The University of Iowa
Iowa City, Iowa

TWENTY FIFTH ANNUAL

Biocatalysis and Bioprocessing Conference

October 17-18, 2016

Iowa Memorial Union
Iowa City, Iowa

First Annual Biocatalysis and Bioprocessing Conference
Center for Biocatalysis and Bioprocessing
The University of Iowa

PROGRAM

Monday. June 10, 1991

6:00 - 9:00 p.m.	Registration and Mixer

Tuesday, June 11, 1991

7:30 a.m.	Breakfast, Coffee and Registration
8:45 - 9:30	Welcoming Remarks
	Hunter R. Rawlings III, President University of Iowa
	Terry E. Branstad, Governor State of Iowa
	"Biocatalysis at The University of Iowa" **Dr. John P. N. Rosazza**, Director Center for Biocatalysis and Bioprocessing
9:30 - 10:00	"New Applications of Biocatalysis" **Dr. Jonathan S. Dordick**, Chemical and Biochemical Engineering
10:00 - 10:30	Break - Coffee and Discussion
10:30 - 11:00	"Biopolymer Structure and Activity" **Dr. Robert J. Linhardt**, Medicinal and Natural Products Chemistry
11:00 - 11:30	"Bioactive Natural Products" **Dr. David F. Wiemer**, Chemistry
11:30 - 12:00	"Catalysis by Alcohol Dehydrogenases" **Dr. Bryce V. Plapp**, Biochemistry
12:00 - 1:30 p.m.	Lunch
1:30 - 2:00	"Biosensors" **Dr. Mark A. Arnold**, Chemistry
2:00 - 2:30	"Biodegradation of Aromatic Compounds" **Dr. Caroline S. Harwood**, Microbiology
2:30 - 3:00	Closing Remarks and Informal Discussion **Dr. David T. Gibson**, Microbiology
3:00 - 3:30	Coffee Break
3:30 - 5:30	Poster Session
5:30 - 6:30	Free Time
6:30 - 7:15	Reception
7:15	Dinner

THE UNIVERSITY OF IOWA

25th Annual

Biocatalysis and Bioprocessing Conference

October 17-18, 2016

"Microbial Factories and Biocatalytic Science"

Sponsored by:

THE UNIVERSITY OF IOWA

Center for Biocatalysis and Bioprocessing

http://cbb.research.uiowa.edu/

25th Annual
Biocatalysis and Bioprocessing Conference

"Microbial Factories and Biocatalytic Science"

Sponsored by:
The University of Iowa
Center for Biocatalysis and Bioprocessing

October 17-18, 2016

Conference Organizing Committee:

Mark Arnold, Ph.D.
Shuvendu Das, Ph.D.
Sridhar Gopishetty, Ph.D.
Amnon Kohen, Ph.D.
Tonya Peeples, Ph.D.
Lisa Kinzenbaw
Troy McCarthy
Mitchell Rotman

Director
Mark Arnold, Ph.D.

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Message from the CBB Director

The 25th CBB Conference is scheduled for October 17 and 18. As in the past, the conference will be held in the beautiful downtown campus of The University of Iowa. Attendees will include world-renowned speakers from both outside and within the CBB, representatives from government, industry and academia, as well as students across science disciplines. Graduate students will present oral and poster presentations on a wide array of topics within the scope of Biocatalytic Sciences. The proceedings are conducted within a friendly, open atmosphere to promote discussions and encourage interactions between attendees.

The dual-theme for this year's conference is Microbial Factories and Biocatalytic Science. Microbial factories represent a major source of manufacturing both in the United States and around the world. Microbial fermentation is central in the production of novel products, including industrial enzymes, food supplements, biotherapeutics, animal health products, and renewal fuels. Research to enhance production yields and overall efficiency of microbial factors is underway as the means to improve manufacturing processes, thereby reducing consumer costs while improving product quality.

Biocatalytic Science broadly covers basic and applied research that centers on enzymes and their function within an array of scientific disciplines, including: 1) discovery of new enzymes and understanding their mechanism of action at the molecular level, 2) exploration of biochemical and cellular pathways as novel drug targets, 3) design and manufacturing of biotherapeutics, such as vaccines for both human and animal health, 4) developing enzyme-mediated chemical transformations for specialty chemicals, 5) metabolic engineering, 6) directed evolution, 7) genomics, 8) agricultural feedstock utilization, and 9) expression, production and purification of protein products. A small fraction of the rich diversity of research ongoing within academia and industry in the area of *Biocatalytic Science* will be highlighted in this year's conference.

It is my pleasure to welcome all the attendees for this conference and also thank its sponsors. Mingling and stimulating scientific discussions are encouraged.

Sincerely,
Mark Arnold, Ph.D.

Director; Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA

Professor and Edwin B. Green Chair in Laser Chemistry, College of Liberal Arts & Sciences, Department of Chemistry, University of Iowa, Iowa, City , IA

25th Annual Center for Biocatalysis and Bioprocessing Conference

"Microbial Factories and Biocatalytic Science"

Iowa Memorial Union, Iowa City, IA

MONDAY, OCTOBER 17, 2016

IOWA THEATER (1ST FLOOR)

12:00 – 1:30 pm	Registration – Hubbard Lobby (outside Iowa Theater, 1st floor IMU)
1:30 – 1:45	Opening Remarks: **Mark Arnold, Ph.D.**, Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA Professor and Edwin B. Green Chair in Laser Chemistry, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa, City, IA
1:45 – 2:30	**David F. Wiemer, Ph.D.,** F. Wendell Miller Professor of Chemistry and Professor of Pharmacology, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa, City, IA *"Biologically Active Compounds Based on the Intermediates of Isoprenoid Biosynthesis"*
2:30 – 3:15	**Robert Kerns, Ph.D.,** John L. & Carol E. Lach Chair in Drug Delivery Technology, Professor and Department Chair, Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA *"New Bioactive Glycoconjugates Derived From Chemical Modification of Sulfated Saccharides"*
3:15 – 3:30	Break
3:30 – 4:15	**Robert J. Linhardt, Ph. D.,** Ann and John H. Broadbent, Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering , Professor of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY *"Chemoenzymatic Synthesis of Heparin and Low Molecular Weight Heparins"*
4:15 – 5:00	**John W. Frost, Ph.D.,** University Distinguished Professor, Department of Chemistry, Michigan State University, East Lansing, MI *"Straight or Bent? Synthesis of Biobased Aromatics"*

BALLROOM (2ND FLOOR)

5:00 – 6:30 pm	Social Gathering and Poster set up – Ballroom, 2nd Floor IMU
5:00 – 6:00	Fermentation Facility Tour– **please register for tour online**
6:30 – 7:30	Welcome Dinner/Buffet – Ballroom, 2nd Floor IMU
7:30 – 8:15	**CBB Past, Present and Future: Comments from CBB Directors** Jack Rosazza, Ph.D., Mani Subramanian, Ph.D., Mark Arnold Ph.D.

TUESDAY, OCTOBER 18, 2016

IOWA THEATER (1ST FLOOR)

7:30 – 8:00 am	Registration – Hubbard Lobby (outside Iowa Theater, 1st floor IMU)
7:30 – 8:30	Continental Breakfast – across from Iowa Theater, 1st floor IMU
8:30– 8:35	**Program** – Iowa Theater, 1st floor IMU **Introduction and Welcome** **Mark Arnold, Ph.D.**, Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA Professor and Edwin B. Green Chair in Laser Chemistry, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa, City , IA
8:35– 8:45	**Joe Hrdlicka,** Executive Director, Iowa Biotechnology Association Des Moines, IA "*Benefits of IowaBio Membership"*
8:45– 9:30	**Maria Spies, Ph.D.,** Associate Professor, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA *"Single-molecule Studies of FeS DNA Helicases: Kinetics, Conformational Dynamics and Molecular Mechanisms"*
9:30– 10:15	**Eric Nuxoll, Ph.D.,** Associate Professor**,** Department of Chemical and Biological Engineering, College of Engineering, University of Iowa, Iowa City, IA *"Wireless Deactivation of Bacterial Biofilms"*
10:15– 10:30	Break – Iowa Theater Lobby, 1st floor IMU
10:30– 11:15	**Ronen Tchelet, Ph.D.,** VP Research & Business Development, Dyadic International Inc. Jupiter, FL *"C1 – the Journey of More Productive Cell Expression"*
11:15– 12:00	**Laura R. Jarboe, Ph.D.,** Associate Professor**,** Department of Chemical and Biological Engineering, Iowa State University, Ames, IA *"Metabolic Engineering of Robust Microbial Cell Factories"*
12:00– 1:30	Lunch – Ballroom, 2nd Floor IMU External Advisory Board-Penn State Room

Afternoon Session – Iowa Theater, 1st Floor, IMU

1:30 – 2:45	**CBB/NIH Fellow Presentations** **Benjamin J. Foust,** Professor David F. Weimer Research Group, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA **"***A New Motif for Inhibitors of Geranylgeranyl Diphosphate Synthase"*

Mark S. Miller, Professor Adrian H. Elcock Research Group, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
"Re-parameterization of Protein Force Fields Guided by Osmotic Coefficient Measurements from Molecular Dynamics Simulations"

Nicholas Vance, Professor M. Ashley Spies Research Group, Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
"Probing Allosteric Regulation of an Executioner Caspase"

2:45 – 3:00 Concluding remarks

3:00 – 5:00 **Poster Session** – Wine/hors d'oeuvres, Ballroom 2nd Floor IMU
Announcement of Usha Balakrishnan Award Winner
Announcement of Director Award Winner
Announcement of Mani Subramanian Award Winner
S^3 Product Show

5:00 **Adjourn**

List of Oral Presentations

ORAL PRESENTATIONS

1. **BIOLOGICALLY ACTIVE COMPOUNDS BASED ON THE INTERMEDIATES OF ISOPRENOID BIOSYNTHESIS**
   David F. Wiemer, Ph.D.
   Wendell Miller Professor of Chemistry and Professor of Pharmacology, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

2. **NEW BIOACTIVE GLYCOCONJUGATES DERIVED FROM CHEMICAL MODIFICATION OF SULFATED SACCHARIDES**
   Robert Kerns, Ph.D.
   John L. & Carol E. Lach Chair in Drug Delivery Technology, Professor and Department Chair, Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

3. **CHEMOENZYMATIC SYNTHESIS OF HEPARIN AND LOW MOLECULAR WEIGHT HEPARINS**
   Robert J. Linhardt, Ph.D.
   Ann and John H. Broadbent, Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering, Professor of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY

4. **STRAIGHT OR BENT? SYNTHESIS OF BIOBASED AROMATICS**
   John W. Frost, Ph.D.
   University Distinguished Professor, Department of Chemistry, Michigan State University, East Lansing, MI

5. **CBB PAST, PRESENT AND FUTURE: COMMENTS FROM CBB DIRECTORS**
   Jack Rosazza, Ph.D. (1994-2005)
   Professor Emeritus, Medicinal and Natural Products Chemistry, Director Emeritus, Center for Biocatalysis and Bioprocessing, University of Iowa, Iowa City, IA

   Mani Subramanian, Ph.D. (2005-2015)
   Professor Emeritus, Chemical and Biochemical Engineering, Director Emeritus, Center for Biocatalysis and Bioprocessing, University of Iowa, Iowa City, IA

   Mark Arnold, Ph.D. (2015-present)
   Director, Center for Biocatalysis and Bioprocessing, University of Iowa
   Professor and Edwin B. Green Chair in Laser Chemistry,
   Department of Chemistry,
   University of Iowa, Iowa City, IA

6. **BENEFITS OF IOWABIO MEMBERSHIP**
   Joe Hrdlicka
   Executive Director of the Iowa Biotechnology Association
   Des Moines, IA

7. **SINGLE-MOLECULE STUDIES OF FES DNA HELICASES: KINETICS, CONFORMATIONAL DYNAMICS AND MOLECULAR MECHANISMS**
   Maria Spies, Ph.D.
   Associate Professor, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

8. **WIRELESS DEACTIVATION OF BACTERIAL BIOFILMS**
   Eric Nuxoll, Ph.D.
   Associate Professor**,** Department of Chemical and Biological Engineering, College of Engineering, University of Iowa, Iowa City, IA

9. **C1 – THE JOURNEY OF MORE PRODUCTIVE CELL EXPRESSION**
   Ronen Tchelet, Ph.D.
   VP Research & Business Development, Dyadic International Inc. Jupiter, FL

10. **METABOLIC ENGINEERING OF ROBUST MICROBIAL CELL FACTORIES**
    Laura R. Jarboe, Ph.D.
    Associate Professor, Department of Chemical and Biological Engineering
    Chair, Interdepartmental Microbiology Graduate Program, Iowa State University, Ames, IA

11. **A NEW MOTIF FOR INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE**
    Benjamin J. Foust
    Ph.D. Candidate, Professor David F. Wiemer Research Group, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

12. **RE-PARAMETERIZATION OF PROTEIN FORCE FIELDS GUIDED BY OSMOTIC COEFFICIENT MEASUREMENTS FROM MOLECULAR DYNAMICS SIMULATIONS**
    Mark S. Miller
    Ph.D. Candidate, Professor Adrian H. Elcock Research Group, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

13. **PROBING ALLOSTERIC REGULATION OF AN EXECUTIONER CASPASE**
    Nicholas Vance
    Ph.D. Candidate, Professor M. Ashley Research Group, Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Speaker's Profiles

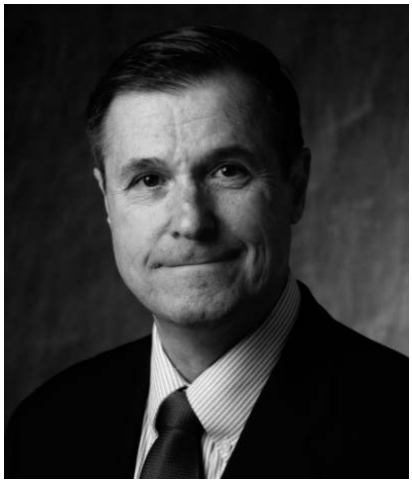
David F. Wiemer, Ph.D.
F. Wendell Miller Professor of Chemistry and Professor of Pharmacology
Department of Chemistry
College of Liberal Arts & Sciences
University of Iowa

David F. Wiemer was born and raised in southeastern Wisconsin. He received a B.S. degree in Chemistry from Marquette University, earned the Ph.D. degree at the University of Illinois, and was an NIH postdoctoral fellow at Cornell University. He joined the faculty in the Department of Chemistry at the University of Iowa as an assistant professor, and now holds the rank of F. Wendell Miller Professor of Chemistry. His research interests include synthetic methodology based on organophosphorus chemistry, synthesis of phosphonate analogues of isoprenoid phosphates as potential enzyme inhibitors, and synthesis of biologically active natural products, especially potential anti-cancer agents.

Robert Kerns, Ph.D.
John L. & Carol E. Lach Chair in Drug Delivery Technology
Professor and Department Chair
Department of Pharmaceutical Sciences & Experimental Therapeutics
College of Pharmacy
University of Iowa

Robert Kerns is the John L. & Carol E. Lach Chair in Drug Delivery Technology at the University of Iowa, College of Pharmacy, where he is also Chair of the Pharmaceutical Sciences and Experimental Therapeutics Department. Professor Kerns received his BS degree in Chemistry from Iowa State University in 1991. After earning his Ph.D. in Medicinal and Natural Products Chemistry from the University of Iowa in 1996 he completed postdoctoral studies in the Department of Chemistry at Princeton University, where he was a National Research Service Award recipient. Dr. Kerns joined the faculty at Wayne State University as Assistant Professor of Pharmaceutical Sciences in 1998, where he also held an associate appointment with the Department of Immunology and Microbiology in the Wayne State University School of Medicine. In 2002 Dr. Kerns joined the faculty in the Division of Medicinal & Natural Products Chemistry at the University of Iowa. His research group is involved in numerous interdisciplinary, translational research projects focused on the design, synthesis and evaluation of novel bioactive small molecule therapeutics. Ongoing projects include optimizing lead structures as chemical probes to validate new drug targets and as lead compounds for further development as possible new therapeutics for the treatment of drug-resistant infectious diseases, inflammatory lung diseases, obesity-related diseases and cancer.

Robert J. Linhardt, Ph.D.
Ann and John H. Broadbent, Jr. ’59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering
Professor of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering
Rensselaer Polytechnic Institute



Robert J. Linhardt received his Ph.D. from Johns Hopkins University (1979), was a postdoctoral fellow at MIT with Professor Langer (1979-1982) and on the faculty of the University of Iowa from 1982-2003. He is currently the Senior Constellation Professor of Biocatalysis and Metabolic Engineering at Rensselaer, holding appointments in Chemistry, Biology, Chemical Engineering & Biomedical Engineering. He is an Adjunct Professor of Orthopaedics at the Icahn School of Medicine at Mount Sinai and an Adjunct Professor of Pharmacy at Albany College of Pharmacy. His honors include the American Chemical Society (ACS) Isbell, ACS Hudson & ACS Wolfrom Awards, the Volwiler Award, an USP Award, is a Fellow of the AAAS, is a Fellow of the National Academy of Inventors (NAI), and one of the Scientific American Top 10. His research in glycoscience has resulted in over 750 peer-reviewed manuscripts and over 50 patents.

John W. Frost, Ph.D.
University Distinguish Professor
Department of Chemistry
Michigan State University

John W. Frost is a University Distinguished Professor in the Department of Chemistry at Michigan State University. He earned his B.S. in Chemistry from Purdue University and his Ph.D. from the Massachusetts Institute of Technology and was a postdoctoral fellow at Harvard University.

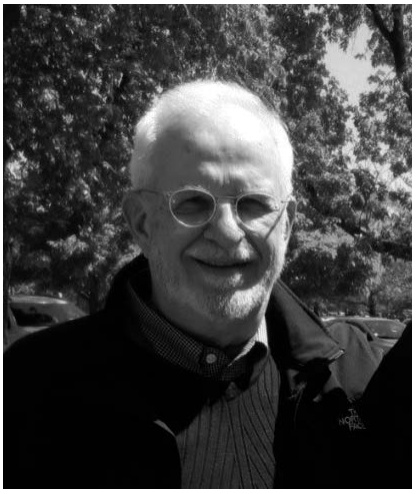
Professor Frost's research group genetically engineers microbes for use as synthetic catalysts and interfaces these biocatalysts with traditional chemical catalysis. Recent research focuses on elaboration of microbe-catalyzed syntheses of starting materials critical to the manufacture of pharmaceuticals as a replacement for the current isolation of these starting materials from exotic natural sources. Hoffmann La Roche has licensed a microbe developed by the Frost group for synthesis of shikimic acid, the starting material used for manufacture of the antiinfluenza drug sold under the trade name Tamiflu.

The Frost group is internationally recognized for its research in the field of green chemistry, where group research is directed toward creation of sustainable, environmentally benign syntheses of a variety of chemicals. These syntheses are catalyzed by genetically engineered microbes and utilize nontoxic starting materials such as glucose derived from renewable feedstocks such as starch or cellulose. In contrast, current chemical manufacture is dominated by the use of toxic starting materials such as benzene, which is derived from petroleum, a nonrenewable feedstock. For their research efforts in this area, Professor Frost and his collaborator and wife, Dr. Karen M. Draths, were awarded the Presidential Green Challenge Award in 1998.

Professor Frost has served in various capacities including consultant and scientific advisory board member for numerous corporations. He and Dr. Karen M. Draths cofounded Draths Corporation in 2005. Draths Corporation was a Michigan-based company spearheading the commercialization of the Frost technology portfolio developed at Michigan State University. Draths Corporation was purchased by Amyris Inc. in 2011. Professor Frost served as Chief Science Officer of Draths Corporation from 2005-2010 and has since returned to Michigan State University to again pursue his academic interests as a University Distinguished Professor.

