controlled temperature. Computational fluid dynamics was used to study the fluid flow patterns and determine the shear strain rate (s^{-1}) at a range of disk speeds. Monomeric antibody concentration and aggregation of the protein in solution were monitored by gel permeation HPLC and turbidity at 350nm. High shear rates were found to cause reduction of protein monomer with exponential decay kinetics, resulting in significant levels of protein aggregation and precipitation. Monomer degradation rate was determined for a range of disk speeds and found to have a non-linear relationship to shear rate.

BIOT 373 - Development and validation of a novel antioxidant capacity assay using sodA::gfp as a living sensor

Wendy A Lea[1], Leah Tolosa[2], and Govind Rao[2]. (1) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, 5200 Westland Blvd., Baltimore, MD 21227, wl1@umbc.edu, (2) Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland at Baltimore County, Baltimore, MD 21250

A novel antioxidant capacity assay was developed based on a reactive oxygen species inducible E. coli strain, which harbored fusion proteins sodA::gfp. These engineered genes report oxidative stress by responding to superoxide anion. Antioxidant capacity was measured by the degree of inhibition of induced fluorescence. At a fixed oxidant concentration, specific fluorescence decreased as the concentration of the antioxidant increased. The assay was validated through linearity, precision and accuracy. There was also a good correlation between the developed assay and currently used standard fruit assays, such as ORAC assay or DPPH assay. The assay was applied to evaluate the antioxidant capacity of some of the common juices. The procedures were easy to follow and cost effective. This assay is able to assess samples with high antioxidant concentrations. Furthermore, since living cells are employed, actual bio-availability is also assessed.

BIOT 374 - Improved solubility and stability of cytochrome c in hydrated ionic liquids

Kyoko Fujita, Department of Biotechnology, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan, Fax: +81-42-388-7467, kyokof@cc.tuat.ac.jp, **Hiroyuki Ohno**, Department of Biotechnology, Tokyo University of Agriculture & Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan, Fax: 81-42-388-7024, ohnoh@cc.tuat.ac.jp, Douglas R MacFarlane, ARC Centre for Electromaterials Science and School of Chemistry, Monash University, Melbourne 3800, Australia, and Maria Forsyth, ARC Centre for Electromaterials Science and Department of Materials Engineering, Monash University, Melbourne 3800, Australia

Hydrated ionic liquids (ILs) were prepared by adding appropriate amount of water to hydrophilic ILs. Some hydrated ILs show excellent solubilizing ability for proteins keeping the basic properties of ILs. The solubility of cytochrome c (cyt. c) depended on the structure of the component ions. When component anions have oxo acid residues, the resulting hydrated ILs solubilize cyt. c quite well. In such hydrated ILs, the structure and activity of cyt. c is influenced by the kosmotropicity of the component ions. The activity of the dissolved cyt. c depends on the permutations of kosmotropicity of the component ions. Cyt. c shows no structural change and retains its activity when dissolved in the hydrated choline dihydrogen phosphate, which is an excellent combination of chaotrope cation and kosmotrope anion. The cyt. c dissolved in this hydrated IL was active even after 18 months storage at room temperature.

BIOT 375 - Surprising results of Pseudomonas aeruginosa heme oxygenase (pa-HO) ortho-19F phenylalanine labeling

An Wang, Department of Chemistry, The University of Kansas, 212 Multidisciplinary Research Building, 2030 Becker Drive, Lawrence, KS 66047, Fax: 785-864-5396, andywang@ku.edu, and Mario Rivera, Department of Chemistry, University of Kansas, Lawrence, KS 66045-7582

Inspection of the pa-HO crystal structure revealed a conserved cluster of Phe residues located near the heme distal pocket. The aromatic cluster plays a key role in maintaining the structure, dynamics and reactivity of pa-HO. Thus the enzyme was labeled with ortho-19F phenylalanine to study the role played by this cluster in the reactivity, stability and dynamics of pa-HO. Ortho-19F-Phenylalanine labeling decreases reactivity as well as increasing protein dynamics while maintaining protein structure intact. Surprisingly, and contrary to the general belief that introducing 19F-Phe will have a negligible influence in protein structure and function, we found that labeling with 19F-Phe alters the dynamic behavior of pa-HO. This in turn, affects its reactivity.

BIOT 376 - Detection of biomolecules by magnetic reporting of labeled protein nanorods

Timothy P Harrah[1], Jin Xie[2], Jeong I Oh[3], Michael J. Naughton[3], Shouheng Sun[2], Robert Guertin[4], and Edward B Goldberg[5]. (1) Department of Biomedical Engineering, Tufts University, c/o Dept

Molecular & Microbiology, A504, 136 Harrison Avenue, Boston, MA 02111, Fax: 617-636-0337, timothy.harrah@tufts.edu, (2) Department of Chemistry, Brown University, Providence, RI 02912, (3) Department of Physics, Boston College, Chestnut Hill, MA 02467, (4) Department of Physics and Astronomy, Tufts University, Medford, MA 02155, (5) Department of Molecular and Microbiology, Tufts University School of Medicine, Boston, MA 02111

Magnetic nanoparticles have recently received increased interest as a feasible basis for the development of rapid and compact biological sensors. Measurement of the AC magnetic susceptibility of nanomagnets is known to yield information about the hydrodynamic behavior of the magnet and has been shown to be an effective way to detect protein binding. Our approach uses sensors constructed of a nanomagnet attached to a rigid protein rod having specific binding site(s) for protein target(s). Target binding events are detected using AC magnetic susceptometry. The protein rod used is derived from the long tail fiber of bacteriophage T4. Purified tail fiber components are a ready source of robust, effectively monodisperse, rod-shaped particles approximately 3nm in diameter and 30 to 150nm in length. Recombinant DNA techniques can be used to create protein rods of different lengths and also to engineer peptide display at specific locations along their length, resulting in a system of nanoscale elements that can be easily produced and site specifically modified. Feasibility data using the anti-v-H-ras antibody-antigen interaction as a model system are presented. This work is supported by a grant from NERCE/BEID (NIAID/NIH).

BIOT 377 - Detection of s-phase cell cycle progression using ethynyl-2'-deoxyuridine incorporation with click chemistry

Suzanne B. Buck[1], Scott T. Clarke[1], Jolene A. Bradford[1], Kyle Gee[1], Brian Agnew[1], and Adrian Salic[2]. (1) Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008, Fax: 541-335-0206, suzanne.buck@invitrogen.com, (2) Harvard Medical School, Boston, MA

Changes in cell proliferation, as measured by incorporation of a nucleoside analog into actively synthesizing DNA, has been the basis for assessing drugs altering or blocking the cell cycle. The standard technique requires incorporation of bromo-deoxyuridine (BrdU) followed by antibody staining. Described is an application of click chemistry, the copper(I)-catalyzed Huisgen [3+2] cycloaddition between a terminal alkyne and an azide, for the detection of S-phase cell cycle progression. Ethynyl-2'-deoxyuridine (EdU) is a nucleoside analog of thymidine which is incorporated into DNA during active DNA synthesis, just like BrdU. The use of this click not only eliminates the need for antibody detection, thereby ridding experimentalists of the need to denature their DNA in order to detect cells in the S-phase of the cell cycle, but also requires less than half the time to conduct and provides the same quality of data as that obtained from the standard arduous protocols.

BIOT 378 - Structure and dynamics of proteins from designed combinatorial libraries

Abigail Go[1], Seho Kim[2], Jean Baum[2], and Michael H. Hecht[1]. (1) Department of Chemistry, Princeton University, Washington Road, Princeton, NJ 08544, ago@princeton.edu, (2) Department of Chemistry, Rutgers University, Piscataway, NJ 08854

Design of proteins *de novo* allows access to diverse, unexplored areas of sequence space. Given the enormity of sequence space, a directed approach is expected to yield a higher fraction of folded and functional proteins than a stochastic sampling of random sequences. One such approach is the design of combinatorial libraries by binary patterning of hydrophobic and hydrophilic amino acids. This approach utilizes hydrophobic collapse to bias protein folding towards specific secondary and tertiary structures. We have previously investigated the possibilities of binary patterning by building a library of *de novo* four-helix bundles. The structure of the most stable protein from the library was solved previously and proved to be consistent with design. However, solving one structure does not fully assess the overall success of the binary patterning strategy, nor does it account for differences in the stabilities of individual proteins. To more fully probe the quality of the binary patterned library, we solved the structure of a second protein, S836, by NMR. Protein S836 proved to be a four-helix bundle consistent with the design. The high similarity of structural features between the two solved structures reinforces previously published experimental evidence that proteins in the library are stable, monomeric, four-helix bundles. Despite their structural similarity and high sequence identity, the two proteins have hydrophobic cores that are packed at different degrees of tightness. This may account for the difference in their stabilities. The relationship between interior packing and protein stability was probed by Modelfree dynamical analysis of both proteins. This analysis showed high frequency of chemical exchange coinciding with less well-packed hydrophobics. We conclude that although the binary patterning may be sufficient to drive protein folding, the overall stability of the fold is moderated by the identity and packing of specific residues in the hydrophobic core.

BIOT 379 - Quantifying the effects of electrostatic forces and microstructure of protein by the change in Gibbs free energy of binding in IMAC

Lakshmi Pathange, Manufacturing Sciences, Bayer Healthcare, Berkeley, CA 94710, lpathang@vt.edu, David Bevan, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, and **Chenming Zhang**, Department of Biological Systems Engineering, Virginia Tech, 800 Dwight Way, Blacksburg, VA 24061, Fax: 540-231-3199, cmzhang@vt.edu

Electrostatic forces play a major role in maintaining both structural and functional properties of proteins. A major component of protein electrostatics is the interactions between its charged or titratable amino acid residues (e.g. His). One way to study the effects of electrostatic forces is by measuring the change in the value of the titratable groups. We have generated ten T4 lysozyme variants, in which the electrostatic environment of the histidine residue was perturbed by altering charged and neutral amino acid residues at various distances from the histidine residue. The electrostatic perturbations were theoretically quantified by

calculating the change in free energy due to the change in electrostatic free energy (deltaE) obtained from simple Coulomb's law. On the other hand, immobilized metal affinity chromatography (IMAC) was used as an experimental tool to quantify these perturbations in terms of protein binding strength or change in free energy of binding due to perturbation. Our results demonstrate a good correlation (R2 = 0.97) between the theoretical and experimental values. NMR experiments were also performed, and the data support the results obtained from IMAC. However, among the ten variants studied, the results from three variants do not follow the trend. The probed histidine residue on these deviant variants was found to be involved in site-specific interactions (e.g. ion pair and steric hindrance), which were further investigated by molecular dynamics simulation. This report demonstrates that IMAC can be used as a simple analytical tool to quantify both electrostatic and microstructural effects in a protein with a surface accessible histidine by measuring its protein binding strength. In addition, IMAC can help in elucidating the unique microstructures around a histidine residue in a protein when abnormal binding behaviors are identified.

BIOT 380 - Engineering of self-assembled vault protein nanocapsules

Marcella Yu[1], Lisa E. Goldsmith[1], Valerie A. Kickhoefer[2], Leonard H. Rome[3], and Harold G. Monbouquette[1]. (1) Chemical and Biomolecular Engineering Department, University of California, Los Angeles, 5531 Boelter Hall, Los Angeles, CA 90095-1592, Fax: 310- 206-4107, marcella@seas.ucla.edu, (2) Department of Biological Chemistry, University of California, Los Angeles, Los Angeles, CA 90095-1737, (3) Department of Biological Chemistry and California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA 90095

Vaults are 40 x 70 nm self-assembled protein nanocapsules, which may be useful for drug/nanomaterial delivery. Although vaults exist in most eukaryotic cells with unknown function, this naturally occurring “organelle” has significant potential as a drug delivery system due to its biocompatibility, large and accessible lumen, and ability to be taken up by mammalian cell lines. We are studying inducible vault conformation changes in solution, covalent modification of vaults, and the controlled loading of material in these nanocapsules. This talk will summarize our current studies of vault nanocapsules in three main areas: (1) Vault conformational change in response to solution conditions; (2) Reversible crosslinking of vaults for controlled vault opening and closing; and (3) Biosynthesis of encapsulated polymer within vaults using an enzyme/vault protein fusion.

BIOT 381 - Enzyme characterization at the nano-bio interface

Jarod L. Kabulski[1], Lance Wollenberg[1], Mingli Yang[2], Nianqiang Nick Wu[2], Timothy S. Tracy[3], David Lederman[4], and Peter M. Gannett[1]. (1) Department of Pharmaceutical and Pharmacological Sciences, West Virginia University, Morgantown, WV 26506-9530, (2) Department of Mechanical and Aerospace Engineering, West Virginia University, Morgantown, WV 26506, (3) Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN 55455, (4) Department of Physics, West Virginia University, Morgantown, WV 26506

Cytochrome P450s represent an important class of heme containing enzymes, responsible for a significant portion of xenobiotic metabolism. There has been interest in immobilizing these enzymes on a surface for studying protein-protein interactions or for construction of chips capable of biosynthesis. P450 kinetics are typically solution-based though it is a membrane-bound protein. The Au-CYP2C9 construct potentially exhibits a system that better represents a membrane-bound protein in its natural environment. However, the methods for studying enzymes, solution-based, are not directly applicable to the construct. To verify attachment and substrate binding activity we have utilized AFM, XPS, SQUID and electrochemical impedance voltammetry. Enzyme metabolic activity of Au-CYP2C9 chips was demonstrated by metabolism of Δ-9-tetrahydrocannibinol and monitored using LC-MS. The work explores the potential for the immobilization of other CYP isoforms on gold substrates, with basic science or industrial applications. (Supported by WVEPSCoR STEM program and NIH GM063215 and GM069753.)

BIOT 382 - Fibrinogen self-assembled fibrils on Clay monolayer templates

Jaseung Koo, Department of Materials Science and Engineering, SUNY at Stony Brook, Old Engineering building, Stony Brook, NY 11794-2275, jkoo@ic.sunysb.edu, Tadanori Koga, Chemical Engineering Program, Department of Materials Science and Engineering, Stony Brook University, Stony Brook, NY 11794-2275, Dennis Galanakis, Department of Pathology, SUNY at Stony Brook, Stony Brook, NY 11794, **Miriam Rafailovich**, Department of Materials Science and Engineering, State University of New York at Stony Brook, Stony Brook, NY 11794-2275, Fax: 631-632-8052, miriam.rafailovich@sunysb.edu, and Jonathan Sokolov, Department of Materials Science, State University of New York, Stony Brook, NY 11794-2275

We obtained unique fibrillary structure of human and bovine fibrinogen on the organoclay Langmuir-Blodgett nano-platelet monolayer using atomic force microscopy (AFM). We prepared two types of clay monolayers, i.e. hydrophilic bare clays (ME-100) and hydrophobic organoclays (MTE), and we found that the polarity of the surface plays an important role in the fibrinogen fibril formation since it affects on the conformational change of fibrinogen molecules at the initial stage. The results show that fibrinogen is gradually assembled into fiber on the MTE surface rather than on the ME-100 surface, which is consistent with the results of the fibrin formation on the surfaces after adding thrombin. We also measured the shear modulus of the fibers to investigate the mechanical property change after thrombin-induced cross-linking of the fibrinogen molecules.

BIOT 383 - Instantaneous distribution of cellular tractions during fibroblast migration

Zhi Pan[1], Yajie Liu[2], Kaustabh Ghosh[3], Toshio Nakamura[2], Richard A. F. Clark[4], and Miriam Rafailovich[1]. (1) Department of Materials Science and Engineering, SUNY at Stony Brook, Stony Brook, NY 11794, zpan@ic.sunysb.edu, (2) Department of Mechanical Engineering, SUNY at Stony Brook, Stony Brook, NY 11794, (3) Harvard Medical School, Harvard University, Boston, MA 02115, (4) Department of Biomedical Engineering,

Dermatology and Medicine, SUNY at Stony Brook, Stony Brook, NY 11794

Cellular traction forces are the physical interaction at cell-substrate interface. They are transmitted to the substrate through focal adhesions, which as they assemble and disassemble generate or release traction forces. This is a dynamic process which directly determines where and how fast cells migrate. Therefore, the instantaneous distribution of cellular tractions is critical to measure in order for us to understand the mechanisms of cell migration. However, the complexity of existing methods for traction force calculation is a major limitation to achieving a precise measurement in real time. Here we demonstrated an effective algorithm to quantify the cellular traction distribution on a 2D surface using optical digital image speckle correlation (DISC) technique in combination with finite element method (FEM). We also constructed an extracellular matrix mimic substrate through cross-linking a thiol-functionalized hyaluronan (HA-DTPH) gel with fibronectin functional domains (FNfds). The cell-substrate adhesiveness could then be controlled by the ligand type or density. We observed the migration of human dermal fibroblasts on these matrices and mapped the corresponding traction distribution during their migration. We found that traction gradients between the front and the rear of the cell directly correlate to cell migration speed rather than the adhesion strength or the magnitude of the traction forces across the whole cell. Furthermore, we found that regardless of the magnitude of the traction forces the cells moved with a nearly constant velocity, which appeared in a pulsed manner, where the pulses were of constant magnitude. We were able to explain this phenomenon by observing a reverse traction gradient across the nucleus, which would increase or decrease, depending on the cell velocity. Hence we concluded that fibroblast migration was a discontinuous process, where the cell could opt to exert a “breaking” force around the nucleus to control its velocity.

BIOT 384 - Integrated platforms for engineered cell-cell communication via cell-directed assembly

Eric C Carnes[1], Carlee E Ashley[1], DeAnna M Lopez[1], Cynthia M Douthit[1], Darren R. Dunphy[2], and C Jeffrey Brinker[2]. (1) Department of Chemical Engineering, University of New Mexico, Advanced Materials Laboratory, 1001 University Blvd. SE, Suite 100, Albuquerque, NM 87106, eccarne@unm.edu, (2) Sandia National Laboratories, Albuquerque, NM 87106

Immobilization of living cells in defined, controllable nano-to-microscale structures is critical for cell-based devices as well as studying cellular physiology. Using our patterning techniques developed for inorganic nanostructures, we can integrate cells into platforms needed for electronic, optical, and spectroscopic interrogation. With this ‘cell-directed assembly' approach (Science, v.313, no.5785, p.337), we are able to create new classes of nominally solid-state materials and devices displaying a symbiotic relationship between the biotic and abiotic components. For example, we present the development of a platform with precise control of cell density and spacing allowing the development and interrogation of cell-to-cell communication networks associated with quorum sensing, biofilm formation, and bacterial virulence. In addition to spatial control of cells and the corresponding effects on communication, we also demonstrate the integration of non-native components to increase functionality of the system, such as nanocrystals for SERS-based sensing and DNA plasmids for patternable, in-situ genetic modification.

