21st Annual
Biocatalysis and Bioprocessing
Conference

“Current Topics in
Industrial Biotechnology”

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21st Annual
Biocatalysis and Bioprocessing Conference

“Current Topics in Industrial Biotechnology”

Sponsored by:

The University of Iowa
Center for Biocatalysis and Bioprocessing

October 15-16, 2012

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21st Annual Center for Biocatalysis and Bioprocessing Conference
“Current Topics in Industrial Biotechnology”
Iowa Memorial Union, Iowa City, IA

**MONDAY, OCTOBER 15, 2012**

4:00 pm Registration – outside Main Lounge, 1st floor IMU
4:00–6:00 Poster set up – Main Lounge, 1st floor IMU
4:30 Tour of CBB – please register for tour on-line
6:00–7:00 Welcome Dinner/Buffet – Main Lounge, 1st floor IMU
7:00–8:00 Charles Abbas, Ph.D., Director, Yeast and Renewables Research, ADM Research
“Recent Progress in the Development of Biocatalysts for Biorefineries”

**TUESDAY, OCTOBER 16, 2012**

7:00–8:00 am Registration – outside Main Lounge, 1st floor IMU
7:30 Continental Breakfast – Main Lounge, 1st floor IMU

*Program* – Bijou, 1st floor IMU

**Introduction and Welcome**
Mani Subramanian, Ph.D., DEO, Center for Biocatalysis and Bioprocessing; Professor, Department of Chemical and Biochemical Engineering

8:30

Hans Liao, Ph.D., Director, Metabolic Engineering, OPX Biotechnologies, Inc.
“Metabolic Engineering for Products Derived from Malonyl-CoA”

9:00

Lawrence Wackett, Ph.D., Professor, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota
“Enzyme-Based Detection Systems for Toxicants”

10:30 Break – Main Lounge, 1st floor IMU

10:45 Lorenz Hasler, Ph.D., Senior Project Evaluation Manager, Global Biological Operations, Lonza AG
“Challenges Facing Biotherapeutics”

11:30 Lunch – Main Lounge, 1st floor IMU

Afternoon Session – Bijou, 1st floor IMU

1:15 pm Ryan Summers, Ph.D., Postdoctoral Research Scholar, Center for Biocatalysis and Bioprocessing, The University of Iowa
“Genetic Intricacies of Caffeine Degradation in P. putida and Metabolic Engineering of E. coli for Alkylxanthine Production”
2:00–3:00  **CBB/NIH Fellow Presentations**
Four 10 minute talks plus 5 minutes for questions by selected graduate students

**Samuel T. Crowley**, Rice Laboratory, Division of Medicinal and Natural Products Chemistry, The University of Iowa
“*Synthesis of a Membrane Disruptive Glycoprotein for Gene Therapy*”

**Tatiana V. Mishanina**, Kohen Laboratory, Department of Chemistry, The University of Iowa
“*Towards Better Antibiotics: Studies of Alternative Thymidylate Synthase*”

**Carolyn B. Rosenthal**, Horswill Laboratory, Department of Microbiology, The University of Iowa
“*Staphylococcus Aureus Hyaluronate Lyase, a Potent Virulence Factor*”

**Veronica S. Wills**, Wiemer Laboratory, Department of Chemistry, The University of Iowa
“*Approaches to Synthesis of the Stellettins*”

3:00–5:00  **Poster Session** – Main Lounge, 1st floor IMU
Wine/hors d’oeuvres
Announcement of Usha Balakrishnan’s Award Winner
Announcement of CBB Director’s Award Winner

5:00   Adjourn
List of Oral Presentations
ORAL PRESENTATIONS

1. **RECENT PROGRESS IN THE DEVELOPMENT OF BIOCATALYSTS FOR BIOREFINERIES**
   Charles Abbas, Ph.D.
   Director, Yeast and Renewables Research, ADM Research, Decatur, IL 62521

2. **METABOLIC ENGINEERING FOR PRODUCTS DERIVED FROM MALONYL-COA**
   Hans Liao, Ph.D.
   Director, Metabolic Engineering, OPX Biotechnologies, Inc., Boulder, CO 80301

3. **ENZYME-BASED DETECTION SYSTEMS FOR TOXICANTS**
   Lawrence Wackett, Ph.D.
   Professor, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St. Paul, MN 55455

4. **CHALLENGES FACING BIOTHERAPEUTICS**
   Lorenz Hasler, Ph.D.
   Senior Project Evaluation Manager, Global Biological Operations, Lonza AG, Visp, Switzerland

5. **GENETIC INTRICACIES OF CAFFEINE DEGRADATION IN P. PUTIDA AND METABOLIC ENGINEERING OF E. COLI FOR ALKYLXANTHINE PRODUCTION**
   Ryan M. Summers, Ph.D.
   Postdoctoral Research Scholar, Center for Bicatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA 52241

6. **SYNTHESIS OF A MEMBRANE DISRUPTIVE GLYCOPROTEIN FOR GENE THERAPY**
   Samuel T. Crowley, Jianfeng Jin and Kevin G. Rice*
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

7. **TOWARDS BETTER ANTIBIOTICS: STUDIES OF ALTERNATIVE THYMIDYLATE SYNTHASE**
   Tatiana V. Mishanina, Eric M. Koehn, John A. Conrad, Bruce A. Palfey, Scott A. Lesley and Amnon Kohen*
   Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242

8. **STAPHYLOCOCCUS AUREUS HYALURONATE LYASE, A POTENT VIRULENCE FACTOR**
   Carolyn B. Rosenthal¹, Crystal L. Jones², Jay K. Moon², Chad C. Black², Daniel V. Zurawski² and Alexander R. Horswill¹*
   ¹Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA 52242
   ²Walter Reed Army Institute of Research (WRAIR), Department of Wound Infections, Silver Spring, MD 20901
9. APPROACHES TO SYNTHESIS OF THE STELLETTINS
Veronica S. Wills and David F. Wiemer
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242
Speaker’s Profiles
Dr. Charles Abbas is Director of Yeast and Renewable Research at Archer Daniels Midland. He oversees research into the bioprocessing of commodity crops and their residues to produce high value-added products as well as the development of yeast strains for large-scale industrial fermentations.

He has spent 25 years working in industry, first as a Senior Scientist in Industrial Microbiology at Difco R & D in Ann Arbor, Michigan, and since 1991 as Group Leader, Manager, and most recently Director at ADM Research and Development.