CBB Past, Present and Future: Comments from CBB Directors

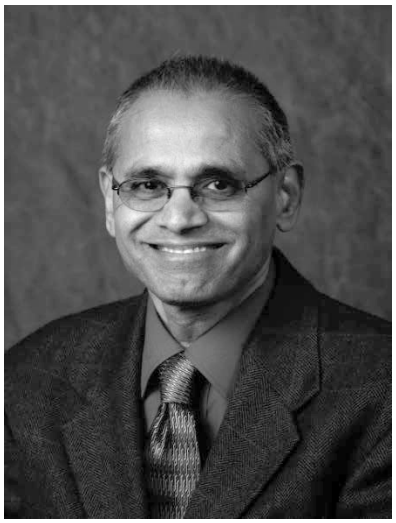
John P. N. Rosazza, Ph.D.
Professor Emeritus, Medicinal and Natural Products Chemistry
Director Emeritus, Center for Biocatalysis and Bioprocessing
University of Iowa
(1994-2005)



Jack Rosazza earned the PhD in Pharmacognosy/Synthetic Organic Chemistry at UConn, was a postdoc at Wisconsin in Pharmaceutical Biochemistry and moved to Iowa in 1969. He achieved full professorship in Medicinal and Natural Products Chemistry (MNPC) in 1977. Jack served as head of MNPC for 18 years. He is co-founder and the first director of the UI, Center for Biocatalysis and Bioprocessing. Jack's research always had a natural products bent focusing on all aspects of the chemistry, biochemistry, and enzymology of biocatalytic transformations of natural and synthetic organic compounds. His lab discovered a host of biocatalytic reactions, new enzymes, new organisms, and applications, and the first bacterial nitric oxide synthase system. He served as mentor for 30 Phds, 10 MSc students and 32 postdocs. Jack and his research group published more than 220 publications and patents during a 38-year career. He was widely sought as a consultant for Pharma, chem and agrochem industries, and the NCI; served on eight editorial boards, was active in ACS, ASP (President, Fellow), SIM; and served widely on collegiate, university, state and national committees.

CBB Past, Present and Future: Comments from CBB Directors

Mani V Subramanian, Ph.D.
Professor Emeritus, Chemical and Biochemical Engineering
Director Emeritus, Center for Biocatalysis and Bioprocessing
University of Iowa
(2005-2015)



Mani Subramanian earned PhD in Biochemistry (1978) at The Indian Institute of Science, Bangalore, India. He was a post-doctoral fellow with Professor David Gibson at The University of Texas, Austin (1978-81). Subsequently, he joined The Dow Chemical Company as a Senior Research Chemist and progressed to Project Leader and Research Leader (1981-1991). At Dow, he was involved in Applications of Enzymes for Production of Chemicals, Discovery of Chemical Leads and Target Site/Mechanism of Action of Agricultural Chemicals including from Natural Products. In the process, he set up micro titer plate-based High Throughput Screening at whole plant and enzyme levels for chemical lead discovery. In 1991, he moved to Sandoz Agro Chemicals in Palo Alto CA, where he continued the lead discovery process for Agricultural Applications, HTP screening, and also took the responsibility for Engineering Herbicide Resistance in Crops, particularly Maize and Soybeans. In 1998, Mani moved to Maxygen where he found new applications for Gene Shuffling in the areas of 'Value-Added Traits' in Crops. In 1999, Mani rejoined The Dow Chemical Company as the Global R&D Director for Biotechnology, Bioinformatics and Bioprocessing and built the Biotechnology R&D Center of 70 scientists. State of the Art Fermentation Technology and Process Laboratories were built in San Diego from ground-up. He also initiated a gemome-based approach for Protein Production in *Pseudomonas fluorescens*. This was commercialized and Trade Marked as *Pfenex*, which 2009 was spun-off as stand-alone company. Mani took early retirement from Dow in 2005 and joined U of Iowa as Director, The Center for Biocatalysis & Bioprocessing (CBB) & Professor, Chemical & Biochemical Engineering. Here expanded and modernized the CBB fermentation and downstream processing operations, and built the GMP-facility for production of proteins for human trials. Mani retired from CBB in June 2015. Mani has won several Awards from Dow and Sandoz for Innovation and commercialization of technology. He has also licensed technology developed in his laboratory at the University of Iowa to two different companies. He started a 'metabolic engineering-based' company from the technology developed in his laboratory in 2013 and sold the company in 2016. Mani continues to do research as Professor Emeritus, in the areas of protein expression and production of chemicals by Synthetic Biology. He has over 100 publications and 20 US and International Patents.

CBB Past, Present and Future: Comments from CBB Directors

Mark Arnold, Ph.D.
Director, Center for Biocatalysis and Bioprocessing
University of Iowa
Professor and Edwin B. Green Chair in Laser Chemistry
Department of Chemistry
University of Iowa
(2015- present)

Mark Arnold is the Director of the Center for Biocatalysis and Bioprocessing as well as the Edwin B. Green Chair Professor in Laser Chemistry at the University of Iowa. He started as an assistant professor in Chemistry at the University in 1982 after completing his doctorate degree at the University of Delaware. His research program focuses on the development of *in situ* chemical sensing technology that is designed to report concentrations of selected chemicals within a system of interest. He is an expert in the use of near infrared absorption spectroscopy for *in situ* analytical measurements in complex biological systems. Examples include noninvasive glucose measurements in people with diabetes and real-time monitoring of hemodialysis during treatments of people with end-stage renal failure. In the spirit of translational research and economic development, Professor Arnold has teamed with others to create ASL Analytical, Inc. for the purpose of commercializing this near infrared sensing technology. ASL's first commercial product is an on-line monitor designed to follow glycerol, methanol, and biomass in real-time as a means to optimize the upstream production of biotherapeutics in *Pichia pastoris.*

Joe Hrdlicka
Executive Director, Iowa Biotechnology Association
Des Moines, IA

As the Executive Director of the Iowa Biotechnology Association (IowaBio) Joe Hrdlicka oversees the industry association and works on behalf of more than 100 members engaged in biotechnology endeavors in Iowa. The Association's mission is to grow Iowa's presence in biotechnology, and position the state as a leader in biotechnology in the global economy.

Joe brings nearly 20 years of public affairs, management and marketing experience to the Association. A St. Louis native, Joe attended the University of Missouri, School of Journalism and worked in various political campaigns before coming to Iowa in 1992 to work for Representative Jim Lightfoot. After his work with Lightfoot, Joe joined the marketing firm Strategic America as Director of Public Relations. Following his tenure at Strategic America, he served 13 years with the Iowa Lottery. First, he was Public Relations Manager, then was promoted to Vice President of Marketing where he served as a member of the Lottery's senior management team. During his tenure at the Iowa Lottery, Hrdlicka managed a team of eight marketing professionals overseeing advertising, promotions, product development and major procurements.

Most recently, Joe served as Vice President of Public Affairs for the Iowa Communications Alliance, an industry organization of telecommunications providers serving Iowa's rural communities. He says working in the association world has been extremely engaging and he feels privileged to have the opportunity to head up such a vibrant industry organization. "I really enjoy working with members and moving an association to bring value and resources to their businesses," says Joe.

Joe is the President-elect of the Rotary Club of Des Moines and he was recently appointed to the West Des Moines Parks and Recreation Advisory Commission.

Maria Spies, Ph.D.
Associate Professor
Department of Biochemistry
Carver College of Medicine
University of Iowa

Dr. Maria Spies is a graduate of St. Petersburg Polytechnic University, Russia (1996 MS diploma with excellence (*cum laude*) in physics/biophysics). After obtaining her PhD in biological sciences at Osaka University, Japan (2000) she was an American Cancer Society postdoctoral scholar with Prof. Steve Kowalczykowski (UC Davis). She started her independent career as an Assistant professor of Biochemistry at the University of Illinois Urbana-Champaign. Currently, Dr. Spies is an Associate Professor of Biochemistry at the University of Iowa Carver College of Medicine.

Spies' laboratory focuses on deciphering the intricate choreography of the molecular machines orchestrating the central steps in homology-directed DNA repair. Her goal is to understand, reconstitute and manipulate an elaborate network of the cellular DNA damage and repair response. The Spies' lab utilizes a broad spectrum of techniques from biochemical reconstitutions of the key biochemical reactions in DNA recombination, repair and replication, to structural and single-molecule analyses of the proteins and enzymes coordinating these reactions, to combined HTS/CADD campaigns targeting human DNA repair proteins.

Work in Spies Lab has been funded by the American Cancer Society (ACS), Howard Hughes Medical Institute (HHMI), and is currently supported by the National Institutes of Health (NIH). She received several notable awards including HHMI Early Career Scientist Award and Margaret Oakley Dayhoff Award in Biophysics. She serves on the editorial board of the Journal of Biological Chemistry, and as an academic editor of the journal Plos-ONE. She is a permanent member and a vice chair of the American Cancer Society "DNA mechanisms in cancer" panel.

Eric Nuxoll, Ph.D.
Associate Professor
Department of Chemical and Biological Engineering
College of Engineering
University of Iowa

Eric Nuxoll is an Associate Professor in the Department of Chemical & Biochemical Engineering at the University of Iowa. He earned bachelor's degrees in Chemistry and Chemical Engineering from the University of Idaho, followed by a Ph.D. degree in Chemical Engineering at the University of Minnesota, where he was an NSF Graduate Research Fellow. After working in the University of Minnesota Pharmaceutics program as an NIH Kirschstein/NRSA Postdoctoral Fellow, he joined the University of Iowa in 2008. His research is in the areas of medical implant infection control and controlled drug delivery.

Ronen Tchelet, Ph.D.
VP Research & Business Development
Dyadic International Inc.

Ronen Tchelet joined Dyadic in May 2013 and has been Dyadic's Vice President of Research and Business Development since January 2016. Dr. Tchelet received his Ph.D. in Molecular Microbiology and Biotechnology from Tel Aviv University in 1993 and did his postdoctoral as EERO fellow at the Institute of Environmental Science and Technology (EAWAG) in Switzerland.

In the late 2000's, he joined the API Division of TEVA Pharmaceutical Industries LTD., where he served as a Chief Technology Officer of Biotechnology. Dr. Tchelet leaded and directed the Research and Development Biotechnology department of TEVA's fermentation plant in Hungary. In his work he managed and organized Biotechnology and Biosimilar projects that combined multidisciplinary areas such as development, operation, and production of API and biologics. In addition, from 2000 – 2005, Dr. Tchelet was the QA manager of COPAXONE® the flag ship TEVA[1]s innovative drug.

From 2007 through 2013, prior to joining Dyadic, Dr. Tchelet became the founder and the Managing Director of Codexis Laboratories Hungary kft. (CLH) and a Vice President of Codexis Inc. At CLH, he established a state-of-the-art laboratory for strain engineering and all aspects of fermentation work including process optimization and scaling up. It was during this time that Dr. Tchelet engaged with the C1 technology that was successfully developed for the Biofuel and the Bio-Industrial enzymes fields.

Laura R. Jarboe, Ph.D.
Associate Professor
Department of Chemical and Biological Engineering
Chair, Interdepartmental Microbiology Graduate Program
Iowa State University

Dr. Laura Jarboe received a BS in Chemical Engineering from the University of Kentucky in 2000 and completed her PhD in Chemical and Biomolecular Engineering at the University of California, Los Angeles, in 2006. Following a two-year term as a postdoctoral researcher at the University of Florida, she joined Iowa State University's Chemical and Biological Engineering department in 2008. She is currently chair of ISU's Interdepartmental Microbiology Graduate program and co-leader of the Metabolic Engineering Thrust of the National Science Foundation Center for Biorenewable Chemicals Engineering Research Center (CBiRC). She 2015, she received the "Leadership in Outreach and Mentoring" award from the Iowa NSF EPSCoR program and the Iowa Energy Center impact award in Bioenergy.

Benjamin Foust
Ph.D. Candidate
David F. Wiemer Research Group
Department of Chemistry
College of Liberal Arts & Sciences
University of Iowa

Benjamin is originally from the small town of Bellevue, Iowa. He obtained his Bachelor of Science degree from the University of Iowa in 2014 and decided to remain at Iowa in the research lab of Dr. David Wiemer for his Ph.D. work. The research group does synthetic organic chemistry focusing on the design and formation of biologically active organophosphorus compounds. More recent efforts have been toward the synthesis of phosphonate containing enzyme inhibitors for their potential use as anti-cancer agents. After obtaining his Ph.D., Benjamin intends to pursue a career in the pharmaceutical industry.

Mark S. Miller
Ph.D. Candidate
Adrian H. Elcock Research Group
Department of Biochemistry
Carver College of Medicine
University of Iowa

Mark grew up in Kalona, Iowa. He earned a Bachelor of Arts in Chemistry with a minor in Mathematics from Goshen College in Indiana. He then went on to pursue a Masters of Science in Neurochemistry from Stockholm University in Sweden. He completed the research portion of this degree as a visiting graduate student at the University of Iowa under the guidance of Dr. Madeline Shea, probing the thermodynamics of the protein Calmodulin binding to voltage-gated sodium channels. From there, he enrolled in the Biochemistry doctoral training program at the University of Iowa, where he eventually joined the Elcock research group. His current research is entirely computational, and focuses on validating protein *force fields* used in molecular dynamics simulations of biomolecules.

Nicholas R. Vance
Ph.D. Candidate
M. Ashley Spies Research Group
Department of Pharmaceutics & Experimental Therapeutics
College of Pharmacy
University of Iowa
Iowa City, IA

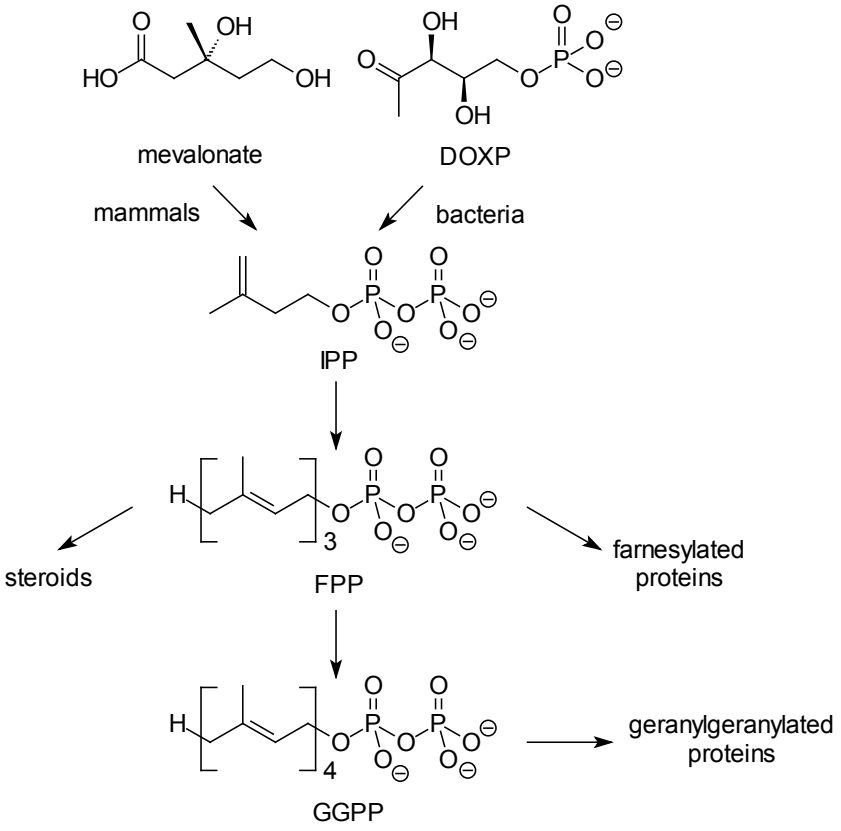
Nick hails from the suburbs of West Des Moines, Iowa. He obtained a Bachelor of Arts in Chemistry and a minor in Mathematics from Central College (Pella, IA). Shortly after graduation, he traveled to Iowa City in pursuit of a Ph.D. in Medicinal and Natural Products Chemistry from the College of Pharmacy at the University of Iowa. After exploring different research groups, Nick landed in the laboratory of M. Ashley Spies. Since joining the Spies lab, Nick has been involved in drug discovery efforts directed towards the development of allosteric caspase inhibitors that could be used to treat neurodegenerative and cardiovascular diseases by prolonging apoptosis. Post-graduation, Nick intends to pursue a post-doctoral research position abroad so he can finally escape Iowa.

Oral Presentation Abstracts

BIOLOGICALLY ACTIVE COMPOUNDS BASED ON THE INTERMEDIATES OF ISOPRENOID BIOSYNTHESIS

David F. Wiemer, Ph.D.
F. Wendell Miller Professor of Chemistry and Professor of Pharmacology, Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Isoprenoid biosynthesis is central to metabolism although the early steps in mammals are quite different from those in bacteria. Historically, efforts have focused on inhibiting the distal steps of this pathway leading to steroid biosynthesis. While inhibition of cholesterol biosynthesis is a common therapeutic goal, other parts of the isoprenoid biosynthesis pathways may offer just as much opportunity for clinical applications. In this presentation the challenges in targeting specific steps in isoprenoid metabolism will be presented, along with our efforts to exploit the opportunities that selective agents may afford.



Presentation 2

NEW BIOACTIVE GLYCOCONJUGATES DERIVED FROM CHEMICAL MODIFICATION OF SULFATED SACCHARIDES

Robert Kerns, Ph.D.
John L. & Carol E. Lach Chair in Drug Delivery Technology, Professor and Department Chair, Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Heparan sulfate (HS) is a cell-surface glycosaminoglycan that plays a profound role in numerous physiological processes. Numerous therapeutic applications have been proposed for molecules to bind specific HS-binding proteins and block or modulate HS-mediated biological activities. The discovery of such molecules, including efforts to chemically modify natural polysaccharides such as the HS-like glycosaminoglycan heparin, has primarily focused on optimizing the degree and spatial orientation of anionic substituents on saccharide based or non-carbohydrate based core structures. Due to the cationic nature of HS-binding sites in HS-binding proteins, the discovery of polyanionic molecules that simply bind to HS-binding proteins is typically not a problem. The problem is identifying molecules that selectively bind with high affinity to the HS-binding site of individual, or a small number of structurally similar, HS-binding proteins. We previously reported the diversity-oriented chemical modification of heparin through a N-desulfonation/N-acylation strategy affords charge-reduced heparin derivatives having increased binding affinity and increased binding selectivity for certain HS-binding proteins. In the work presented here, the unique structural requirements for select N-desulfonated/N-acylated heparin derivatives and heparin amides to bind HS-binding proteins will be discussed. Extension of this approach to the design and synthesis of N-arylacyl O-sulfonated aminoglycosides has led to the identification of comparatively lower molecular weight structures that bind with high affinity to select HS-binding proteins, and that possess unique profiles for the inhibition of serine proteases.

Presentation 3

CHEMOENZYMATIC SYNTHESIS OF HEPARIN AND LOW MOLECULAR WEIGHT HEPARINS

Robert J. Linhardt, Ph.D.
Ann and John H. Broadbent, Jr. '59 Senior Constellation Professor of Biocatalysis and Metabolic Engineering , Professor of Chemistry and Chemical Biology, Biology, Chemical and Biological Engineering and Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY

Heparin, a highly sulfated polysaccharide anticoagulant, commands worldwide market of ~$7B. Currently, heparin and related low molecular weight heparins (LMWHs) require an extraction from pig intestine. The ultra-low molecular weight heparin (ULMWH), a chemically synthesized pentasaccharide, is expensive and has limited clinical applications. This presentation describes the efficient chemoenzymatic synthesis of heparin, LMWH, and ULMWH. Our goals are to prepare ULMWH at reduced cost, LMWH with improved pharmacological properties (*i.e.,* defined pharmacokinetics, liver clearance, reduced heparin-induced-thrombocytopenia, and protamine reversibility), and bioengineered heparin without the use of animal derived starting material. Our approach relies on chemoenzymatic synthesis chemical relying on Golgi-derived biosynthetic enzymes, including heparosan synthases, sulfotransferases and epimerase. We are also exploring metabolic engineering for heparin production. Such biotechnological processes should allow the cGMP preparation of these critical drugs, affording improved products reducing impurities, contaminants and adulterants that resulted in the 2008 heparin contamination crisis.