BIOT 385 - Nanobiobased electrochemical biosensors for cisplatin detection

John E Jett[1], Nianqiang Nick Wu[2], Huaqing Li[2], and Peter M. Gannett[3]. (1) Department of Basic Pharmaceutical Sciences, (2) Department of Mechanical and Aerospace Engineering, (3) Dept of Pharmaceutical and Pharmacological Sciences West Virginia University, 1 Medical Center Drive, Morgantown, WV 26506, jjett@hsc.wvu.edu

Cisplatin is a common chemotherapeutic drug used to treat such malignancies as lung, bladder, testicular, and ovarian cancer, among others. Following tumor excision, the drug is given as a means to kill any cancerous cells remaining in the tumor bed. The current method of monitoring cisplatin concentration is through blood analysis. However, physicians can only infer from these values the concentration that is being delivered to the site of tumor removal and inter-patient variability in vasculature in and around the tumor bed can account for large differences in delivery rates to this site. The lack of response that occurs in certain patients may be attributed to the fact that equal blood concentrations don't necessarily mean equal tumor bed concentrations. Therefore, the long-term goal of this project is to build an implantable, nanowire-based electrochemical biosensor capable of measuring cisplatin concentration at the site of action in a real-time, concentration-dependent manner.

BIOT 386 - Nanotube-assisted protein inactivation

Amit Joshi[1], Supriya Punyani[1], Theodorian Borca-Tasçiuc[2], and Ravi S. Kane[3]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, (2) Department of Mechanical, Aerospace and, Nuclear Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, (3) The Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

There is currently substantial interest in understanding and controlling protein function on nanomaterials. Carbon nanotubes represent a particularly attractive class of nanomaterials for such investigations, given their unique electrical, optical, and mechanical properties, and the numerous applications of carbon nanotube-protein conjugates. We demonstrate the remote and specific nanotube-mediated deactivation of proteins. We have used this phenomenon in multiple contexts: to design nanotube-peptide conjugates that selectively destroy a target biological toxin – anthrax toxin – and to design nanotube-based "self-cleaning" coatings. Nanotube-assisted inactivation represents a facile strategy for the targeted destruction of proteins, pathogens, and cells, with applications ranging from antifouling coatings to proteomics and novel therapeutics.

BIOT 387 - One pot synthesis and biotinylation of CdS nanocrystals by incorporating intein-mediated protein splicing

U Loi Lao[1], Seung Hyun Kang[2], Ashok Mulchandani[2], and Wilfred Chen[2]. (1) Department of Chemical and Environmental Engineering, University of California, Riverside, Riverside, CA 92521, (2) Department of Chemical and Environmental Engineering, University of California at Riverside, Riverside, CA 92521

Semiconductor nanocrystals, often referred as quantum dots (QDs), have gained extraordinary momentum in biological applications as compelling alternatives to conventional organic fluorescent dyes. Although the current chemical method to produce QDs is relatively robust and reproducible to meet the demand of high quality QDs for biological applications, the involvement of boiling organic solvents raises the safety and environmental concerns. Moreover, the nanocrystal product is not readily water soluble. Additional capping exchange reaction is required which might compromise the quantum yield of QDs. A rising alternative is to utilize evolutionarily selected and engineered peptides to specifically recognize and grow inorganic "building blocks". Unfortunately, this approach does not yield the necessary motif for easy conjugation to biomolecules.

Herein, we present a one-pot biological approach that not only incorporates peptide sequences to direct CdS nanocrystal nucleation and growth but also facilitates the direct conjugation mediated by intein. Biotin-derivatives are used to biofunctionalize the nanocrystals by directly reacting with the thioester moiety generated by the mini-intein gryA. Additionally, the purification of the conjugates is greatly simplified by insertion of an elastin like protein (ELP) domain to take advantage of stimuli-triggered separation. The resultant biotinylated CdS nanocrystals are subjected to fluorescence property characterization and transmission electron microscopy for size distribution. The functionality of the biotinylation of QDs will be confirmed by their affinity toward avidin- functionalized surfaces. The potential applications for immuno-analysis will also be discussed.

BIOT 388 - Phytochelatin-mediated synthesis of cadmium chalcogenide semiconductor nanocrystals

Seung Hyun Kang, Ashok Mulchandani, and Wilfred Chen, Department of Chemical and Environmental Engineering, University of California at Riverside, Riverside, CA 92521

Semiconductor nanocrystals, known as quantum dots, have received significant attention as novel fluorescent dyes in biological field due to their unique optical characteristics. Cadmium chalcogenide quantum dots such as CdS, CdSe or CdTe are widely used for biolabelling. The major route to produce high quality quantum dots is organometallic synthesis using hydrophobic stabilizing ligand, trioctyl phosphine oxide (TOPO), at high temperature. However, since quantum dots should be water-soluble and biocompatible for biological applications, an additional reaction for ligand exchange with hydrophilic ligands like thiols is accompanied. Another way to obtain water-soluble quantum dots is to directly synthesize using hydrophilic ligands in aqueous solution. Although monothiols such as cysteine or glutathione are one of the most popular stabilizing agents, they often fail to stabilize quantum dots. Enhanced stability has been achieved by using other dithiol ligands, dihydrolipoic acid, suggesting that phytochelatins, multithiol peptides, can be a better stabilizer. Our strategy for making semiconductor nanocrystals is to construct a metabolically engineered E. coli synthesizing the cysteine-rich peptides, phytochelatins. In-vitro synthesis using cell extract will be explored for phytochelatin-directed synthesis of cadmium chalcogenide quantum dots.

BIOT 389 - Proteomic characterization of *E. coli* metal binding proteins

Rajaramesh Varakala[1], Ryan Haley[2], **Robert R. Beitle**[1], and Mohammad M Ataai[3]. (1) Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, AR 72701, Fax: 479-575-7926, rvaraka@uark.edu, rbeitle@engr.uark.edu, (2) University of Arkansas, Department of Chemical Engineering, Fayetteville, AR 72701, (3) Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15261

Advances in proteome-describing technologies have allowed for the rapid identification of proteins present in a sample. This poster highlights in detail the use of mass spectroscopy and database analysis to determine the contaminant profile from the *E. coli* genome that are present during Immobilized Metal Affinity Chromatography (IMAC) capture step. Genetic level modifications and manipulation of the IMAC step will be presented that lead to a decrease in the concentration of host contaminants. Thus, the methodology described improves the efficiency of IMAC in terms of column capacity and process time.

BIOT 390 - Screening the transcriptional machinery for potent artificial transcriptional activators

Chinmay Y. Majmudar and Anna K. Mapp, Department of Chemistry, University of Michigan, 930 N University, Ann Arbor, MI 48109-1055, chinmay@umich.edu

Gene-specific transcription relies on a series of events that includes activator proteins recruiting the transcriptional machinery to the gene promoter. These proteins are often mutated and give rise to various diseases; activator artificial transcription factors (activator ATFs) are being developed to replace these malfunctioning activators and to boost gene expression levels for biotechnology applications. Activators are thought to function by making redundant contacts with the transcriptional machinery, complicating the design of activator ATFs. To probe the importance of each interaction, we used a screening strategy to isolate ligands specific for the activator targets, Med15 and Tra1. The functional evaluation of these ligands as activator ATFs in yeast is discussed. We have also used ligand combinations to investigate the functional effect of targeting multiple transcription proteins. Although this work relies on peptidic activators, the screening strategy is easily amenable to small molecule libraries to generate a new class of activator ATFs.

BIOT 391 - Single molecule analysis of DNA/protein interactions: The nanopore shift assay

Meni Wanunu[1], Devorah Cohen-Karmi[2], Yong Yu[1], Zhiping Weng[1], and Amit Meller[3]. (1) Biomedical Engineering, Boston University, 44 Cummington St, Boston, MA 02215, Fax: 617-358-2835, wanunu@bu.edu, (2) Boston University, Boston, MA 02215, (3) Department of Biomedical Engineering, Boston University, Boston, MA 02215

Transcription factors (TFs) are an important class of regulatory proteins for gene expression in living cells. To date, the most common method for analyzing DNA/TF interactions is the electrophoretic mobility shift assay (EMSA), a highly time-consuming method which typically requires radioactive labeling and the production of TF antibodies. We present here a novel, high-throughput approach for analyzing DNA/TF interactions at the single-molecule level. The method involves fabrication of solid-state nanopores of sizes on the order of the DNA cross-section, followed by electrophoretic threading of the DNA molecules through the nanopores in electrolyte solution. Free DNA threads through the nanopore with different mobility than TF-bound DNA molecules, enabling ultra fast screening of TF binding. We present results demonstrating the feasibility of our approach and its application to map the interaction of a number of human TFs with genomic DNA fragments.

BIOT 392 - Targeting gold probes to vault nanocapsule interiors using mINT "shuttles"

Lisa E. Goldsmith[1], Leonard H. Rome[2], and Harold G. Monbouquette[1]. (1) Chemical and Biomolecular Engineering Department, University of California, Los Angeles, 5531 Boelter Hall, Los Angeles, CA 90095-1592, Lisa.Goldsmith@ucla.edu, (2) Department of Biological Chemistry and California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA 90095

Vaults are hollow, ribonucleoprotein capsules (72.5 x 41 x 41 nm) comprised primarily of 96 self-assembled copies of one 97 kDa protein. The biological function for these nanocapsules, which are ubiquitous intracellular components of eukaryotes, is unknown; yet they may prove useful for material entrapment and drug delivery. If vaults are to serve as protective compartments for the transport of drugs, it is necessary to target material to the vault nanocapsule interior in order to optimize drug loading. Current experiments aim to attach Nanogold clusters to the mINT protein, which is known to enter and interact with the vault interior via vault "breathing", therefore "shuttling" the gold inside the vaults. Loaded vaults are separated from unbound probes via immunoprecipitation methods. Successful encapsulation of the gold-mINT complex within vaults may be verified with parallel western blot and silver development (to detect gold) analyses, and by TEM imaging after Nanogold enhancement.

BIOT 393 - The mechanism of arginine interaction with proteins

Kouhei Tsumoto, Department of Medical Genome Sciences, The University of Tokyo, 301 FBS-Bldg, 5-1-5 Kashiwanoha, Kashiwa 277-8562, Japan, Fax: +81-4-7136-3601, tsumoto@k.u-tokyo.ac.jp, Daisuke Ejima, Ajinomoto Inc, Kawasaki 210-8681, Japan, and Tsutomu Arakawa, Alliance Protein Laboratories, Inc, Thousand Oaks, CA 91360

An attempt to was made to understand the mechanism of the wide range of arginine effects on protein-protein interactions. The fact that relatively high concentration of arginine, e.g., at least 0.1 M, is required indicates that weak interaction of arginine with the proteins is involved. Such weak interaction can only be determined by equilibrium technique. Both equilibrium dialysis and amino acid solubility measurements were used to determine the binding of arginine to the proteins. Equilibrium dialysis showed limited amount of arginine binding to the native proteins, as is evident from comparing extensive binding of guanidine hydrochloride. The binding was nevertheless significant, as manifested by deviation from the interaction results of protein-stabilizing and association-enhancing amino acids, which showed no apparent binding to the proteins. On the amino acid level, arginine binding was similar to the binding of guanidine hydrochloride, which showed favorable interactions with a majority of amino acid side chains. We propose that while arginine has potential to favorably interact with both non-polar and polar amino acid side chains, the binding of arginine to the proteins is highly restricted, which may be responsible for the observed effects on proteins as an aggregation suppressor, but not as a protein denaturant. Application of arginine to several biotechnological methods, including chromatography procedures, would be described.

BIOT 394 - Ultrafast sensing platform based on surface plasmon-coupled emission

Derek S. Smith, Yordan Kostov, Ramachandram Badugu, and Govind Rao, Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland at Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, Fax: 410-455-6500, dsmith4@umbc.edu

Conventional fluorescence-based sensors typically have thick sensing matrices that have diffusion-limited response times. Thick layers are necessary due to poor collection efficiency of the isotropic fluorescence observed under free space conditions. In order to achieve ultra-fast response times, fluorescence observation from ultra-thin sensing layers, even surface bound probes, is required. However, this is difficult without expensive equipment. High sensitivity detection can be simplified via the phenomenon of surface plasmon-coupled emission (SPCE). By modifying the free space condition with nanometer thick metal films, SPCE directional fluorescence can be observed enhancing the collection efficiency when compared to conventional fluorescence observation. This allows for the detection of fluorescence from nanometer-thick sensing layers. Consequently, SPCE-based sensors have rapid, non-diffusion limited response times based solely on the analyte-probe interaction. The schematic for SPCE-based sensors will be discussed along with several practical examples including rapid oxygen and pH sensing.

BIOT 395 - A new strategy for acylation of tRNA with unnatural substrates

Hiroki Masu, Masayoshi Hayami, Shinsuke Sando, and Yasuhiro Aoyama, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University,

Katsura, Nishikyoku, Kyoto 615-8510, Japan, Fax: +81-75-383-2767, masuzo1112@yahoo.co.jp

There has been much recent challenge on applying the ribosomal decoding system for the synthesis of unnatural molecules or biopolymers. Fully purified translation system has enabled a rewriting of the codon table with unnatural substrates beyond the twenty canonical amino acids. Chemical misacylation of tRNA has been widely used to attach unnatural substrates at 3'-terminus of tRNA. This method has an advantage in that a variety of substrates can be misacylated on tRNA without the limitation of its structure. However, enzymatic aminoacylation of tRNA by aminoacyl-tRNA synthetases (aaRS) is much more suitable for multiple incorporations of unnatural substrates, as revealed by protein synthesis in naturally occurring translation system. Here we show a new strategy to expand the acceptable substrates of aaRS. Our approach enables to enzymatically acylate unnatural substrates onto tRNA.

BIOT 396 - Direct measurement of binding interactions among split inteins

Mirco Sorci[1], Bareket Dassa[2], Gaurav Anand[1], Shmuel Pietrokovski[3], and Georges Belfort[4]. (1) Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, sorcim@rpi.edu, (2) Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel, (3) Weizmann Institute of Science, Rehovot, Israel, (4) Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590

Inteins are well-known self-splicing and self-cleavage protein elements. They exist in nature as whole or split inteins. Split inteins are inactive but exhibit high-affinity for each other and can rapidly assemble and refold into an active intein. How split inteins find their partners and combine into an active splicing intein which can remove itself and ligate flanking peptides or proteins is of fundamental interest. Simulation molecular modeling of this process is in progress. However, what is lacking is experimental measurements of the binding energies between separate split inteins. Atomic Force Microscopy (AFM) is a relatively new tool used to investigate structure and biological function of biological molecules. AFM is well known for its high resolution imaging capabilities, but it is also a powerful technique for intermolecular force measurements. Furthermore, the possibility to coat the cantilever tips and substrates with different materials, allows one to study the interaction between different combinations of bound molecules on each surface. In this work we have used AFM in force-mode to analyze the interaction between three naturally split-inteins from diverse cyanobacteria: Oscillaria limnetica, Thermosynechococcus vulcanus and Nostoc species PCC7120. The binding energy measurements show that endogenous pairs of split inteins exhibit twice the binding energies than do exogenous pairs.

BIOT 397 - Development of a novel method for direct nanopatterning of bacteriophage using dip-pen nanolithography

Shaun F Filocamo, Macromolecular Sciences Team, Natick Soldier Research, Development and Engineering Center (NSRDEC), Natick, MA 01760, shaun.filocamo@us.army.mil, Chung-Yi Chiang, Department of Materials Science & Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, Angela M. Belcher, Department of Materials Science and Engineering and Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, and Charlene M. Mello, Macromolecular Science Team, Natick Soldier Research, Development and Engineering Center (NSRDEC), Natick, MA 01760

Nanoscale organization of biological macromolecules, such as viruses, has been accomplished mainly through affinity binding onto prepatterned substrates. Direct patterning would eliminate the need for prepatterning, provide a mechanism for controlling the orientation of biological macromolecules on the surface, and present a template for multi-dimensional nanopatterning using these macromolecules as building blocks. Dip-pen nanolithography (DPN) has demonstrated this level of patterning control on a variety of materials, and is amenable to many biological macromolecules. We are utilizing DPN and a M13 bacteriophage "ink" solution to form nanopatterns on inorganic substrates, in which the bacteriophage has been genetically-modified at the pVIII coat protein to preferentially bind Au. The bacteriophage solution and DPN conditions are currently being optimized for maximal control over nanopattern formation. Optical and electronic analyses will be performed on these inorganic-biological composite nanowires to determine future application directions. Progress towards these goals will be presented.

BIOT 398 - Facile and rapid direct gold surface immobilization with controlled orientation for diverse carbohydrate types

Jeong Hyun Seo[1], Dong Gyun Kang[1], Yeon Kyu Kim[1], Kyoung Ro Kim[1], Hea Yeon Lee[2], and Hyung Joon Cha[1]. (1) Department of Chemical Engineering, POSTECH (Pohang University of Science and Technology), Pohang 790-784, South Korea, Fax: 82-54-279-5528, nexist9@postech.ac.kr, (2) The Institute of Scientific and Industrial Research, Osaka University, Osaka 567-0047, Japan

Carbohydrates encode information for specific molecular recognition, help determine protein folding, stability, and pharmacokinetics, play critical roles in determining biological functions, and affect diverse physiological processes. In addition, carbohydrate-protein interactions have been used to elucidate fundamental biochemical processes and identify new pharmaceutical substances in living cell systems. We herein demonstrated that the amine group of thiol-bearing aminophenyl disulfide was successfully coupled with the aldehyde group of the terminal reducing sugar in three types of carbohydrates, namely glucose (monosaccharide), lactose (disaccharide), and GM1 pentasaccharide. The proposed modification method has several advantages over previously reported methods, including direct and rapid one-step immobilization onto a gold surface without surface pretreatment(s) by thiol group coupling in mild reaction environment, and exposure of functional carbohydrate moieties through oriented immobilization of the terminal reducing sugar. This novel modification and immobilization method should prove useful for diverse biomimetic studies in carbohydrates, including carbohydrate-biomolecule interaction and carbohydrate sensor or array development for diagnosis and screening.

BIOT 399 - Impact of target mRNA structure on silencing efficiency and immune stimulation in RNA interference

Joseph A. Gredell, Angela Berger, and **S. Patrick Walton**, Department of Chemical Engineering and Materials Science, Michigan State University, Michigan State University, 2527 Engineering Building, East Lansing, MI 48824, Fax: 517-432-1105, gredellj@msu.edu, spwalton@egr.msu.edu

RNA interference (RNAi) is mediated by short interfering RNAs (siRNAs) and results in potent gene silencing. RNAi efficiency has been shown to depend on the characteristics of the siRNAs and on the target mRNA structure. In mammalian cells, siRNA duplex features have also been implicated in activating an innate immune response. Our goal is to define design rules for siRNAs accounting for target mRNA structure that predict silencing efficiency and/or immune stimulation. We describe our findings that siRNAs targeting different predicted structures of the enhanced green fluorescent protein transcript differ in their silencing efficiencies. Furthermore, the correlation between mRNA structure accessibility and silencing efficiency agrees well with the current understanding of biased siRNA strand loading into the RNA-induced silencing complex. We will also discuss the structural bases of immune activation as mediated by Protein Kinase R and Toll-like Receptor 3. These results have broad implications for experiments employing RNA interference.