In addition to working at ADM, Charles has maintained ties with academic programs. He holds an adjunct appointment in the Department of Food Science and Human Nutrition at the University of Illinois at Urbana-Champaign, where he oversaw a joint research project with ADM. He is also an adjunct Professor in the Department of Bioproducts and Biosystems Engineering at the University of Minnesota-St. Paul campus, where he is also a member of that department’s advisory board.

Previously at the University of Illinois, Charles was an adjunct Professor with the Department of Animal Sciences, where he oversaw several joint research projects with ADM and an affiliate to the renewables theme at the Institute of Genomics Biology. He also served for over six years as a representative of college-wide departmental research to the College of Agricultural, Consumer and Environmental Sciences.

At the International Congress on Yeasts (ICY) held in Kyiv, Ukraine, in August 2008, Charles was elected as the new commissioner representing the United States. At the ISSY 28 meeting in Bangkok, Thailand, September 2010, Charles was also elected as Chair of the U.S. Organizing Committee of the ICY 2012 meeting being held in Madison, Wisconsin, August 26-31, 2012. In this role, Charles serves as the only industry representative to the Commission among leaders in the research on yeasts.

Charles received the Alltech Medal of Excellence for Alcohol Production in 2001. He served in 2005 as Division O Chairman for Fermentation and Biotechnology of the American Society for Microbiology. He has given numerous talks at national and international meetings and has chaired sessions on topics including yeast biotechnological applications, emerging biorefineries, food security, and system biology. He has authored or co-authored more than 30 publications, several review articles and book chapters, over 100 scientific abstracts, and over 30 patents and patent applications.

He holds a Bachelor of Science in Microbiology from the University of Minnesota, the Master of Science in Microbiology from the University of Montana, and the Ph.D. in Microbiology and Cell Science with Biochemistry as a supporting area from the University of Florida. His undergraduate and graduate training emphasized the industrial application of a wide range of enzymes and microbial systems in fermentations.
Hans Liao, Ph.D.
Director, Metabolic Engineering, OPX Biotechnologies, Inc.

Dr. Hans Liao has over 30 years of experience in using and developing molecular tools to generate novel metabolic capabilities. He carried out one of the first experiments in directed evolution, to generate thermostable enzyme variants, and lead the technical team that developed a biocatalyst for the production of 3-hydroxypropionic acid, a new-to-the-world platform chemical made from renewable resources. Prior to joining OPX, Hans worked on industrial biotechnology at small firms (Draths Corporation), large companies (Cargill Incorporated), and academic institutions (the University of Wisconsin Biotechnology Center). Hans holds a Ph.D. in biochemistry from the University of California, Berkeley.

Lawrence Wackett, Ph.D.
Professor, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Dr. Lawrence Wackett is Distinguished McKnight University Professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. He holds joint appointments in the Biotechnology Institute and the Department of Microbiology. He is co-founded the University of Minnesota Biocatalysis/ Biodegradation Database (UM-BBD) http://umbbd.msi.umn.edu/. Professor Wackett studies microbial biodegradation and biocatalysis in his research.

Wackett obtained his B.S. degree in Biology & Chemistry from SUNY at Brockport. He was employed as an analytical chemist prior to his graduate work. He obtained his M.S. degree in Microbiology at Louisiana State University in Baton Rouge and his Ph.D. in Microbiology at the University of Texas at Austin with David Gibson. Wackett was an NIH Postdoctoral Fellow with Christopher Walsh in the Chemistry Department at MIT from 1984-7. Dr. Wackett started as an Assistant Professor at the University of Minnesota in 1987. He became the Distinguished McKnight Professor of Biochemistry at Minnesota in 2001.

He is a Fellow of the American Association for the Advancement of Science, the American Academy of Microbiology, and is an ASM Foundation Lecturer. Wackett co-authored the textbook Biocatalysis and Biodegradation, sold by ASM Press. The book has been translated into Chinese and is now also available from Chemical Industry Press in Beijing.
Dr. Lorenz Hasler is Senior Project Evaluation Manager for Lonza AG. In his role within the Lonza AG, a world leading Contract Manufacturing Organization, he is responsible for the evaluation of research and development, technology transfer, and manufacturing activities as requested by customers. While performing these evaluations, Dr. Hasler reviews all aspects of the manufacturing process and identifies gaps (asset & process) to ensure ultimate success with the project.

Dr. Hasler obtained his PhD degree in Biophysics at the Biozentrum in Basel, Switzerland in 1999. The focus during his diploma and doctorate thesis was the purification of membrane proteins, their two-dimensional crystallization, and the subsequent structural analysis. After a two years Postdoctoral fellowship at the Harvard Medical School, he moved into the field of engineering which had always been an underlying passion. He joined the Pharmaplan Engineering AG based in Basel, Switzerland and started as a qualification/validation engineer for pharmaceutical and biotechnology manufacturing facilities. His understanding in biotechnology process alongside his “on the job” acquired engineering background, allowed him to function as process technology expert in the plant design for a broad spectrum of cGMP facilities. In 2009 Lorenz became a Senior Project Evaluation Manager at Lonza AG where he combines the multiple aspects of development and production of active pharmaceutical ingredients.
Oral Presentation
Abstracts
RECENT PROGRESS IN THE DEVELOPMENT OF BIOCATALYSTS FOR
BIOREFINERIES

Charles A. Abbas, Ph.D.
Director Yeast Research, ADM Research, Decatur, IL 62521

Over the past two decades, the increased interest in biofuels and industrial chemicals from renewable feedstocks has accelerated research for the development of biological catalysts for use in biorefinerries. These developments have been aided by the exploitation and harnessing of the metabolic repertoire of a wide range of microorganisms through the deployment of a wide range of genomic tools aided by protein engineering technologies and system wide biology approaches. The use of new tools has resulted in development of a wide range of microbial platforms (bacterial, fungal, algal) by a number of research groups at universities, federal and private laboratories. While the different microbial platforms are of great academic and basic research interest, there still exists a preference for microorganisms that are robust and tolerant to industrial scale up conditions such as yeasts and other fungi that are currently in use. These organisms are increasingly being developed to produce biofuels, chemical intermediates, and enzymes. In this presentation, I aim to highlight some of the new developments on the production of enzymes and biofuels from renewable feedstocks and to predict future research in these two areas.
METABOLIC ENGINEERING FOR PRODUCTS DERIVED FROM MALONYL-CoA