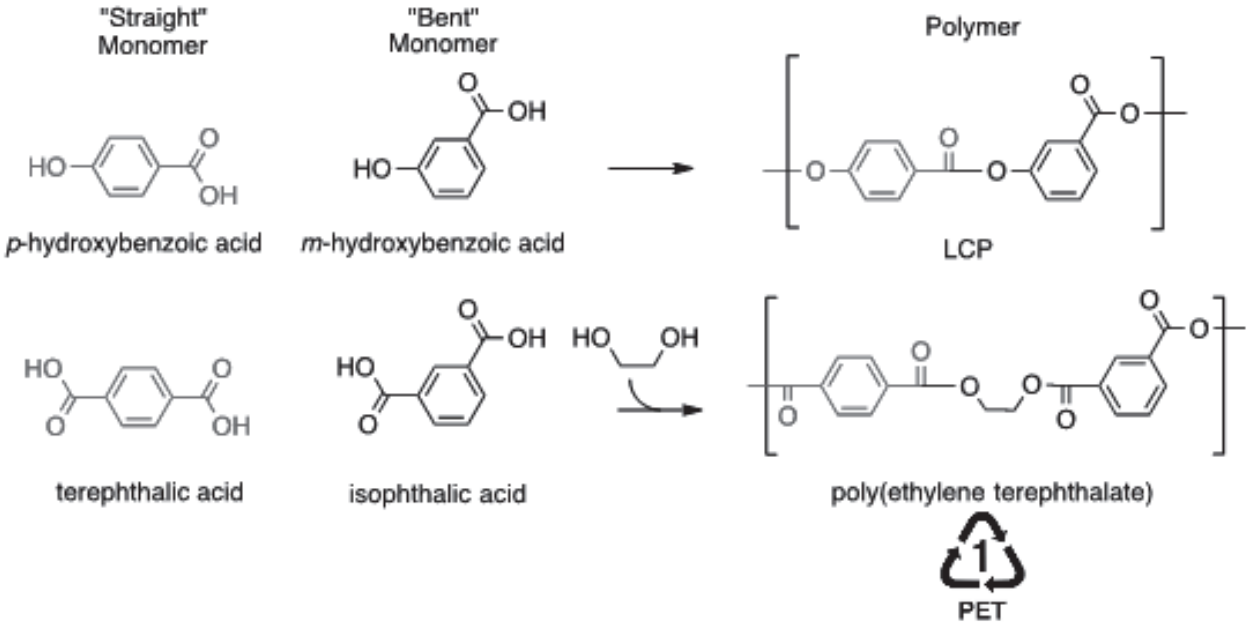
Presentation 4

STRAIGHT OR BENT? SYNTHESIS OF BIOBASED AROMATICS

John W. Frost, Ph.D.
University Distinguish Professor, Department of Chemistry, Michigan State University, East Lansing, MI

Liquid crystalline polymers (LCPs) and poly(ethylene terephthalate) (PET) represent opposite ends of the polyester performance/volume spectrum. LCP polyesters are used in value-added applications requiring low melt viscosity to form intricate structures that are dimensionally stable even at elevated temperatures. PET is prized for its clarity and finds widespread use in consumer container applications. The key "straight" monomer (1,4-disubstituted benzene) in all LCP formulations is *p*-hydroxybenzoic acid (21 x 10^6 kg/yr) while the critical "straight" monomer in PET manufacture is terephthalic acid (50 x 10^9 kg/yr). Smaller amounts of "bent" monomers (1,3-disubsituted benzenes) such as *m*-hydroxybenzoic acid enable melt processability of LCPs. Inhibition of crystallization of PET using "bent" isophthalic acid is critical to achieving the polymer clarity that is the defining physical property of PET.

Microbial synthesis of shikimic acid under fermentor-controlled conditions, isolation of shikimic acid from fermentor broth, and synthesis of biobased *p*-hydroxybenzoic acid from shikimic acid will be detailed. A synthesis of biobased *m*-hydroxybenzoic acid from shikimic acid will also be presented. A sequence of three reactions enables the synthesis of both terephthalic acid and isophthalic acid from biobased acrylic acid and biobased isoprene. The key steps in this reaction sequence include $BOB(OAc)_4$-catalyzed cycloaddition of acrylic acid with isoprene under solvent-free conditions, vapor-phase, Pd/SiO_2-catalyzed aromatization of the cycloadducts, and Amoco-MidCentury oxidation of *m*-toluic acid and *p*-toluic acid to isophthalic acid and terephthalic acid. Purification of terephthalic acid to 99.994 wt% polymer-grade purity will be discussed



Presentation 5-7

CBB PAST, PRESENT AND FUTURE: COMMENTS FROM CBB DIRECTORS

Brief comments from:

John P. N. Rosazza, Ph.D.
Professor Emeritus, Medicinal and Natural Products Chemistry
Director Emeritus, Center for Biocatalysis and Bioprocessing
University of Iowa
(1994-2005)

Mani V Subramanian, Ph.D.
Professor Emeritus, Chemical & Biochemical Engineering
Director Emeritus, Center for Biocatalysis and Bioprocessing
University of Iowa
(2005-2015)

Mark Arnold, Ph.D.
Director, Center for Biocatalysis and Bioprocessing
Professor and Edwin B. Green Chair in Laser Chemistry
Department of Chemistry
University of Iowa
(2015-present**)**

Presentation 9

SINGLE-MOLECULE STUDIES OF FES DNA HELICASES: KINETICS, CONFORMATIONAL DYNAMICS AND MOLECULAR MECHANISMS

Maria Spies, Ph.D.
Associate Professor, Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

DNA helicases are integral components of molecular machines that orchestrate and regulate a broad range of processes vital to cellular DNA metabolism. These enzymes use ATP to fuel two important biochemical activities (i) strand separation, where dsDNA is unwound to produce transient single-stranded intermediates of DNA replication, recombination and repair; and (ii) translocation, which can be coupled to remodeling of the nucleoprotein complexes. I will discuss our single-molecule studies that leverage total internal fluorescence microscopy to understand structural and mechanistic features that enable structurally similar enzymes within the XPD family of the FeS-containing DNA helicases to perform drastically different tasks in the cell. I will focus on our findings regarding the mechanism by which XPD auxiliary domains define translocation polarity and the ability to signal the presence of DNA damage. The mechanism of XPD will be compared to that of the related FeS-containing helicase FANCJ, a caretaker of genomic integrity, which supports DNA replication through difficult to replicate sequences (such as G-quadruplexes) and ensures fidelity of homologous genetic recombination.

Wu, C.G., and Spies, M., G-quadruplex recognition and remodeling by the FANCJ helicase (2016) *NAR, gkw574 in press* [PMID: 27342280; PMCID: *in progress*]

Ghoneim, M. and Spies, M., Direct Correlation of DNA Binding and Single Protein Domain Motion via Dual Illumination Fluorescence Microscopy (2014) *Nano Lett* 14(10):5920-31 [PMID: 25204359; PMCID: PMC4189620]

Qi, Z., Pugh, R.A., Spies, M., and Chemla, Y.R., Sequence-dependent base-pair stepping dynamics in XPD helicase unwinding (2013) *eLife 2:e00334* [PMID: 23741615; PMCID: PMC3668415]

Pugh, R. A., Wu, C. G., and Spies, M., Regulation of translocation polarity by helicase domain 1 in SF2B helicases. (2012) *EMBO J* 31: 503 – 514

Honda, M., Park, J., Pugh R.A., Ha, T. and Spies, M., Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase. (2009) Mol. Cell 35 (5), 694-703

Support: R01 GM108617

Presentation 10

WIRELESS DEACTIVATION OF BACTERIAL BIOFILMS

Eric Nuxoll, Ph.D.
Associate Professor**,** Department of Chemical and Biological Engineering, College of Engineering, University of Iowa, Iowa City, IA

Bacterial biofilms infect 2 – 4 % of medical devices upon implantation, resulting in multiple surgeries and increased recovery time due to the tremendous increase in antibiotic resistance in the biofilm phenotype. To tackle this problem, we are investigating the feasibility of thermal mitigation of biofilms at physiologically accessible temperatures. *Pseudomonas aeruginosa* biofilms were cultured to high bacterial density (1.7×10^9 CFU/cm^2) and subjected to thermal shocks ranging from 50 °C to 80 °C for durations of 1 to 30 minutes. The decrease in viable bacteria was closely correlated with an Arrhenius temperature dependence and Weibull-style time dependence, demonstrating up to six orders of magnitude reduction in bacterial load. Bacteria grown under different conditions demonstrate different quantitative thermal susceptibility at modest temperatures, though effects at higher temperatures are uniform across all growth conditions, suggesting that the trade-off between temperature and exposure time should favor higher temperatures. Concurrently, we have developed magnetic nanoparticle / polymer composite coatings which we can wirelessly heat in the presence of an alternating magnetic field, providing a mechanism for delivering heat precisely to the biofilm.

Presentation 11

C1 – WHY IT IS EXCELLENT PRODUCTIVE CELL EXPRESSION

Ronen Tchelet and Mark A. Emalfarb
Dyadic International Inc., Jupiter, FL

For over 30 years Dyadic has proven itself, commercially and scientifically, as a high quality and highly productive producer of enzymes and proteins using a proprietary and patented expression system based on the *Myceliopthora thermophila fungus,* nicknamed C1. The C1 platform technology is a hyper-productive fungal expression system used to develop & manufacture large quantities of desired proteins at industrial scale at significantly lower CapEx and OpEx costs. So far Dyadic has shown that by using its C1 expression system they can produce vaccines at high level with potentially better immune response. Dyadic has also demonstrated the ability to easily express mAb's. Key aspects of C1's gene expression, protein development and fermentation processes will be discussed.
The C1 technology has the potential to change the way in which both animal health and human biotech and pharmaceutical companies bring their biologic vaccines and drugs to market faster, in greater volumes, at lower cost, and with newer beneficial properties, and most importantly save lives.

Presentation 12

METABOLIC ENGINEERING OF ROBUST MICROBIAL CELL FACTORIES

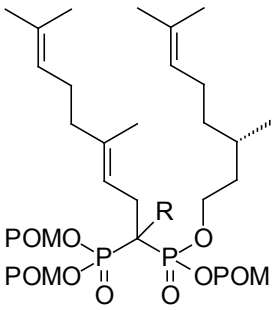
Laura R. Jarboe, Ph.D.
Associate Professor, Department of Chemical and Biological Engineering Chair,
Interdepartmental Microbiology Graduate Program, Iowa State University, Ames, IA

Production of biorenewable fuels and chemicals by microbial cell factories is often limited by product toxicity or inhibitory components of the biomass-derived sugars. This toxicity can be addressed by detoxification of the feed stream, in situ removal of the product or increasing the resistance of the microbial biocatalyst. The Jarboe group uses evolutionary and rational strain improvement methods to increase microbial robustness, specifically in *Escherichia coli*. This talk will discuss our work with membrane engineering to increase robustness to a variety of membrane-damaging compounds, production of the bulk biorenewable chemicals styrene and fatty acids, and utilization of thermochemically depolymerized biomass.

A NEW MOTIF FOR INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE

Benjamin J. Foust[1], Cheryl Allen[2], Sarah A. Holstein[2], and David F. Wiemer[1,3]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]Division of Oncology and Hematology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE
[3]Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA

Geranylgeranyl diphosphate synthase (GGDPS) is a key enzyme in a later stage of the human mevalonate pathway, which is of increasing interest for the treatment of a variety of different conditions including malaria, osteoporosis, and malignant bone diseases such as myeloma. The enzyme receives the substrate farnesyl diphosphate and catalyzes production of geranygeranyl diphosphate. Both substrate and product seem to have different lipophilic channels allowing access to this enzyme terminating in an area capable of stabilizing the high charge density of the natural pyrophosphates. Previously studied bisphosphonates with two isoprenoid chains positioned on the α-carbon have proven to be effective inhibitors of this enzyme. Recent efforts have afforded a new motif (1) with one isoprenoid chain on the α-carbon, a second included as a phosphonate ester, and the potential for a third at the α-carbon. The pivaloyloxymethyl (POM) prodrugs of several compounds based on this motif have been prepared and the resulting compounds have been tested for their ability to disrupt protein geranylgeranylation and induce cytotoxicity in myeloma cells. The initial bioassays show activity consistent with GGDPS inhibition, and demonstrate a structure-function relationship dependent on the nature of the third alkyl substituent on the α-carbon.



1

R =	Disruption of cellular geranylgeranylation (μM)
H	≥ 5.0
Methyl	≥ 0.5
Allyl	≥ 2.5
Prenyl	≥ 1.0
Geranyl	≥ 2.5

POM = $CH_2OC(O)C(CH_3)_3$

Presentation 14

RE-PARAMETERIZATION OF PROTEIN FORCE FIELDS GUIDED BY OSMOTIC COEFFICIENT MEASUREMENTS FROM MOLECULAR DYNAMICS SIMULATIONS

Mark S. Miller, Wesley K. Lay, Shuxiang Li, William C. Hacker, Jiadi An, Jianlan Ren, and Adrian H. Elcock*
Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

Our group and others have recently described the use of osmotic coefficient measurements as a means of validating solute-solute interactions in molecular dynamics (MD) simulations of biomolecules. In particular, in simulations of amino acids, we and others found that intermolecular interactions are often too attractive; these disagreements with experiment were attributed to problems with the side chain functional groups, the charged termini, and interactions with counter-ions. We have since attempted to understand the cause of these problems more fully, using MD simulations to measure osmotic coefficients for a variety of amino acids and small molecules modeled with four very commonly used protein force fields. We then improved agreement with experiment by adjusting the van der Waals parameters assigned to given atom types, either using modifications recently reported in the literature or by optimizing the values in-house. These improvements highlight the potential utility of osmotic coefficient data in efforts to describe interactions between biological macromolecules accurately.

Presentation 15

PROBING ALLOSTERIC REGULATION OF AN EXECUTIONER CASPASE

Nicholas R. Vance[1], Lokesh Gakhar[1] and M. Ashley Spies[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

The caspase family of cysteine-dependent, aspartate-specific endoproteases have attracted widespread attention as drug targets due to their fundamental roles in apoptosis and inflammation pathways. Caspases employ a catalytic cysteine-histidine dyad to judiciously hydrolyze peptide bonds, in order to carry out their essential cellular functions. Apoptotic caspases are further segregated into initiator and executioner caspases. Initiator caspases (-8, -9) activate the executioner caspases (-3, -6, -7) in order to carry out the final steps of apoptosis. Cell death resulting from aberrant caspase activation has been implicated in numerous cardiovascular and neurodegenerative diseases.

Numerous screening efforts have been directed towards the development of caspase inhibitors. To date, the overwhelming majority of these compounds target the active site through a form of mechanism-based inhibition. However, due to the aspartate preference of the executioner caspases, these molecules are inherently charged and lack drug-like properties. Targeting a known allosteric site at the dimer interface is proposed to yield molecules with superior drug properties. We also hypothesize that current HTS libraries do not contain the complementary chemical space for the allosteric site of the caspases, and that fragment screening will provide the necessary diversity to discover allosteric caspase inhibitors.

In order to maximize screening chemical diversity, we leveraged fragment-based drug discovery. A fragment library was screened against caspase-7 by differential scanning fluorimetry (DSF). Compounds identified from the screen were further tested by DSF, functional assays, and surface plasmon resonance. Two non-competitive inhibitors from the screening effort have been characterized by X-ray crystallography and were confirmed to bind at the aforementioned allosteric site. Further work is being done to understand the mechanism by which these compounds are allosterically inhibiting caspase-7.

List of Posters and Authors

Posters

1. **QUINAZOLINE-2,4-DIONE DERIVATIVES TO OVERCOME FLUOROQUINOLONE RESISTANCE**
   Arturo L. Aguirre and Robert J. Kerns*
   Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

2. **DIRECT MICROBIAL N-DEMETHYLATION OF CAFFEINE TO THEOBROMINE BY METABOLICALLY ENGINEERED *E. COLI***
   Khalid H. R. Algharrawi[1], Ryan M. Summers[2] and Mani Subramanian[1]*
   [1]Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA
   [2]Department of Chemical and Biological Engineering, University of Alabama, Tuscaloosa, AL

3. **INVESTIGATION OF STRUCTURE ACTIVITY RELATIONSHIP OF PEG-PEPTIDE SCAVENGER RECEPTOR INHIBITORS**
   Rondine Allen and Kevin Rice*
   Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy University of Iowa, Iowa City, IA

4. **16S METAGENOMIC PROFILING OF FRESHWATER MUSSEL BED MICROBIAL COMMUNITIES REVEALS INFLUENCE ON NITROGEN-CYCLING BACTERIA**
   Ellen M. Black and Craig L. Just*
   Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

5. **BIOCHEMICAL AND STRUCTURAL STUDIES OF A NOVEL METAL DEPENDENT DIMETHYLSULFONIOPROPIONATE LYASE**
   Saumya M. De Silva, Nicholas J. Schnicker and Mishtu Dey*
   Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

6. **TIME AND TEMPERATURE DEPENDENCE OF ATZ GENE EXPRESSION IN PSEUDOMONAS SP. ADP BIOFILMS**
   Michael A. Delcau and Tonya L. Peeples*
   Department of Chemical and Biochemical Engineering, College of Engineering University of Iowa, Iowa City, IA

7. **NOVEL ACTIVITY OF N-1 FLUOROQUINOLONE DERIVATIVES**
   Justine Delgado and Robert J. Kerns*
   Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

8. **USING ULTRASOUND TO ENHANCE TUMOR CELL KILLING BY ANTI-PD1 AND DOXORUBICIN-LOADED PARTICLES**
Anh-Vu Do[1], Kee W. Jang[2], Dongrim Seol[2], Phillip Tobias[1], Daniel Carlsen[1], Ino Song[2], James A. Martin[2], and Aliasger K. Salem[1]*
[1]Department of Pharmaceutics & Translational Therapeutics, College of Pharmacy, University of Iowa, IA
[2] Department of Orthopedics and Rehabilitations, University of Iowa Hospitals and Clinics, University of Iowa, IA

9. **CHARACTERIZING AND QUANTIFYING POLYCHLORINATED BIPHENYNL BIODEGRADERS IN ALTAVISTA LAGOON**
Jessica Ewald, Yi Liang, Jerald Schnoor, and Timothy Mattes*
Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

10. **A NEW MOTIF FOR INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE**
Benjamin J. Foust[1], Cheryl Allen[2], Sarah A. Holstein[2], and David F. Wiemer[1,3]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]Division of Oncology and Hematology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE
[3]Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA

11. **NOVEL ANTIBIOTIC FORMULATIONS FOR ERADICATION OF BIOFIOLMSOF *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES**
Sachin Gharse[1]and Jennifer Fiegel[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

12. **EFFECTS OF MASS MODULATION ON HYDRIDE TRANSFER IN THYMIDYLATE SYNTHASE**
Ananda K Ghosh, Michael J Christoperson and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

13. **PARTICLE SURFACE PROPERTIES DETERMINE PARTICLE-LUNG SURFACTANT INTERACTIONS**
Bharath Kumar Gowdampally[1] and Jennifer Fiegel[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2] Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

14. **THE ROLE OF ASPARAGINE 177 IN THE CATALYTIC CYCLE OF THYMIDYLATE SYNTHASE**
Ilya Gurevic, Zahidul Islam, Sobia Rasool, Tasnia Iqbal, Kai Trepka, Ananda Ghosh, Chethya Ranasinghe, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

15. THE MECHANISM OF CHEMOTHERAPY-RESISTANT CANCEROUS THYMIDYLATE SYNTHASE

Zahidul Islam, Ilya Gurevic, Muhammad Saeed, Sobia Saeed, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa. Iowa City, IA