BIOT 400 - Influence of α-gliadin and poly(hydroxyethyl methacrylate-*co*-4-styrenesulfonic acid) concentrations on the structure of α-gliadin

Li Liang, Department of Food Science and Nutrition, University Laval, Pavillon Paul Comtois, Quebec, QC G1K 7P4, Canada, liliang_bm@yahoo.com, Jean-Christophe Leroux, Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Montreal, QC H3C 3J7, Canada, and Muriel Subirade, Canada Research Chair in proteins, bio-systems and functional food, Department of Food Science and Nutrition, University Laval, Quebec, QC G1K 7P4, Canada

α-Gliadin, a protein fraction of wheat, involved in celiac disease, can be efficiently complexed by the copolymer of hydroxyethyl methacrylate and 4-styrenesulfonic acid, poly(HEMA-*co*-SSt), currently investigated for the treatment of celiac disease. In this work, the influence of α-gliadin and poly(HEMA-*co*-SSt) (43:57 mol%) concentrations on the structure of α-gliadin was studied using circular dichroism and fluorescence. The experiments were conducted in 1) 70% ethanol; 2) aqueous solution of pH 1.2 and 3) 0.1 M acetic acid. The results showed that the secondary structure changed from α-helix to β-turn when its concentration increased, possibly due to the self-association of the protein. At pH 1.2, poly(HEMA-*co*-SSt) induced the structural transition of the protein from β-turn to α-helix with a change in polymer concentration. Hence, the influence of poly(HEMA-*co*-SSt) on the structure of α-gliadin can simulate that of α-gliadin concentration on its structure.

BIOT 401 - Noncovalent immobilization of proteins on a solid surface by cucurbit[7]uril-ferrocenemethylammonium pair, a potential replacement of biotin-avidin pair

Kimoon Kim, Ilha Hwang, **Kangkyun Baek**, Minseon Jung, Youngkook Kim, Kyeng Min Park, Don-Wook Lee, and Narayanan Selvapalam, Department of Chemistry, National Creative Research Initiative Center for Smart Supramolecules, Pohang University of Science and Technology, San 31 Hyojadong, Pohang 790-784, South Korea, Fax: 82-54-279-8129, kkim@postech.ac.kr, uranus@postech.ac.kr

A novel noncovalent method to immobilize a protein on a solid surface using the cucurbit[7]uril (CB[7])-ferrocenemethylammonium ion (FA) pair, which exhibits exceptionally high binding affinity ($K \sim 10^{12}\ M^{-1}$), is reported. This method involves (1) anchoring CB[7] units on an alkanethiolate self-assembled monolayer (SAM) on gold, (2) attachment of FA units to a protein to be immobilized, and (3) immobilization of the "ferrocenylated" protein to the CB[7]-attached SAM on gold. As a proof of concept, the immobilization of ferrocenylated glucose oxidase (GOx) on a CB[7]-anchored gold substrate, and its use as a glucose sensor were demonstrated. In principle, this approach can be applied to the immobilization of any biomolecules including nucleic acids on any surfaces including glass, silicon, silica and polymers. The synthetic host-guest pair with exceptional affinity, chemical robustness, simple preparation, and easy handling may replace the biotin-avidin system not only in the immobilization of biomolecules on solid surfaces, but also in other applications such as affinity chromatography and immunoassay.

BIOT 402 - Research of BODIPY fluorescence dyes-NHS derivates with high biochemical activity

Wang Dongchuan, **Peng Xiaojun**, and Fan Jiangli, State Key Laboratory of Fine Chemicals, Dalian University of Technology, P.R. China, 158 Zhongshan Road, Dalian 116012, China, Fax: 086-411-8899-3906, wdong818@163.com, pengxj@dlut.edu.cn

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (boron-dipyrromethene, BDP or BODIPY) based fluorescence dyes have attracted a lot of attention in most recent years, for their high fluorescence quantum yields (&Phi f), relatively high molar absorption coefficients (&epsilon), good photostability, insensitivity towards solvent and pH, narrower spectrum in absorption and emission, and amenable to structural modification et al. This kind of dyes is suitable for probing the anions, cations and small molecules, and was applied to the biosensor, nucleic acid detection, pathological analysis and cell imaging also widely in present work. This research was undertaken to obtain a series of new BODIPY dyes' derivatives by substituted aldehydes or carboxylic acid anhydrides and pyrroles, which were further treated with N-hydroxysuccinimide. As we all known, fluorescence detection is widely used in analysis field for its high sensitivity and efficiency. One-pot reaction was applied and obtained the carboxylic BODIPY dyes in relatively high yield with different conditions. What's more, the activated BODIPY-NHS esters can be further used in biochemical analysis by its fluorescence spectrum properties. It should be noted that, the BODIPY dyes are well proper probes for investigating dye-DNA

interactions, More research work has been taken on application in protein-labeling, such as BSA(bovine serum albumin) and so on.

BIOT 403 - Sensing of nucleic acid sequence using unmodified DNA/RNA as a probe

Atsushi Narita, Shinsuke Sando, and Yasuhiro Aoyama, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyoku, Kyoto 615-8510, Japan, Fax: +81-75-383-2767, narita@sbchem.kyoto-u.ac.jp

As the number of identified genetic information continues to grow, there has been much current interest in rapid and simple gene sensing with a high selectivity. Among various homogeneous sensing methods so far reported, several new strategies have enabled sensing of target sequence using an unmodified nucleic acid as a probe. We report a new strategy of molecular beacon-mRNA for sensing genotyping, which was designed based on the system of naturally occurring or engineered hairpin-shaped RNAs for a control of translation frequency, so that the sensing of nucleic acid sequence, even at single nucleotide resolution, can be carried out using a genetically-encodable unmodified RNA as a probe. The other sensing approach using an unmodified nucleic acid probe will also be discussed.

BIOT 404 - Sterilizable optical sensor for alcohol measurements

Yordan Kostov and Govind Rao, Center for Advanced Sensor Technology, Department of Chemical and Biochemical Engineering, University of Maryland at Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, kostov@umbc.edu

The need for low-cost, robust alcohol sensors has increased with the renewed interest in alternative fuels. They are needed for monitoring and control of ethanol production bioprocesses, as feedback sensors in methano/ethanol fuel cells, as well as for fuel identification in the FlexFuel cars. In this work, two fluorescence ethanol sensors based on immobilization of Nile Blue in poly(ethylene glycol) dimethacrylate are presented. The first one employs physical immobilization of the dye in the hydrogel, while the second one uses covalent bonding. When in solution, the dye exhibits a single fluorescence peak. Surprisingly, a dual emission peak is observed upon immobilization of the dye in the hydrogel. The sensor also exhibits well defined isobestic point. The sensitivity, alcohol selectivity, response time, and cross-sensitivity to pH, polarity, and ionic strength of the sensor were investigated. The sensor has very broad range – 0 to 100%v/v. Due to the hydrogel's restrictive pore size, the sensor is sensitive to short-chain alcohols such as methanol, ethanol, and propanol, but does not respond to longer-chain alcohols, such as butanol and hexanol. The sensor maintains full functionality after autoclaving. Sensor sensitivity to alcohol in solutions of varying ionic strength is negligible, whereas the solvent's polarity must be controlled to maintain meaningful results. The sensor is most sensitive in acidic and neutral environments, indicating promising use for yeast-based alcohol fermentations or for monitoring solutions for fuel-cell applications.

BIOT 405 - Tyrosinase immobilization on carbon nanoparticles

Kyoung Seon Min, **Jae Yeon Park**, Jeong Soo Cho, and Young Je Yoo, School of Chemical and Biological Engineering, Seoul National University, San 56-1, Shillim-dong, Gwanak-gu, Seoul 151-744, South Korea, Fax: 82-2-887-1659, min4605@hanmail.net, albert7eng@snu.ac.kr

Enzymes are highly selective and efficient natural catalysts because of their substrate specificity, mild operating conditions, and reduced waste. For large scale applications in many cases, enzymes need to be immobilized. Recently, the combination of nano-scaled materials and enzymes is the focus of interest in the biotechnology due to incredibly large surface area of nanomaterials for immobilization. Although many papers related to immobilization of enzymes on nanomaterials have been published for last few years, there is little report analyzing how much enzyme is immobilized quantitatively and what mechanism governs the immobilization process. Tyrosinase was selected as a model enzyme and it was immobilized onto a carbon nanoparticle. The maximum amount of enzyme was obtained by introducing covalent bonds between enzyme and nanoparticle. The amount of enzyme immobilized was the highest one ever reported. The details including an immobilization mechanism, a long-term stability, and a comparison with simple adsorption will be presented.

BIOT 406 - Evaluation of UVC reactors using photochemically active fluorescent microspheres

Amanda Lewis, Purification Process Development, Amgen, 4000 Nelson Road, AC24A, Longmont, CO 80503, Fax: 303-401-4402, alewis@amgen.com

Treatment of fluid with short wavelength ultraviolet light (UVC) has been shown to be an effective method for inactivating infectious agents such as bacteria and viruses. Treatment efficacy is a function of device design and is strongly influenced by parameters such as mixing, fluid depth and the optical properties of the fluid. UVC treatment has been exploited in a number of industries, such as water treatment and food processing, to reduce the risk of potential pathogen transmission. However, application of UVC to the biotechnology industry has been minimal, with the exception of air and water treatment applications. Broader application of UVC technology in the biotechnology industry will likely require devices that produce a consistent, narrow residence time distribution to ensure homogeneous exposure of potential pathogens and therefore consistent pathogen inactivation. We examine 3 different UVC devices for their performance characteristics with respect to fluency distribution, which is a function of residence time distribution, using fluorescent microspheres capable of photobleaching on UVC exposure. The fluency distribution for each device was calculated using the post-treatment changes in the fluorescence distribution of the beads, correlated with a standard curve generated using a collimated UV beam device. Device performance and design differences will be discussed.

BIOT 407 - Deregulation of CAP dependent translation can play a role in the malignant transformation of eukaryotes

Satyaprakash Nayak, Jalal K Siddiqui, and Jeffrey D. Varner, School of Chemical and Biomolecular Engineering, Cornell University, 244 Olin Hall, Cornell University, Ithaca, NY 14853, sn248@cornell.edu

Cap dependent translation processes greater than 95% of mammalian mRNA and is closely linked with cell cycle progression. In this study, we explore the hypothesis that deregulation of Cap dependent translation is a pathway to malignancy. A comprehensive mathematical model of cap dependent translation in mammalian cells was formulated that includes both the positive regulation by TOR kinase and the PI3K/PTEN/AKT pathway. Model parameters were selected to qualitatively mimic physiological time profiles taken from literature. A monte-carlo sensitivity analysis was preformed where Overall State Sensitivity Coefficients (OSSC) were calculated over a family of random parameters constructed by perturbing the nominal set. The fragile mechanisms predicted by the sensitivity analysis were consistent with known biology indicating that monte-carlo sensitivity analysis can provide qualitative insights into network function, despite model uncertainty.

BIOT 408 - Identifying toxicity pathways through analysis of global transcriptional dynamics

Zheng Li, Chemical Engineering and Materials Science Department, Michigan State University, 2527 Engineering building, East Lansing, MI 48824, Fax: 517-432-1105, lizheng1@msu.edu, Shireesh Srivastava, Dept of Chemical Engineering and Material Science, Michigan State University, East Lansing, MI 48824, and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824

The mechanism of free fatty acid (FFA) and tumor necrosis factor (TNF)-a induced cytotoxicity was studied in this paper using a transcriptional dynamic analysis. A global functional gene mapping analysis [1] was applied to the cDNA microarray data (15 conditions*4 replicates) at three time points (24, 48, 72 hours of exposure to FFA). It was found that unsaturated free fatty acids exerted transcriptional regulation mainly within the first 24 hours, while saturated free fatty acid, palmitate, regulated metabolic pathways such as the electron transport chain and TCA cycle within the first 24 hours. In the next 24 hours palmitate upregulated 36 cell death relevant signaling pathways and downregulated several protective pathways such as the PPP and glutathione pathways. In the final 24 hours the free fatty acids did not induce significant transcriptional regulation. Therefore we hypothesized that palmitate induced cytotoxicity by first disturbing the metabolic pathways to induce changes in metabolites or signaling molecules, which subsequently triggered cell death relevant signaling pathways on day 2. To further elucidate the metabolite changes, we measured 27 metabolites and applied metabolic flux analysis to obtain the intracellular metabolic flux profiles. Altered metabolic fluxes were identified in the palmitate as compared with the control and unsaturated free fatty acid conditions. We inferred the interactions between the metabolites and the gene regulatory pathways triggered by palmitate in the first and second 24 hours by integrating the gene expression profiles, metabolic data with literature information using Bayesian network analysis. Reference: 1. Segal E. et al. (2004) A module map showing conditional activity of expression modules in caner, Nature Genetics, 36 (10):1090-1098.

BIOT 409 - Systems analysis of cellular development and apoptosis in taxus cell lines

Ying-Jin Yuan, Department of Pharmaceutical Engineering, School of Chemical Engineering and Technology, Tianjin University, Weijin Road 92#, Nankai district, Tianjin 300072, China, Fax: 86-22-27403888, yjyuan@tju.edu.cn

LC/ESI/MSn and GC-TOF tools were employed to investigate the changes in metabolites and membrane phospholipids during the procession of cellular development and apoptosis of plant cell lines, Taxus cuspidata and Taxus chinensis var. mairei. The results showed that Phosphatidic acid (PA),phosphatidylcholine (PC) and lysophosphatidylcholine (LysoPC) were three important lipid groups that were responsible for the discrimination between the apoptotic cells and normal cells. The results suggest that the alternation of membrane phospholipids might regulate apoptosis, triggering an increase in taxol production of T. chinensis var. mairei cells.

BIOT 410 - Unsupervised classification and identification of physiologically relevant features by integrating ontology information and expression data using Bayesian regression mixture models

Shireesh Srivastava, Dept of Chemical Engineering and Material Science, Michigan State University, 2527 Engineering Building, East Lansing, MI 48824, Fax: 517-432-1105, srivas14@egr.msu.edu, Rong Jin, Dept of Computer Science and Engineering, Michigan State University, East Lansing, MI 48824, and Christina Chan, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824

Here, we present a method that integrates the gene ontology (GO) information and expression data using Bayesian regression mixture models to perform unsupervised classification of multiple subpopulations and identify functionally relevant genes regulating desired responses of the cells. This method offers many advantages over current methods. For example, it allows one to regulate the extent of incorporation of prior information, which is essential for optimal classification and has the ability to simultaneously analyze multiple cellular responses and classify multiple sub-populations. As a model application, we applied this method to identify the genes that regulate the cytotoxicity of saturated fatty acid (SFA) and TNF-alpha to human hepatoblastoma cell line (HepG2). Adenylate cyclase 9 and lysosomal ATPases were identified as two most important genes which separated the cytotoxic from the non-toxic phenotypes. The important roles of these genes in the cytotoxicity of SFA were verified based on available literature and experimentally.

BIOT 411 - Coculture of human embryonic stem cells with murine embryonic fibroblasts on microwell patterned substrates

Ali Khademhosseini[1], Lino Ferreira[1], James Blumling[1], Judy Yeh[1], Jeffrey M. Karp[1], Junji Junji Fukuda[1], and Robert Langer[2]. (1) Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, alik@mit.edu, (2) Department of Chemical Engineering and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139

Human embryonic stem (hES) cells are a potentially valuable source of cells for regenerative medicine. These cells are generally expanded on top of a feeder layer of mitotically inactivated feeders (MEFs). This co-culture system presents several challenges since large cell clusters tend to differentiate at the borders, and clusters with different sizes may lead to heterogeneous differentiation patterns within embryoid bodies. We present an approach to culture hES cells with controlled cluster size and number. Polymeric microwells were fabricated and used to control the size and uniformity of hES cell clusters in co-culture with MEFs. The results show that it is possible to culture hES cells in patterned co-cultures while keeping their undifferentiated state as confirmed by the expression of stem cell markers Oct-4 and ALP. In addition, these clusters can be recovered from the microwells to generate homogeneous aggregates for differentiation experiments.

BIOT 412 - An imaging chamber for cultures of human islets of Langerhans

Duane Moogk[1], Stephen Hanley[2], John Ramunas[1], A. Blaylock[1], Jana Skorepova[1], Lawrence Rosenberg[2], and Eric Jervis[1]. (1) Department of Chemical Engineering, University of Waterloo, 200 University Ave. West, Waterloo, ON N2L 3G1, Canada, ericjj@cape.uwaterloo.ca, (2) Department of Surgery, McGill University, Montréal, QC H3G 1A4, Canada

A means of expanding islet cell mass is urgently needed to supplement the limited availability of donor islets of Langerhans for transplant. Live cell imaging of human islets in culture has the potential to identify specific cells and processes involved in islet expansion. An imaging chamber was developed to facilitate long-term three-dimensional imaging of human islets during transformation to duct-like cystic structures – allowing us to construct a high resolution recorded history of each cell. A mathematical model was developed to validate the chamber design by determining chamber dimensions required to avoid oxygen and glucose transport limitations. Differential interference contrast time-course images were obtained of human islets. Immunofluorescent imaging confirmed that islet phenotype can be maintained in the chamber environment. Islet transformation was induced and the ability to identify and track individual cells and to observe cell death and phenotype transformation was confirmed.

BIOT 413 - System to study colony-colony interactions in murine embryonic stem cells

Somponnat Sampattavanich, Harvard-MIT Division of Health Science and Technology, Department of Electrical Engineering and Computer Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue Bld.36-854, Cambridge, MA 02139, ssampat4@mit.edu, and Joel Voldman, Department of Electrical Engineering and Computer Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

Many efforts have been made to characterize the necessary regulatory factors involved in self-renewal of embryonic stem cells (ESCs). Recent studies showed that self-renewal and proliferation of murine embryonic stem cells (mESCs) are affected by autoregulatory signalings intrinsic to the cells, predominantly in serum-free cultivating conditions. These observations were investigated based on a comparison of different groups of mESCs that were plated at varying cell densities. To study these interactions in a better-controlled fashion, we have developed a system to study the role of colony-colony interactions in mESCs. We have used stencil cell patterning to precisely localize mESC colonies on the culture substrate. This technique allows the formation of mESC colonies with precise shape and controllable inter-colony distances. To automate phenotype analysis, we have developed image analysis algorithms to analyze colony morphology and motility, along with a method for registering images to each other over space and time. We are using this system to investigate the role of colony-colony interactions in stem cell self-renewal.