Hans Liao, Ph.D.
Director, Metabolic Engineering, OPX Biotechnologies, Inc., Boulder, CO 80301

As the demand for fuels and chemicals increases, their production from renewable feedstocks by fermentation becomes a more critical means of supplementing or even replacing traditionally petroleum-based products. Many of these products may be derived from the core metabolic precursor, malonyl-CoA, including fatty acids (and hence long chain alkanes and fatty alcohols), polyketides (including pharmaceuticals), and 3-hydroxypropionic acid (3-HP), a precursor of the industrial monomer acrylic acid. We use metabolic engineering to construct microorganisms with the ability to produce compounds not normally the end-product of metabolism and to redirect metabolic flow towards the desired endpoint. Using these tools, we have engineered platform microorganisms to increase metabolic flux to malonyl-CoA, and biocatalysts based on these platforms to generate products from this versatile metabolic precursor. Production of 3-HP has been developed first and has been taken through pilot scale. We are also constructing biocatalysts that produce free fatty acids from malonyl-CoA. In addition, we are implementing these metabolic engineering approaches to microorganisms capable of utilizing CO2 and H2 as feedstocks, significantly reducing the cost of production. Continued optimization of bioprocesses using this core platform will enable biological production of a large number of valuable commercial products.
The food supply is vulnerable to real and perceived threats from toxic chemical agents and adulterants. This became manifest in 2008 when rogue suppliers amended milk with melamine to increase the apparent protein content. This act sickened 400,000 children in China and alerted everyone to the vulnerability of the global food supply. We cloned, expressed, and provided the enzyme melamine deaminase to Bioo Scientific who manufactured and marketed a test kit to rapidly detect melamine in foods. The methods used for the melamine test kit are modular. Melamine deaminase reacts exclusively with melamine to release ammonia and that, in turn, reacts with phenol and hypochlorite to produce an intense blue dye. This general principle can be expanded to theoretically test for any one of thousands of different nitrogen-containing chemicals. The major requirement for the test is a specific enzyme(s) that metabolizes any given chemical to release ammonia. The enzyme must be specific and not react with chemicals that are commonly in the food matrix. In another example, we have identified a nitrilase enzyme that releases ammonia from ricinine, a known biomarker for the highly toxic protein ricin. The ricinine nitrilase was identified by whole genome sequencing and the genes were synthesized chemically and expressed in the presence of chaperone proteins. The ricinine nitrilases are currently being characterized. The development of tests for new toxins that are based on an already established platform will decrease development time in the event of a crisis. Learning from the melamine example, it is critical to move quickly in the event of an unforeseen food adulteration.
CHALLENGES FACING BIOHERAPEUTICS

Lorenz Hasler, Ph.D.
Senior Project Evaluation Manager, Global Biological Operations, Lonza AG, CH-3930 Visp, Switzerland

Where would modern medicine be without biotherapeutics? Serious illness including cancer, heart disease, arthritis are targets for biotherapeutic treatment. The latter treatments have an excellent track record for patient safety and are often more efficient in their application than conventional drugs. The rational for biotherapeutical application is that they specifically target the actual root cause whereas with traditional and broadly known chemical drugs such as ibuprofen, aspirin, and general antibiotics, the scope of application is rather vague.

While chemical drugs are small molecules which are manufactured by synthesis, biotherapeutics are complex and large compounds in structure and size. They are commonly sensitive to environmental conditions leading in reduced stability. This requires elaborate process development strategies, starting with the selection of the host cell of living organisms and ending with the right final formulation of the active pharmaceutical ingredient (API).

The API biotherapeutics pathway is filled with roadblocks, both small and large. While microbial and mammalian expression systems are readily available, choosing which one is the best is not always easy. When developing and reviewing the process, one has multiple options for harvesting and purification.

After the excitement of finding a potential candidate for illness treatment: where are potential hurdles and where can bottlenecks be hidden? A list of extracted points of interest should shed lights on a chosen set of discussions:

- Choice of the expression host – is the path forward microbial or mammalian based?
- Development of the process – is the process scalable and robust for manufacturing at larger scale?
- Analysis of the potential candidate – are the methods to analyse and characterize the potential drug candidate applicable?
- Market demand and application – is there indeed a demand, is there a competition, and do the Cost-of-Goods correlate with the expectations?
- Timeline – what timelines do apply to bring the product through the clinical phases which milestones are to be targeted?
- Regulatory – is the projected overall system suitable to be filed to the authorities?

Lessons learned on a broad variety of products in various status including research and development, technology transfer approaches, and scale-up activities shall therefore be presented with the aim to have the audience keep thoughts in the back of their mind during the excitement search for new biotherapeutics.
GENETIC INTRICACIES OF CAFFEINE DEGRADATION IN P. PUTIDA AND METABOLIC ENGINEERING OF E. COLI FOR ALKYLXANTHINE PRODUCTION

Ryan M. Summers^2, Chi-Li Yu^{1,2} and Mani Subramanian^{1,2*}
^1Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA 52242
^2Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA 52241
SYNTHESIS OF A MEMBRANE DISRUPTIVE GLYCOPROTEIN FOR GENE THERAPY

Samuel T. Crowley, Jianfeng Jin and Kevin G. Rice*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

Gene therapy has the potential to treat a wide variety of illnesses and disorders, including hereditary disease, cancer, viral infections, and more. Gene therapy is normally divided into two categories, viral and nonviral. Viral gene therapy uses viruses to deliver DNA to target tissues, and has the advantage of higher expression. However, viral gene therapy has higher risk of side effects such as inflammation and immune response. Nonviral gene therapy can potentially avoid these side effects, but suffers from much lower efficiency than viral methods.

Two of the major obstacles to efficient nonviral gene therapy have been escaping from the endosome and entering the nucleus. Both of these steps require crossing cellular membranes. Several viruses have been discovered to have phospholipase A2 activity in their capsid proteins, and if this activity is removed, the viruses become much less infective. Phospholipase A2 is an enzyme that hydrolyzes phospholipids and can disrupt membranes, potentially allowing the virus to deliver its genetic cargo across cellular membranes. Therefore, we hypothesize that PLA2 activity will improve nonviral gene transfer by disrupting membranes and allowing DNA to escape the endosome and enter the nucleus.