16. KINETIC SOLVENT ISOTOPE EFFECTS AS A MECHANISTIC TOOL IN FLAVIN DEPENDENT THYMIDYLATE SYNTHASE (FDTS)

Kalani Karunaratne[1], Mitchell LeFebvre[2], Jose Villalobos[1], and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]Carver College of Medicine, University of Iowa, Iowa City, IA

17. SPECIALIZED METABOLITES AFFECTING THE INTERSPEICES INTERACTIONS BETWEEN *MYXOCOCCUS XANTHUS* AND *BACILLUS SUBTILIS*

Paige Kies, Susanne Müller* and John R. Kirby*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

18. SYNTHESIS OF CHoP-MA COATINGS VIA PHOTOINITIATED RAFT POLYMERIZATION AS A TARGETED, STEALTH DRUG DELIVERY SYSTEM

Benjamin King and Jennifer Fiegel*
Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

19. ESTABLISHING THE ROLE OF GLOBAL NETWORKS ON COUPLED MOTIONS IN HUMAN DIHYDROFOLATE REDUCTASE

Jiayue Li and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

20. ACCELERATION OF PROTEIN DESIGN THERMODYNAMICS USING A DUAL FORCE FIELD APPROACH

Jacob M. Litman[1], Stephen D. LuCore[2], Young Joo Sun[1], Ernesto Fuentes[1], and Michael J. Schnieders[1,2]*
[1]Departments of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Biomedical Engineering, College of Engineering, University of Iowa, Iowa City, IA

21. FEMTOSECOND DYNAMICS IN THE ACTIVE SITE OF FORMATE DEHYDROGENASE: A MOLECULAR DYNAMICS APPROACH TO 2DIR SPECTROSCOPY

William K. Marquardt, Amnon Kohen* and Christopher M. Cheatum*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

22. RE-PARAMETERIZATION OF PROTEIN FORCE FIELDS GUIDED BY OSMOTIC COEFFICIENT MEASUREMENTS FROM MOLECULAR DYNAMICS SIMULATIONS

Mark S. Miller, Wesley K. Lay, Shuxiang Li, William C. Hacker, Jiadi An, Jianlan Ren, and Adrian H. Elcock,*
Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

23. PHOTOCROSSLINKER NON-CANONICAL AMINO ACIDS FOR PROTEIN INTERACTION STUDIES

Steven M. Molinarolo, Jason D. Galpin and Christopher A. Ahern*
Department of Molecular Physiology and Biophysics, Carver College of Medicine, University of Iowa, Iowa City, IA

24. PROBING THE LIKELINESS OF PROPOSED INTERMEDIATE OF FDTS CATALYZED REACTION

Dibyendu Mondal, Jiajun Yao, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

25. DOUBLE STRANDED mRNA NANOPARTICLES FOR NON-VIRAL GENE DELIVERY

Jacob A. Poliskey and Kevin G. Rice*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

26. MASS MODULATED EFFECTS ON FORMATE DEHYDROGENASE

Chethya U. Ranasinghe[1], Qi Guo[1], Paul J. Sapienza[2], Andrew L. Lee[2], Christopher Cheatum[1], and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts & Sciences University of Iowa, Iowa City, IA
[2]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC

27. THE SYNERGISTIC EFFECTS OF ANTIBIOTICS AND HEAT ON *PSEUDOMONAS AERUGINOSA* BIOFILMS

Erica N.B. Ricker and Eric Nuxoll*
Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

28. SYNTHESIS OF NOVEL PAWHUSKIN AND SCHWEINFURTHIN ANALOGUES

David P. Stockdale, Alyssa M. Hartung and David F. Wiemer*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

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Young Joo Sun[1], Xu Liu[1], Titus Hou[1], Lokesh Gakhar[1,2], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Crystallography Facility, Carver College of Medicine, University of Iowa, Iowa City, IA

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Nitija Tiwari[1], Lotte Van Den Goor[3], Young Joo Sun[1], Patrick M. Schlievert[2], John R. Kirby[2], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA
[3]Loyola University, Chicago, IL

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Nicholas R. Vance[1], Lokesh Gakhar[1] and M. Ashley Spies[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Biochemistry, Carver College of Medicine, University of Iowa Iowa City, IA

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Jennifer L. Welch and Chioma M. Okeoma*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

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Christopher W. White and Kevin G. Rice*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

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Shuvendu Das and Mark A. Arnold*
Center for Biocatalysis and Bioprocessing, Office of Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

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Shuvendu Das[1, 2] and Mani Subramanian[1,3]*
[1]Center for Biocatalysis and Bioprocessing, Office of Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA
[2]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[3]Department of Chemical & Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

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Elizabeth R. Gibson[1], Kaylee Lanz[1], Edwin Koerperick[1], Daniel Cooley[1], Jonathon T. Olesberg[1], Gary W. Small[1,2], and Mark A. Arnold[1,2]*
[1]ASL Analytical, Inc., Coralville, IA
[2]Optical Science and Technology Center and Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

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Rachel L. Grenell[1], Gary W. Small[1,2], Kaylee Lanz[2], Kevin L. Weiss[3], William B. O'Dell[3,4], and Mark A. Arnold [1,2]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]ASL Analytical, Inc., Coralville, IA
[3]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN
[4]Department of Molecular and Structural Biochemistry, NC State University, Raleigh, NC

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Yi Liang, Xikun Liu and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

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Wadie D. Mahauad-Fernandez and Chioma M. Okeoma*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

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Priyanka Singh and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

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Zhen Xu[1], Lokesh Gakhar[2], Fletcher Bain[1], Maria Spies[1], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]X-ray Crystallography Core Facility, Carver College of Medicine, University of Iowa, Iowa City, IA

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Atsushi Yahashiri, Matthew A. Jorgenson, Eric M. Ransom, Jon C. Houtman, and David S. Weiss*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

Poster Abstracts

Poster 1

QUINAZOLINE-2,4-DIONE DERIVATIVES TO OVERCOME FLUOROQUINOLONE RESISTANCE

Arturo L. Aguirre and Robert J. Kerns*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Fluoroquinolones are a class of antibiotics that target type II topoisomerases in bacteria (DNA gyrase and topoisomerase IV) for their antibacterial activity. These small molecules block function of bacterial type II topoisomerases by forming a ternary complex, which is comprised of the fluoroquinolone, enzyme (topoisomerase IV or DNA gyrase), and nicked DNA; formation of the ternary complex inhibits the ability of the topoisomerase to religate the nicked DNA. Fluoroquinolone binding to type II topoisomerases is primarily governed by a critical water bridge where the keto acid moiety of the fluoroquinolone is in complex with a divalent magnesium ion, and as a result, a magnesium-water bridge is formed with a serine and an acidic residue (aspartate or glutamate), on helix-4 of the enzyme (topoisomerase IV or DNA gyrase). Resistance arises through mutations that lead to substitutions in the serine or the acidic residues, which in turn, abolishes formation of the magnesium-water bridge and ternary complex is not formed. Quinazoline-2,4-diones bind to type II topoisomerases in a similar fashion, but do not require magnesium-water bridge formation, and notably, have equipotent activity against both wild-type and fluoroquinolone resistant topoisomerases that contain mutations in helix-4. These discoveries imply that the quinazoline-2,4-dione chemical structure can be exploited to facilitate drug-enzyme binding, without the requirement of the magnesium-water bridge that is critical in fluoroquinolone binding. Furthermore, the C*2* carbonyl oxygen of a quinazoline-2,4-dione is believed to form a hydrogen bond with a conserved arginine residue in GyrA of the type II topoisomerase; nonetheless, this binding interaction is not as strong as the magnesium-water bridge. Additional binding interactions are needed to increase quinazoline-2,4-dione binding to type II topoisomerases, with the aim to increase ternary complex formation and improve overall antibacterial potency. In this presentation, the design and synthesis of novel quinazoline-2,4-diones, which are predicted to have additional binding contacts with bacterial type II topoisomerases, will be discussed.

Poster 2

DIRECT MICROBIAL N-DEMETHYLATION OF CAFFEINE TO THEOBROMINE BY METABOLICALLY ENGINEERED *E. COLI*

Khalid H. R. Algharrawi[1], Ryan M. Summers[2] and Mani Subramanian[1]*
[1]Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA
[2]Department of Chemical and Biological Engineering, the University of Alabama, Tuscaloosa, AL

In this study, we report the production of theobromine directly from caffeine. The N-demethylation reaction of caffeine to theobromine was catalyzed by *E. coli*, metabolically engineered with N-demethylases genes (*NdmA* and *NdmD*). First, five strains of *E. coli* engineered with multiple combinations of *ndmA* and *ndmD* genes, were tested for activity. Strain pAD1dDD produced the highest amount of theobromine from caffeine with 98.5% conversion. Then, three different media were used to examine cell growth and activity, and super broth medium was found to be the best to produce the largest amount of cells. After that, cell growth and N-demethylation reaction were scaled up in which the N-demethylation reaction was carried out at a temperature of 30 ^{o}C, shaker speed of 250 rpm, and pH of 7.5. Finally, theobromine was separated from the reaction mixture by preparative chromatography and purified by drying. The final biologically produced theobromine was highly pure and the overall molar yield was 80%.

Poster 3

INVESTIGATION OF STRUCTURE ACTIVITY RELATIONSHIP OF PEG-PEPTIDE SCAVENGER RECEPTOR INHIBITORS

Rondine Allen and Kevin Rice*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy
University of Iowa, Iowa City, IA

The rapid capture and degradation of viral and non-viral gene delivery nanoparticles by scavenger receptors (SR) on Kupffer cells and fenestrated endothelial cells in the liver results in a decreased half-life of all nanoparticles in an *in vivo* system, as well as a lower percentage of the dose available for delivery to the target organ. Current attempts to achieve SR inhibition involves co-administering high molecular weight, polyanionic inhibitors. Polyinosinic acid (Poly-I), the most widely used and potent inhibitor, competes for SR binding and successfully inhibits the uptake and metabolism of viral gene delivery nanoparticles in the liver. However, Poly-I also activates the immune system, resulting in toxicity in mice, making Poly-I clinically unacceptable.

We have discovered peptide based SR inhibitors that block SR uptake of DNA nanoparticles and improves their metabolic half-life by forming protein nanoparticles in the blood. These Polyethylene glycol (PEG) polylysine peptides potently inhibit SR and allow DNA to transfect hepatocytes up to 12 hours after administration in mice.

Radio-iodinated PEG-peptides were used to study the pharmacokinetics and bio distribution to understand the structural properties which influence transfection competency and potency. We hypothesize that the *in vivo* potency and activity is influenced by the stability of protein nanoparticles formed in the blood. Peptides which are able to form stable nanoparticles are able to effectively block liver uptake of DNA nanoparticles by saturating SRs on Kupffer cells in the liver. The results of this study provide a framework for the design and synthesis of future PEG-peptide scavenger receptor inhibitors, as well as an assay to quickly determine the activity of each inhibitor.

Poster 4

16S METAGENOMIC PROFILING OF FRESHWATER MUSSEL BED MICROBIAL COMMUNITIES REVEALS INFLUENCE ON NITROGEN-CYCLING BACTERIA

Ellen M. Black and Craig L. Just*
Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

The Upper Mississippi River (UMR) contains highly dense native freshwater mussel (Unionioida) populations of 4 mussels/m^2, on average. Mussel assemblages collectively have a water filtration rate of 53.1 million m^3/d and deposit up to 25 kg-N/d into sediment for subsequent use by benthic organisms. Bacteria in the phylum Nitrospirae were five times more abundant in the UMR mussel bed ($p=0.0004$), and Crenarchaeota archaea, responsible for oxidizing NH_4^+ to NO_2^-, were significantly decreased ($p=0.03$) in the presence of mussels. Additionally, anaerobic ammonium oxidizing (anammox) bacteria (phylum Planctomycetes) facilitate a significant and unique nitrogen removal mechanism in freshwater ecosystems. Greater anammox bacteria abundance was identified 3 cm below the sediment-water interface when mussels were present ($P< 0.001$), whereas anammox were more prominent at a sediment depth of 5 cm without mussels ($P=0.002$). 16S rRNA amplicon-sequenced data of these sediment microbial communities showed that mussel presence suppresses the intra-sample (alpha) species diversity while inter-sample species diversity (beta) variability was partially explained by mussel presence. This research indicates that freshwater mussels may be responsible for enhancing the niche of specific microbes while lowering overall microbial diversity, perhaps through bioturbation and excretion of nitrogen substrates. Future research should address real-time microbial activity in mussel beds to determine if the biotic coordination between mussels and mussel bed microbiomes play a large role in freshwater nitrogen cycling

Poster 5

BIOCHEMICAL AND STRUCTURAL STUDIES OF A NOVEL METAL DEPENDENT DIMETHYLSULFONIOPROPIONATE LYASE

Saumya M. De Silva, Nicholas J. Schnicker and Mishtu Dey*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Dimethylsulfoniopropionate (DMSP) produced by marine phytoplankton is degraded by marine bacteria to form acrylate and a climatically active gas dimethylsulfide (DMS). Approximately 50 million tons of this biogenic volatile sulfur gas is released to the atmosphere annually. The mechanism of DMS production by these marine bacteria is not yet clearly understood primarily due to the lack of sufficient biochemical and structural data. There are six known DMSP cleavage enzymes, DddD, DddL, DddP, DddQ, DddW and DddY, that degrade DMSP via the lyase pathway. A vast majority of DMSP is thought to be degraded by a demethylation pathway and the pathway genes and enzymes exist in a SAR11 clade of marine bacteria known as *Pelagibacter ubique*. Whereas, recently, a novel enzyme has been identified from *Pelagibacter ubique* that cleaves DMSP via the lyase pathway to produce DMS and acrylate. This novel enzyme DddK shares some sequence similarity with various other DMSP lyases and contains conserved sequence and common residues of the cupin superfamily of proteins. Our crystal structures of DddK show the presence of a conserved β-barrel fold with residues involved in binding a metal ion. Site-directed mutagenesis studies reveal the key residues of the active site are necessary for enzyme activity. Since the cupin-fold containing proteins use various metal ions for their diverse activity, we determined the metal-binding properties of DddK in order to understand the selectivity for catalysis. Among the DMSP lyases, metal selectivity properties have been characterized only for DddW and DddW has been shown to be catalytically most competent in presence of Fe(II). Based on our structural and biochemical studies of DddK, we propose a new mechanism of DMSP cleavage reaction forming DMS and acrylate.

Poster 6

TIME AND TEMPERATURE DEPENDENCE OF ATZ GENE EXPRESSION IN PSEUDOMONAS SP. ADP BIOFILMS

Michael A. Delcau and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering
University of Iowa, Iowa City, IA

The maximum contaminant level set of the commonly used pesticide, atrazine, is set to 30 parts per billion by the Environmental Protection Agency. However, soil and ground water in the mid-western United States frequently exceeds this limit. To mitigate the resulting high concentrations, the use of a bacterial strain capable of metabolizing atrazine, *Pseudomonas* sp. ADP, is considered as an environmentally friendly and cost-effective method of remediation. The genes encoding for the enzymatic degradation of atrazine, *AtzA-AtzF*, are dispersed on the 108 kilo base pair plasmid, pADP-1. Reverse transcription real-time quantitative polymerase chain reaction was used to determine the relative expression of the atrazine-degrading gene set in 3-day, 5-day, and 10-day biofilms grown in a drip-flow reactor. Furthermore, the relative gene expression was determined at 25^{o}C, 30^{o}C, and 37^{o}C to demonstrate temperature dependence. At 37^{o}C, genes *AtzA*, *AtzB*, and *AtzC* were significantly downregulated whereas *AtzD*, *AtzE*, and *AtzF* were upregulated compared to the control (30^{o}C) and low temperature (25^{o}C) conditions. The expression of all atrazine-degrading genes for a 10-day old biofilm is expected to be induced compared to 5-day old biofilm, whereas the genes for a 3-day old biofilm are expected to be repressed. Upregulation of atrazine-degrading genes in higher temperature environments provides insight into environmental conditions at which the bacteria may thrive to degrade atrazine. Furthermore, determination of the optimal age of a *Pseudomonas* sp. ADP biofilm demonstrates differential gene expression of mature and young biofilms in the degradation of recalcitrant contaminants.

Poster 7

NOVEL ACTIVITY OF N-1 FLUOROQUINOLONE DERIVATIVES

Justine Delgado and Robert J. Kerns*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Fluoroquinolones are broad spectrum antibiotics used to treat a variety of bacterial infections. Bacterial resistance to clinically used antibiotics is a worldwide problem. The need for novel agents able to retain activity against resistant mutants continues to grow. Fluoroquinolones activity is derived from their ability to selectively inhibit bacterial type II topoisomerases, DNA gyrase and/or topoisomerase IV. Type II topoisomerases regulate the topology of DNA and are necessary for replication and transcription. Fluoroquinolones fall into a class of compounds termed topoisomerase poisons. Fluoroquinolones inhibit topoisomerase activity by trapping of the topoisomerase-DNA cleavage complex, preventing re-ligation of DNA. This leads to an increase in double strand breaks and eventually cell death. Mutations to genes encoding these two target enzymes are the main cause of fluoroquinolone resistance. To overcome fluoroquinolone resistance new derivatives, need to be designed to retain activity against mutated enzyme. Through the use of crystallographic investigation, the N-1 and C-7 positions of fluoroquinolones were identified as targets for structural modifications. These positions were chosen because of their potential to acquire additional binding contacts not utilized in current fluoroquinolones. New interactions with amino acids in the active site not known to mutate would overcome key mutations that result in a loss of activity. During this study it was found that incorporation of certain aryl groups at the N-1 position of the fluoroquinolone core resulted in loss of activity toward the bacterial enzyme. However, these fluoroquinolones do not act as poisons. Further characterization of binding to determine the mechanism of action of these fluoroquinolones was pursued and is presented here.

Poster 8

USING ULTRASOUND TO ENHANCE TUMOR CELL KILLING BY ANTI-PD1 AND DOXORUBICIN-LOADED PARTICLES

Anh-Vu Do[1], Kee W. Jang[2], Dongrim Seol[2], Phillip Tobias[1], Daniel Carlsen[1], Ino Song[2], James A. Martin[2], and Aliasger K. Salem[1]*
[1]Department of Pharmaceutics & Translational Therapeutics, College of Pharmacy, University of Iowa, IA
[2]Department of Orthopedics and Rehabilitations, University of Iowa Hospitals and Clinics, University of Iowa, IA

Metastatic melanoma is currently an incurable disease for which alternative treatments to just chemotherapy alone are sought. The use of ultrasound with microbubbles is a promising regimen for tumor treatment. In this study, the addition of ultrasound provided a means for controlled drug delivery through inertial cavitation. In vitro experiments involving the use of ultrasound with blank PLGA particles showed higher cytotoxicity in B16.F10 melanoma cells compared to either blank PLGA particles or ultrasound alone. Further in vitro experiments demonstrated an ability to control the release kinetics of doxorubicin from doxorubicin-loaded PLGA particles through the delivery of different ultrasound doses. Utilizing a melanoma murine model, the application of ultrasound to doxorubicin-loaded particles, injected intratumorally, and/or αPD-1 yielded higher tumor regressions and survival rates compared to either treatment alone. Exploiting the phenomenon of inertial cavitation, ultrasound can provide a noninvasive and effective way to deliver drugs and genes to combat many different diseases.
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Poster 9

CHARACTERIZING AND QUANTIFYING POLYCHLORINATED BIPHENYNL BIODEGRADERS IN ALTAVISTA LAGOON

Jessica Ewald, Yi Liang, Jerald Schnoor, and Timothy Mattes*
Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

Polychlorinated biphenyls (PCBs) are persistent contaminants known to have a profound impact on humans, animals, and the environment. The recalcitrant nature of these compounds makes then a prime candidate for bioremediation techniques. Microorganisms take advantage of these contaminants for use in their metabolism, and in turn degrade them. Studying the presence of these microorganisms and their effects on overall PCB contamination levels in the environment can reveal interesting dynamics of the environmental systems and suggest means of remediation.