BIOT 414 - Effects of fiber alignment on the behavior of human dermal fibroblasts on the 3-D Poly (methyl methacrylate) (PMMA) electrospun scaffold

Ying Liu[1], Yuan Ji[1], Kaustabh Ghosh[2], Richard AF. Clark[2], Jonathan C. Sokolov[1], and Miriam H. Rafailovich[1]. (1) Department of Material Science and Engineering, SUNY at Stony Brook, Stony Brook, NY 11794-2275, (2) Department of Biomedical Engineering, SUNY at Stony Brook, Stony Brook, NY 11794-8181

The development of tissue engineering requires the biomaterial scaffolds not only support cell growth, but also regulate cell spreading and orientation. To test the response of adult human dermal fibroblasts (AHDFs) to PMMA fibers, PMMA fiber meshes were electrospun under conditions that produced mean fiber diameters of 0.16-8.64μm onto rigid support. Examination of cell morphology revealed Compared to flatter and dendritic structures on the nano-scale fibers, AHDF cells oriented well on the micro-scale fibers.Then the rectangle aligned PMMA scaffolds were fabricated. Their bulk architecture and mechanical property was compared with randomly aligned scaffold. The interaction between ADHF and the two scaffolds such as attachment and proliferation were compared with cells on the spin cast film. The results came out as the rate of fibroblast differentiation was independence of fiber alignment, however, the substrates influenced the distribution of the cells focal adhesion points and thus the distribution of the actin filament in the cell body; therefore, they influence the stiffness of the cells on the substrates. These studies show that topographical factors designed into biomaterial scaffolds can regulate spreading, orientation, and proliferation of AHDF cells.

BIOT 415 - Hill colonies, a putative endothelial progenitor cell derived population, are non-clonal and result from aggregation of multiple cell types as determined using long term imaging

C. S. Russell[1], A. Blaylock[2], J. Ramunas[2], L. Kelly[2], E. Clarke[1], C. G. Pereira[1], A. W. Wognum[1], T. Thomas[1], A. Eaves[1], and Eric Jervis[2]. (1) StemCell Technologies Inc, Vancouver, BC, Canada, ericjj@cape.uwaterloo.ca, (2) Department of Chemical Engineering, University of Waterloo

Prior work by others has shown that readily identifiable colonies ("Hill" colonies) that arise in cultures of peripheral blood correlate with cardiovascular risk and function – leading to the hypothesis that endothelial progenitor cells are involved in Hill colony formation. However, little was known about the mechanism and dynamics of colony formation. Large-field long term live cell imaging was used to investigate colony formation. Two distinct cell types were identified: abundant, small, fast-moving podiated cells and less abundant, adherent spindle-shaped cells. Enumeration of spindle shaped cells indicated positive correlation with Hill colony formation. However it was not clear whether the spindle shaped cells originated from the colonies or migrated towards the Hill colonies. Long term imaging studies showed that transient podiated cell aggregates are stabilized when spindle cells appear in the culture, showing that the timing of cell differentiation and migration is important in the formation of these mixed cell colonies.

BIOT 416 - Long-term expansion of embryonic stem cells in serum-free media containing plant protein hydrolysates

Ning Liu and Shang-Tian Yang, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Avenue, columbus, OH 43210, Liu.681@osu.edu

To use ES cells for clinical purposes, a reproducible and well defined culture system free of animal products is required. Several low-cost plant protein hydrolysates, including hydrolyzed wheat protein, soy protein, and hydrolyzed cottonseed were tested as substitutes for FBS in culture media, and their effects on the growth of ES cells cultured in three-dimensional (3D) fibrous scaffolds in microbioreactors were screened. A serum-free medium containing plant protein hydrolysates was then used in culturing ES cells in a fibrous bed bioreactor (FBB). It was found that the serum-free medium with hydrolysates as additives could be used for mass production of undifferentiated ES cells in the FBB. The ES cells produced from this process maintained a high pluripotency. Repeated batch and perfusion culture systems were also developed to further improve the long-term expansion of ES cells in the serum-free culture system.

BIOT 417 - Application of centrifugal force to enhance cell seeding in tissue engineering scaffold

Robin Ng[1], Xudong Zhang[2], and Shang-Tian Yang[2]. (1) Department of Chemical Engineering, Ohio State University, 140 West 19th Ave., Koffolt Lab, Columbus, OH 43210, Fax: 614-292-3769, ng.128@osu.edu, (2) Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210

Tissue engineering requires culture of cells in a three-dimensional pattern. Seeding anchorage-dependent cells to support matrices is one of the most important steps in tissue engineering. In this study, a novel centrifugal seeding method was investigated to improve the cell seeding efficiency. Two different scaffolds were studied: a 93% and a 88% porosity. Compared to other methods, our seeding method achieved a high 90% seeding efficiency in 3 - 5 minutes. Although centrifugal force and time affected seeding efficiency, the seeding efficiency was not affected by the scaffold porosity. The maximum cell number entrapped in 100 ƒÝL scaffold was 0.7 million cells. Post seeding cell viability was similar to those before centrifugation. The cells proliferated for over five days in multi wells. This novel centrifugal seeding method offers a higher seeding efficiency at a relatively short time, which is an advantageous in tissue engineering and 3-D cell culture.

BIOT 418 - Biomechanical properties of tissue engineered cartilage

Ferenc Horkay[1], David C. Lin[1], Iren Horkayne-Szakaly[1], Emilios K. Dimitriadis[2], Candida Silva[1], and Peter J. Basser[1]. (1) Laboratory of Integrative and Medical Biophysics, National Institutes of Health, NICHD, 13 South Drive, Bethesda, MD 20892, horkay@helix.nih.gov, (2) Instrumentation and Research Development Resource, Division of Bioengineering and Physical Science, ORS, National Institutes of Health, Bethesda, MD 20892

The physical properties of articular cartilage depend on the structure and organization of the macromolecules in the extracellular matrix. The collagen gives it tensile strength and produces a tissue that is not only strong in tension but also resistant to compression. The tensile strength of the collagen network balances the swelling pressure of the charged glycosaminoglycan molecules enmeshed in the collagen network. Our emphasis is on new experimental techniques in testing mechanical and osmotic properties of cartilage. We constructed a tissue micro-osmometer to measure the swelling pressure of very small tissue samples. We developed a high-throughput automated method to generate high-resolution elastic modulus maps based on force measurements made by the atomic force microscope. It will be demonstrated that results obtained by biophysical and biochemical techniques for tissue engineered cartilage specimens shed light on the function of the major polymeric constituents of the cartilage matrix.

BIOT 419 - Characterization and isolation of neurons differentiated from mouse ES cells by DRG-conditioned medium

Ayako Kitazawa and Norio Shimizu, Graduate School of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Ora-gun, Gunma 374-0193, Japan, Fax: +81-276-82-9210, gx0300046@toyonet.toyo.ac.jp

The *in vitro* differentiation of mouse embryonic stem (ES) cells was expected new perspectives for studying the potential applications in neuroscience and regenerative medicine. Therefore, experiments have been carried out to control the differentiation of ES cells into neurons and isolate neurons from a variety of

differentiated cells. We previously reported the differentiation of ES cells into neurons using chick dorsal root ganglia (DRG) conditioned medium (CM). We investigated to characterize the types of neurons differentiated from ES cells by the addition of DRG-CM. Neurons differentiated from ES cells were mainly motor neurons, GABAergic neurons, and serotonergic neurons. Especially, the proportion of motor neurons appeared to be 40-60% of neurons. We investigated to isolate neurons from a variety of differentiated cells using nanoscale magnetic beads coated with nerve growth factor and an 11-mm magnet. Neurons were collected in the particular area of a culture plate and promoted neurite outgrowths.

BIOT 420 - Culture under low oxygen conditions markedly enhances differentiation of murine embryonic stem cells into cardiomyocytes

Jeffrey R. Millman, Daryl E. Powers, Joao Paulo M. Mattos Almeida, and Clark K. Colton, Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, Fax: 617-252-1651, jmillman@mit.edu

Cardiomyocytes derived from differentiated embryonic stem (ES) cells hold promise for treating heart disease, but generation of sufficient quantities of differentiated cells is a challenge. Here, we show that control of pO_{2cell} to the range 0 to 36 mmHg, which is experienced by the developing embryo, enhances differentiation of ES cells into cardiomyocytes. Cells were differentiated under 142, 36, or 7 mmHg oxygen for 10 days. Cardiomyocytes were identified by flow cytometry of dispersed cells immunostained with an antibody to sarcomeric myosin heavy chain (MF-20) and confirmed with MF-20 immunostaining of tissue sections. Differentiation for 6 days at 7 followed by 4 days at 142 mmHg resulted in a cardiomyocyte number (45 per initial ES cell) and number fraction (33%) that were 4-5 times larger than for culture at 142 mmHg. Culture at low pO_{2cell} markedly increases differentiation into cardiomyocytes, and low pO_{2cell} may be important in embryonic development.

BIOT 421 - Engineering polymerized hemoglobins for use in transfusion medicine

Andre F. Palmer, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, OH 43210, palmer.351@osu.edu

Artificial blood substitutes based on glutaraldehyde polymerized hemoglobin (PolyHb) are currently being developed for use in human subjects needing blood transfusions. Despite the commercial development of PolyHb dispersions, a systematic study of the effect of varying the glutaraldehyde to hemoglobin (G-Hb) molar ratio on the resulting PolyHb physical properties (molecular weight distribution and oxygen binding parameters) has not been conducted to date. The results of this study show that increasing the G-Hb molar ratio elicits a general decrease in the P-50 (partial pressure of oxygen at which Hb is half saturated with oxygen) and cooperativity and a simultaneous increase in the weight averaged molecular weight of the PolyHb dispersion and methemoglobin (MetHb) level. Strategies are presented to lower the MetHb level and increase the P-50 of PolyHb. We also assessed the ability of various PolyHbs to transport oxygen in capillaries via the development of an oxygen transport model.

BIOT 422 - Enhanced albumin production by hepatic lineage cells derived from mouse embryonic stem cells

Chang-Chun Hsiao, Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, 123, Ta-Pei Road, Niao-Sung, Kaohsiung 83305, Taiwan, Fax: +886-7-7324841, cchsiao@mail.cgu.edu.tw, Chih-Hsiu Yin, Department of Chemical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan, Wannhsin Chen, Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Chutung, Hsinchu 310, Taiwan, Chao-Long Chen, Liver Transplant Program, Chang Gung Memorial Hospital- Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung 833, Taiwan, and Wen-Teng Wu, Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan

Embryonic stem (ES) cells are capable of differentiating into hepatic lineage cells in vitro . These cells can potentially be a source of hepatocytes for research and for use in regenerative medicine. In particular ES cell-derived hepatocytes would alleviate the shortage of quality donor liver cells in cell transplantation or in bioartificial liver for treatment of liver failure. This in vitro study explores the use of mouse ES cells to derive hepatic lineage cells capable of producing a higher levels albumin. We developed a refined culture method by adjusting the composition of culture medium dynamically. MES medium was replaced with HD medium from day 5 to 7 of ES cell differentiation. Supplementing the culture medium with dexamethasone and ITS resulted in an enhanced albumin secretion by differentiated embryoid bodies (EBs). The EBs cultured by this method produced albumin at a high rate of 1.90 ± 0.198 pg/hxcell. In addition the hepatic lineage cells exhibited mature hepatic characteristics. These cells were able to take up indocyanine green, expressed the hepatic-specific genes α1-anti-trypsin,α-fetoprotein, albumin, carbamoyl-phosphate synthetase 1,cytochromeP450 7A1,glucose-6-phosphatase,tyrosine aminotransferase, tryptophan 2,3-dioxygenase, and transthyretin by RT PCR. In conclusion, the differentiation method we developed allows for high albumin-producing hepatic lineage cells to be derived from mES cell for experimental use.

BIOT 423 - Efficient ethanol production from glucose and xylose mixture using growth-arrested Corynebacteria

Shinsuke Sakai[1], Miho Sasaki[1], Masako Suda[1], Yoshiki Tsuchida[2], Shohei Okino[1], Toru Jyojima[1], Masayuki Inui[1], and Hideaki Yukawa[1]. (1) Microbiology Research Group, Research Institute of Innovative Technology for the Earth (RITE), 9-2 Kizugawadai, Kizu-shi, Kyoto 619-0292, Japan, (2) Honda R&D Co., Ltd, Saitama 351-0193, Japan

Ethanol production from corn and sugarcane continues to increase rapidly but it is expected that the supply of these feedstocks will be limits in the near future. Therefore, the utilization of

lignocellulosic biomass as an attractive feedstock is currently investigated. Because the typical lignocellulose hydrolysate contains not only hexoses but also pentoses (6—25%), the utilization of pentoses is a necessity for efficient production of ethanol from lignocellulosic biomass. In a previous study, we demonstrated ethanol production from glucose by recombinant *Corynebacterium glutamicum* R under growth-arrested conditions. Growth-arrested conditions were achieved through oxygen deprivation of cells in a reactor, resulting in high volumetric ethanol productivity. A recombinant *C. glutamicum* R strain harboring *Escherichia coli xylA* and *xylB* genes as well as *Zymomonas mobilis pdc* and *adhB* genes was not only an efficient ethanol producer, but it simultaneously utilized glucose and xylose in the process.

BIOT 424 - Evaluation of production of bioethanol and biodiesel from renewable resources using process simulation tools

Dimitris Tsangaris, Intelligen Europe, Dimitriou Ralli 52, 2nd Floor, Marousi 15124, Greece, dtsangaris@intelligen.com, Charles Siletti, INTELLIGEN, INC, Mt. Laurel, NJ 08054, and Demetri Petrides, INTELLIGEN, INC, Scotch Plains, NJ 07076

Over the past three decades there has been intense investigation on the development of fuel producing processes that are based on the use of renewable agricultural materials as feedstock. This activity is driven primarily by the quest for fuel self-reliance and carbon oxides emission reductions. The main effort has been concentrated on bio-ethanol and bio-diesel which have been shown to give motor engine performance similar to that of conventional petroleum based fuels. In addition to product characteristics, however, process economics play an equally important role in any successful product commercialization. In this work, realistic process simulation models have been developed in order to analyse the economics of "corn-to-ethanol" and "soybean-oil to bio-diesel" production. The models developed include process flow diagrams, material and energy balances, equipment sizes and estimates of capital and operating costs. This presentation will illustrate how such models can facilitate the design of new manufacturing facilities and the optimization of existing ones.

BIOT 425 - Heterologous expression of Hydrogenovibrio marinus NiFe-hydrogenase in Escherichia coli for biohydrogen production

Jaoon Kim and Hyung Joon Cha, Department of Chemical Engineering, POSTECH (Pohang University of Science and Technology), Pohang 790-784, South Korea, jaoon@postech.ac.kr

Hydrogenase plays an important role in biological hydrogen production. Many efforts have paid attention to this enzyme in attempts to enhance the efficiency of biological hydrogen production. In contrast to the well-characterized Fe-hydrogenases, NiFe-hydrogenase, a heterodimeric metalloenzyme composed of two subunits, has not been examined in detail for biotechnological application. In this study, we designed a universal degenerate primer pair based on highly conserved motifs at the N-terminus of the small subunit and C-terminus of the large subunit, and successfully performed degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) to amplify 2.9~3.0 kb NiFe-hydrogenase genes from various microorganisms. After confirmation of validity for this technique, we detect unknown sequences from the large subunit of oxygen-tolerant NiFe-hydrogenase in Hydrogenovibrio marinus. We performed heterologous expression of this enzyme in Escherichia coli. Its cellular localization was analyzed with the change of signal peptide of small subunit and effect of some accessory protein on the maturation of this protein was studied for further investigation on what is necessary for functional expression of H. marinus Ni-Fe hydrogenase in E. coli.

BIOT 426 - Lignin blockers to reduce costs of enzymatic hydrolysis of pretreated cellulose

Charles E. Wyman, Chemical and Environmental Engineering, University of California, Riverside, CA 92521, cewyman@engr.ucr.edu, Bin Yang, University of California Riverside, Riverside 92507, and Deidre Willies, Mascoma Corporation, West Lebanon 03784

Cellulosic biomass must be pretreated to realize high yields of fermentable sugars via enzymatic digestion and achieve low enough costs to be economically viable for production of fuels and commodity chemicals that could open up major new agricultural markets with powerful societal benefits. Furthermore, pretreatment is the most single expensive step followed closely by the cost of enzymatic hydrolysis of the pretreated substrate and the cost of the enzymes themselves. Thus, a better understanding of factors that control interactions of pretreated substrates and enzymes is invaluable in identifying pathways to low cost systems. Addition of non-catalytic proteins such as BSA has been shown to enhance cellulose hydrolysis by enzymes or lower the amount of enzyme required to realize a particular conversion, potentially lowering costs. Thus, we are determining interactions among substrate features, enzymes, and BSA to better understand mechanisms by which these additives influence hydrolysis and identify promising routes to reduce enzyme use and speed rates. Adsorption results revealed that cellulase has a higher mass affinity than either β-glucosidase or BSA for Avicel cellulose, dilute acid pretreated corn stover, and corn stover lignin. In addition, β-glucosidase and BSA were found to have very little affinity for cellulose and higher affinity for pretreated corn stover and lignin, consistent with greater absorption of each on the lignin fraction. BSA improved hydrolysis of dilute acid pretreated corn stover in a manner similar to Tween 80, with the most dramatic benefits being at low enzyme loadings. Specifically, additives could significantly reduce the need for β-glucosidase supplementation, lowering enzyme costs proportionately. A plausible explanation is that these additives particularly reduce nonproductive adsorption of β-glucosidase on lignin.

BIOT 427 - Preparation of microbial oil by fermentation for biodiesel production

Yuxin Mo[1], Hongjuan Liu[1], **Jianan Zhang**[1], and Xiaohui Chen[2]. (1) Division of Green Chemistry and Technology, Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, China, Fax: 86-10-69771464, zhangja@tsinghua.edu.cn, (2) College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China

In biodiesel production by transesterification, it is important to get an appropriate raw material. Microbial oil will be considered to be promising. In our research, production of microbial oil by fermentation was studied and effects of operation parameters on fermentation were investigated. The results showed that the optimal fermentation conditions were glucose 5%, (NH4)2SO4 0.25%, MgSO4 0.2%, CaCl2 0.01%, temperature 30°C and pH 6. The components of microbial oil produced by fermentation were made up of palmitic acid, steric acid, oleic acid and linoleic acid, which were 17.80%, 10.27%, 53.02%, and 5.29%, respectively, which were similar to those of vegetable oil. Furthermore, using microbial oil as raw material, production of biodiesel by transesterification with catalyst was discussed. The result also indicated that the microbial oil can be used to produce biodiesel. The properties and components of biodiesel produced by microbial oil were similar to those produced by vegetable oil.

BIOT 428 - Study on mild alkaline/oxidative pretreatment of bagasse

Zhigang Zhao[1], Keke Cheng[1], **Jianan Zhang**[1], and Feng Gao[2]. (1) Division of Green Chemistry and Technology, Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, China, Fax: 86-10-69771464, zhangja@tsinghua.edu.cn, (2) College of Materials Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China

In order to improve the efficiency of bagasse hydrolysis, a mild alkaline/oxidative pretreatment with NaOH and H2O2 was carried out. The results showed that the optimal pretreatment condition was that reaction time was 20h, ratio of liquid to solid was 125:5, NaOH concentration was 1% and H2O2 concentration was 0.6 %. In addition, the recycling utilization of the pretreatment solution was discussed. The NaOH can be reduced by 34% after recycling the filtrate four times. Moreover, the structure after pretreatment was analyzed by SEM. The results made it clear that the structure of bagasse was destroyed after pretreatment by mild alkaline/oxidative method compared with bagasse raw material, and its smooth and contiguous surface was perforated. These pores may increase the enzyme-accessible surface area which increases the enzyme digestibility of the bagasse during hydrolysis.