This research has focused on two strategies, recombinant PLA2 expression and chemical modification of PLA2. Recombinant expression allows for the addition of nuclear targeting sequences to PLA2 and other highly controlled mutation of the enzyme. Chemical modification, while much less controllable, is necessary for adding certain moieties to a protein, such as a carbohydrate.

An attractive carbohydrate for protein modification is the Triantennary Oligosaccharide, or Tri. Tri binds to the asialoglycoprotein receptor of hepatocytes and can be imported into the cell. Tri was reacted with iminothiolane and dithiodipyridine to create Tri-thiopyridine, a carbohydrate that would add to free thiols.

Bee venom PLA2 was then modified with iminothiolane to add free thiols. Tri-thiopyridine was then added to those thiols to create an enzyme that retained PLA2 activity and could be purified by affinity chromatography using a lectin gel.

This technique can be used to attach Tri to recombinant PLA2 enzymes, allowing for the addition of hepatocyte targeting and nuclear targeting moieties to the same molecule. PLA2 modified in such a manner could prove useful for delivery of DNA or RNA to cells in vitro and in vivo.

\[ \text{Tri-Thiopyridine} \]
TOWARDS BETTER ANTIBIOTICS: STUDIES OF ALTERNATIVE THYMIDYLATE SYNTHASE

Tatiana V. Mishanina, Eric M. Koehn, John A. Conrad, Bruce A. Palfey, Scott A. Lesley and Amnon Kohen
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242

The enzyme thymidylate synthase (TSase) is responsible for de novo production of thymidylate, one of DNA building blocks. To date, two different classes of TSase enzymes are known: the well-characterized classical TSase and a less understood flavin-dependent TSase (FDTS). Although the two enzymes catalyze the same net chemical conversion, they differ greatly in their structure, cofactor requirement and mechanism. Several mechanisms have been proposed for FDTS catalysis, but identification of any intermediate(s) that may support a specific mechanism has never been reported. In this presentation, I report the chemical trapping and identification of such intermediates in the FDTS-catalyzed reaction, using labeling of the substrates with radioactive isotopes and rapid-quenching techniques. The identity of the trapped intermediates underlines the uniqueness of FDTS chemistry and narrows down the possible chemical mechanisms. Furthermore, experiments conducted in deuterated solvent distinguish between the proposed mechanisms and elucidate the timing of events in FDTS catalysis. The described experimental approach provides an important tool for future studies of flavin-dependent thymidylate synthesis. Because many human pathogens depend on FDTS for thymidylate, knowledge of FDTS chemistry may assist the efforts to rationally design inhibitors as leads for antibiotics.
STAPHYLOCOCCUS AUREUS HYALURONATE LYASE, A POTENT VIRULENCE FACTOR

Carolyn B. Rosenthal1, Crystal L. Jones2, Jay K. Moon2, Chad C. Black2, Daniel V. Zurawski2 and Alexander R. Horswill1*

1Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA 52242
2Walter Reed Army Institute of Research (WRAIR), Department of Wound Infections, Silver Spring, MD 20901

Staphylococcus aureus is the causative agent of both acute and chronic bacterial infections ranging from mild skin and soft tissue infections to more serious conditions such as pneumonia, endocarditis, toxic shock syndrome, and sepsis. The ability to cause a wide range of infections in multiple tissue types is due in part to the vast array of virulence factors produced by this bacterial pathogen. Some of the most important factors are secreted proteins such as the hemolysins, superantigens, and exo-enzymes. One of these exo-enzymes, called hyaluronate lyase, cleaves the β-1,4 glycosidic bond of hyaluronic acid, a host matrix polymer composed of repeating disaccharide subunits of N-acetyl-glucosamine and D-glucuronic acid. Hyaluronate lyases have been implicated as virulence determinants in a number of bacterial pathogens, facilitating the dissemination of bacterial cells and other secreted factors during infection. Here we report the construction and characterization of a hyaluronate lyase (hysA) null mutant in a USA300 community-associated methicillin-resistant S. aureus (CA-MRSA). Using quantitative enzyme assays, we confirmed the absence of hyaluronate lyase activity in the USA300 hysA mutant and we observed that activity increased significantly in USA300 sigB and sarA global regulatory mutants. To assess hyaluronate lyase as a USA300 virulence factor, we performed a neutropenic murine model of pulmonary infection and determined that the USA300 hysA mutant is significantly attenuated. Altogether, these results indicate that the S. aureus hyaluronate lyase is a potent virulence factor and future studies will further elucidate the regulation and pathogenic role of this enzyme.
The stellettins (e.g. 1–4), a small family of natural products isolated from marine sponges, have been of interest because they exhibit potent and differential cytotoxicity in the National Cancer Institute’s (NCI) 60 cell–line assay. Furthermore their pattern of activity does not correlate to any known mechanism of action. For some years our group has pursued synthesis of the schwarzfurthins, and many of the goals of that program have been met. While there is little similarity of structure between the schwarzfurthins and the stellettins, there is a strong correlation between their profiles of biological activity, with the most prominent activity against CNS, leukemia, and renal cancer cell lines. This strong correlation suggests that both families of compounds affect the same target or at least the same biological pathway, but their target remains unknown. Moreover, the stellettins are both more potent and more selective than the schwarzfurthins. Because the stellettins are not readily available from their natural source, we have decided to pursue a chemical synthesis of these complex natural products.

Synthesis of stellettin A has been divided into a left and right half and poses two main challenges: stereochemistry of the left half ring system and the polyene tail in the right half. The stellettins feature a \textit{trans–syn–trans} tricyclic core that causes the B ring to be in a strained twisted boat conformation. Therefore, care must be taken to control the stereochemistry around this ring so that the \textit{trans–anti–trans} system is not formed. Installation of the polyene tail late in the synthesis of stellettin A is imperative because it isomerizes and decomposes when exposed to light, so our initial efforts have focused on preparation of the tricyclic core. Key steps in the formation of the left half include oxidation of farnesol, Wittig olefination, hydroboration-oxidation to provide homofarnesol, bromination of the alcohol, addition of trimethyl(propargyl)silane via an alkylation reaction of the bromide, and an asymmetric epoxidation to introduce a stereogenic center. The epoxidation will be followed by a cascade cyclization initiated by a Lewis acid, which opens the epoxide to afford the desired carbocycle. Our synthesis of the stellettin family will allow a more in-depth exploration of the biological activity of these rare, but very potent compounds.