This study focuses on a lagoon in Altavista, VA which contains significant levels of PCB contamination. To develop a better understanding of the existing microbial community 16s rRNA sequencing was preformed utilizing the Illumina sequencing technology at Argonne National Laboratory. Illumina sequencing revealed an abundance of known anaerobic and aerobic PCB degraders. Dominant phyla include Proteobacteria, Firmicutes, and Cholroflexi among others. The phylum Chloroflexi, which includes known anaerobic PCB degraders such as *Dehalococcoides* spp. and *Dehalogenimonas* spp., was detected in the samples. Other genera detected which contain known chlorinated compound degraders include *Geobacter*, and *Clostridium*. The presence of these microorganisms suggests *in situ* PCB degradation.

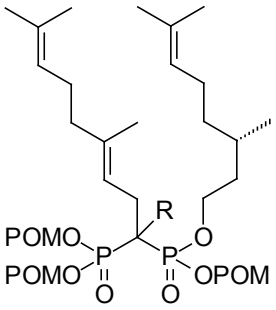
To further investigate suspected PCB biodegradation qPCR was performed on extracted DNA from the Altavista samples. The enzyme *bphA* is known to participate in the aerobic degradation of PCBs, which makes it an ideal biomarker. The qPCR analysis revealed *bphA* present in abundances of 10^4-10^7 gene copies per gram of sediment, suggesting the occurrence of aerobic PCB degradation.

Poster 10

A NEW MOTIF FOR INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE

Benjamin J. Foust[1], Cheryl Allen[2], Sarah A. Holstein[2], and David F. Wiemer[1,3]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]Division of Oncology and Hematology, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE
[3]Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA

Geranylgeranyl diphosphate synthase (GGDPS) is a key enzyme in a later stage of the human mevalonate pathway, which is of increasing interest for the treatment of a variety of different conditions including malaria, osteoporosis, and malignant bone diseases such as myeloma. The enzyme receives the substrate farnesyl diphosphate and catalyzes production of geranygeranyl diphosphate. Both substrate and product seem to have different lipophilic channels allowing access to this enzyme terminating in an area capable of stabilizing the high charge density of the natural pyrophosphates. Previously studied bisphosphonates with two isoprenoid chains positioned on the α-carbon have proven to be effective inhibitors of this enzyme. Recent efforts have afforded a new motif (1) with one isoprenoid chain on the α-carbon, a second included as a phosphonate ester, and the potential for a third at the α-carbon. The pivaloyloxymethyl (POM) prodrugs of several compounds based on this motif have been prepared and the resulting compounds have been tested for their ability to disrupt protein geranylgeranylation and induce cytotoxicity in myeloma cells. The initial bioassays show activity consistent with GGDPS inhibition, and demonstrate a structure-function relationship dependent on the nature of the third alkyl substituent on the α-carbon.



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R =	Disruption of cellular geranylgeranylation (μM)
H	≥ 5.0
Methyl	≥ 0.5
Allyl	≥ 2.5
Prenyl	≥ 1.0
Geranyl	≥ 2.5

POM = $CH_2OC(O)C(CH_3)_3$

Poster 11

NOVEL ANTIBIOTIC FORMULATIONS FOR ERADICATION OF BIOFIOLMSOF *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

Sachin Gharse[1] and Jennifer Fiegel[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Purpose: The purpose of our research is to develop antibiotic formulations consisting of an antibiotic and a nutrient dispersion compound for eradication of pulmonary *Pseudomonas aeruginosa* biofilms. Previous work done in our laboratory on *P. aeruginosa* lab strain PAO1 established that nutrient dispersion compounds entice bacteria out of the biofilm, making them more susceptible to antibiotics. In our current investigation, we study the efficacy of novel antibiotic formulations against both young and mature *in vitro* biofilms of *P. aeruginosa* clinical isolates obtained from cystic fibrosis patients.

Methods: Clinical isolates of *P. aeruginosa* were obtained from the University of Iowa Hospitals and Clinics. Minimum biofilm eradication concentration (MBEC™) assays were carried out by treating young biofilms (grown over 24 hours) of the clinical isolates as well as the lab strain PAO1 with either an antibiotic or combinations containing an antibiotic and a nutrient dispersion compound. The antibiotic concentrations were used in the range from 1 to 1000 μg/mL, while the dispersion compound concentration was kept constant. The residual biofilms on the pegs post-treatment were dispersed in sterile growth medium and incubated for 24 hours, following which optical density was measured at 650 nm. Sodium citrate at concentrations of either 10 mM or 20 mM was used as the nutrient dispersion compound in these studies. Similarly, mature biofilms (grown over 4 days) of the lab strain PAO1 and the clinical isolates were treated with either an antibiotic or combinations containing an antibiotic and a nutrient dispersion compound. The residual biofilms were then evaluated by measuring the optical density at 650 nm.

Results: The combination of ciprofloxacin hydrochloride and 10 mM sodium citrate eradicated young biofilms of lab strain PAO1 at lower antibiotic concentration of 50 μg/mL as compared to the antibiotic alone (125 μg/mL). The combination of colistin methanesulfonate and 20 mM sodium citrate also displayed a lower MBEC (125 μg/mL) as compared to the antibiotic alone (250 μg/mL). However, both these combinations failed to better eradicate young biofilms of *P. aeruginosa* clinical isolate P2 compared to the antibiotic alone. For clinical isolate P2, treatment with the combination of tobramycin sulfate and 10 mM sodium citrate resulted in an MBEC value of 50 μg/mL, compared to 250 μg/mL for tobramycin sulfate alone. Mature *P. aeruginosa* biofilms demonstrated lesser susceptibility to the treatments as compared to young biofilms, as was expected. However, combinations containing an antibiotic and a dispersion compound managed to eradicate the mature biofilms at lower antibiotic concentrations than the antibiotic alone.

Conclusion: Combination formulations consisting of an antibiotic and a nutrient dispersion compound were successful in eradicating both young and mature *in vitro P. aeruginosa* biofilms at lower antibiotic concentrations than the antibiotic alone. Our future studies will aim to develop an animal model mimicking pulmonary CF bacterial infection, and test the efficacy of our proposed formulations in these animal models.

Poster 12

EFFECTS OF MASS MODULATION ON HYDRIDE TRANSFER IN THYMIDYLATE SYNTHASE

Ananda K Ghosh, Michael J Christoperson and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Enzymes display a hierarchy of characteristic motions varying from very fast and localized at femtosecond-picosecond timescale to slow motions that occur on millisecond-second timescales. Understanding the active role of protein dynamics in enzyme catalysis is a controversial contemporary topic.[1] Many of these motions have an important role in the catalytic cycle of an enzyme. Different approaches have been used to unravel the involvement of these protein motions on catalysis, including nuclear magnetic resonance spectroscopy (NMR), vibrational spectroscopy and temperature dependence of kinetic isotope effects (KIEs).[2] One recently developed tool designed to address this question is the use of enzymatic isotopic substitution to alter the bond vibration frequencies at the faster femtosecond (fs) – picosecond (ps) time scale.[3] In the present work, we studied the effect of altered mass vibrations of the enzyme on different steps in a complex reaction using thymidylate synthase form *Escherichia coli* (TSase) as a model system. The results of the comparative studies of temperature dependence of intrinsic KIEs of light (unlabeled), half heavy (^{15}N and ^{13}C labelled) and heavy enzyme (^{15}N, ^{13}C and ^{2}H labelled) suggest a direct involvement of fast vibrational frequencies to bond activation in the light enzyme.

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Poster 13

PARTICLE SURFACE PROPERTIES DETERMINE PARTICLE-LUNG SURFACTANT INTERACTIONS

Bharath Kumar Gowdampally[1] and Jennifer Fiegel[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Purpose: Lung surfactant, a mixture of phospholipids and proteins, is present as a lining at the air-water interface of alveoli. Lung surfactant makes breathing process easy by maintaining low surface tension at the alveolar interface. When foreign particles are deposited on alveoli they interact with lung surfactant and could inhibit surfactant functions resulting in respiratory diseases. The purpose of this research was to investigate the effect of particle surface properties on Infasurf (natural lung surfactant) surfactant function to understand particle-lung surfactant interactions.

Methods: Particle-lung surfactant interactions were investigated by studying surfactant phase behavior and microstructure using Langmuir-Wilhelmy tensiometer and fluorescent microscopy, respectively. Surfactant phase behavior was studied by conventional single compression and physiologically relevant multiple compression-expansions. The surface area was compressed either slowly at 10 mm/min to characterize equilibrium behavior or at 150 mm/min to mimic physiologically-relevant compressions and expansions. During microscopy studies a fluorescent probe, TexasRed DHPE, was added to the Infasurf to observe condensed domains at the surface during compression. Images of the surface were captured and analyzed to determine the size of condensed domains and extent of condensation of Infasurf.

Results: During single compression studies decreased surface pressures were observed for Infasurf only in the presence of plain polystyrene particles indicating loss of Infasurf at the air-water interface, likely due to surfactant adsorption onto the particle surface. In lung relevant studies, hysteresis area was decreased initially only in the presence of carboxyl modified particles. However, by the end of 10 compression-expansion cycles, no significant change was observed. In microscopy studies, more smaller condensed Infasurf domains were observed in the presence of carboxyl modified particles, likely due to increased repulsion between negatively charged carboxyl groups and anionic phospholipid molecules. Fewer larger condensed domains were observed in the presence of aliphatic amine particles. The positively charged amine groups may decrease the repulsion among negatively charged phospholipid molecules, thus resulting in theformation of large condensed domains.

Conclusion: The studies provide new information on the effects of nanoparticle surface properties on fundamental surfactant behavior of natural lung surfactant. The results indicate that the particles may have the ability to inhibit surfactant functionality based on particle surface chemistry. This knowledge will aid our understanding of how to design nanoparticles for applications in lungs, such as pulmonary drug delivery, where potential toxic effects are a concern.

Poster 14

THE ROLE OF ASPARAGINE 177 IN THE CATALYTIC CYCLE OF THYMIDYLATE SYNTHASE

Ilya Gurevic, Zahidul Islam, Sobia Rasool, Tasnia Iqbal, Kai Trepka, Ananda Ghosh, Chethya Ranasinghe, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

An adequate pool of nucleotides including thymidylate must be present for proper replication of DNA prior to cell division. In most organisms, thymidylate synthase (TSase) is the primary or sole route to the production of thymidylate (dTMP) from uridylate (dUMP)[1]. After fifty years of study, the reaction mechanism of TSase is undergoing re-evaluation. Recently a new reaction intermediate composed of a covalently bridged combination of both substrates has been predicted on the basis of computational[2] studies, and the existence of that intermediate was verified experimentally[3]. A related question – the formation of an enolate involving C5 and O4 of dUMP along the reaction pathway – has also been examined, and recent reports[2,4] weigh in on whether this species is formed after two of the important chemical steps of the reaction: proton abstraction and hydride transfer. Asparagine 177 appears positioned favorably to make hydrogen bonds to O4 from X-ray crystallographic evidence[5] and presumably stabilize the charge on the oxyanion. Building on previous reports suggesting disrupted interactions of N177 mutants with substrate,[6,7] here, we seek to obtain a comprehensive insight into the role of N177 in catalysis, particularly the hydride transfer. We employ kinetic isotope effect (KIE) techniques to make inferences regarding the role of N177 in the crucial hydride transfer step by employing an activated tunneling model[8].

Our outcomes with N177 mutants indicate that there are significant disruptions in steady-state kinetics. Our kinetic studies with isotope effects indicate perturbation to the mutants' active site, suggesting that N177 is a part of the reaction coordinate for the hydride transfer in the WT, indicating the presence of charge accumulation on the carbonyl oxygen during the hydride transfer.

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Poster 15

THE MECHANISM OF CHEMOTHERAPY-RESISTANT CANCEROUS THYMIDYLATE SYNTHASE

Zahidul Islam, Ilya Gurevic, Muhammad Saeed, Sobia Saeed, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa,
Iowa City, IA

In most organisms, including human, Thymidylate Synthase (TSase) catalyzes a reaction that provides the sole *de novo* source of the DNA base thymidylate. Because of its importance in DNA production, human (*hs*) TSase is a common target for several anticancer drugs. 5-fluorouracil is a mechanism-based pro-drug that have long been used as chemotherapeutic drug for colorectal and ovarian cancers. However, cancer cells have developed resistance mechanisms, such as overexpression of *hs* TSase and introducing mutations in the TSase gene, leading to drug-resistant cancers.[1]

Berger et al reported a variant of *hs* TSase (Y33H), resistant to 5-fluorouracil, that was derived from a primary culture of human colonic tumor.[2] Although several studies tried to locate the origin of the resistance profile, its molecular mechanism has not been established yet. Thereby, to investigate the molecular origin behind the resistance profile, we expressed and purified recombinant Y33H and characterized it structurally and kinetically. Our studies indicate that the recombinant Y33H exhibits similar inhibitory kinetics with 5F-dUMP (the actual drug naturally derived from the pro-drug 5-fluorouracil) as were found for the natural variant isolated from human colonic tumor. Interestingly, although residue Y33 is remote from the active site and has no contact with the substrates, our studies found that the drug-resistant variation Y33H perturbed the enzymes structure, kinetics, isotope effects, affinity for the anticancer drug, and other parameters that explain the molecular and mechanistic source of the cancer resistance to 5-fluorouracil. Implications in combating drug-resistant cancers will be discussed.

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Poster 16

KINETIC SOLVENT ISOTOPE EFFECTS AS A MECHANISTIC TOOL IN FLAVIN DEPENDENT THYMIDYLATE SYNTHASE (FDTS)

Kalani Karunaratne[1], Mitchell LeFebvre[2], Jose Villalobos[1], and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]Carver College of Medicine, University of Iowa, Iowa City, IA

Thymidylate (dTMP, one of the four building blocks of DNA) is synthesized by methylation of dUMP (2'-deoxyuridine 5'-monophosphate) at the C5 position. In most eukaryotes, including humans, the enzyme thymidylate synthase (TSase) encoded by *thy*A gene catalyzes the reductive methylation of dUMP to form dTMP. (1, 2) However, a novel gene named *thy*X coding for a new class of thymidylate synthase was discovered (3). This new class of TSases named as flavin-dependent thymidylate synthases (FDTSs) makes use of a noncovalently bound flavin adenine dinucleotide (FAD). Several disease-causing bacteria such as *Rickettsia prowazekii* (typhus-causing), *Mycobacterium tuberculosis*, and *Bacillus anthracis* rely on FDTS (3). Classical TSase and FDTS are substantially different in structure (4) and chemical mechanism (5, 6, 7). Hence, understanding the FDTS mechanism may allow the development of mechanism-based antibiotics with minimal toxicity to humans. In this work we have utilized solvent isotope effects to probe the mechanism of this unique enzyme. Both quench flow and stopped flow experiments were carried out in H_2O and in D_2O. Rate of product formation and substrate consumption followed by quench flow experiments done in isotopic solvents were compared to calculate the solvent isotope effect. Stopped flow experiments in two solvents reveal the oxidation state of Flavin with respect to reaction time. Information obtained by these techniques will provide insight into the complex mechanism of FDTS catalyzed thymidylate synthesis.

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Poster 17

SPECIALIZED METABOLITES AFFECTING THE INTERSPEICES INTERACTIONS BETWEEN *MYXOCOCCUS XANTHUS* AND *BACILLUS SUBTILIS*

Paige Kies, Susanne Müller* and John R. Kirby*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

Myxococcus xanthus and *Bacillus subtilis*, two soil dwelling bacteria, are capable of multicellular development and sporulation. Both organisms are thought to affect the composition and dynamics of microbial communities within the soil. Under starvation conditions, *M. xanthus* acts as a predator and secretes specialized metabolites and lytic enzymes in order to consume a wide variety of prey (1, 2). The ancestral strain of *B. subtilis,* NCIB3160, resists *M. xanthus* predation utilizing the specialized metabolite bacillaene and ultimately escapes predation by creating unique biofilms, designated as megastructures. Within these dense matrices mature spores are embedded, thus ensuring the survival of the *B. subtilis* community. We could show that megastructure formation is genetically distinguishable from *B. subtilis* colony biofilm formation and is specifically a predation-induced stress response (3). *M. xanthus* is capable of making a diverse selection of specialized metabolites; we have demonstrated one specialized metabolite, myxoprincomide, to be an active component in the interspecies interaction between *M. xanthus* and *B. subtilis* NCIB3160. A myxoprincomide mutant is significantly deficient in killing of *B. subtilis* NCIB3160 without affecting megastructure formation. We are interested in the spatio/temporal regulation of specialized metabolites during predation by *M. xanthus* which are synthesized by NRPS/PKS clusters. We also show that deletions in two *sfp* genes in *M. xanthus* whose protein products regulate the NRPS/PKS clusters are defective in predation of *B. subtilis,* indicating additional specialized metabolites with potential roles in this interspecies interaction.

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Poster 18

SYNTHESIS OF CHoP-MA COATINGS VIA PHOTOINITIATED RAFT POLYMERIZATION AS A TARGETED, STEALTH DRUG DELIVERY SYSTEM

Benjamin King and Jennifer Fiegel*
Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Nanoparticle-based systems are an attractive solution to the problem of delivering drug and gene therapies to specific cell populations. Two key features of effective delivery systems are stealth, the ability to evade immune response, and targeting, the ability to include features that deliver the therapy only into desired locations. Using phosphorylcholine (CHoP), a chemistry commonly used by bacteria to invade host cells that exhibits key features commonly associated with stealth, we have developed a polymer whose properties are expected to provide stealth and targeting in the lung environment for treatment of infectious diseases. These polymers have been synthesized as brushes grafted onto gold nanoparticles as a means of studying the properties of the coating.

The polymers are made of methacrylates functionalized with phosphorylcholine side-groups. The reactions are performed in the presence of a reversible addition-fragmentation chain-transfer (RAFT) polymerization agent, which help control molecular weight. The RAFT agent forms end groups that acts as anchors that allow the polymer brushes to form self-assembled monolayers onto the gold nanoparticles.

Poster 19

ESTABLISHING THE ROLE OF GLOBAL NETWORKS ON COUPLED MOTIONS IN HUMAN DIHYDROFOLATE REDUCTASE

Jiayue Li and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Enzymologists have been working for decades understanding the roles of protein dynamics in an enzyme catalyzed C-H (hydride, proton or atom) transfer reactions. Although some enzyme functions are well understood, the chemical transformation at the active site is not as well defined. The isolation of chemical step is challenging since chemistry is rarely fully rate-limiting to enzymatic turnover. Examination of intrinsic kinetic isotope effects (KIEs) partly circumvents this challenge and serves as an important tool for understanding the transitional state of enzyme reactions. Dihydrofolate reductase (DHFR) from *Escherichia coli* has long been a benchmark model system for structural, kinetic and evolutionary studies. One can examine the role of protein motions in DHFR by measuring the temperature dependence of the intrinsic KIEs of the DHFR catalyzed reaction and analyzing the data within the context of activated tunneling models of hydrogen transfer. For *ec*DHFR, a global dynamic network of coupled motions has been mapped out using this methodology. To verify whether this phenomenon is conserved along the evolution from relatively flexible bacterial enzyme (*ec*DHFR) to the rigid human DHFR (*h*DHFR), site directed mutagenesis is used to alter the equivalent residues in *h*DHFR and the temperature dependence of the intrinsic KIEs of the mutants and their double mutants are determined. This study can help generate a more comprehensive understanding about reactivity of this enzyme and potentially shed light on drug design or biomimic studies.