Program by Day

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

THURSDAY MORNING

8:00 - 11:00 am	**Biophysical and Biomolecular Symposium: Protein Engineering**	**G. Makhatadze and R. S. Rajan, Organizers**	**Papers 429-436**	**BCEC 108**
8:00 - 11:00 am	**Downstream Processing: Non-Chromatographic Separation Techniques: Improving Process Throughput**	**G. Vedantham and D. W. Wood, Organizers**	**Papers 437-444**	**BCEC 107 B**
8:00 - 11:00 am	**Commercialization of Biologics: Scale-up & Process Design for Commercial Facilities: Debottlenecking and Facility Fit**	**R. Kiss and N. Titchener-Hooker, Organizers**	**Papers 445-452**	**BCEC 106**
8:00 - 11:00 am	**Upstream Processing: Advances in Tissue Engineering**	**J. B. Leach and A. Coury, Organizers**	**Papers 453-460**	**BCEC 107 C**

THURSDAY AFTERNOON

2:00 - 5:00 pm	**Biophysical and Biomolecular Symposium: Protein Engineering**	**G. Makhatadze and R. S. Rajan, Organizers**	**Papers 461-468**	**BCEC 108**
2:00 - 4:20 pm	**Downstream Processing: High Throughput Screening in Process Development**	**J. Coffman and G. Jagschies, Organizers**	**Papers 469-474**	**BCEC 107 B**
2:00 - 4:20 pm	**Downstream Processing: Disposable Bioprocessing**	**D. D. Frey and S. Ramanan, Organizers**	**Papers 475-480**	**BCEC 106**
2:00 - 5:25 pm	**Biophysical and Biomolecular Symposium: Protein Aggregation**	**T. Przybycien and M. Ricci, Organizers**	**Papers 481-487**	**BCEC 107 C**

THURSDAY MORNING

8:00 – 11:00 am BCEC 108

Biophysical and Biomolecular Symposium: Protein Engineering

G. Makhatadze and R. S. Rajan, Organizers Papers 429 - 436

BIOT 429 - Engineering and design of repeat proteins

Lynne Regan, Departments of Molecular Biophysics & Biochemistry and Department of Chemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06511, Fax: 203-432-5175, lynne.regan@yale.edu, Aitziber Lopez Cortajarena, MB&B, Yale University, New Haven, CT 06511, and Gilad Haran, Department of Chemical Physics, Weizman Institute, Rehovot, Israel

Tetratricopeptide Repeat (TPR) proteins provide a perfect scaffold of the engineering and design of proteins with enhanced properties, including stability, supramolecular structure and binding activities. The modular, repetitive nature of repeat proteins in general, and TPR proteins in particular, make them especially suited for rational re-engineering for a variety of applications. Here we present our recent work on the folding and stability of repeat proteins, which allows us to re-design their properties in a ration fashion. We also presnt our recent work on the incorporation of novel activities onto the TPR framework, and show how such novel proteins can be developed for a variety of applications.

BIOT 430 - Enzymatic activity from a designed artificial proteome

Shona C. Patel, Department of Chemical Engineering, Princeton University, Princeton, NJ 08540, scpatel@princeton.edu, and Michael H. Hecht, Department of Chemistry, Princeton University, Princeton, NJ 08544

Enzyme engineering seeks to generate proteins with an enhanced ability for catalysis or binding and often begins with naturally occurring proteins. However, natural proteins have evolved for specific purposes and are already biased towards particular tasks. Proteins that are designed de novo have the potential to be used in a wider variety of bioengineering applications since they have not evolved for specific functions. In order to probe the potential for enzymatic activity within unselected proteins, we are studying a designed artificial proteome comprised of a combinatorial library of de novo, four-helix bundle proteins. By screening proteins that were not designed for any particular function, we determined the likelihood of finding rudimentary activity within the model proteome. Once activity was discovered within the proteome, we implemented directed evolution on selected sequences to improve activity. This approach yielded novel proteins with several types of catalytic activity.

BIOT 431 - Computational procedure for transferring new binding sites into existing protein scaffolds

Hossein Fazelinia, Patrick C. Cirino, and Costas D. Maranas, Department of Chemical Engineering, The Pennsylvania State University, 149 Fenske Lab, University Park, PA 16802

Computer simulations play an increasingly significant role in understanding the underlying physical principles that dictate protein folding, stability and function, and computational advances in this area have greatly improved protein design predictions. While it is not yet possible to robustly predict structure and function de novo, it is possible to assess the impact of mutations on existing, well-characterized proteins. In this study we are developing and testing a computational framework to systematically transfer a binding site to a protein with known scaffold. In response to these design challenges we put forth a two-level procedure where we first identify where are the most appropriate locations to graft the new binding pocket into the protein structure. This challenge gives rise to a high dimensional search problem which we tackle using combinatorial optimization to identify promising locations to place the new binding site. Once a set of promising grafting sites is identified the next step involves the identification of mutations in the neighboring residues around the grafted binding site such that the geometry of the binding site is preserved upon energy minimization. Detailed atomistic energy calculations are employed to identify what mutations, if any, are needed to ensure that the minimum energy conformation of the binding pocket coincides with the configuration desired for function. This computational framework is benchmarked against the results available in the literature for transferring metal binding site for catalytic antibody and azurin-thioredoxin systems as well as transferring an active site into a protein of known scaffold.

BIOT 432 - Dynamics in computational protein engineering

Nikolay V. Dokholyan, Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Campus Box #7260, Chapel Hill, NC 27599-7260, dokh@med.unc.edu

Some of the emerging goals in modern medicine are to uncover the molecular origins of human diseases, and ultimately contribute to the development of new therapeutic strategies to rationally abate disease. Of immediate interests are the roles of molecular structure and dynamics in certain cellular processes leading to human diseases and the ability to rationally manipulate these processes. Despite recent revolutionary advances in experimental methodologies, we are still limited in our ability to sample and decipher the structural and dynamic aspects of single molecules that are critical for their biological function. Thus, there is a crucial need for new and unorthodox techniques to uncover the fundamentals of molecular structure and interactions. We follow a hypothesis-driven approach which is based on tailoring simplified protein models to the systems of interest. Such an approach allows significantly extending the length and time scales for studies of complex biological systems. I will describe several recent studies that signify the predictive power of simplified protein models within the hypothesis-driven modeling approach.

BIOT 433 - Protein engineering of extremophilic chaperones for molecular self-assembly

Timothy A. Whitehead and Douglas S Clark, Department of Chemical Engineering, University of California, Berkeley, 210 Gilman Hall, Berkeley, CA 94720, taw@berkeley.edu

Self-assembled proteins hold tremendous promise for creating a range of useful nanoscaled templates. Advantages of protein templates include processing in aqueous environments and functionalization through standard genetic engineering to incorporate new chemistries, to embed enzymes, or to incorporate nucleating peptides for a diverse range of inorganic materials. In this talk, we describe the creation of specifically interacting proteins for ordered architectures through the application of powerful genetic selection technology. These architectures are based on the quaternary structure of a new type of filament protein (the γ prefoldin, or γ-PFD) from the hyperthermophile *Methanocaldococcus jannaschii* that we have previously discovered and characterized. These engineered filaments are to be used as building blocks for 3-D architectures of arbitrary geometry, and progress towards this end will be discussed.

BIOT 434 - Rational approaches to protein design and engineering

Philippa J. Reeder[1], Christopher Bystroff[2], and Jonathan S. Dordick[1]. (1) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th St, Troy, NY 12180, reedep@rpi.edu, (2) Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12180

Efforts to understand protein function at the molecular level have yet to fully capture the general rules that govern protein design. One important aspect of protein design is the prediction and optimization of enzyme stability over long time periods and under various harsh industrial operating conditions. This work focuses on data-driven methods for design and improvement of functional protein properties, primarily protein stability. A sequence-based study of the Subtilase superfamily is presented, revealing a sequence motif responsible for thermodynamic stability in that family. We demonstrate that subsequence insertion of that motif into a mesophilic family member results in increased thermal stability in vitro. A structure-based protein redesign method is presented for the direct improvement of protein kinetic stability with minimal effects on protein activity. We hypothesize in this work that enzyme function is primarily conferred by intramolecular contacts between secondary structural units. By maintaining such interactions (and therefore the majority of residue-residue contacts) and intelligently changing the way in which those structural units are connected (i.e., changing the topology of the protein), we demonstrate that it is possible to change the rate of unfolding of a model protein, GFP, in vitro. This method may be universal and therefore applied to any protein with multiple structural units.

BIOT 435 - Structure-based humanization of therapeutic antibodies

Karl Hanf[1], You Li[2], Kenneth Simon[2], Ling Ling Chen[1], Matthew Jarpe[3], Ellen Garber[1], Fred Taylor[2], Laura Silvian[1], Joseph Arndt[1], and Alexey Lugovskoy[1]. (1) Drug Discovery, Biogen Idec Inc, 14 Cambridge Center, Cambridge, MA 02142, karlhanf@comcast.net, (2) Molecular Engineering, Biogen Idec Inc, Cambridge, MA 02142, (3) Physical Biochemistry, Biogen Idec Inc, Cambridge, MA 02142

Antibodies are well-established protein therapeutic agents. Typically, high-affinity antibodies are obtained by immunization of rodent species and need to be humanized to reduce their immunogenicity, which could otherwise lead to adverse reactions in patients or inferior pharmacokinetic behavior. We have developed a novel structure-based method of antibody humanization through remodeling of complementarity-determining regions (CDRs). Poor interactions between mature murine CDRs and the chosen human framework are resolved by changing the back side of murine CDRs to fit the human framework, which results in excellent retention of binding to antigen. Further, we redesigned antibodies for improved stability and affinity using structure-based computational optimization with dead-end elimination followed by Poisson-Boltzmann electrostatics.

BIOT 436 - Molecular strategy for designing robust proteins: Looking deeper at the surface

George Makhatadze, Department of Biochemistry and Molecular Biology, Penn State University College of Medicine, 500 University Drive, Hershey, PA 17033, Fax: 717-531-7072, makhatadze@psu.edu

The progress in understanding the forces responsible for the protein stability has been enormous, largely through the combination of experimental and theoretical approaches. It has been shown that the hydrophobic effect, hydrogen bonding and packing interactions between residues buried in the protein interior are dominant factors that define protein stability. The role of surface residues for protein stability received much less attention. It was believed that surface residues are not important for protein stability particularly because their interactions with the solvent should be similar in the native and unfolded states. However, our experimental data using different model proteins shows that the surface residues contribute to protein stability through a variety of factors. These factors can be operationally divided into long-range interactions (charge-charge interactions between ionizable groups) and short-range local interactions (salt-bridges, hydrophobicity and packing, peptide bond hydration, a-helical propensity, helix capping). Quantitative analyses of the contribution of these different factors to the protein stability will be presented and their applicability to the design of thermostable enzymes will be discussed. In addition, several examples providing experimental validation for these computational protein design approaches will be given.

Supported by a grant from the National Science Foundation (MCB 0416746).

THURSDAY MORNING

8:00 – 11:00 am BCEC 107 B

Downstream Processing: Non-Chromatographic Separation Techniques: Improving Process Throughput G. Vedantham and D. W. Wood, Organizers Papers 437 - 444

BIOT 437 - Crystallization of therapeutic proteins and antibodies at Genentech: Purification and bulk storage applications

Bryan A. Bean, Process Development Engineering, Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, beanb@gene.com, and Timothy C. Matthews, Process Development Engineering, Genentech, South San Francisco, CA 94080

Genentech, Inc. has recently developed a process to purify a therapeutic recombinant protein via crystallization. The step yield, crystal morphology, and crystal size distribution were optimized at laboratory scale by studying the effects of salt species, salt concentration, temperature, pH, and anti-solvents. The crystallization unit operation was scaled up and integrated into a manufacturing purification process where we achieved both high yields and excellent purity. Genentech is also investigating the crystallization of a monoclonal antibody for purification and bulk drug substance storage applications. The crystallization conditions were optimized and the molecule's stability was evaluated using various crystalline storage methods. These applications are extremely novel to the biotechnology industry. Data will be presented on solubility, metastable zone, crystal morphology and size distribution, crystal filtration characteristics, purity, molecule stability, and equipment scale-up.

BIOT 438 - Detergent-mediated liberation of intracellular recombinant virus-like particles (VLPs) from S. cerevisiae homogenate

Gaik Sui Kee[1], Hari Pujar[2], and Nigel Titchener-Hooker[1]. (1) Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom, Fax: +44 (0) 20 7916 3943, ucbegsk@ucl.ac.uk, (2) Bioprocess R & D, Merck & Co. Inc, West Point, PA 19486

Virus-like Particles (VLPs), such as the hepatitis B surface antigen, are expressed intracellularly in S. cerevisiae. In the recovery of VLPs, the detergent-facilitated release from the host membrane components is a critical process step, responsible for setting the backdrop for subsequent purification. Based on a lipoprotein VLP model, the effect of different detergent conditions were investigated based on tradeoffs between maximal product recovery and preservation of particle antigenicity. Our studies identified that higher concentrations of detergent favours VLP recovery only to a specific threshold. Beyond this, further increase in detergent results in delipidation of VLP, leading to antigenicity loss. Release of contaminating proteins and host lipids into the process stream is also influenced by conditions of the detergent step. The effects of resulting process stream on subsequent unit operations were also characterized.

BIOT 439 - Elastin-like polypeptide-intein mediated protein purification

Alison R. Gillies, Department of Chemical Engineering, Princeton University, A-217 Engineering Quadrangle, Olden St., Princeton University, Princeton, NJ 08544, agillies@princeton.edu, and David W. Wood, Departments of Chemical Engineering and Molecular Biology, Princeton University, Princeton, NJ 08544

An alternative to conventional affinity-tag separations has been developed for the purification of recombinant proteins. This method replaces the affinity resins found in conventional systems with elastin-like polypeptide tags. In this system, an ELP gene is attached to a gene for a self-cleaving protein linker, and the two are then joined to the product protein gene. The resulting expressed fusion protein is then purified via salt and temperature-dependent aggregation of the ELP tag, combined with sequential rounds of centrifugation and resuspension to wash the product protein. Self-cleavage of the ELP tag is induced by a pH shift and the purified target protein is recovered following precipitation of the cleaved ELP tag. This method has been very successful in E. coli. In principle, the ELP system will be applicable to a wide range of expression systems and hosts, and early modeling suggests applications in biologics manufacturing at very large scale.

BIOT 440 - Large-scale antibody purification using non-chromatographic methods

Sundar Ramanan, Purification process development, Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, sramanan@amgen.com, and Rosalind Stenson, Purification Process Development, Amgen, Inc, Thousand Oaks, CA 91320

Recent advances in cell culture technology for monoclonal antibody production has resulted product (antibody) titers greater than 5 g/L. The increased titer coupled with a crude feed stream poses a challenge to the downstream steps consisting only of chromatography unit operations. We explored alternative purification processes using precipitation. Here we present results comparing the two purification schemes. The comparison attributes will include parameters such as product quality (aggregates/lower-molecular weight impurities by size-exclusion, host cell contaminant, DNA, etc.), scale-up as well as overall productivity.

BIOT 441 - Salt-dependent transmission of plasmid DNA through ultrafiltration membranes

David R. Latulippe and Andrew Zydney, Department of Chemical Engineering, The Pennsylvania State University, 219 Fenske Laboratory, University Park, PA 16802, Fax: 814-865-7846, drl193@psu.edu

Recombinant plasmid DNA can be used as a biotherapeutic for gene therapy and DNA vaccination. Although various chromatographic methods have been examined, these are expensive and time-consuming for large scale DNA purification

due to the low binding capacity. The objective of this work was to evaluate the use of membrane ultrafiltration for plasmid DNA purification including the effects of flux and solution conditions on plasmid transmission. Stirred-cell sieving experiments were conducted with Ultracel membranes using a 3 kbp plasmid. At constant flux, the plasmid transmission increased over 80-fold as the NaCl concentration increased from 0 to 150 mM or as the $MgCl_2$ concentration increased from 0 to 10 mM. Higher $MgCl_2$ concentrations (up to 40 mM) showed no significant differences in the transmission behavior compared to 10 mM. Plasmid transmission was also a strong function of filtrate flux due to DNA elongation. These results provide important insights into the factors governing plasmid ultrafiltration.

BIOT 442 - Selective precipitation using polyelectrolytes: A novel approach to the purification of monoclonal antibodies

Paul J McDonald, Jayme Carter-Franklin, and Robert Fahrner, Bioprocess Development, Genentech, Inc, 1 DNA Way, South San Francisco, CA 94080, pmcd@gene.com

The purification of monoclonal antibodies typically involves multiple chromatography steps exploiting orthogonal modes of separation. These chromatography steps use resins consisting of a support matrix onto which particular functional chemistries are immobilized. Using polyelectrolytes, it may be possible to exploit these same functional chemistries to achieve protein purification in solution. Through the manipulation of solution pH and ionic strength, an antibody-polyelectrolyte complex can form leading to antibody precipitation. Purification occurs through selective partitioning of the antibody and impurities into the solid or liquid phases. The potential for polyelectrolyte induced precipitation of antibodies to replace traditional chromatography steps was evaluated using polyvinylsulfonic acid (PVS). PVS precipitation was evaluated as a replacement for the initial capture step as well as an intermediate polishing step in the purification of a humanized IgG1 monoclonal antibody. PVS precipitation separated the antibody from host cell proteins, leached protein A, small molecules such as insulin and gentamicin as well as antibody fragments and aggregates. PVS was subsequently removed from antibody pools to < 1ug/mL using anion exchange chromatography. PVS precipitation did not impact the biological activity of the re-suspended antibody.

BIOT 443 - Selective precipitation-assisted recovery of immunoglobulins from bovine serum

Adith Venkiteshwaran, Patrick Heider, and Georges Belfort, Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180-3590, **venkia@rpi.edu**

A large proportion of protein therapeutics in the product pipelines of biotechnology firms are monoclonal antibodies (IgG). The high binding specificity of protein A affinity ligands for monoclonal antibodies is usually exploited in their downstream recovery. However, high costs associated with these ligands, along with requirements such as high pH for cleaning and problems involved with leaching of the ligand from the column, have prompted the development of alternative strategies for selective monoclonal antibody separation. Exploiting physicochemical properties with synthetic membrane processes to recover IgG from bovine serum has not proved very successful in our laboratory. As a result we have developed a novel method to selectively recover IgG from a bovine serum. The key steps involve the selective precipitation of IgG, by addition of an optimum amount of ammonium sulfate, combined with crossflow membrane microfiltration to retain the precipitate and remove the undesirable soluble bovine serum albumin (BSA). High transmission of the soluble BSA was obtained with minimal fouling of the 0.1 micron hydrophilized poly(vinylidene flouride) (PVDF) membrane with retention of all the IgG. A subsequent diafiltration step resulted in the removal of about 95% of the BSA, and a purity of 90% for the IgG. The effect of wall-shear rate and protein concentration on the filtration of the above precipitate-containing feed were also investigated in total recycle mode to obtain a further understanding of the behavior of the system. The above process could greatly impact plasma and serum fractionation and diagnostic proteomics, and potentially be extended to other biological feeds containing recombinant IgG, at the very least, it could reduce the dependence on protein A affinity chromatography. Work with this feed is in progress.