\begin{equation}
\begin{align*}
1 \text{ R=R'=O, 13-E = stellettin A} \\
2 \text{ R=R'=O, 13-Z = stellettin B} \\
3 \text{ R=H, R'=OAc, 13-E = stellettin C} \\
4 \text{ R=H, R'=OAc, 13-Z = stellettin D}
\end{align*}
\end{equation}
List of Posters and Authors
1. **EFFECTS OF Y209W ON THE HYDRIDE TRANSFER STEP IN THYMIDYLATE SYNTHASE**
   Thelma Abeyesinghe, Zhen Wang and Amnon Kohen
   Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242
   Authors contributed equally to this work

2. **PRODUCTION OF METHYLXANTHINES BY METABOLICALLY ENGINEERED E. COLI**
   Khalid H. R. Al-Gharrawi, Ryan M. Summers, Sridhar R. Gopishetty and Mani Subramanian
   Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA 52242
   Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA 52241
   Authors contributed equally to this work

3. **DETERMINING PROTEIN BINDING SELECTIVITY OF N-ARYLACYL O-SULFONATED AMINOGLYCOSIDE DERIVATIVES**
   Ioana Craciun, Amanda M. Fenner and Robert J. Kerns
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

4. **SYNTHESIS OF A MEMBRANE DISRUPTIVE GLYCOPROTEIN FOR GENE THERAPY**
   Samuel T. Crowley, Jianfeng Jin and Kevin G. Rice
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

5. **DESIGN OF MULTIFUNCTIONAL RESURRECTING AGENTS FOR AGED ACHE-OP ADDUCTS**
   Jacob D. Frueh and Daniel M. Quinn
   Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242

6. **SOLVENT-ENHANCED BIOTRANSFORMATIONS OF CHEMICALS BY BEAUVERIA BASSIANA AS BIOCATALYST**
   Richard Gonzalez and Tonya L. Peeples
   Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City IA 52242

7. **MUTANT FORMATE DEHYDROGENASE STUDIES REVEAL ALTERED ACTIVE SITE DYNAMICS**
   Qi Guo, Christopher Cheatum and Amnon Kohen
   Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242
8. DETECTING ATRAZINE DEGRADATION IN PSEUDOMONAS SP. STRAIN ADP BIOFILM WITH IN-SITU REVERSE TRANSCRIPTION AND RAMAN SPECTROSCOPY
Victoria A. Henry, Julie L. Jessop* and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA 52242

9. EFFECTS OF THE MUTATION OF Arg166 IN THE HYDRIDE AND PROTON TRANSFERS IN THYMIDYLATE SYNTHASE
Zahidul Islam, Zhen Wang and Amnon Kohen
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA 52242

10. TOWARD DETERMINING THE FUNCTION OF RLPA, A CELL DIVISION PROTEIN FROM PSEUDOMonas AERUGINOSA
Matthew A. Jorgenson1,2, David L. Popham3 and David S. Weiss1,2*
1Interdisciplinary Program in Genetics, The University of Iowa, Iowa City, IA 52242
2Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA 52242
3Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061

11. SMALL MOLECULE OXIDATIVE METABOLISM INHIBITORS FOR METASTATIC MELANOMA THERAPY
Department of Radiation Oncology (Free Radical Radiation Biology Program), Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA 52242

12. DESIGN AND SYNTHESIS OF NOVEL N-1 FLUOROQUINOLONES
Chaitanya A. Kulkarni, Tyrell R. Towle and Robert J. Kerns*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

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Tara A. Ladie, Nolan R. Mente and David F. Wiemer
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Poster Abstracts
EFFECTS OF Y209W ON THE HYDRIDE TRANSFER STEP IN THYMIDYLATE SYNTHASE

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Thymidylate synthase (TSase) catalyzes the *de novo* biosynthesis of the DNA building block thymidine, 2′–deoxythymidine 5′–monophosphate (dTMP) in most eukaryotes including human. The essential role of TSase makes it a common target for chemotherapeutic and antibiotic drugs. Recent studies with *Escherichia Coli* (ecTSase), showed that a highly conserved residue, Tyr-209, plays a key role in stabilizing the closed conformation. Although the crystal structures of wt ecTSase and its Y209W mutant are nearly identical at a 1.3 Å resolution, the anisotropic B factors of the phosphate-binding loop of Y209W mutant are not uniformly oriented as in the wt enzyme. To assess whether this dynamic effect alters on the hydride transfer step (the rate determining step at the physiological temperatures), we have compared the kinetic Isotope affects (KIEs), other kinetic properties, and the ability to trap reaction intermediate between the wt and the Y209W mutant.

Experiments suggested that thiols from the media can trap an intermediate in the reaction suggesting Y209W mutation reduces the stability of the ternary complex prior to the hydride transfer. Intrinsic KIEs throughout the temperature range (5 °C to 35 °C) indicated alteration of the nature of that step. Together these findings emphasize the importance of the relationship between long range interactions and the chemical reactivity of enzymes.
PRODUCTION OF METHYLXANTHINES BY METABOLICALLY ENGINEERED *E. coli*

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Alkylxanthines are naturally occurring compounds that are used in pharmaceutical preparations as diuretics, asthma treatments, and cardiac, pulmonary, and neurological stimulants. Mono- and dimethyl xanthines are key intermediates in synthesis of various alkylxanthines drugs. Using a novel biocatalytic approach, we can produce these valuable intermediates in large quantities using cheap substrates such as caffeine, theobromine, and theophylline.

We have previously reported\(^1,2\) that *Pseudomonas putida* CBB5 degrades caffeine via sequential N-demethylation to theobromine or paraxanthine, then 7-methylxanthine, and then xanthine. CBB5 contains five novel N-demethylase genes, *ndmA*, *ndmB*, *ndmC*, *ndmD*, and *ndmE*, which are responsible for caffeine degradation. These genes have been isolated, cloned, and heterologously expressed to determine their biochemical properties. N-demethylase A (NdmA) and N-demethylase B (NdmB) are Rieske monooxygenases that catalyze positional-specific \(N_1\)- and \(N_7\)-demethylation, respectively. Both enzymes receive reducing equivalents from NADH via a redox-center-dense Rieske reductase, NdmD. NdmC is an \(N_7\)-demethylase with activity specific for 7-methylxanthine in conjunction with NdmD and NdmE, a glutathione-S-transferase homolog.