Poster 20

ACCELERATION OF PROTEIN DESIGN THERMODYNAMICS USING A DUAL FORCE FIELD APPROACH

Jacob M. Litman[1], Stephen D. LuCore[2], Young Joo Sun[1], Ernesto Fuentes[1], and Michael J. Schnieders[1,2]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Biomedical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Computational design of proteins to achieve enhanced thermodynamic stability and/or binding affinity to a target is inherently dependent on precision-accuracy tradeoff. Overall simulation accuracy depends on both simulation time scale (i.e. the precision afforded by sampling convergence) and the accuracy of the underlying potential energy function (i.e. a force field). Polarizable atomic multipole force fields, such as AMOEBA, improve the accuracy of each simulation time step compared to fixed partial charge force fields (i.e. AMOEBA atomic forces are more accurate than Amber, CHARMM or OPLS-AA), but reduce the sampling time scale and precision achieved due to a more computationally expensive functional form. To mitigate this, we describe a novel dual force field based approach that computes the free energy change for moving between force field resolutions. This approach allows long time scale transitions to be sampled with an inexpensive fixed partial charge force field, followed by short time scale thermodynamic corrections to account for moving from the fixed charge potential to the polarizable potential. Although this approach has been demonstrated for small molecule crystal deposition free energies (i.e. ~10-30 atoms), here we show that it scales up to larger peptides and discuss the path forward to full scale protein thermodynamics in the context of computational protein design.

Poster 21

FEMTOSECOND DYNAMICS IN THE ACTIVE SITE OF FORMATE DEHYDROGENASE: A MOLECULAR DYNAMICS APPROACH TO 2DIR SPECTROSCOPY

William K. Marquardt, Amnon Kohen* and Christopher M. Cheatum*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

It is expected that the motions of a protein have a relationship with catalysis, but current models do not reasonably predict how perturbations to dynamics on the timescale of bond vibrations – femtoseconds to picoseconds – correlate with effects on the kinetics of the chemical step. Formate dehydrogenase (FDH) serves as an excellent model system to explore this question, because it catalyzes a hydride transfer from formate to the nicotinamide ring of NAD^+. Kinetic isotope effect measurements readily provide kinetics information on hydride transfer reactions. Likewise, two-dimensional infrared (2DIR) spectroscopy provides a means to probe femtosecond dynamics, and azide anion is both a strong infrared chromophore and an analogue of the H-transfer transition state. 2DIR measurements of the FDH-azide-NAD^+ ternary complex thus arguably probe the structure of the reaction transition state.

2DIR measurements of FDH showed oscillatory components in the frequency-frequency correlation function with frequencies of 24 ± 1 cm^{-1} and 9.9 ± 0.4 cm^{-1}, suggesting periodic motion of the azide environment at the same frequency [1]. Such low frequency modes should be overdamped by thermal fluctuations of the protein and solvent. These oscillations were not observed in 2DIR measurements of the ternary complex with the reduced cofactor, suggesting that NAD^+ is the source of the oscillating behavior. The exact nature of these oscillations is the focus of this study.
Current work focuses on modeling a 2DIR experiment on the FDH-azide-NAD^+ ternary complex. Instantaneous frequencies are approximated by application of an empirical frequency correlation map to a molecular dynamics trajectory, as has been done for azide in water, and 2DIR experiments are being simulated using a numerical integration of the Schrödinger equation approach [2,3].

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Poster 22

RE-PARAMETERIZATION OF PROTEIN FORCE FIELDS GUIDED BY OSMOTIC COEFFICIENT MEASUREMENTS FROM MOLECULAR DYNAMICS SIMULATIONS

Mark S. Miller, Wesley K. Lay, Shuxiang Li, William C. Hacker, Jiadi An, Jianlan Ren, and Adrian H. Elcock*
Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA

Our group and others have recently described the use of osmotic coefficient measurements as a means of validating solute-solute interactions in molecular dynamics (MD) simulations of biomolecules. In particular, in simulations of amino acids, we and others found that intermolecular interactions are often too attractive; these disagreements with experiment were attributed to problems with the side chain functional groups, the charged termini, and interactions with counter-ions. We have since attempted to understand the cause of these problems more fully, using MD simulations to measure osmotic coefficients for a variety of amino acids and small molecules modeled with four very commonly used protein force fields. We then improved agreement with experiment by adjusting the van der Waals parameters assigned to given atom types, either using modifications recently reported in the literature or by optimizing the values in-house. These improvements highlight the potential utility of osmotic coefficient data in efforts to describe interactions between biological macromolecules accurately.

PHOTOCROSSLINKER NON-CANONICAL AMINO ACIDS FOR PROTEIN INTERACTION STUDIES

Steven M. Molinarolo, Jason D. Galpin and Christopher A. Ahern*
Department of Molecular Physiology and Biophysics, Carver College of Medicine, University of Iowa, Iowa City, IA

Nonsense suppression allows for novel approaches to identify and characterize protein complexes. The photoreactive crosslinking non-canonical amino acid (ncAA), p-benzoyl-**L**-phenylalanine (Bpa), can be genetically encoded in a target protein via the co-expression of an evolved orthogonal synthetase/tRNA pair. This approach has been used successfully in capturing and identifying transient protein interactions in reconstituted complexes purified from *E. coli* and mammalian cells. One shortcoming of Bpa approach is steric clash whereby the bulky benzyl groups of Bpa can hinder encoding of the ncAA or disrupt protein interaction. To overcome this shortcoming in addition to Bpa we are utilizing azide (AzF) and diazirine (Dz) photoreactive crosslinkers. Here we show encoding of AzF, Bpa and Dz into the human voltage-gated sodium channel β1 subunit. Future studies will employ the photocrosslinkers to determine locations of interaction between the pore subunit and auxiliary β-subunits in voltage-gated sodium channels.

PROBING THE LIKELINESS OF PROPOSED INTERMEDIATE OF FDTS CATALYZED REACTION

Dibyendu Mondal, Jiajun Yao and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Flavin-dependent thymidylate synthase (FDTS) encoded by *thyX* provides an alternative biosynthetic path for the *de novo* biosynthesis of thymidylate (dTMP) in several human pathogens (e.g., those causing anthrax, tuberculosis, typhus, and more,). The gene *thyX* is absent in humans thus making FDTS an attractive antibiotic target.[1,2] This enzyme utilizes a noncovalently bound flavin adenine dinucleotide (FAD) prosthetic group to catalyze the chemistry and unlike human TSase, FDTS produces tetrahydrofolate (H_4folate) rather than dihydrofolate (H_2folate), thus FDTS dependent organisms do not contain the *folA* gene encoding for dihydrofolate reductase.

Several published mechanisms has proposed the formation of some putative intermediate along the catalytic cycle to explain the mechanism of FDTS catalyzed reaction. We are interested in synthesizing these intermediate and probe the likeness of these intermediates.

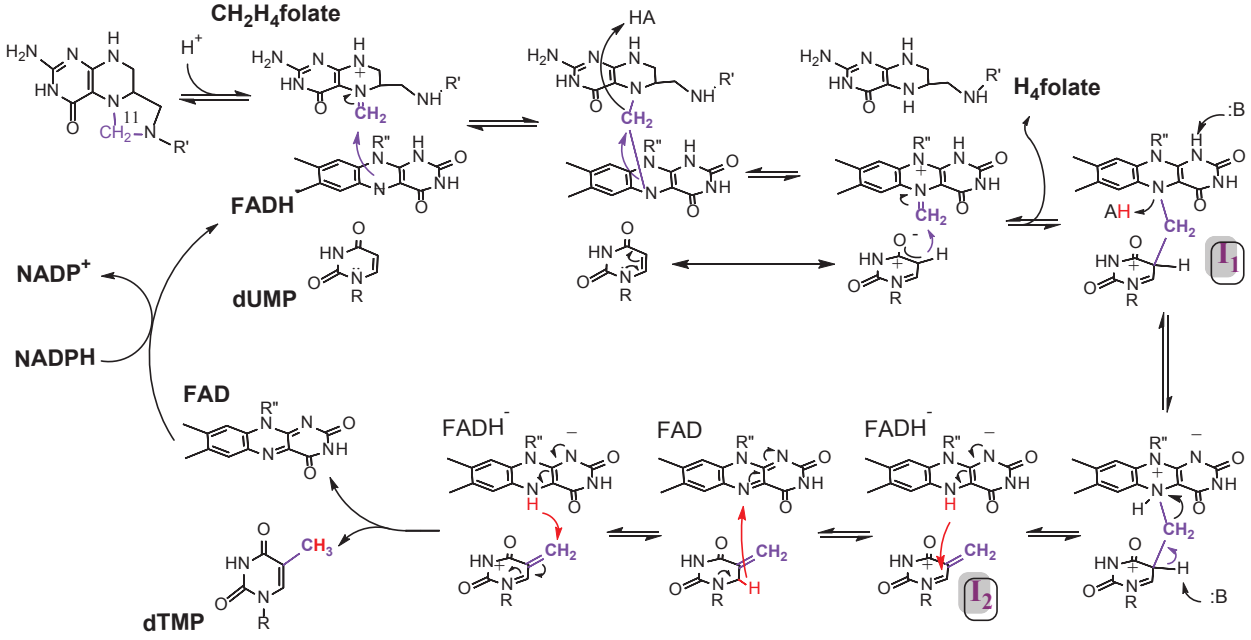

Figure **1** present proposed chemical mechanism for FDTS.

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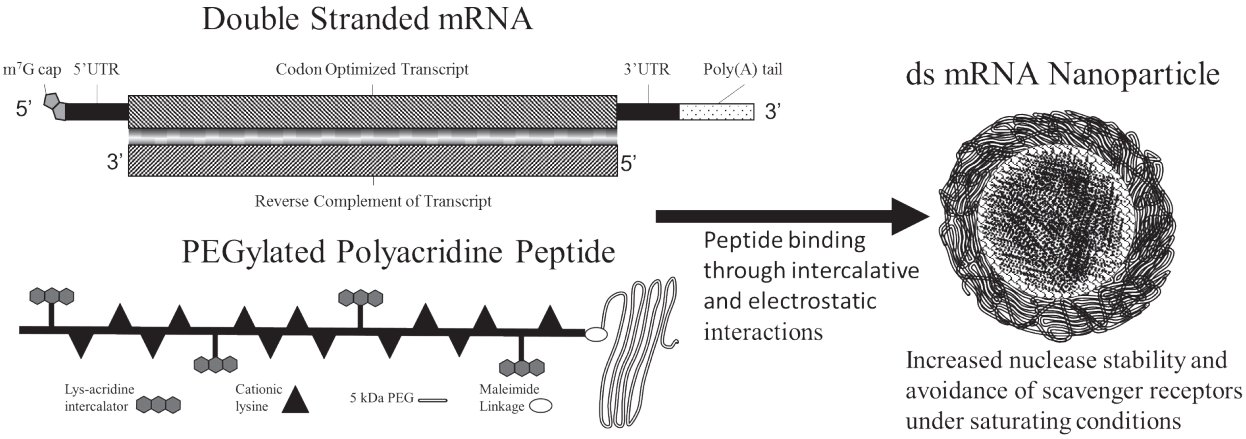
DOUBLE STRANDED mRNA NANOPARTICLES FOR NON-VIRAL GENE DELIVERY

Jacob A. Poliskey and Kevin G. Rice*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Gene therapy may one day be the first option for the treatment for many acquired and genetic diseases. By delivering genes directly, dysfunctional proteins can be replaced. We have previously developed a PEGylated polyacridine peptide (PEG-peptide) that complexes with and stabilizes DNA in circulation for up to 12 hours[1]. However, successful delivery of stabilized DNA to the nucleus of cells in animals remains highly challenging. The primary barrier to DNA delivery is the nuclear membrane. Therefore, mRNA offers an advantage over DNA because delivering mRNA bypasses the need for nuclear translocation. Yet active mRNA persists in mouse circulation for less than five minutes, even when complexed with PEG-peptide that increases its activity by 100-fold in vivo[2]. We discovered that double stranded mRNA (ds mRNA) is more stable to RNase challenge than single stranded mRNA (ss mRNA). ds mRNA withstands 100-fold greater RNase upon complexing with PEG-peptide than its uncomplexed counterpart. When hydrodynamically dosed in a mouse, ds mRNA results in similar level of luciferase expression compared to ss mRNA. Optimizing the reverse strand to preserve the structure of the untranslated regions (UTRs) increases the persistence of expression upon hydrodynamic dosing versus the full-length reverse strand. Applying scavenger receptor inhibition recovers over ten-fold luciferase expression upon hydrodynamic stimulation. Progress toward ds mRNA formulations for non-viral gene delivery will be presented.



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Poster 26

MASS MODULATED EFFECTS ON FORMATE DEHYDROGENASE

Chethya U. Ranasinghe [1], Qi Guo [1], Paul J. Sapienza [2], Andrew L. Lee[2], Christopher Cheatum[1], and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts & Sciences University of Iowa, Iowa City, IA
[2]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC

Enzymes exhibit dynamics on time scales ranging from milliseconds to femtoseconds. Investigation of possible correlations between fast (femtosecond (fs) to picosecond (ps)) protein motions and catalyzed chemistry is an area of great interest in contemporary enzymology. The current work employs isotopically labeled enzymes, in which its fast dynamics are dampened by the heavier mass of the protein. We examined the effects of the above phenomenon on catalysis by following the temperature dependence of intrinsic kinetic isotope effects (KIEs), a tool that probes the nature of the catalytic transition state and quantum mechanical (QM) tunneling, along the reaction coordinate. Mass effects on the chemical step of two distinct isotopic substitutions of formate dehydrogenase (FDH) were examined in which the protein's mass had been modulated by 5.5% and 11.1% via $^{13}C;^{15}N$ (half heavy) and $^{13}C;^{15}N;^{2}H$ (heavy) substitutions, respectively and were compared to those of isotopically unlabeled FDH (light). Steady state and substrate isotope effects on single turn-over studies together with examination of the temperature dependence of intrinsic KIEs indicated that millisecond kinetics were not significantly altered but that the temperature dependence of the intrinsic KIEs was proportional to the mass of the protein. The results suggest that femtosecond dynamics contribute to catalysis in the native enzyme, while there's complex effects from mass modulation on slower timescale kinetics. These studies collectively give a better understanding of possible coupling between protein dynamics and enzyme catalysis.

Poster 27

THE SYNERGISTIC EFFECTS OF ANTIBIOTICS AND HEAT ON *PSEUDOMONAS AERUGINOSA* BIOFILMS

Erica N.B. Ricker and Eric Nuxoll*
Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Each year in the United States tens of thousands of people have medical implants removed due to bacterial biofilm infections growing on the device. These biofilms form on the surfaces of the implants and are difficult for doctors to treat and for the immune system to eradicate. Therefore, the current standard of care is for a surgeon to perform invasive surgery to remove the implant and surrounding infected tissue, creating significant local tissue damage and a prolonged healing process. The patient then undergoes a high course of antibiotics until the infection is gone, followed by another surgery to implant a new device. This second implantation has twice the risk of infection as the first implantation. The current method for dealing with bacterial biofilm implant infections has a low patient quality of life and incurs billions of dollars in health care costs in the U.S. alone.

We have proposed a new approach to solve this problem: localized, wireless heat shock to kill the biofilm *in situ* without surgical intervention. By coating the implant with a polymer encasing iron oxide nanoparticles, an alternating magnetic field can induce remote heating that conductively transfers to the surface of the implant where the biofilm infection is located. Since antibiotics are normally the first step in treatment for these patients a series of experiments were performed to study the combined effects of heat application in conjunction with antibiotics. *Pseudomonas aeruginosa* biofilms grown in 96-well MBEC reactors for 24 hours were exposed to a variety of antibiotics and concentrations for 24 hours after the growth phase. The antibiotics explored were ciprofloxacin, tobramycin, and erythromycin chosen for their stability at higher temperatures and their efficacy against planktonic *P. aeruginosa.* Four hours into the antibiotic exposure the biofilms were also heat shocked at temperatures ranging from 37 °C to 80 °C for 1 to 30 minutes.

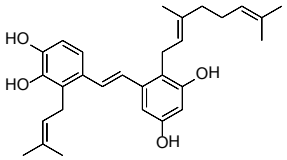
The biofilms proved to be remarkably resilient to the heat and antibiotics alone, however, at specific temperatures and exposure times the combined treatment decreased the viable bacteria significantly. At 60 °C for 5 minutes all of the antibiotics showed an increased effect including some of the low concentrations of antibiotics which appeared to be ineffective otherwise against the biofilms. Tobramycin additionally had an increased efficiency at 50 °C for 30 minutes. This combined therapy will allow for lower temperatures to be reached for shorter amounts of time and lower antibiotic concentrations for improved biofilm mitigation on implanted devices. With this knowledge the polymer coatings could be designed to heat only as much as needed for complete mitigation. The introduction of such a novel coating in combination with antibiotics could obviate thousands of surgeries, improve patient quality of life, and save billions of dollars spent on ex-plantation, recovery, and re-implantation.

Poster 28

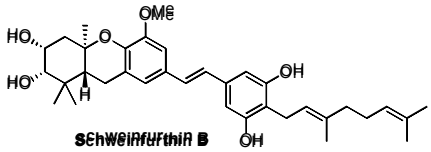
SYNTHESIS OF NOVEL PAWHUSKIN AND SCHWEINFURTHIN ANALOGUES

David P. Stockdale, Alyssa M. Hartung and David F. Wiemer*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

The pawhuskins are a family of natural compounds that were isolated from the purple prairie clover (*Dalea purpurea*) in 2004 by Belofsky *et al*.[1] This family of compounds is comprised of prenyl- and geranylated phenols joined by a stilbene linkage. They are non-nitrogenous opioid receptor modulators and thus represent a unique class of biologically active compounds. A second family of natural stilbenes would be the schweinfurthins, a family of natural products that were isolated from *Macaranga schweinfurthii* by Beutler *et al*. in 1998. They are pharmacologically intriguing because they have shown strong anti-proliferative activity against a variety of human-derived tumor cell lines.



Pawhuskin A



Schweinfurthin B

The unique activities of both the pawhuskins and schweinfurthins have led our group to do extensive structure-activity relationship investigations. It is known that the stilbene linkage in both classes of natural products is essential for activity. A goal of my research has been to prepare isosteric substitutions of the stilbene moiety in both the pawhuskins and schweinfurthins, to improve their water solubility and hopefully increase their activity. The latest results in this effort will be presented.

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Poster 29

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF CASK PDZ DOMAIN INTERACTIONS WITH PROTEIN AND LIPID BINDING PARTNERS

Young Joo Sun[1], Xu Liu[1], Titus Hou[1], Lokesh Gakhar[1,2], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Crystallography Facility, Carver College of Medicine, University of Iowa, Iowa City, IA

PSD-95/Dlg/ZO-1 (PDZ) domains are protein-protein interaction modules that generally interact with the C-terminus of partner scaffold proteins to participate in signal transduction processes. A subset of PDZ domains also interact with phophoinositides (PtdIns) and/or cholesterol. Our study focuses on the calcium/calmodulin-dependent serine protein kinase (CASK) PDZ domain that has been shown to synergistically interact with both protein binding partners and PtdIns. First, we quantitate the interaction between the CASK PDZ domain and known interaction partners. We show that the CASK PDZ domain binds to neurexin1 (NRXN1) and phosphorylated syndecan1 (pSDC1) with micromolar affinity, and only weakly (>100 uM) with unphosphorylated syndecan isoforms (SDC1-4). Second, we determined the crystal structure of the CASK PDZ domain alone and complexed with several peptides. A comparison of the CASK PDZ/NRXN1 structure with the CASK PDZ/SDC structures indicates that peptide-binding shifts the alpha-helix adjacent to the binding pocket resulting in a more expanded binding groove. Moreover, the CASK/pSDC1 crystal structure shows an interaction between residue R517 and the phosphoryl group of pSDC1, suggesting that his new interaction provides specificity and affinity. Third, using our own tertiary structure based sequence alignment tool, specialized for PDZ domains, we analyzed the crystal structures of 156 PDZ domains deposited in PDB data bank. Based on observations found in this data, we made several variants of the CASK PDZ domain at specific positions the peptide binding pocket expecting to manipulate the binding affinity and specificity. The CASK PDZ I503F mutant was capable of binding Caspr4 with the highest affinity (8.2 uM) of any known peptide, whereas the WT CASK PDZ had no measurable affinity for this peptide. Consistent with previous literature, protein-lipid blot analysis data showed that the CASK PDZ binds to various PtdIns molecules. However, NMR-based titration data indicated that this interaction is weak. Future experiments will explore the cooperativity of peptides and PtdIns in binding the CASK PDZ in the context of cell membranes.