BIOT 444 - Can affinity-sinking become an alternative to affinity-chromatography?

Guy Patchornik, Department of protein purification, Affisink Biotechnology, Rehovot, Israel, Fax: 972-8-9302565, guy@affisink.com

Affinity-Sinking is a novel purification approach based on free non-immobilized modified ligands. The non-immobilized state of the ligand circumvents the need to immobilize ligands to polymeric supports, hence; all polymeric components are excluded from the process and purification is accomplished without the use of affinity columns.

The mechanism of purification requires three sequential steps: I - The target is specifically precipitated in the presence of a non-immobilized modified ligand whereas impurities are left soluble in the supernatant and are removed by centrifugation. II - The resulting pellet is then washed once with minute volume of buffer to remove traces of impurities. III - The target is eluted from the pellet while keeping the modified ligand insoluble in the precipitate. The approach was implemented in two distinct platforms each exploiting a different complexing chemistry, either the: [Desthiobiotin : Avidin] complex or the [Catechol : Fe3+] complex and has been demonstrated with four distinct ligands (Protein A, Protein G, Concanavalin A and Fluorescein) leading to relatively high yields (75-94%) and purity (88-97%) within 20-25 minutes overall time. The purity obtained was shown not to be affected by substantial increase in the contamination background.
In addition, greater purity and yield, as well as a significantly faster isolation process were observed when free rather than immobilized Protein A was used in a comparative study. This provided direct evidence of the advantages derived from binding under homogenous solutions together with the exclusion of polymeric resins from the process. Preliminary results and possible implementation of the approach for: Fusion proteins (e.g. His-tag proteins, GST-proteins); Membrane proteins (e.g. Na,K-ATPase); Eukaryotic mRNA and Cells will be presented and discussed.

THURSDAY MORNING

8:00 – 11:00 am BCEC 106

Commercialization of Biologics: Scale-up & Process Design for Commercial Facilities: Debottlenecking and Facility R. Kiss and N. Titchener-Hooker, Organizers Papers 445 – 452

BIOT 445 - Biopharmaceutical portfolio management and capacity planning: A stochastic multiobjective optimization approach

Edmund D. George, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom, Fax: +44 (0) 20 7916 3943, e.george@ucl.ac.uk, and Suzanne Farid, Department of Biochemical Engineering, University College London, London WC1E 7JE, United Kingdom

Optimizing the structure and development pathway of biopharmaceutical drug portfolios are core concerns that come with several attached complexities. These include strategic decisions for the choice of drugs, the scheduling of critical activities, and the possible involvement of third parties at various stages for each drug. Additional complexities that must be considered are the uncertain environment, dependency relationships between decisions, and rationalization between multiple and conflicting objectives. This research presents the development of a stochastic, multi-objective, combinatorial optimization framework designed to address these issues. The framework has two main components: one which simulates the drug development pathway with associated manufacturing, and another that harnesses the ability of Bayesian networks to achieve optimality by characterizing the probabilistic structure of superior decisions via machine learning. Industrially-relevant examples are presented that focus on evaluating options for a portfolio of therapeutic antibodies. The impact of budgetary constraints on the optimal decisions is also illustrated.

BIOT 446 - Gauging the technical, regulatory and financial implications of process changes

Inass Hassan[1], Mark Bulmer[2], John More[2], and Suzanne Farid[1]. (1) Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, United Kingdom, Fax: +44(0)-207-916-3943, i.hassan@ucl.ac.uk, (2) Bio Products Laboratory, Hertfordshire, United Kingdom

Pressures from product competition, regulatory authorities and customer requirements are driving biologics companies to make process changes during a product's lifecycle so as to enhance factors such as yields, purity, robustness or cost-effectiveness. The influence of regulatory hurdles as well as possible changes to product quality are but a few considerations that need to be taken into account when assessing process changes. This presentation will provide a hierarchical framework for gauging the potential cost and risk implications of implementing process changes, which encompasses both the technical and regulatory activities involved. Industrial case studies focusing on changes to a plasma fractionation process will demonstrate the insights from such an approach. For example, the impact of transforming of a process side-fraction into a commercial product will be presented where the market potential of new products is weighed-up against processing costs, development activities, time-delays and risks.

BIOT 447 - Industrial case studies of debottlenecking and future proofing through discrete event simulation

P. A. Sinclair, Biopharm Services Ltd, Lancer House, East Street, HP5 1DG, Chesham, United Kingdom, Fax: +441494 785 954, a.sinclair@biopharmservices.com

The biomanufacturing industry faces increasing pressure to reduce capital and operating costs whilst maximizing flexibility and reliability over the lifetime of the facility. Two industrial case studies will explore how discrete event simulation can provide insight into people and plant constraints, and evaluate process design and technology options to guide business and design decisions. The first case study illustrates how biomanufacturing simulation has allowed a contract manufacturing organization to predict future bottlenecks, assess de-bottlenecking options, and reduce risk when introducing new products. The second case study shows how simulation can be integrated into the design phase to screen alternative technologies, and explore the effects of future developments over the lifetime of the facility. The integration of simulation into the design process allowed the design team to develop solutions that support the current and predicted client product portfolios, and identify factors that influence long term capital and operating costs.

BIOT 448 - Prototype software methodology for the rapid evaluation of biomanufacturing process options

Sunil Chhatre[1], Richard Francis[2], Kieran O'Donovan[2], Nigel J. Titchener-Hooker[1], Anthony R. Newcombe[2], and Eli Keshavarz-Moore[1]. (1) The Advanced Centre for Biochemical Engineering, University College London, Gower Street, London WC1E 7JE, United Kingdom, sunil.chhatre@ucl.ac.uk, (2) Process Development Group, Protherics U.K. Limited, Llandysul Wales SA44 5JT, United Kingdom

A novel modelling framework is described that rapidly evaluates bio-manufacturing process options. The framework consists of three layers, each containing its own dynamic simulation. In each layer, inferior options are screened out, while the more promising candidates are passed into the next layer for further evaluation. Successive layers incorporate greater accuracy and detail, thereby becoming more computationally intensive and requiring more detailed, time-consuming experimental studies to obtain input data. By screening out options, however, the lower layers are only challenged with the most promising options, minimizing the total computational time and focusing detailed experimentation on those options showing the greatest potential. This approach enables the efficient identification and selection of the most favourable manufacturing strategies. The utility of the framework was illustrated by evaluating alternative options for an industrial

process producing a Fab–based rattlesnake anti–venom (Protherics U.K. Limited). Currently, a 500 L ovine serum feed containing rattlesnake anti-venom IgG is subjected to sodium sulphate precipitation, after which the sedimented antibodies are separated from the contaminating supernatant by centrifugation. Papain digestion is then used to produce Fab and Fc fragments. Fc is removed by an ion exchanger and a Fab-specific affinity chromatography step is used finally to produce purified anti-venom-specific Fab. Alternative process options considered, both individually and in combination, were (A) increasing the initial feed volume to 1000 L, (B) varying the concentration of precipitating agent, (C) using a microfiltration step instead of the centrifuge and (D) using a Protein G step to capture the IgG instead of precipitation and centrifugation, followed optionally by (E) a concentration step. Results show that out of all options assessed, using a 1000 L feed volume and a Protein G step instead of precipitation and centrifugation (options A/D) resulted in the most superior alternative to the current process.

BIOT 449 - Facility fit, when should it happen?

Jean D. Harms, Amos M. Tsai, and Anurag S Rathore, Process Development, Amgen Inc, 18S -1-A, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-499-6819, jingjinh@amgen.com

In the race to reach market, expedited drug development timelines have become a key component for success in the biotech industry. Accelerated timelines require not only rapid initial process definition but also speedy and seamless transfer of a process from the bench scale into clinical and commercial manufacturing scales. Fitting new processes into existing manufacturing facilities in a timely and cost-efficient manner becomes critical. As companies continue to build or acquire more facilities at different scales and difference sites, moving products between these facilities also becomes necessary. Described herein is a strategy we have developed to address these challenges. This strategy centers around performing preliminary facility fit analysis prior to the initial process definition and thus avoiding major facility fit hurdles later on during process transfer. A specific case study on the development of a monoclonal antibody process and fitting this process into a commercial facility is presented as the benefits and drawbacks of this strategy are discussed.

BIOT 450 - Facility fit: Ensuring process performance comparability for commercial antibody processes when transferring to differently designed equipment

Keith L. White[1], Roderick Garcia[2], Kathleen Carswell[1], Eric Ordonez[1], Anuj Bhartiya[1], Brandy Jones[3], Todd Renshaw[4], and Robert Kiss[1]. (1) Process Development (LSCC), Genentech, 1 DNA Way, South San Francisco, CA 94080-4990, whitek@gene.com, (2) Process Development (LSP), Genentech, South San Francisco, CA, (3) Automation Engineering, Genentech, South San Francisco, CA 94080-4990, (4) Manufacturing Collaborations and Contract Manufacturing, Genentech, South San Francisco, CA 94080-4990

To ensure successful transfer of commercial bulk manufacturing processes to different manufacturing sites, our strategy is to align process equipment with Genentech's historical experience where possible and use common engineering principles to minimize the potential impact of scale or unit operation differences. From that basis, minor adjustments can then be made to achieve process performance comparability. The critical first step is a detailed gap analysis to identify equipment and control strategy differences. Equipment can then be modified or replaced where feasible. Significant gaps have included bioreactor geometry, impeller and sparger styles, harvest equipment, buffer and pool tank sizes, chromatography and UF/DF skid configurations, and process control algorithms. To ensure consistent performance between different scales of bioreactor operation, constant agitator specific power input and volumetric flow rates are used as initial targets to establish operating parameters. Constant linear flow rates and volumetrically proportional buffer volumes are maintained in purification unit operations. Results from recent transfers are included to demonstrate successful process performance comparability.

BIOT 451 - Strategies for increasing downstream capacity

David H Peers and Meliana Ratna, Manufacturing Sciences, Genentech, 1000 Horizon way, Vacaville, CA 95688, dhp@gene.com

The pressure to reduce costs and improve manufacturing efficiency has resulted in cell culture process innovations that provide both increasing titers as well as more challenging feedstocks for downstream processes. This in turn is requiring facilities to operate at the limits of existing purification plant capacity and capabilities. This presentation will discuss approaches used to de-bottleneck and optimize a purification train to increase recovery capacity by more than 50% above the original plant design. Emphasis will be placed on the techniques and strategies used for rapid process evaluation to optimize facility fit while maintaining plant throughput.

BIOT 452 - Can modular construction help to address key bottlenecks in the retrofit projects?

Thorsten Kimmel, Front End Design, Pharmadule AB, Danvik Center 28, 13130 Nacka, Sweden, Fax: +46-8-58742888, thorsten.kimmel@pharmadule.com

There are increasing pressures on biopharmaceutical manufacturers to achieve rapid retrofits of existing facilities. Each such design represents a unique series of challenges. This paper will address how bottlenecks associated with changes in process design and process scale-up can be tackled and solved from a facility design point-of-view. Various ways to troubleshoot space limitations that arise from new and extended process operations will be highlighted and the use of modular facility design and construction approaches singled out as a powerful route that enables speed to manufacture. Case studies and real examples will be used to demonstrate the philosophy and to generate key metrics for the technology.

THURSDAY MORNING

8:00 – 11:00 am BCEC 107 C

Upstream Processing: Advances in Tissue Engineering J. B. Leach and A. Coury, Organizers Papers 453 - 460

BIOT 453 - Dual growth factor loaded PEGylated fibrin gels to control SMC phenotype from human MSC

Charles T. Drinnan and Laura J. Suggs, Department of Biomedical Engineering, University of Texas at Austin, 1 University Station, Mail Code C0800, Austin, TX 78712, Fax: 512-232-5803, c.drinnan@mail.utexas.edu

The aim of the current study is to admix PDGF-BB and sequester TGF-β within a fibrin gel crosslinked with a bifunctional succinimidyl α-methylbutanote PEG (PEG-$(SMB)_2$). This system can be utilized to modulate expression of smooth muscle phenotypes from a population of mesenchymal stem cells (MSC) by temporally controlling growth factor release. Gels are formed by thrombin addition to non-PEGylated gels, gels PEGylated with PEG-$(SMB)_2$, and gels PEGylated with monofunctional mPEG-SMB. Controls consist of gels without growth factors. Release curves are quantified with commercial ELISA kits. TGF-β and PDGF-BB bioactivity is assessed with the Mv1Lu growth inhibition assay and a MSC migration assay, respectfully. Differentiation potential is measured using rtPCR with primers for smooth muscle α-actin and calponin. Results indicate that TGF-β is sequestered within the PEGylated gels while PDGF-BB is completely released by day 2. Further results demonstrate that released TGF-β maintains bioactivity.

BIOT 454 - A 3-D tissue model for high-throughput drug discovery

Xudong Zhang, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Ave., Columbus, OH 43210, zhangx@chbmeng.ohio-state.edu, Robin Ng, Department of Chemical Engineering, Ohio State University, Columbus, OH 43210, and Shang-tian Yang, Dept. of Chemical Engineering, Ohio State University, Columbus, OH 43210

3D tissue model can mimick in-vivo tissue environment and provide bioactivity information in HTS fashion, facilitating selection of better drug candidates for further animal experiments. A high-throughput, real-time, bioactivity assay based on 3D cultures of green fluorescent protein (GFP)-expressing cells was developed. The system containing 40 microbioreactors on a 384-well plate can be used for cytotoxicity and proliferation assays in both high-density and low-density 3D cultures. To test this new 3D microbioreactor array for HTS drug screening, a colon cancer cell line was used to study the efficacy of several commonly used cancer drugs, including 5-fluorouracil and gemcitabine. The results from high-density 3D cultures matched with clinical effects of these drugs, whereas conventional 2D monolayer cultures errorneously predicted a much lower effective drug dosage. Thus, the 3D microbioreactor array has a great potential for improving the drug discovery process.

BIOT 455 - Intestinal epithelial cell response to crypt-like substrate topography

Lin Wang, Shashi K. Murthy, and Rebecca L. Carrier, Department of Chemical Engineering, Northeastern University, 360 Huntington Ave., 342 Snell Engineering Center, Boston, MA 02115, wang.li@neu.edu

Modification of biomaterial scaffolds used in tissue engineering have traditionally focused more on chemical and mechanical properties than on topography. Microfabrication was used to produce topographic analogs of intestinal basal lamina crypts. A test pattern of micro-wells with dimensions (50-500 μm diameter, 120 μm depth) similar to those of native crypt structures was produced in polydimethylsiloxane (PDMS). PDMS surfaces were coated with 50 μg/ml fibronectin and seeded with Caco-2 intestinal epithelial cells. Cells attached mostly to the bottoms of micro-wells and were able to migrate/proliferate along the steep side walls of well structures. Overall, cells attached better on flat surfaces. Substrate topography also affected viability, with cells on side-walls having greater viability than at the bottom or tops of wells. These studies will aid in understanding the unique role intestinal topography plays in cell development and provide design parameters for a scaffold for tissue engineered intestine to treat intestinal disease.

BIOT 456 - Novel cell adhesion biomaterial based on mussel adhesive protein fused with RGD peptide

Dong Soo Hwang, Youngsoo Gim, and **Hyung Joon Cha**, Department of Chemical Engineering, POSTECH (Pohang University of Science and Technology), Pohang 790-784, South Korea, Fax: 82-54-279-5528, pang@postech.ac.kr, hjcha@postech.ac.kr

Previously, we designed and constructed a hybrid of the mussel adhesive protein (MAP) fp-151, which is a fusion protein with six type 1 (fp-1) decapeptide repeats at each type 5 (fp-5) terminus. Through various cell-adhesion analyses, we previously demonstrated that fp-151 has the potential to be used as a cell or tissue bioadhesive. In the present study, to improve the cell-adhesion properties of fp-151, we designed a new cell-adhesive protein, fp-151-RGD, which is a fusion with the GRGDSP residues, a RGD peptide sequence that has previously been identified at the cell-attachment site of fibronectin, at the C-terminus of fp-151. Although recombinant fp-151-RGD maintained the advantages associated with fp-151, such as a high production yield in Escherichia coli and simple purification, it showed superior spreading ability as well as better cell-adhesion ability compared with other commercially produced cell-adhesion materials such as poly-L-lysine (PLL) and the naturally extracted MAP mixture Cell-Tak. The excellent adhesion and spreading abilities of fp-151-RGD might be due to the fact that it utilizes three types of cell-binding mechanisms: DOPA adhesion of Cell-Tak, cationic binding force of PLL, and RGD sequence-mediated adhesion of fibronectin. Therefore, the new recombinant fp-151-RGD is suitable for use as a cell-adhesion material in cell culture

or tissue engineering, and in any other area where efficient cell adhesion is required.

BIOT 457 - Tissue-like self assemblance of hepatocytes in vitro by manipulation of collagen

Qin Meng[1], Jing Dai[1], and Guoliang Zhang[2]. (1) College of Materials Science and Chemical Engineering, Zhejiang University, 38 Zheda Road, Hangzhou 310027, China, Fax: 86-571-87951227, mengq@zju.edu.cn, (2) Institute of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, China

Hepatocyte spheroids or cuboids, possessing high liver-specific functions and tissue-like structure, are desirable for construction of a bioartificial liver. Previously, many extracellular matrixes have been verified to enhance spheroid formation. However, collagen, the most extensively used extracellular matrix in dispersed hepatocye culture including monolayer culture and gel entrapment culture, has never been reported to promote tissue-like assemblance in vitro. Our current work showed that hepatocytes could form either spheroids or cuboids in presence of collagen with a specific concentration within hollow fibers. To illustrate the possible mechanis on the interaction between collagen and hepatocytes, the tissue-like assemblance of hepatocytes were firstly investigated on by varying both collagen concentration, hepatocyte density and the configuration of the hollow fibers. The morphologies of formed spheroids and cuboids were assayed together with liver-specific functions. The expression of specific genes by RT-PCR was further conducted to interpret the manipulation of collagen on self assemblance.

BIOT 458 - Anticancer drug test in tissue engineered perfusion microbioreactor array

Yuan Wen[1], Xudong Zhang[2], and Shang-Tian Yang[2]. (1) Chemical and Biomolecular Engineering, The Ohio State University, 125A Koffolt Labs, 140 West 19th Avenue, Columbus, OH 43210, wen.33@osu.edu, (2) Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210

In vitro tissue culture provides a physiologically relevant platform for disease models and drug discovery. We have developed a 4-times-6 microfluidic bioreactor array which enabled perfusion culture of tissue constructs with the capability of real-time quantification of cell proliferation using EGFP (enhanced green fluorescent protein)-expressing cells. The effect of a commonly used chemotherapeutical drug 5-fluorouracil was investigated against a human colon cancer cell line HT-29. The drug was tested with clinically relevant modes, either in continuous perfusion or high dose bolus administration followed by perfusion culture. With this system, 6 concentrations of the drug could be investigated with 4 replicates plus controls simultaneously for hundreds of hours. Besides the real-time cell proliferation assay, direct access to the tissue constructs after the drug test also allowed further analysis of the anti-cancer mechanisms of the drug.