A library of metabolically engineered *E. coli* strains has been created using different combinations of *N*-demethylase genes from CBB5\(^3\). We have initiated screening of these *E. coli* strains for production of 1-, 3-, and 7-methylxanthines. This work summarizes screening results from two *E. coli* strains, pAD1dDD and pBD2dDB. Resting cells experiments with fresh cells of *E. coli* pAD1dDD, which contains both *ndmA* and *ndmD*, converted 1 mM caffeine to theobromine (95% conversion) and 1 mM theophylline to 3-methylxanthine (75% conversion). The activity dropped by 70% with frozen cells of *E. coli* pAD1dDD. Similarly, resting cells with fresh cells of *E. coli* pBD2dDB, containing both *ndmB* and *ndmD* converted 1 mM theobromine to 7-methylxanthine (70% conversion), while frozen cells showed substantial drop in the conversion theobromine to 7-methylxanthine.

References:

3. Summers *et al.*, 2012 CBB Conference; Metabolic Engineering of *E. coli* for Production of High-Value Methylxanthines from Caffeine, Theophylline, and Theobromine.
DETERMINING PROTEIN BINDING SELECTIVITY OF N-ARYLACYL O-SULFONATED AMINOGLYCOSIDE DERIVATIVES

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Heparan sulfate (HS) is a cell surface glycosaminoglycan that plays a profound role in numerous physiological processes. HS chains are comprised of repeating disaccharide units, with regions of high anionic content that are important for the selective and/or nonselective binding to diverse proteins. Over 200 proteins have been identified to bind HS and the structurally similar glycosaminoglycan heparin. Over twenty therapeutic applications have been proposed for molecules to bind specific HS-binding proteins and block or modulate HS-mediated biological activities. In our work, synthetic modifications of glycosaminoglycans has been undertaken toward identifying molecules that selectively, if not specifically bind HS-binding site of individual HS-binding proteins.

Aminoglycosides are small, naturally occurring saccharides originally used as antibiotics. This class of compounds provides an untapped resource as a carbohydrate scaffold for designing new therapeutic agents. The hydroxyl and amino functional groups are synthetically useful handles for designing compound libraries, and the saccharides core provides structural support for positioning structural moieties to enhance binding contacts with specific proteins.

The Kerns lab has previously identified certain N-arylacylated and carboxamide derivatives of glycosaminoglycans and N-arylacyl O-sulfonated aminoglycosides that selectively bind HS-binding proteins. In the studies presented here, this work was furthered by molecular modeling studies to elucidate the reason for binding selectivity of these N-arylacyl O-sulfonated aminoglycosides to HS-binding proteins. Establishing a binding model is expected to facilitate future in silico screening of N-arylacyl O-sulfonated aminoglycosides to identify structural derivatives that selectively bind and inhibit different HS-binding proteins.
SYNTHESIS OF A MEMBRANE DISRUPTIVE GLYCOPROTEIN FOR GENE THERAPY

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Gene therapy has the potential to treat a wide variety of illnesses and disorders, including hereditary disease, cancer, viral infections, and more. Gene therapy is normally divided into two categories, viral and nonviral. Viral gene therapy uses viruses to deliver DNA to target tissues, and has the advantage of higher expression. However, viral gene therapy has higher risk of side effects such as inflammation and immune response. Nonviral gene therapy can potentially avoid these side effects, but suffers from much lower efficiency than viral methods.

Two of the major obstacles to efficient nonviral gene therapy have been escaping from the endosome and entering the nucleus. Both of these steps require crossing cellular membranes. Several viruses have been discovered to have phospholipase A2 activity in their capsid proteins, and if this activity is removed, the viruses become much less infective. Phospholipase A2 is an enzyme that hydrolyzes phospholipids and can disrupt membranes, potentially allowing the virus to deliver its genetic cargo across cellular membranes. Therefore, we hypothesize that PLA2 activity will improve nonviral gene transfer by disrupting membranes and allowing DNA to escape the endosome and enter the nucleus.

This research has focused on two strategies, recombinant PLA2 expression and chemical modification of PLA2. Recombinant expression allows for the addition of nuclear targeting sequences to PLA2 and other highly controlled mutation of the enzyme. Chemical modification, while much less controllable, is necessary for adding certain moieties to a protein, such as a carbohydrate.

An attractive carbohydrate for protein modification is the Triantennary Oligosaccharide, or Tri. Tri binds to the asialoglycoprotein receptor of hepatocytes and can be imported into the cell. Tri was reacted with iminothiolane and dithiodipyridine to create Tri-thiopyridine, a carbohydrate that would add to free thiols.

Bee venom PLA2 was then modified with iminothiolane to add free thiols. Tri-thiopyridine was then added to those thiols to create an enzyme that retained PLA2 activity and could be purified by affinity chromatography using a lectin gel.

This technique can be used to attach Tri to recombinant PLA2 enzymes, allowing for the addition of hepatocyte targeting and nuclear targeting moieties to the same molecule. PLA2 modified in such a manner could prove useful for delivery of DNA or RNA to cells in vitro and in vivo.
DESIGN OF MULTIFUNCTIONAL RESURRECTING AGENTS FOR AGED AChE-OP ADDUCTS

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Organophosphates (OP) are effective transition state inhibitors of acetylcholinesterase (AChE). Due to the effectiveness of these compounds to act as inhibitors of AChE, particularly as chemical warfare agents, new remedies must be developed in order to avoid the high number of mortality cases being reported each year due to unintended or intended exposure. The most common used antidote, 2-PAM, has several deficiencies, most significantly its failure to reactivate the “aged” AChE-OP adducts. To solve this dilemma, several multifunctional methylating agents, containing a skeletal structure similar to the Alzheimer medication Aricept, are being developed to help resurrect “aged” AChE by methylating the AChE-OP adducts. Presented here are the initial results of employing thiomethyl ethers as methyl transfer agents and the kinetic analysis of a model system.
SOLVENT-ENHANCED BIOTRANSFORMATIONS OF CHEMICALS BY BEAUVERIA BASSIANA AS BIOCATALYST