Poster 30

STUDY OF THE REGULATORY MECHANISMS OF THE *S.AUREUS* SRRAB TWO-COMPONENT SYSTEM

Nitija Tiwari[1], Lotte Van Den Goor[3], Young Joo Sun[1], Patrick M. Schlievert[2], John R. Kirby[2], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA
[3]Loyola University, Chicago, IL

Antibiotic resistant *Staphylococcal aureus,* commonly known as Methicillin-Resistant *Staphylococcus aureus* (MRSA) are a major cause of hospital infections. Thus, there is an urgent need for identifying novel targets/mechanisms for antibiotic development. Bacterial two-component systems (TCS) contribute to infections by regulating toxin production, antibiotic resistance and survival. TCSs are composed of a membrane bound histidine kinase (HK) and a cytoplasmic response regulator (RR). The HK senses extracellular stimuli through its extracellular sensor domain and undergoes autophosphorylation at a conserved histidine residue in the cytoplasmic kinase domain. The phosphorylated HK then transfers the phosphoryl group to a conserved aspartate residue in the RR, which in turn binds DNA to control gene expression. SrrAB TCS is a regulator of *S. aureus* virulence factors including toxic shock syndrome toxin-1 (TSST-1), which causes toxic shock syndrome. SrrB is a dual function kinase/phosphatase capable of regulating the level of phosphorylation of SrrA. Here, we present data showing that the PAS domain effects SrrB kinase and phosphatase function. Preliminary data indicates that the presence of the PAS domain increases the rate of autophosphorylation and has an inhibitory effect on SrrB phosphatase activity. In addition to this, our data shows that the phosphorylation of SrrA affects its DNA binding function. We also identified heme as a ligand for the PAS domain, which could be a potential mechanism of regulating SrrAB TCS. Crystal structure of the apo form of the PAS domain shows a putative binding pocket which provides insights into potential residues involved in heme binding. Together, our data suggest that heme binding to the SrrB PAS domain may be involved in redox regulation of the SrrAB TCS.

Poster 31

PROBING ALLOSTERIC REGULATION OF AN EXECUTIONER CASPASE

Nicholas R. Vance[1], Lokesh Gakhar[1] and M. Ashley Spies[1,2]*
[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
[2]Department of Biochemistry, Carver College of Medicine, University of Iowa
Iowa City, IA

The caspase family of cysteine-dependent, aspartate-specific endoproteases have attracted widespread attention as drug targets due to their fundamental roles in apoptosis and inflammation pathways. Caspases employ a catalytic cysteine-histidine dyad to judiciously hydrolyze peptide bonds, in order to carry out their essential cellular functions. Apoptotic caspases are further segregated into initiator and executioner caspases. Initiator caspases (-8, -9) activate the executioner caspases (-3, -6, -7) in order to carry out the final steps of apoptosis. Cell death resulting from aberrant caspase activation has been implicated in numerous cardiovascular and neurodegenerative diseases.

Numerous screening efforts have been directed towards the development of caspase inhibitors. To date, the overwhelming majority of these compounds target the active site through a form of mechanism-based inhibition. However, due to the aspartate preference of the executioner caspases, these molecules are inherently charged and lack drug-like properties. Targeting a known allosteric site at the dimer interface is proposed to yield molecules with superior drug properties. We also hypothesize that current HTS libraries do not contain the complementary chemical space for the allosteric site of the caspases, and that fragment screening will provide the necessary diversity to discover allosteric caspase inhibitors.

In order to maximize screening chemical diversity, we leveraged fragment-based drug discovery. A fragment library was screened against caspase-7 by differential scanning fluorimetry (DSF). Compounds identified from the screen were further tested by DSF, functional assays, and surface plasmon resonance. Two non-competitive inhibitors from the screening effort have been characterized by X-ray crystallography and were confirmed to bind at the aforementioned allosteric site. Further work is being done to understand the mechanism by which these compounds are allosterically inhibiting caspase-7.

Poster 32

SEMEN EXOSOMES: EFFECT ON HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) RNA

Jennifer L. Welch and Chioma M. Okeoma*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

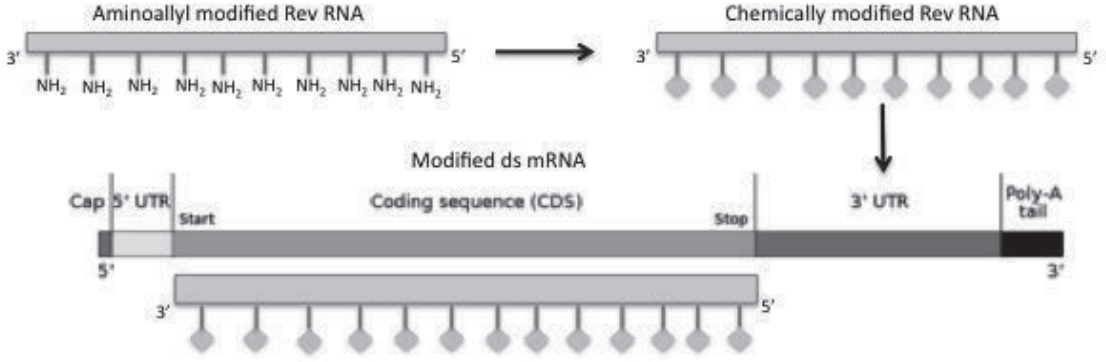
The AIDS pandemic remains a significant global problem with about 2 million people infected with HIV-1 in 2014 and 1.2 million HIV-infected people died from AIDS-related illnesses the same year. It is estimated that more than 80% of HIV cases result from sexual transmission and semen is the primary vector. However, only about 0.1% of acts of heterosexual coitus with an infected individual result in HIV infection, suggesting that factors contained in semen may impair the infectivity/fitness of HIV in semen. Recently, we demonstrated the presence of exosomes with anti-HIV activity in human semen. Semen exosomes (SE) potently impair infectivity of wild type HIV through reduction in (i) viral reverse transcriptase activity, (ii) viral RNA expression, and (iii) progeny fitness. It is known that control of HIV RNA expression is critical for the maintenance of a reservoir of infected cells in the host and that HIV RNA species are present in infected cells at different points of the viral replication cycle.
Here we show that SE decrease the levels of all HIV RNA species (multiply spliced; singly spliced; and unspliced). Additionally, we show that the reduction in HIV RNA is conserved in progeny virions in the presence of SE. Indeed, quantitation of progeny RNA copy numbers using droplet digital PCR revealed significant decrease in virion-associated RNA in the presence of SE. Similar to the trend in virion-associated RNA, additional data show that the presence of SE result in significant reduction in the amount of viral RNA present in the cytoplasm. Remarkably, viral RNA encapsidation efficiency defined as: the ratio of virion-associated and cytoplasmic RNA, is significantly reduced in the presence of SE compared to vehicle treated cells. Since SE do not reduce cell- and virion-associated Gag protein levels, the effect of SE on HIV infectivity could not be attributed to modulation of virus production, but to potential impairment of RNA transcription and/or encapsidation efficiency.

Poster 33

USE OF 384 WELL CELL-FREE TRANSLATION ASSAY TO TEST THE EXPRESSION OF NOVEL DS MRNA CONSTRUCTS

Christopher W. White and Kevin G. Rice*
Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

We have demonstrated that direct hydrodynamic stimulation of an optimized ss mRNA construct gives expression levels of a luciferase reporter gene that are equivalent to an equal weight dose of pDNA in mice.[1] However, ss mRNA is inherently susceptible to degradation by RNases, and thus serum stability of ss mRNA is low. Recent unpublished work demonstrates that when ss mRNA is annealed with a complimentary reverse strand, to form ds mRNA, an ~100-fold increase in stability to RNase A is achieved and expression levels in vivo are maintained. Being that the reverse strand of ds mRNA is not involved in translation, we hypothesized that in vivo gene expression could be enhanced by chemically modifying the reverse strand. Reactive primary amine containing reverse strand RNA was synthesized by the total substitution of unnatural aminoallyl (aa) containing uridine (U) and cytosine (C) for their natural counterparts during in vitro transcription to create either aa-U, aa-C or aa-U/C double modified reverse RNA. Reverse strands were then modified with various chemical functionalities as activated N-hydroxysuccinimide esters, purified and annealed with Forward mRNA to form modified ds mRNA, figure 1. To directly test whether these chemical modifications are tolerated by eukaryotic translational machinery, we have adapted the cell free rabbit reticulocyte lysate system into a miniaturized 384 well assay.



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Poster 34

SINGLE-USE BIOREACTORS FOR MAMMALIAN CELL CULTURES IN THE BIOPHARMACEUTICAL INDUSTRY

Shuvendu Das and Mark A. Arnold*
Center for Biocatalysis and Bioprocessing, Office of Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

The global market for biopharmaceuticals increases each year and is expected to reach $1.671 trillion by the end of 2020, according to Mordor Intelligence, a market research and consulting firm. Biopharmaceuticals are proteins or other biomolecules that must be manufactured by complex cellular processes. In many cases, FDA approval requires the production of "human like" or humanized proteins which involves post-transcriptional modification of the basic protein structure. Humanized proteins are best created in mammalian cells and, at present, CHO cells are the most popular cellular platform within the pharmaceutical industry for the production of commercial biotherapeutics. Complexities associated with manufacturing therapeutic proteins via mammalian cell cultivation processes are responsible for high production costs and, correspondingly, high consumer costs.

Single-use bioreactors promise to reduce costs and enhance flexibility in CHO cell manufacturing of therapeutic proteins. Single-use bioreactors consist of disposable plastic containers engineered for manufacturing of proteins from either fermentation or cultivation processes. These plastic containers are multi-layer structures composed of at least three layers designed to: 1) provide mechanical stability, 2) control permeability of gases, and 3) create a cell friendly surface. The cell expansion and protein expression environment can be controlled in terms of temperature, dissolved oxygen levels, and pH. Agitation is provided by either stirred or wave-induced convection. Single-use bioreactors offer several advantages over conventional stainless steel bioreactors, including: 1) shorter times for setup and takedown, 2) reduced risk of cross-contamination between processes, 3) less complexity for qualification and validation procedures, 4) lower energy costs for preparation and operation, and 5) improved biological safety record.

This presentation will summarize the state-of-the-art single-use bioreactors designed for CHO cell manufacturing of biotherapeutics. Systems offered by the principal suppliers of single-use technologies will be summarized, including single-use products by GE Healthcare, Sartorius, ATMI, Millipore, Pall Life Sciences, PBS Biotech, and Hyclone Thermo Fisher. Material details pertaining to bag designs as well as scale-ability will be featured. Recent efforts to improve function will also be presented along with efforts to make single-use bioreactor systems more convenient, suitable for large-scale production, and compliant with the guidelines of regulatory agencies.

Poster 35

FUNCTIONAL EXPRESSION OF HUMAN CYTOCHROME P450S (CYPS) IN METABOLICALLY ENGINEERED YEASTS

Shuvendu Das[1,2] and Mani Subramanian[1,3]*
[1]Center for Biocatalysis and Bioprocessing, Office of Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA
[2]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[3]Department of Chemical & Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Our larger vision is to metabolically engineer a microbe to "mimic" drug metabolism of human liver. Also, the engineered microbes must be scalable to obtain tens of kilograms of cells within 2-4 days via high cell density fermentation. This will enable rapid screening for metabolite identification of new chemical entities and biocatalytic prep-scale synthesis of drug metabolites. Every drug in development needs to be fully characterized with respect to all the metabolites produced in humans. We have chosen yeasts as our preferred hosts due to their capability of high cell density fermentation and post-transcription.

Human CYPs have been expressed as recombinant proteins in *E. coli*, *S. cerevisiae*, and the baculovirus-insect cells. These cloned enzymes require expensive cofactor NADPH (and sometimes cytochrome b5) for activity. The baculovirus-insect cell system is quite expensive to cultivate and difficult to scale-up. Human CYPs are also commercially available in the form of liver microsomes, but the presence of multiple CYPs in liver microsomes hinder the production of a specific drug metabolite since hepatic CYPs have broad substrate specificity. Also, preparation, storage, and using microsomes in enzyme reactions are also cumbersome and very expensive. Availability of large amounts of human liver, variability from batch to batch adds to the problem. Our work overcomes these problems. Moreover, our yield is better than any methods reported in the literature.
There are approximately 60 human CYP genes published in the literature. Among them, only eight genes are responsible for the metabolism of more than 95% of the drugs. Hence, we have engineered and expressed CYP2D6, CYP3A4, CYP2C9, CYP2C19, CYP1A2, CYP2E1, CYP2B6, and CYP1A1 in yeast, optimized high-cell density fermentation and made stable dried powder (SDP) of these cells for the catalytic reaction. We have scaled up reaction to several 100s of ml to produce several 10s of mg metabolites. As an example, 400 ml of CYP 2C9 catalyzed reaction of diclofenac (DN) produced 53.6 mg of 4-hydroxydiclofenac (HDN) from 59.2 mg of DN in only 1.5 hours. This SDP can be used for 2-cycles of catalysis, to further increase the yield of metabolites.

Our method including prep-scale synthesis is extremely simple; add 20-100 mg of SDP/ mL of drug-candidate (20-1000 μM) of interest in 50 mM phosphate or Tris-HCl buffer at 30°C. Incubate for 1 to 4 h and analyze metabolites by HPLC or LCMS. SDP is easily separated from reaction mixture just by centrifugation. The detailed results and the comparison with other hosts are presented in the poster.

Poster 36

NOVEL ON-LINE TECHNOLOGY FOR UPSTREAM BIOPROCESS MONITORING AND CONTROL IN REAL-TIME

Elizabeth R. Gibson[1], Kaylee Lanz[1], Edwin Koerperick[1], Daniel Cooley[1], Jonathon T. Olesberg[1], Gary W. Small[1,2], and Mark A. Arnold[1,2]*
[1]ASL Analytical, Inc., Coralville, IA
[2]Optical Science and Technology Center and Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

Analytical sensing technologies that can measure key chemicals in real-time during cell expansion and protein expression phases of upstream processes have been of interest to the biotechnology community for some time. The driving goal for the development of these technologies, in addition to enhanced process efficiency, is consistent product quality through discovery, process development, scale up, and manufacturing. Real-time chemical monitoring is recognized as being particularly important during upstream processes that are, by their very nature, complex multicomponent environments that continuously change throughout the growth and expression phases.

A novel on-line bioprocess monitor is presented for the simultaneous, real-time measurement of critical nutrients and byproducts during fermentation and cell culture processes. This novel system provides simultaneous multi-analyte quantitation, non-destructive automated and continuous operation for extended time periods, robust and repeatable performance across multiple batches, requires little operator time, and is insensitive to normal operating conditions (agitation rates, pH changes, etc.). Data from this robust on-line bioprocess monitor includes results from industrial processes using yeast-based expression platforms as well as mammalian cell cultures. Bioprocess monitor performance for multiple batches conducted over months of industrial operation will be shown. Results from protein expression in the yeast *Pichia pastoris* demonstrate the reliable, accurate measurement of glycerol and methanol during more than a year of operation in bioprocess development. Critical performance criteria will also be shown for CHO cell cultures where glucose and lactate concentrations are quantitated in real-time for runs in excess of 400 hours. The unique and high value-add information that real-time, continuous monitoring provides will be highlighted.

Poster 37

FEASIBILITY OF NIR BIOPROCESS MONITORING IN PRODUCTION OF DEUTERIUM LABELED BIOMOLECULES FOR NEUTRON STRUCTURAL BIOLOGY

Rachel L. Grenell[1], Gary W. Small[1,2], Kaylee Lanz[2], Kevin L. Weiss[3], William B. O'Dell[3,4], and Mark A. Arnold [1,2]*
[1]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA
[2]ASL Analytical, Inc., Coralville, IA
[3]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN
[4]Department of Molecular and Structural Biochemistry, NC State University, Raleigh, NC

Neutron scattering techniques are valuable tools for investigating the structure and dynamics of materials. Small-angle neutron scattering studies can resolve the sizes and shapes of macromolecular complexes and deuteration of an individual component within a complex can provide a selective scattering enhancement. Neutron crystallographic analyses can uniquely determine hydrogen atom positions and the use of deuterium labeling can improve data quality by increasing the diffraction intensities while reducing the background. Fed-batch cultivations using deuterium-enriched minimal media are often used to produce sufficient quantities of material for these studies.[1]

The aim of this work is to assess the feasibility of real-time monitoring of *Pichia pastoris* fermentations in deuterium-labeled media. This preliminary analysis focuses on the feasibility of monitoring the concentrations of glycerol and methanol with the RTBio® monitor offered by ASL Analytical, Inc. ASL's RTBio® *Pichia* fermentation monitor is designed for measurements in proton-based aqueous solutions by using nondestructive near-infrared spectroscopy over the 5000-4000 cm^{-1} spectral range.[2] Molecular absorptions over this spectral range correspond to O-H, C-H, and N-H combinations of stretching and bending vibrations. Our principal question is: how does replacing protons with deuterons impact this spectroscopy from an analytical standpoint?

Near-infrared spectra were collected with a Nicolet Nexus 670 Fourier transform spectrometer. Spectra were collected for a series of solutions prepared in deuterium oxide (D_2O) and composed of H8-glycerol, D8-glycerol, H4-methanol, D4-methanol and minimal *Pichia* fermentation medium. Results illustrate the heavier deuterium atom red-shifts the vibrational absorption frequencies for O-D, C-D, and N-D bonds so first overtones of stretching vibrations are located within the 5000-4000 cm^{-1} spectral window. An 8-fold increase in the optical throughput results when using deuterium oxide as the solvent compared to water. Such a higher optical throughput for the solvent can be exploited to give higher signal-to-noise ratios when proton-based substrates (H8-glycerol and H4-methanol) are monitored in D_2O based solutions. Longer optical path lengths are possible under such conditions, thereby improving analytical sensitivity relative to conventional water-based fermentations.

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Poster 38

ABUNDANCE AND ACTIVITY OF VINYL CHLORIDE DEGRADERS IN CONTAMINATED GROUNDWATER

Yi Liang, Xikun Liu and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

Vinyl chloride (VC) is a common and persistent groundwater contaminant formed primarily from incomplete anaerobic dechlorination of chloroethenes. VC-degrading microorganisms offer a sustainable and cost-effective approach for *in situ* VC clean-up. Some organohalide-respiring bacteria such as *Dehalococcoides mccartyi* can reduce VC to ethene under anaerobic conditions. Under aerobic conditions, microorganisms such as etheneotrophs (ethene oxidizing bacteria) and methanotrophs (methane oxidizing bacteria) could oxidize VC via metabolic or cometabolic processes.

In this study, we used quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) to estimate the *in situ* abundance and activity of anaerobic and aerobic VC degraders in the groundwater at six VC contaminated sites. VC concentrations at different locations of those sites ranged from non-detectable to 10^4 μg/L. Strong positive correlations were observed between VC concentrations and etheneotroph functional genes (etnC and etnE) and their transcripts with a multilevel model (etnC and etnE: $p<0.001$, etnC and etnE transcripts: $p<0.01$). VC-dechlorination functional genes (bvcA and vcrA) and their transcripts were also found positively correlated to VC concentrations in the groundwater (bvcA and its transcript: $p<0.001$, vcrA and its transcript: $p<0.01$). No correlation was found between methanotroph functional genes (mmoX and pmoA) and their transcripts ($p>0.05$). The positive correlations between VC concentrations and etheneotrophs and VC-dechlorinators suggested that VC is an influential factor for the growth of etheneotrophs and VC dechlorinators, but not for methanotrophs. It is likely that VC was used as a growth substrate by etheneotrophs and VC dechlorinators.