BIOT 459 - Targeted oxygen delivery to hepatic hollow fiber bioreactors via hemoglobin-based oxygen carriers

Andre F. Palmer, Department of Chemical and Biomolecular Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, OH 43210, palmer.351@osu.edu

Hepatic hollow fiber bioreactors are considered a promising class of bioartificial liver assist device (BLAD). Unfortunately, limited oxygen transport to hepatocytes within this device hinders further development. Hepatocytes in vivo (in the liver sinusoid) experience a wide range of oxygen tensions (25-70 mmHg) which is important for development of proper differentiated function (zonation). We hypothesize that altering the kinetics of oxygen binding/release to/from hemoglobin-based oxygen carriers (HBOCs) could potentially target oxygen delivery to cultured cells. Low oxygen affinity HBOCs preferentially targeted oxygen delivery at high inlet oxygen tensions. Conversely, high oxygen affinity HBOCs targeted oxygen delivery at low inlet oxygen tensions. Additionally, inlet oxygen tension, flow rate, and HBOC concentration were varied to find optimal bioreactor operating conditions. Our results demonstrate that HBOCs can enhance oxygen delivery to cultured hepatocytes, while exposing them to in vivo-like oxygen tensions which is critical in developing a fully functional BLAD.

BIOT 460 - Novel systems for alginate-immobilized mammalian cell culture in bioreactors

Corinne A Hoesli[1], Kamini Raghuram[1], Minh Luu[2], Jill Osborne[3], Gregory Korbutt[4], and James M. Piret[1]. (1) Michael Smith Laboratories & Department of Chemical and Biological Engineering, University of British Columbia, 251 - 2185 East Mall, Vancouver, BC V6T 1Z4, Canada, Fax: 604-822-2114, choesli@chml.ubc.ca, (2) Process Development (LSCC), Genentech, Inc, South San Francisco, CA 94080, (3) Department of Chemical Engineering and Applied Chemistry, University of Toronto, (4) Department of Surgery, University of Alberta, Edmonton, AB T6G 2N8, Canada

Immobilization in 3D matrices is increasingly used for stem cell differentiation and tissue engineering applications, including for the generation of islets for diabetes therapy. Two novel processes for alginate-immobilized mammalian cell culture are being developed to make clinical and larger scale implementation more practical: an alginate-filled hollow fiber bioreactor (AHFBR) and alginate beads formed by emulsion/internal gelling. The cell lines chosen for proof-of-concept experiments formed discrete pancreatic islet-sized aggregates in alginate. The AHFBR allowed 25-fold expansion of CHO cells in 8 days and 10-fold expansion of Min6 cells was achieved in 11 days in the emulsion beads. Using primary human pancreatic cells, ~ 50% live cell recovery was obtained immediately after the emulsion bead process. The losses observed were mainly attributed to the acidic condition required for bead gelling. No significant detriments to scale-up were found after 10 days of culture of primary neonatal porcine pancreatic cells in the AHFBR.

THURSDAY AFTERNOON

2:00 – 5:00 pm BCEC 108

Biophysical and Biomolecular Symposium: Protein Engineering G. Makhatadze and R. S. Rajan, Organizers Papers 461 - 468

BIOT 461 - Beyond biology: Engineering new structure and function with computational protein design

Jeffrey G. Saven, Department of Chemistry, University of Pennsylvania, 231 South 34th St, Philadelphia, PA 19104, saven@sas.upenn.edu

Protein design opens new ways to probe the determinants of folding, to facilitate the study of proteins, and to arrive at novel molecules, materials and nanostructures. Recent computational methods for identifying the properties of amino acid sequences consistent with a desired structure and function will be discussed. Computationally designed protein-based molecular systems will be presented, including proteins tailored to accommodate nonbiological cofactors and their novel functional properties.

BIOT 462 - Directed evolution of conformational changes in peptides

Mark Blenner, Karuppiah Chockalingam, and **Scott Banta**, Chemical Engineering, Columbia University, 500 W 120th St, New York, NY 10027, mab2134@columbia.edu, sbanta@cheme.columbia.edu

The ability to change conformations in response to environmental perturbations is a central feature of many proteins. Stimulus-responsive peptides have been used to advance many important applications including: biomaterials, nanodevices, biosensors, bioseparations, tissue engineering, and drug delivery. Directed evolution is a powerful technique for the engineering of proteins and peptides with novel phenotypes, but the evolution of peptides to exhibit conformational changes in response to prescribed stimuli is especially challenging. We are developing new methods for the detection and separation of peptides based on their conformational states. The first method uses a single chain antibody coupled with affinity chromatography, and the second method uses sensitivity to proteolysis combined with phage display. By screening peptide libraries under different environmental conditions, we are finding novel peptides that exhibit alternative structures, and thus are structurally responsive to unique environmental cues such as reactive oxygen species, glucose, heavy metals, or TNT.

BIOT 463 - Engineering allosteric inteins for use as biosensors or in bioseparations

Alison R. Gillies, Department of Chemical Engineering, Princeton University, A-217 Engineering Quadrangle, Olden St., Princeton University, Princeton, NJ 08544, agillies@princeton.edu, and David W. Wood, Departments of Chemical Engineering and Molecular Biology, Princeton University, Princeton, NJ 08544

Inteins are intervening protein sequences that self-excise from various host proteins. Usually, the host protein's activity is dependent on the splicing reaction. It is possible to modify the intein's terminal residues so that it cleaves rather than splices. This project focuses on engineering allosteric inteins that can be controlled by the presence of small molecule ligands. Ligand-dependent splicing inteins have been created by inserting a hormone or sugar binding domain into the intein at a strategic site. The binding domain-intein complex is coupled to a thymidylate synthase selection system. Bacterial cells in which the thymidylate synthase gene has been knocked out are transformed with intein-controlled thymidylate synthase genes on plasmids and grown in thymineless medium. The cells are only able to grow when the intein is actively splicing, creating an effective biosensor for ligands that target the receptor. Ligand-controlled cleaving is currently being investigated for use in bioseparations.

BIOT 464 - Insights into protein splicing and the engineering of inteins

Brian Pereira[1], Georges Belfort[1], Shekhar Garde[2], Victoria Derbyshire[3], and Marlene Belfort[3]. (1) Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, pereib@rpi.edu, (2) Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, (3) Wadsworth Center, New York State Department of Health, Albany, NY 12208

An intein is an in-frame genetic insertion that is removed post-translationally through the process of protein splicing. Protein splicing is a highly complex series of reactions; by catalyzing and coordinating the reactions, the intein mediates the ligation of two protein fragments. Because of its properties, the intein has become a popular tool for a wide variety of applications. In order to gain insight into the engineering of inteins for applications, we have explored the details of the protein splicing reaction mechanism. Through site-directed mutagenesis and molecular modeling of the intein, we have identified the roles for well-conserved residues in the reaction mechanism. From these results, we have developed a model pathway for the different activities that the intein is capable of and demonstrated that competing reactions are responsible for which pathway is followed. With this knowledge, we have gained insight into how to engineer an intein for use in applications.

BIOT 465 - Picomolar affinity fibronectin antibody mimics by yeast display

Ben Hackel and **K. Dane Wittrup**, Chemical Engineering and Biological Engineering Departments, Massachusetts Institute of Technology, MIT, Cambridge, MA 02139, wittrup@MIT.EDU, wittrup@MIT.EDU

The tenth type III domain of human fibronectin (Fn3) is a small, stable protein with a beta-sandwich fold and three solvent-exposed loops (termed BC, DE, and FG). It has been demonstrated as an effective protein scaffold to engineer high affinity binders to multiple targets using mRNA display, phage display, and yeast two-hybrid methods. While variability in the length of

complementarity-determining regions is recognized as a critical factor in antibody diversity, past Fn3 libraries had fixed loop lengths. The current work addresses if loop length diversity can improve the affinity and recognition capacity of the Fn3 scaffold. A second question is if recurrent mutagenesis of selected populations, already a necessary component of in vitro display methods through PCR, enables rapid isolation of high affinity binders using yeast surface display libraries.

Oligonucleotides of multiple lengths containing NNB degenerate codons were used to diversify the length and amino acid composition of the BC, DE, and FG loops. The yeast surface display library had 2x10^7 Fn3 clones with four possible lengths of each loop. Clones that bound lysozyme were selected by multiple rounds of fluorescence-activated cell sorting. Every 2-3 rounds the enriched population was diversified by error-prone PCR, loop shuffling, and homologous recombination using a simple one-day protocol. Isolated clones were sequenced and characterized in terms of affinity and association and dissociation kinetics.

Multiple clones with picomolar equilibrium dissociation constants were identified. The majority of high affinity clones had BC and DE loops one amino acid shorter than wild-type suggesting that loop length diversity is a valuable element of Fn3 library design. Isolation of such high affinity clones from a relatively small initial library was enabled by affinity maturation via continued selection and diversification as demonstrated by parallel selections without mutagenesis and shuffling. The recurrent population diversification should be generally advantageous to protein engineering.

BIOT 466 - Intracellular ribosome display for selection of synthetic antibodies in the bacterial cytoplasm

Lydia M. Contreras Martinez and Matthew P. DeLisa, School of Chemical and Biomolecular Engineering, Cornell University, 304 Olin Hall, Ithaca, NY 14853, Fax: 607-255-9166, lmc67@cornell.edu

Ribosome display is a powerful *in vitro* selection tool to identify proteins with enhanced functional properties (i.e., affinity, specificity, thermal stability) from large combinatorial libraries. However, one limitation of ribosome display is that it only works *in vitro*, where efficient translation is challenging and where cellular factors (i.e., chaperones and isomerases) that might be required for efficient protein folding are absent. Additionally, *in vivo* verification is typically needed to ensure that any functional improvements discovered *in vitro* are reproducible inside host cells, where engineered proteins will eventually be manufactured. A final limitation of ribosome display and several other *in vitro* platforms (e.g., phage display) with respect to antibody engineering is the need for purification and immobilization of the target antigen. To address these shortcomings, we have engineered a modified ribosome display strategy for engineering antibodies that operates entirely inside of living cells, which is advantageous because: (1) expression and solubility of displayed proteins are intrinsic selection parameters imposed by the assay; and (2) the 'biopanning' process occurs entirely in the cytoplasm via co-expression of the antigen, thereby obviating the need for purification and immobilization. In particular, we will discuss how our ribosome display system has enabled stability maturation of functional antibody fragments directly in the *E. coli* cytoplasm. Such intracellular antibodies that are solubly expressed and function in the cytoplasm are known as intrabodies and have great potential in functional genomics/proteomics efforts and in molecular medicine.

BIOT 467 - Structure-guided optimization of a therapeutic bispecific antibody

Alexey Lugovskoy[1], Karl Hanf[1], Brian Miller[1], Steve Demarest[1], Jennifer Michaelson[2], Xin Wang[1], Ellen Garber[1], Jeff Browning[2], and Scott Glaser[1]. (1) Drug Discovery, Biogen Idec Inc, 14 Cambridge Center, Cambridge, MA 02142, Fax: 617-679-3635, alexey.lugovskoy@biogenidec.com, (2) Immunobiology, Biogen Idec Inc, Cambridge, MA 02142

Engineered bispecific antibodies that engage two different target epitopes represent a promising new class of therapeutic agents. Typically a single chain variable (scFv) fragment, which is linked to a monoclonal antibody to achieve dual specificity, displays low intrinsic stability and high aggregation propensity. To address these limitations, we applied structure-guided protein engineering techniques to increase thermostability of anti-LTbR scFv by over 15oC without compromising its affinity towards antigen. Further, we demonstrated that incorporation of a stabilized scFv into a full-length bispecific antibody led to a molecule with markedly improved properties.

BIOT 468 - Engineering protein stability using polyethylene glycol conjugation: GCSF as a case study

Rahul S. Rajan[1], Tiansheng Li[1], Christopher Sloey[2], Mohini Aras[3], Weston Sutherland[2], and David N. Brems[4]. (1) Department of Pharmaceutics, Amgen, Inc, One Amgen Center Drive 2-1-A, Thousand Oaks, CA 91320, Fax: (805)-498-8674, rrajan@amgen.com, (2) Amgen, Inc, Thousand Oaks, CA 91320, (3) University of California at Berkeley, Berkeley, CA 94720, (4) Pharmaceutics Department, Amgen Inc, Thousand Oaks, CA 91320

Kindly refer to abstract text in:
Protein Sci. 2006 May; 15 (5):1063-75

THURSDAY AFTERNOON

2:00 – 4:20 pm BCEC 107 B

Downstream Processing: High Throughput Screening in Process Development J. Coffman and G. Jagschies, Organizers Papers 469 – 474

BIOT 469 - Chromatographic HTS and simulation for improved process development and optimization

Arne Staby[1], Matthias Bensch[2], Jacob Nielsen[1], Janus C. Krarup[1], Thomas Budde Hansen[3], Steffen Kidal[4], Lars Sejergaard[5], Ernst Hansen[6], and Jürgen Hubbuch[2]. (1) Protein Separation, Novo Nordisk A/S, Hagedornsvej 1, DK-2820, Gentofte, Denmark, ast@novonordisk.com, (2) Institute of Biotechnology 2, Research Center Jülich, Julich, Germany, (3) Department of Protein Separation, Novo Nordisk A/S, Gentofte DK-2820, Denmark, (4) HAD1.175, Ge, Novo Nordisk A/S, DK-2820 Gentofte, Denmark, (5) Novo Nordisk, Denmark, (6) Protein Separation, CMC API Production, Novo Nordisk A/S, DK-2820 Gentofte, Denmark

Increased demand of material for clinical trials and handling of an increasing number of projects in the biopharmaceutical industry are calling for different ways of performing process development. This paper will present true examples of implementation of high-throughput screening techniques as stand alone tool and in combination with mathematical modelling for separation development, trouble shooting, and batch release for chromatographic purification steps. Further, these techniques will be discussed in relation to scale-up, general accuracy of results, and PAT.

BIOT 470 - Development and application of an automated, small-volume chromatography system for resin and condition screening

Mark Teeters, Daniel Bezila, Patricia Alred, and Ajoy Velayudhan, Purification Development, Centocor R & D, Inc, 145 King of Prussia Road, Radnor, PA 19087, mteeters@cntus.jnj.com

A small-volume chromatography system was developed for rapid resin and condition screening and applied in the purification of a monoclonal antibody from a key product-related impurity. Accounting for constraints in peripheral volume, gradient formation, column integrity, and fraction collection in microtiter plates, the resulting system employed 2.0 mL columns and was successfully integrated with plate-based methods for rapid sample analysis (e.g., use of automated liquid handlers, plate readers, and HPLC). Several cation-exchange chromatography resins were screened using automated programs and tailored gradients for the combination of a particular resin and a given antibody feedstock. Results from the tailored gradient runs were used to select a resin, and to arrive at efficient stepwise elution schedules for the chosen resin. By maintaining a constant residence time, final operating conditions were successfully scaled to representative bed heights and column diameters up to 2.6 cm. This approach significantly improved throughput while reducing development time and material consumption.

BIOT 471 - Fast acquisition of bioseparation process development data from crude protein mixtures

Tangir Ahamed[1], Beckley K. Nfor[1], Emile J. A. X. van de Sandt[2], Michel H. M. Eppink[3], Peter D. E. M. Verhaert[1], Gijs W. K. van Dedem[1], Luuk A. M. van der Wielen[1], and Marcel Ottens[1]. (1) Department of Biotechnology, Delft University of Technology, Julianalaan 67, Delft 2628 BC, Netherlands, t.ahamed@tudelft.nl, (2) DSM Anti-infectives B.V, Delft 2600 AK, Netherlands, (3) Biotechnology Operations, N.V. Organon, Oss 5340 BH, Netherlands

In this study, a novel two-dimensional (2-D) liquid chromatography (LC) system was developed for the fractionation and characterization of crude protein mixtures, in which a pH-gradient anion-exchange chromatography (AEC) was followed by a salt-gradient hydrophobic-interaction chromatography (HIC). This 2-D-LC fractionation system provided a complete profile of the major proteins present in a crude mixture and made pure protein fractions available. The pure fractions were analyzed in PAGE and MS to obtain the required process development data. In addition, the elution-pH and hydrophobicity of the components were readily available from the fractionation system, which are essential parameters for the development of downstream processes. For example, process scale IEC can be designed and optimized in a fast and rational way using the elution-pH data obtained from pH-gradient IEC.

Acknowledgement: This project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public private NWO-ACTS program.

BIOT 472 - High throughput process development: Determination of dynamic binding capacity using microtiter plates filled with chromatography resins

Karol M. Lacki, Tryggve Bergander, Anna Grönberg, and K. Öberg, R&D, GE Healthcare Bio-Sciences AB, Björkgatan 30, Uppsala, Sweden, Fax: +46(0)18 6121844, karol.lacki@ge.com

Reduction of R&D cost associated with the introduction of new drugs to the market should lead to less expensive bio-molecule based therapies. For a constant probability of success, this cost can be reduced if a larger number of drug candidates are considered in a given time, which puts higher demands on process development work. Introduction of high throughput techniques for process development are thus very important for improving the overall economy of drug manufacturing. Currently, process development related to downstream processing is performed using packed bed chromatography. Typical parameters studied include determination of dynamic binding capacity (DBC), efficient wash and elution protocols. These studies are both time and resources consuming. Therefore, faster and scaled-down methods that could significantly reduce time necessary for developing a chromatography step are needed. This presentation will describe an alternative approach to

column based investigations for generating DBC versus residence time data. The approach is based on collecting necessary data using microtiter filter plates filled with chromatographic resin and using the information obtained to predict dynamic binding capacities at various residence times. The proposed approach is less time consuming and requires significantly smaller sample size compared to traditional column based investigation. Analysis and evaluation of the proposed experimental method will be presented. Optimal conditions for different adsorption system will be discussed and recommendations will be given. Experimental results obtained with a model adsorption system consisting of MabSelect SuRe™ and hIgG, as well as obtained with a real feed containing monoclonal antibody will be presented. Experimental approaches for screening of load, wash and elution conditions will also be presented. The data obtained using the high throughput format will be compared with results obtained using the traditional column based approach.