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This research evaluates the oxidative capacity of n-alkane-induced fungal Beauveria bassiana; a versatile whole cell biocatalyst used in the biotransformation of steroids, and in the production of valuable organic compounds. n-alkane solvents like dodecane, and hexadecane serve as carbon sources during growth of B. bassiana, inducing the expression of oxidative enzymes, and affecting the growth rate. n-Piperidinylacetophenone is a classic substrate used for the hydroxylation of an unfunctionalized carbon; an attractive reaction that results in valuable intermediates for specialty chemical synthesis. As well, steroid 7-alpha-hydroxy-4-cholesten-3-one (NS) is hydroxylated to 7-alpha-12 alpha-dihydroxy-4-cholesten-3-one, to provide metabolites of interest for medical purposes. Our research targets the hydroxylated products of these chemicals. Reactions with n-alkane adapted strains (ATCC 90517, ATCC 90518, ATCC 7159) on a resting cell environment; were grown in media containing different nitrogen sources (Corn Step Liquor, Soybean flour, peptone, and ammonium nitrate). 1H-NMR, and HPLC were used to characterize, and isolate the biotransformation products. Results include biotransformation selectivity, reaction yield, n-alkane effect analysis in biocatalyst performance, and correlations between biomass and reaction yields.
MUTANT FORMATE DEHYDROGENASE STUDIES REVEAL ALTERED ACTIVE SITE DYNAMICS

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One hotly debated topic in enzymology is the role of fast protein structural fluctuations in enzyme-catalyzed reactions. Our research is focused on characterizing enzyme active-site motions on the femtosecond to picosecond time scale, and elucidating their functional relevancies. Formate dehydrogenase (FDH) is selected as a model system because its transition state analogue inhibitor, azide, enables us to study the fs to ps time scale dynamics using two-dimensional infrared spectroscopy (2D IR). Previous kinetic isotope effects (KIE) and 2D IR studies on the commercial \textit{wt}-FDH from \textit{Candida boidinii} suggest that the active site structure in the transition state (TS) is rigid reflecting the fact that the native tunneling ready state is well organized for efficient hydride transfer. On the other hand, mutant enzyme (expressed in \textit{E. coli}) showed significant different 2D IR result, suggesting that the active site undergoes a different dynamic process. While none of the mutations is in the vicinity of the active site and most of them are reported as ‘natural variants’, combination of these mutations has an impact on the active site. Understanding the structural and kinetic differences and probing the dynamic motions between the commercial and the recombinant may clarify the dynamic network of the distal mutations in FDH.
DETECTING ATRAZINE DEGRADATION IN *PSEUDOMONS* SP. STRAIN ADP BIOFILM WITH IN-SITU REVERSE TRANSCRIPTION AND RAMAN SPECTROSCOPY

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Within a biofilm, organisms are protected by a complex matrix and are able to survive longer than free cells against environmental challenges. This persistence may inflict havoc on the human body by encouraging resistance to antibiotics, but may be beneficial in bioremediation. In a biofilm, organisms are able to trap contaminants within their extracellular sticky matrix and encourage formation of catabolic pathways via transfer of plasmids amongst organisms in the biofilm. For the purpose of this research, *pseudomonas* sp. strain ADP was grown as a biofilm with atrazine as the model contaminant. Atrazine, an endocrine disruptor compound and group C-possible carcinogen, is the second most widely used herbicide in the US, often resulting in ground water and surface water runoff contamination. Biofilms were grown in open flow chambers. In-situ reverse transcriptase and in-situ hybridization were applied to probe presence and expression of genes which reside on the plasmid responsible for atrazine degradation. Surface enhanced Raman spectroscopy was used to monitor atrazine and its metabolites. Success of this research will lead to a firmer understanding of scientific fundamentals associated with the capacity of microbial biofilms for pollutant degradation and retention of key degradative genes. This understanding will ultimately lead to improved remediation applications, reduced pollutant-associated illness, and general methodologies for biofilm analysis.
EFFECTS OF THE MUTATION OF Arg166 IN THE HYDRIDE AND PROTON TRANSFERS IN THYMIDYLATE SYNTHASE

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Thymidylate synthase (TSase) catalyzes the reductive methylation of 2’-deoxyuridine-5’-monophosphate (dUMP) to form 2’-deoxythymidine-5’-monophosphate (dTMP). Being crucial for biosynthesis of DNA, TSase is a common target of antibiotic and chemotherapeutic drugs. The proposed mechanism of TSase involves an initial attack of a highly conserved cysteine residue (Cys146 in *E. Coli* TSase) to dUMP, which forms a covalent C-S bond between the enzyme and the substrate. Our QM/MM calculations suggested that the Arg166 residue facilitates the departure of Cys146 from dUMP by stabilizing the resulting sulfur anion in the rate-limiting hydride transfer step. Recent calculations also implicate the same phenomena for a faster proton transfer step in the catalytic cascade, suggesting a novel intermediate that is not covalently bound to the enzyme. This novel intermediate provides a new direction for designs of drugs that can take advantage of the liability of the C-S bond under study.

In order to experimentally examine these calculations, we are currently studying the kinetics of the lysine mutant of Arg166 (R166K), where the proposed C-S bond cleavage is expected to be less favorable. In addition, R166K is still active, which allows functional comparison with the *wt* enzyme. Our preliminary experiments suggest differences between the kinetic properties of the mutant and *wt* enzymes. To test our theoretical predictions, future experiments will measure intrinsic kinetic isotope effects and their temperature dependence for both the proton and hydride transfers in R166K to probe the effect of R166 in both C-H bond activation steps.

References:
Cell division is an essential process, requiring the concerted efforts of at least 30 proteins in a Gram-negative bacterium like *Escherichia coli*. Together, these proteins form a complex structure at the midcell known as the septal ring to mediate the simultaneous inward growth of the three layers of the cell envelope: the outer membrane, the peptidoglycan cell wall, and the inner membrane. Recently, our lab identified three new septal ring proteins—DamX, DedD and RlpA—in *E. coli*, all of which contain a ~75 amino acid SPOR domain that is sufficient for localization to the septal ring. Loss of DamX or DedD resulted in cell division defects. Conversely, loss of rare lipoprotein A (RlpA) did not cause a cell division defect in *E. coli*. However, we found that a transposon mutant of *rlpA* in *Pseudomonas aeruginosa* has defects in cell separation when grown in media of low ionic strength, forming chains of 4-8 cells. We constructed a non-polar deletion of *rlpA* in *P. aeruginosa* and confirmed the chaining phenotype. We also constructed an RlpA-mCherry fusion and showed septal ring localization during cell division. Interestingly, the SPOR domain is necessary for RlpA localization but not for function. Utilizing the RlpA-mCherry fusion, we also suggest that RlpA is an outer membrane lipoprotein. Peptidoglycan analysis revealed some structural differences between wild type and the *rlpA* mutant, suggesting RlpA is an enzyme involved in peptidoglycan turnover or maturation. Data from an in vitro assay combining purified RlpA with isolated peptidoglycan supports this hypothesis.
SMALL MOLECULE OXIDATIVE METABOLISM INHIBITORS FOR METASTATIC MELANOMA THERAPY