To investigate the possible effect of VC degrading microorganisms on *in situ* VC degradation, the six VC-contaminated sites were categorized into three groups: slow, moderate, and fast VC degradation group. With a categorical multilevel model, it is found that the abundances of etheneotrophs and VC-dechlorinators were higher at moderate and fast VC degradation sites than slow VC degradation sites ($p<0.05$). Etheneotrophs and VC-dechlorinators could both contribute significantly to VC degradation in the contaminated groundwater.

Poster 39

B49, A NOVEL BST-2-TARGETING PEPTIDE FOR THE TREATMENT OF METASTATIC BREAST CANCER

Wadie D. Mahauad-Fernandez and Chioma M. Okeoma*
Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA

Breast cancer is the second cause of cancer-related deaths in women. While localized and regional breast cancers have high 5-year survival rates; only 26% of all metastatic breast cancer patients will survive past 5 years. This is partially due to lack of effective treatments. Chemotherapy is the main treatment for metastatic cancer; however, it has limited efficacy and it is highly toxic. Thus, there is a need to discover new therapeutic molecules and targets. B49 targets the protein Bone marrow stromal antigen 2 (BST-2) that has been newly discovered as cancer-promoting gene. BST-2 is overexpressed in several cancers including breast cancer where it promotes the migration, invasion, adhesion and growth in suspension of cancer cells. Overexpression of BST-2 in an orthotopic model of breast cancer resulted in poor host survival due to metastasis. BST-2 can mediate the adhesion between cells found in the tumor microenvironment and to promote cancer cell growth in suspension. Mechanistically, this protein was found to bind to itself and this interaction was shown to be necessary for cancer cells to adhere to each other and survive in suspension. In vivo, when using a mutant form of BST-2 which cannot bind to itself results in decreased primary tumor growth, reduced metastasis and increased survival of cancer-bearing mice. Furthermore, using a recombinant form of BST-2, we were able to reduce cancer cell-to-cancer cell adhesion in vitro suggesting that BST-2 interactions with itself could be targeted therapeutically to treat BST-2^{+} breast cancers. Our technology, B49, inhibits BST-2 interactions with itself preventing cancer cell adhesion in vitro and slowing tumor growth in vivo. Future experiments will aim at stabilizing B49 to increase is stability and effectiveness. Our peptide biologic is an innovative peptide that we expect to target all breast cancers that express BST-2 (~70%) regardless of their subtype/grade classification or chemotherapeutic-resistance status.

Poster 40

STUDIES OF CHEMICAL STEPS IN ENZYME CATALYSIS: NEW INSIGHTS INTO ENZYME EVOLUTION

Priyanka Singh and Amnon Kohen*
Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

The molecular basis of enzyme evolution is unknown, which limits our ability to rationally engineer these biocatalysts for new functions in industrial and environmental applications. Our objective is to gain a better understanding of how covalent bond activation in enzymes has evolved. We trace the evolving nature of the chemical step in dihydrofolate reductase (DHFR), specifically a C-H→C hydride transfer in a primitive form of the enzyme evolving to a mature catalyst. Our hypothesis is that as a primitive enzyme evolves: (i) the rate of the chemical step will increase to become non-rate-limiting and (ii) the active site of the enzyme will more effectively be able to reorganize to achieve an ideal transition state, although it is currently unclear which happens first. To investigate this hypothesis, we have to start from a primitive enzyme, in which the chemical step is rate-limiting and far from ideal, and follow that step as the enzyme evolves. The model system for our study is a circularly permuted DHFR (cpDHFR) as a primitive starting point for directed evolution and the chemical step is followed through measurements of temperature dependence of intrinsic kinetic isotope effects (KIE)1-3. KIEs were measured to follow the evolution of the chemical step from a poor transition-state ensemble in the primitive enzyme to an accurate transition-state ensemble for mature variants. The cpDHFR appears to be quite primitive, not only because its kcat/KM is slower by 5 orders of magnitude, but also because of the steep temperature dependence of its intrinsic KIEs. Furthermore, the catalytic efficiency increased by 300 fold in evolved DHFR and the steepness in temperature dependence of intrinsic KIEs decreased, hence supporting our hypothesis. It will be interesting to continue the accelerated evolution, and see whether the chemical step remains perfect as the rates increase. Since very little is known about the changing physical nature of the chemistry along the evolutionary path of an enzyme, our study will shed light on this interesting biological question and broaden our understanding of the evolution of drug resistance in pathogens and cancer.

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Poster 41

AUTOINHIBITION OF GUANINE NUCLEOTIDE EXCHANGE ACTIVITY OF TIAM1 VIA THE PH_n-CC-EX DOMAIN

Zhen Xu[1], Lokesh Gakhar[2], Fletcher Bain[1], Maria Spies[1], and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]X-ray Crystallography Core Facility, Carver College of Medicine, University of Iowa, Iowa City, IA

The T-cell lymphoma invasion and metastasis 1 (Tiam1) is a multi-domain Dbl-family guanine nucleotide exchange factor (GEF) that is specific for the Rho-family GTPase Rac1 though its C-terminal catalytic Dbl-homology (DH) and pleckstrin homology (PH) domain. Mutations or aberrant regulation of Tiam1 have been implicated in oncogenic transformation of cells and linked to several kinds of invasive and metastatic forms of cancer. In addition to the DH-PH_c domain, Tiam1 contains several protein-protein interaction domains (PH_n-CC-Ex, RBD and PDZ) at the N-terminus that are thought to contribute to the regulation of its GEF activity (the catalytic DH-PH_c domain) through inter-domain interactions. However, the mechanism by which the GEF function of Tiam1 is regulated remained poorly understood. Here, we showed that truncation of N-terminal domains activates Tiam1 GEF function of the catalytic DH-PH_c domain, but adding of the PH_n-CC-Ex domain reconstructs the inhibition of Tiam1 GEF activity *in vitro*. In addition, the auto-inhibition of Tiam1 GEF function is determined to be a competitive inhibition that N-terminus decreases the substrate (GTPase Rac1) binding affinity but does not change the maximum exchange activity by enzymatic kinetics experiments. We also quantified the protein-protein interaction between Rac1 and activated Tiam1 DH-PH_c or inhibited Tiam1 PH_n-PH_c fragment by single molecule total internal reflection fluorescence (TIRF) microscopy. Moreover, structural model of Tiam1 PH_n-PH_c in solution revealed an auto-inhibited conformation in which the N-terminal PH_n-CC-Ex domain and linker regions block the substrate-binding site as evidenced by small angle X-ray scattering (SAXS). These data began to elucidate the biochemical and structural mechanisms of Tiam1 GEF regulation.

Poster 42

BINDING STUDIES OF *E. coli* CELL DIVISION PROTEIN SPOR DOMAINS WITH GLYCAN STRANDS THAT LACK STEM PEPTIDES

Atsushi Yahashiri, Matthew A. Jorgenson, Eric M. Ransom, Jon C. Houtman, and David S. Weiss*
Department of Microbiology, Carver College of Medicine, The University of Iowa, Iowa City, IA

Bacterial SPOR domains bind peptidoglycan (PG) and are thought to target proteins to the cell division site by binding to "denuded" glycan strands that lack stem peptides, but uncertainties remain, in part because septal-specific binding has yet to be studied in a purified system. Here we show that fusions of the green fluorescent protein (GFP) to SPOR domains from the *Escherichia coli* cell division proteins DamX, DedD, FtsN and RlpA all localize to septal regions of purified PG sacculi obtained from *E. coli* and *Bacillus subtilis*. Treatment of sacculi with an amidase that removes stem peptides enhanced SPOR domain binding, while treatment with a lytic transglycosylase that removes denuded glycans reduced SPOR domain binding. Isothermal Titration Calorimetry has been used to study the binding of SPOR domains to denuded glycans, and the results indicate SPOR domain proteins bind amidase-treated sacculi with a *K*d of l ~15 μM. These findings demonstrate unequivocally that SPOR domains localize by binding to septal PG, that the physiologically relevant binding site is indeed a denuded glycan and that denuded glycans are enriched in septal PG rather than distributed uniformly around the sacculus. Accumulation of denuded glycans in the septal PG of both *E. coli* and *B. subtilis*, organisms separated by 1 billion years of evolution, suggests that sequential removal of stem peptides followed by degradation of the glycan backbone is an ancient feature of PG turnover during bacterial cell division. Linking SPOR domain localization to the abundance of a structure (denuded glycans) present only transiently during biogenesis of septal PG provides a mechanism for coordinating the function of SPOR domain proteins with the progress of cell division.

CBB/NIH Fellowships

YEAR	CBB Fellowship	*NIH Trainee Fellowship
1990-91	10	4
1991-92	12	8
1992-93	12	6
1993-94	12	6
1994-95	13	10
1995-96	13	6
1996-97	12	6
1997-98	12	6
1998-99	12	6
1999-00	13	7
2000-01	14	7
2001-02	12	7
2002-03	11	8
2003-04	14	8
2004-05	12	7
2005-06	11	7
2006-07	10	7
2007-08	10	7
2008-09	10	8
2009-10	6	8
2010-11	7	8
2011-12	6	8
2012-13	5	6
2013-14	4	5
2014-15	4	6
2015-16	5	6
2016-17	6	0
TOTAL	268	178 *Partially Sponsored by CBB

The Center for Biocatalysis and Bioprocessing gratefully acknowledges the following contributors for their support of the 25th Biocatalysis and Bioprocessing Conference "Microbial Factories and Biocatalytic Science" October 17-18, 2016

Jo Dickens

and

Usha Balakrishnan

Please join us!

The 26th Biocatalysis and Bioprocessing Conference

will be held on

October 16-17, 2017

CBB FACULTY, RESEARCH GROUP AND STAFF

BIOCHEMISTRY

♦Brenner, Charles	4-403 BSB	5-7934
DeMali, Kris	4-470 BSB	5-7882
Elcock, Adrian	4-530 BSB	5-6643
Fuentes, Ernesto	4-362 BSB	3-4244
Musselman, Catherine	4-452 BSB	5-8888
Montgomery, Rex Emeritus	4-772 BSB	5-7897
Plapp, Bryce, Emeritus	4-550 BSB	5-7909
Schnieders, Michael	4-516 BSB	5-7891
Shea, Madeline	4-450 BSB	5-7885
Washington, M. Todd	4-516 BSB	5-7518

BIOLOGY

Cheng, Chi-Lien	143 BB	5-2583
Fassler, Jan	324 BBE	5-1542
Gussin, Gary, Emeritus	318 BB	5-1113
Shih, Ming-Che, Emeritus	200 BBE	5-2071
Soll, David	302 BBE	5-1117

BIOMEDICAL ENGIMEERING

Schnieders, Michael	5013 SC	5-7891

CHEMICAL & BIOCHEM ENG

Aurand, Gary	3324SC	4-0970
Carmichael, Greg	4140SC	5-5191
Fiegel, Jennifer	4124 SC	5-8830
Murhammer, David	4132SC	5-1228
♦Nuxoll, Eric	4140SC	3-2377
♦Peeples, Tonya	4128SC	5-2251
Rethwisch, David	4139SC	5-1413
Subramanian, Mani Emer.	C100 MTF	5-4900

CHEMISTRY

◊♦Arnold, Mark	230 IATL	5-1368
Cheatum, Christopher	326 IATL	3-0379
Dey, Mishtu	W285 CB	4-1319
Geng, Lei	319 CB	5-3167
Gloer, Jim	E515 CB	5-1361
Grassian, Vicki	244 IATL	5-1392
Hass, Amanda	204ATL	4-3695
♦Kohen, Amnon	E274 CB	5-0234
MacGillivray, Leonard	E555 CB	5-3504
Margulis, Claudio	118 IATL	5-0615
Messerle, Louis	E45 CB	5-1372
Nguyen, Hein	E457 CB	4-1887
♦Quinn, Dan	W333 CB	5-1335
Tivanski, Alexei	E272 CB	4-3692
Wiemer, Dave	%531 CB	5-1365

CIVIL&ENVIRONMENTAL ENG

Lefevre, Gegory	540EMRB	5-7783
♦Mattes, Tim	4105 SC	5-5065
Parkin, Gene	4106 SC	5-5655
Schnoor, Jerald	4112 SC	5-5649
Valentine, Richard	4118 SC	5-5653

MICROBIOLOGY

Apicella, Michael ,Emeritus	3-403B BSB	5-7807
Cox, Charles, Emeritus	3752 BSB	5-7779
Feiss, Michael Emeritus	3-352 BSB	5-7782
Horswill, Alexander	540F EMRB	5-7783
Kirby, John	3-632 BSB	5-7818
McCarter, Linda	3-430 BSB	5-9721
♦Okeoma, Chioma	3-612 BSB	5-7906
Stauffer, George Emeritus	3-315A BSB	5-7791
Salgado-Pabon, Wilmara	4-450 BSB	5-7790
Weiss, David	3-452 BSB	5-7785
Yahr, Timothy	540B EMRB	5-9688

MOLECULAR PHYSIOLGY & BIOPHYICS

Ahern, Christopher	4256 CBRB	5-6964

PHARMACEUTICAL SCIENCES & EXPERIMENTAL THERAPEUTICS

Doorn, Jonathan	S328 PHAR	5-8834
Duffel, Michael	S325 PHAR	5-8840
Fiegel, Jennifer	S215 PHAR	5-8830
Jin, Zhendong	S315 PHAR	3-5359
♦Kerns, Robert J.	S321 PHAR	5-8800
Olivo, Horacio	S319 PHAR	5-8849
Rice, Kevin	S300 PHAR	5-9903
Rosazza, Jack, Emeritus	C106 MTF	5-4908
Salem, Aliasger	5228 PHAR	3-8810
Spies, M. Asley	S313 PHAR	3-5645

PHARMACEUTICS/TRANSLATIONAL THERAPY

An, Guohua	S227 PHAR	7-4600
Brogden, Nicole	S421 PHAR	5-8752

RADIOLOGY

♦Schultz, Michael	B180 ML	5-8017

CBB STAFF

Main	C100MTF	5-4900
Arnold, Mark		Director
Gopishetty, Sridhar		Technical Director
Das, Shuvendu		Research Lead
Rotman, Mitchell		Administrator
Gardner, Mary		Quality Assurance Manager
McCarthy, Troy		Quality Assistant
Kinzenbaw, Lisa		Administration Support
Bustos, Arvin		Research Assistant
Coeur, Melissa		Research Assistant
Dostal, Larry		Research Assistant
Ehler, Jolene		Research Associate
Kasperbauer, Sarah		Research Associate
Lashmit, Philip		Research Associate
Lettington, Deanna		Research Assistant
Liu, Wensheng		Research Assistant
Railsback, Michelle		Research Associate
Steward-Thorp, Heather		Research Assistant
Xu, Jingying		Research Assistant

Notes

THE UNIVERSITY OF IOWA
Center for Biocatalysis and Bioprocessing

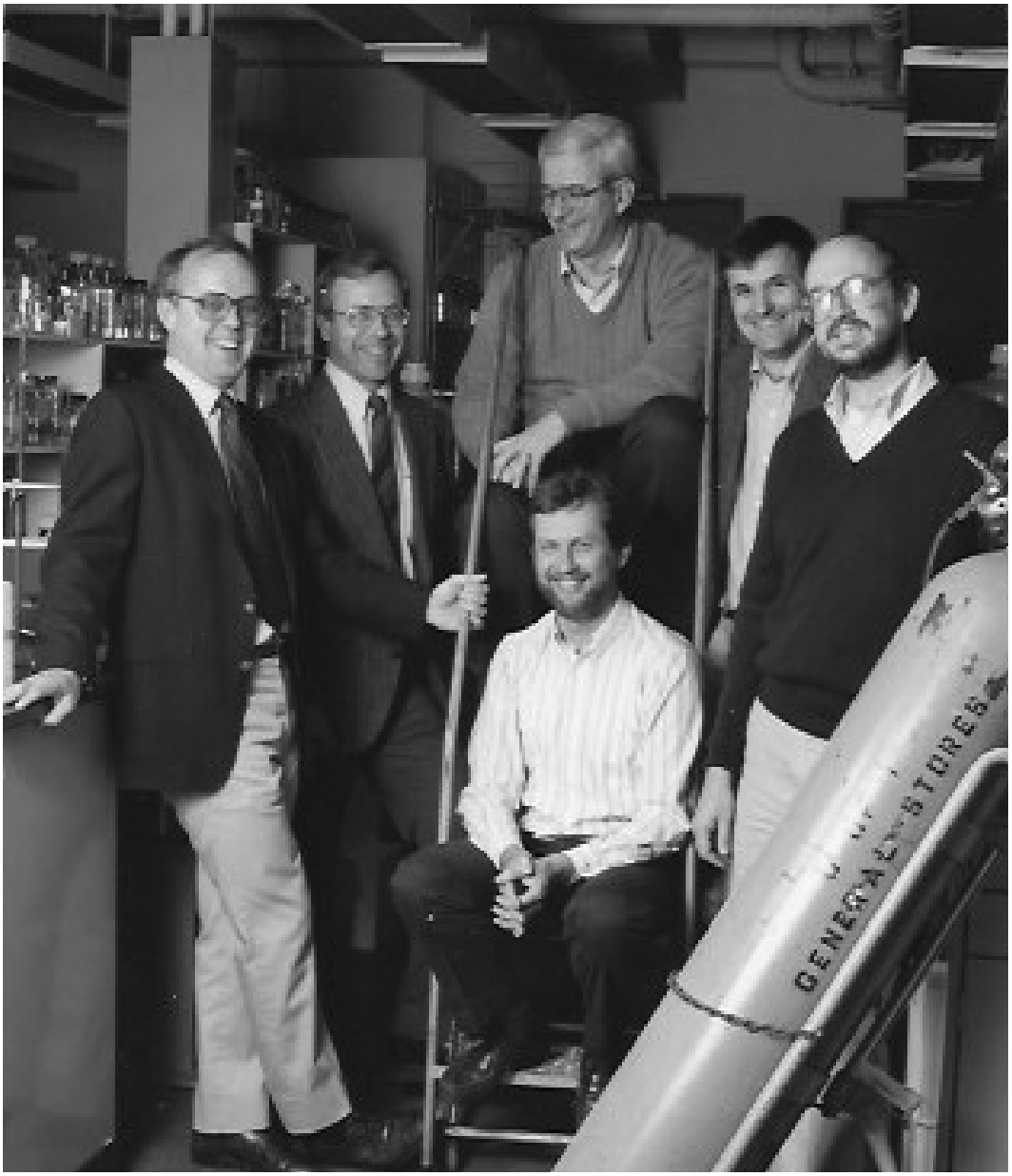
CBB Offers a Course in Upstream Biotechnology Processing

Sartorius Biostat-A 5-L Fermentors used in the Upstream Biotechnology Processes course

The biotechnology industry is rapidly growing in the U.S. and beyond and represents a major manufacturing arm for the production of novel products, including industrial enzymes, food supplements, biotherapeutics, animal vaccines, and renewal fuels. The CBB is pleased to offer a course specifically focused on microbial fermentation.

The course, entitled Upstream Biotechnology Processes, is designed to provide students with *hands-on* experience in basic upstream fermentation processes. Basic concepts and general designs of upstream fermentations are covered through a series of lectures. The bulk of the course, however, is spent in the laboratory where students have an opportunity to perform fermentation on a 5-liter scale including reactor preparation, inoculation methods, reactor operation, control, and termination of the process. All the fundamental steps used within the fermentation industry are experienced, including basic cloning, selection of clones, growth of microorganism, preparation of microbial glycerol stock, monitoring purity of the culture, harvest of cells, and bioassay to document product yield.

Upstream Biotechnology Processes (CHEM: 4850) is a two-semester hour course offered each year during both the summer and winter terms.



Left to right are: Dan Quinn(Chemistry), Bryce Plapp(Biochemistry), Jack Rosazza(Medicinal/ Natural Products Chemistry), Bob Linhardt (MNPC), Lacy Daniels (Microbiology) and in front Jonathan Poulton (Biological Sciences)