BIOT 473 - High throughput screening of protein A resin for purification process development

James Booth[1], Jonathan Coffman[1], and Brian Kelley[2]. (1) Drug Substance Development, Wyeth BioPharma, 1 Burtt Road, Andover, MA 01810, jbooth@wyeth.com, (2) Wyeth BioPharma, Andover, MA 01810

Protein A chromatography has been used as a capture step in most antibody and Fc-fusion purification processes. The step concentrates the product, removes host cell protein and media derived impurities, and provides a product pool with an appropriate ionic strength, pH, and buffer for the following step. The step often suffers from precipitation, the level of which depends upon the strength and type of wash, the ionic strength of the elution buffer, the pH of neutralized peak, and the buffer used in elution and neutralization. A high throughput Protein A screen has been developed that quickly identifies acceptable operating conditions within a large experimental space. The screen evaluates 288 combinations of wash conditions, elution conditions, and neutralization conditions to allow facile optimization of the step in column mode. The screen has been an important factor that allows Phase I/II development of the Protein A step with a small number of lab-scale chromatographic runs.

BIOT 474 - High throughput screening in downstream processing – from solubility studies to packed bed chromatography systems

Jürgen Hubbuch, Institute of Biotechnology 2, Research Center Jülich, Stetternicher Forst, Juelich, Germany, j.hubbuch@fz-juelich.de

The development of protein purification processes has become a major bottleneck in the production of biopharmaceuticals. In order to tackle this situation robotic techniques for the screening of parameters which are relevant for the design of downstream processes, which could significantly reduce development time, have recently been developed. In this theme we developed strategies and miniaturized systems on a TECAN Evo Freedom platform covering the evaluation important process variables as well as the major unit operations in downstream processing. Next to batch operations such as solubility screens, ATPS, isothermal and kinetic finite bath data, we extended the systems capability to column screens operated under constant flow conditions. It is thus possible to use scale-down models of chromatographic columns as well as membrane stacks under controlled fluid flow conditions. A newly developed fraction collection device further enables us to take automatically fractions ranging from drop size to several hundred micro liters. In its current form 8 systems are operated in parallel. In this paper we introduce the technique, hardware and its application on HTS platforms. Finally, parameter estimation procedures for model based process development as well as a comparison to typical lab scale systems are addressed. The techniques are presented using industrial model systems.

THURSDAY AFTERNOON

2:00 – 4:20 pm BCEC 106

Downstream Processing: Disposable Bioprocessing D. D. Frey and S. Ramanan, Organizers, Papers 475 - 480

BIOT 475 - Regulatory considerations for using disposable technologies in bioprocessing

Antonio Moreira, Chemical and Biochemical Engineering, University of Maryland Baltimore County, Baltimore, MD 21250

Disposable technologies are becoming prominently used in the bioprocessing manufacturing industry by contract manufacturing organizations (CMOs) and for in-house manufacturing. This presentation will include a discussion of the regulatory issues and requirements concerning the use of disposable systems in biomanufacturing under CGMP compliance. A comparison of positive impacts (such as less intensive validation) and perceived increased burden (such as leachables/extractables issues and system integrity) will be addressed.

BIOT 476 - A comparison of conventional and controlled freezing methods

Matt Olsen, Stedim Biosystems North America, 445 Devlin Rd., Napa, CA 94558, **molsen@integratedbio.com**

Conventional freeze thaw techniques at production scale can present challenges to biologics during processing due to macroscopic freeze concentration of solutes. This effect results in solute concentration changes occurring differentially throughout the frozen volume. Controlled freezing is presented as a method capable of minimizing this macroscopic freeze concentration in production volume equipment. The effects of each method of freezing on the distribution of protein and solutes present in freezing containers have been examined. The concentration distribution has been found to vary significantly depending upon the type of freezing employed as well as the process scale.

BIOT 477 - Disposable chromatography in antibody manufacturing: The good the bad and the ugly

Uwe Gottschalk, VP Purification Technologies, Sartorius AG, Weender Landstr. 217, Goettingen 37075, Germany, Fax: +49-551-308-2835, Uwe.Gottschalk@sartorius.com

Virtually everything in bioprocessing can be made disposable and its implementation is simply a matter of overall process economy. With state of the art cost models and simulation tools disposable strategies can be easily assessed. Economy of scale effects allow for viable solutions in single use unit operations such as in buffer preparation and storage, harvesting, concentration and also chromatography. In chromatography disposability is at the doorstep. Polishing steps – operated in flow-through mode – are going to be dominated by membrane chromatography devices and provide the basis for two column processes – the next paradigm shift in bioseparation. The presentation will focus on case studies to assess the technical and economical feasibility of disposable unit operations in biomanufacturing.

BIOT 478 - Scalability of disposable Mixer system with a single-use levitating impeller

Alex Terentiev, LevTech, Inc, 1509 Bull Lea Road, Suite 300, Lexington, KY 40511, **aterentiev@levtech.net**

A reliable, efficient and scalable disposable mixing technology is required to transition single-use storage bags to disposable processing vessels. The LevTech LevMixer System (DB-200) incorporates a sterile single-use impeller which is levitated and rotated inside of biobags without the use of troublesome shafts, seals or bearings. This technology is based on a detachable superconductive drive unit that provides levitation and rotation of a magnetic impeller located inside of bags of varied sizes, shapes and configurations. This superconductive technology provides a completely disposable mixing platform with the convenience and torque of conventional bottom driven magnetic mixers as well as unparalleled grind-free and friction-free cleanliness. Unlike the impeller of traditional magnetically driven mixers, the levitating impeller requires no component that comes in physical contact with the bag and thus eliminates any particle shedding and mechanical stress to the bag film. In this presentation the results of powder dissolution tests and fluid-in-fluid mixing are presented for mixing bag volumes ranging from 50L to 2,000L with varied impeller sizes and locations. In these studies it is found that aspect ratio of the retaining tank (H/D) produces great effect on the mixing time. As a result of these studies a set of optimal powder mixing bag designs ranging form 50L to 1,000L have already been commercialized.

BIOT 479 - Use of a disposable tangential flow filtration skid to manufacture monoclonal antibodies

Harish Santhanam, Bioprocess Manufacturing, Eli Lilly, Lilly Technology Center, Drop Code 5613, Indianapolis, IN 46285, Fax: 317-276-1403, santhanam_harish_k@LILLY.COM

The use of disposable skids in downstream operations has many advantages. Ease of cleaning, flexibility and lack of capital expenditure being some of them. There is also an operational trade off with the use of disposable systems. The unit operations that utilize disposable systems become less automated and more operator dependant. There is less control over the processing conditions and there is an inability to monitor and acquire data that might be useful to troubleshoot the unit operation. A good strategy to overcome some of these drawbacks is the ability to use disposable systems with the capability to control and collect data. This presentation will outline the technologies used to optimally operate and control a disposable tangential flow filtration skid. The technologies used will enable data historian collection and analysis in a manufacturing environment without sacrificing the advantages of using disposable systems.

BIOT 480 - Viral clearance using disposable systems in Mab commercial downstream process

Joe Zhou, Process Development, Amgen, Inc, Mail Stop 30W-2-A, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-447-6212, **joez@amgen.com**

Disposable systems are becoming routine operations in Mab downstream manufacturing production due to their high product throughput, cost efficiency, and user friendly with no cleaning, life time and storage validations.
Once highly selective Protein A affinity is chosen for robust Mab downstream process, the major role of polishing steps is to remove product related impurities, trace amount of host cell proteins, DNA/RNA, and potential viral contaminations. Disposable systems can take place as powerful alternatives either to replace polishing column chromatography or as additions to ensure product purity and excellent viral clearance power for patents' safety.
In this presentation, the implementation of three disposable systems such as depth filtration, membrane chromatography, and nanometer filtration technology in a commercial process is introduced. The excellent data set of viral clearance with these systems is presented. Application advantages and disadvantages including cost analysis are further discussed.

THURSDAY AFTERNOON

2:00 – 5:25 pm BCEC 107 C

Biophysical and Biomolecular Symposium: Protein Aggregation

T. Przybycien and M. Ricci, Organizers Papers 481 - 487

BIOT 481 - Does one folding defect lead to another? The importance of quaternary structure in transthyretin-mediated inhibition of beta-amyloid aggregation

Lin Liu, Jie Hou, and Regina M. Murphy, Department of Chemical and Biological Engineering, University of Wisconsin, 1415 Engineering Drive, Madison, WI 53706, linliu@wisc.edu

Aß aggregation and its subsequent deposition as fibrils is the underlying cause of Alzheimer's disease. Transgenic mice studies suggest that transthyretin (TTR), a homotetrameric protein in blood and cerebrospinal fluid, interacts directly with Aß to inhibit its toxicity. We report results from biophysical analysis of Aß aggregation kinetics in the presence of plasma-derived TTR (pTTR). At substoichiometric ratios, pTTR drastically decreased the rate of aggregation. The data support a hypothesis wherein pTTR preferentially binds to aggregated Aß and arrests further growth. Recombinant TTR (rTTR), produced in *E. coli*, folded into the correct secondary structure and assembled into stable tetramers. However, rTTR failed to inhibit Aß aggregation and showed no binding to Aß. pTTR had higher thermal and acid stabilities and fewer accessible hydrophobic sites than rTTR. We concluded that rTTR, folding *in vitro* without the aid of chaperones, had subtle folding defects that damaged its ability to inhibit Aß aggregation.

BIOT 482 - Folding, unfolding, and fibril formation of human eye lens γC-crystallin

Yongting Wang and Jonathan King, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, ytwang@MIT.EDU

Human γC crystallin (HγC-Crys) is a major protein of the lens nucleus. Aggregated and oxidatively damaged forms are recovered from the insoluble fraction of nuclear cataracts. HγC-Crys is a two domain all β-sheet monomer, homologous to γD- and γS-crystallins. Mutations in the genes encoding these proteins are associated with juvenile-onset cataracts[1]. Equilibrium unfolding and refolding experiments at pH 7, 37°C, revealed that wild-type HγC-Crys exhibited a transition midpoint of 2.7M GuHCl. The transition was reversible above 1.0 M GuHCl. An aggregation pathway competed with productive refolding at near physiological conditions, with denaturant concentrations below 1.0M GuHCl. Similar aggregation observed in HγD-Crys were previously observed to have branched fibril structure using AFM[2]. When incubated without denaturant at pH 3 and 37°C, HγC-Crys assembled into non-branched fibril structures *in vitro*. Characterization by dye binding assays, polarized microscopy, and transmission electron microscopy showed that these non-branched species have characteristics of fibrils associated with amyloid diseases. The formation of HγC-Crys fibrils under destabilizing conditions may be significant for the development of cataract with aging.

1. Liang, J.J., Interactions and Chaperone Function of αA-Crystallin with T5P γC-Crystallin Mutant. Protein Sci, 2004. 13(9): 2476-82. 2. Kosinski-Collins, M.S. and King, J., In Vitro Unfolding, Refolding, and Polymerization of Human γD Crystallin, a Protein Involved in Cataract Formation. Protein Sci, 2003. 12(3): 480-90.

BIOT 483 - Molecular insights into self-perpetuating conformational changes of prions and amyloids

Peter M Tessier, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, tessier@wi.mit.edu, and Susan Lindquist, Whitehead Institute for Biomedical Research & Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

Protein conformational changes drive many biological processes. Few are as dramatic in terms of the structural changes that occur, or the biological consequences they produce, as those governing the formation of amyloid fibers. These highly ordered, beta-sheet rich structures were initially linked to several neurodegenerative disorders such as Alzheimer's disease, but more recently have also been linked to normal biological functions such as cell adhesion. Prions represent a unique class of amyloid-forming proteins capable of switching to self-perpetuating conformations that are infectious. We have recently found that several outstanding questions related to the biology of amyloids and prions can be investigated using arrays of short, surface-bound peptides. In this presentation I will discuss the use of peptide arrays to identify small segments within the sequences of these proteins that drive amyloid nucleation and propagation, and show how these results can be used to elucidate several questions related to their biology.

BIOT 484 - Folding, misfolding, and aggregation of polyglutamine peptides and proteins

Matthew Tobelmann, Christine Lee, Robert Walters, and Regina M. Murphy, Department of Chemical and Biological Engineering, University of Wisconsin, 1415 Engineering Drive, Madison, WI 53706, tobelmann@wisc.edu

In Huntington's and related diseases, proteins with expanded polyglutamine domains are expressed; these proteins aggregate into inclusions that are believed to cause neuronal degeneration. Synthetic polyglutamine peptides are useful models of polyglutamine-containing proteins. A widely accepted hypothesis is that polyglutamine aggregation follows a nucleation-elongation mechanism characterized by a lag time and a monomeric nucleus. We re-examined this hypothesis by measuring the aggregation kinetics of $K_2Q_{23}K_2$, using light scattering and size exclusion chromatography. During the putative lag time there is substantial organization of the peptide into soluble linear aggregates. We propose that polyglutamine assembles first via hydrophobic interactions, which then convert into insoluble ß-sheet fibrils via

slow conformational changes. Once fibrils form, monomer loss is accelerated, possibly through templated assembly. We established a model system using apomyoglobin as the host protein, and generated a library of mutants containing 16 - 102 glutamines. Detailed characterization of folding, stability, and aggregation of this library is underway.

BIOT 485 - Refolding aggregation-prone GPCRs and G-protein α subunits from inclusion bodies expressed in Escherichia coli

Emily C. McCusker[1], Steven E. Bane[2], and **Anne Skaja Robinson**[2]. (1) Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19711, mccusker@udel.edu, (2) Department of Chemical Engineering, University of Delaware, Newark, DE 19711, asr@udel.edu

G protein-coupled receptors (GPCRs) are seven-helix transmembrane proteins involved in numerous cellular process and diseases in humans; they are the targets of over 30% of drug discovery efforts. Heterotrimeric G-proteins are membrane-associated signaling partners of GPCRs that initiate the signaling cascades, and therefore both GPCR and G-proteins are of great interest for biophysical characterization and high-resolution structural studies, necessitating their large-scale production. We have expressed the neurokinin NK_1R, a class A (rhodopsin-like) GPCR, and representative members of the G_α family as inclusion bodies in *Escherichia coli* and determined best-case refolding strategies to minimize aggregation. We found that NK_1R expression activated the stress response of *E. coli*, but that NK_1R was not degraded, unlike many other GPCR that were expressed as inclusion bodies. We have purified milligram amounts of inactive NK_1R from detergent-solubilized inclusion bodies for use in characterization and refolding studies. We have also developed a novel strategy for refolding G_α subunits expressed as *E. coli* inclusion bodies. The refolded G_α subunits are monomeric, bind GDP, and have native-like α-helicity.

BIOT 486 - Nonnative protein polymers: Structure, morphology, and relation to nucleation and growth rates

William F. Weiss IV, Christopher J. Roberts, and Abraham M. Lenhoff, Department of Chemical Engineering, University of Delaware, Newark, DE 19716, wweiss@udel.edu

A rigorous, mechanistic understanding of protein aggregation is critical both to prevent losses in the biopharmaceutical industry and to combat aggregation-based disease. To this end we have characterized thermally-induced, soluble aggregates from two model protein systems, α-chymotrypsinogen A and bovine granulocyte-colony stimulating factor. Static and dynamic light scattering, cryo-TEM and circular dichroism measurements indicate that soluble aggregates of these proteins are linear, semi-flexible chains of non-native monomers with relatively low polydispersity. Although they are non-fibrillar, aggregates from both systems bind thioflavine T, indicating this is not a conclusive assay for amyloid fibrils. Manipulating incubation time and initial monomer concentration provides control over the length of the aggregates. A mechanistic basis for these observations is offered by a novel analysis based on a nucleated-polymerization model, which allows separate nucleation and growth time scales, the predominant nucleus size, and the number of monomers per polymerization step to be quantified.

BIOT 487 - Osmolyte controlled nucleation of insulin amyloid fibrillation

Arpan Nayak, Howard Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th street, Troy, NY 12180, Fax: 518-276-4030, nayaka@rpi.edu, Chuang-Chung Lee, Department of Chemical Engineering, Massachussets Institute of Technology, Cambridge, MA 02139, Gregory J. McRae, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139-4307, and Georges Belfort, Howard P. Isermann Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590

Amyloid fibrillation is the process of native soluble proteins misfolding into insoluble fibrils comprising of cross-β-sheets and has received wide attention due to its substantial physiological relevance and the complexity of the underlying physical and chemical reactions. At present, more than 20 amyloidogenic diseases including Alzheimer's disease, Parkinson's disease, and prion–associated encephalopathies have been found to share fibril formation as a common phenomenon. Human insulin is chosen as a model molecule for our study because (i) it is associated with a clinical syndrome, injection-localized amyloidosis, (ii) it is a member of the class of fibril forming proteins that loses its zinc-coordinated hexameric structure to form oligomers and then fibrils, (iii) of its well-characterized in vitro fibrillation kinetics under well-defined solution conditions (2 mg/ml, pH 1.6 and 65ºC), and (iv) fibril formation is a problem in commercial isolation and purification of insulin at low pH values of 1-3. Here, we investigate the influence of dissolved osmolytes on the kinetics of insulin fibrillation. While sugars (stabilizing osmolytes) delay the onset of fibrillation, urea and guanidine (destabilizing osmolytes) accelerate it. The inhibiting effect of sugars is correlated with their heats of solution and is explained by the theory of preferential exclusion of sugar molecules from protein surfaces. We show that osmolytes with higher neutral surface area have a better protecting effect on the native protein. A mathematical mechanistic model that simulates the phenomena by incorporating the chemical reactions of nucleation and growth dynamics is presented. Using model fits of the experimental data, the rate constants and Gibbs free energy for nucleation are estimated. Both increased or decreased in presence of destabilizing and stabilizing osmolytes, respectively. Taken all together, these results provide a thermodynamic basis for the mechanism of amyloid fibrillation and provide insight into the role of sugar-based excipients for pharmaceutical formulations.

American Chemical Society
Division of Biochemical Technology
234th ACS National Meeting, Boston, MA, August 19-23, 2007

W. Chen, W. Wang, A. S. Rathore, Program Chairs

Author Index

Massachusetts Bay Transportation Authority

Subway Fares & Passes (effective January 2007)

The T subway system is comprised of the Blue, Orange, Green, Red, and even part of the Silver Line. Riders can travel anywhere on the system in any direction for the same low fare.

Get the Plastic CharlieCard and Save!

http://www.mbta.com/fares_and_passes/subway/

CharlieCard	**$1.70** FREE transfer to Local Bus DISCOUNTED transfer to Express Bus
CharlieTicket/ Cash-on-board	**$2.00**
Monthly LinkPass	**$59**/month Unlimited travel on Subway plus Local Bus.
Day/Week LinkPass	**$9.00** for 1 day/**$15.00** for 7 days Unlimited travel on Subway, Local Bus, Commuter Rail Zone 1A, and Inner-Harbor Ferry.
Seniors and Persons with Disabilities (Blind persons ride for free)	**$.60**/ride/**$20**/month Unlimited travel on Local Bus and Subway. Requires a Senior/T.A.P ID or Blind Access Card.
Students (Junior High and High School)	**$.85**/ride/**$20**/month Unlimited travel on Bus, Subway, Express Bus, and Commuter Rail Zones 1, 1A and 2 until 8p.m. on school days. Requires Student ID Badge.
Children 11 and under	**Free** When accompanied by a paying adult.