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Metastatic melanoma incidence is increasing faster than any other form of cancer worldwide. Despite advances in chemotherapeutic, biological, and targeted therapies, no treatment affords durable benefit to melanoma patients. The current study explores a novel approach to melanoma therapy utilizing a small-molecule triphenylphosphonium (TPP) platform technology that targets fundamental differences in oxidative metabolism and response to oxidative stress in melanoma cells relative to normal cells. Based on the mitochondrial targeting properties of TPP, a small library of TPP derivatives were examined to determine if the molecular composition of TPP variants could be modified to affect melanoma cell oxidative metabolism and viability. In vitro measurements of cell viability, mitochondrial membrane potential, electron transport chain complex activity, oxidative stress, and oxygen consumption rates were conducted. In vivo studies were performed in mice bearing melanoma tumor xenografts to determine if TPP treatment can be tolerated and induce a reduction in melanoma tumor growth via increased oxidative stress. Results indicate that TPP derivatives can be designed to disrupt oxidative metabolism and lead to melanoma cell death via increased oxidative stress. Further, melanoma tumor bearing mice treated with TPP exhibited decreased melanoma tumor growth rates compared to untreated mice. Importantly, TPP treatment was well tolerated and tumor lysates showed increased oxidative stress markers, thus providing evidence that TPP derivatives can be designed to disrupt oxidative metabolism and selectively kill melanoma cells via increased oxidative stress.
Fluoroquinolones are synthetic broad spectrum antibiotics that target the bacterial type-II topoisomerases DNA gyrase and topoisomerase IV. These enzymes are responsible for maintaining the topology of DNA during replication and transcription, through an ATP driven process that involves relaxing the double helix, introducing supercoils and relieving torsional strain. Fluoroquinolones inhibit these processes by forming a ternary complex with DNA and topoisomerase. In recent years there has been a rise in the number of bacterial strains that are resistant to fluoroquinolones. One important reason for the development of resistance is mutation of the target enzyme(s), which results in decreased binding of the fluoroquinolones to form the ternary complex. The goal of my current research is to develop novel fluoroquinolones that exhibit activity against quinolone-resistant mutants. Study of crystal structures of the ternary complex of clinically established fluoroquinolones, like moxifloxacin, shows that modifications at the \( N-1 \) site of the fluoroquinolone scaffold can afford analogues capable of interacting with new sites in the ternary complex, in addition to making conventional binding contacts. By performing molecular modeling and docking studies on the crystal structure of moxifloxacin, DNA and \textit{Acinetobacter baumannii} topoisomerase IV (ParE-ParC fusion truncate) [2XKK], we have developed a panel of novel \( N-1 \) fluoroquinolones that are designed to make new contacts. Compounds showing promising \textit{in-silico} binding scores in the docking studies are included in the panel. Substitution of benzylic groups at the \( N-1 \) position intends to increase the hydrophobic interactions. Amine and guanidine groups connected to the \( N-1 \) position by a carbon chain of suitable length are anticipated to form new ionic interactions. Finally, groups like napthyl and phenyl linked to the \( N-1 \) position of the fluoroquinolone scaffold may orient to form \( \pi \) stacking.

Presented here is representative data from molecular modelling and docking studies performed to design the panel of novel \( N-1 \) fluoroquinolones. The synthetic methodology employed to make these fluoroquinolones will also be described.
A SYNTHETIC APPROACH TO MEROTERPENOID PHOMOARCHERIN C

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The initial characterization of Phomoarcherin C was reported in 2011 and it was identified as the tetracyclic aldehyde 1 on the basis of its spectroscopic data. Its antimalarial activity and interesting skeleton have drawn our attention to a synthesis of this natural product. With the readily available orcinol (2) as a starting material, we have hypothesized that a cationic cyclization can be used to assemble the three additional rings of the tetracyclic system. Our progress toward synthesis of this meroterpenoid, and the use of \(E,E\)-farnesol as a model system for the cascade cyclization, will be presented.
CO-CRYSTALS OF COMPLEMENTARY BIFUNCTIONAL TEMPLATES

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Templates provide a means to assemble reactants for chemical reactions. Our group utilizes organic templates to direct photostable olefins into photoreactive geometries in the solid state. The templates operate through hydrogen bonding between the template and olefin. The templates function as either hydrogen bond donors (e.g. 1,3-dihydroxyl benzene), or the hydrogen bond acceptors (e.g. 1,8 bis(4-pyridyl) naphthalene). Both type of templates exhibit similar geometries to direct the [2+2] photodimerization in solids. A key feature of template systems encountered in biology is an ability of complementary template to assemble (e.g. DNA Helix). With this in mind, we hypothesized the ability to cocrystallize two different templates to form discrete hydrogen-bonded structures. In this poster we will present our first studies on the cocrystallization of hydrogen bond donor and acceptor templates to form complementary assemblies in solids. Discussions on the crystal structures and local packing will be reported.
MICROBIAL POLYCHLORINATED BIPHENYL DEGRADATION IN SWITCHGRASS PLANTED RHIZOSPHERE

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Polychlorinated biphenyls (PCBs) are a group of 209 synthetic chemicals that were widely used in industry during the 20th century. They pose a possible threat to both human and environmental health, because certain PCB congeners are toxic, carcinogenic and recalcitrant. Phytoremediation is a promising and cost-effective strategy for cleaning up PCB contaminated sites, in which plants and associated microbes remove and detoxify PCBs. Microbes could degrade PCBs via two general processes: aerobic oxidative biphenyl ring cleavage and anaerobic reductive dechlorination. And plants can provide an amenable environment for microorganisms thus improving microbial PCB biodegradation in the root zone.

Previous results have shown significantly higher reductions of PCB-52, 77 and 153 in switchgrass planted systems compared to the unplanted systems. In this study, we are analyzing microbes' role in PCB biodegradation in a switchgrass planted system with PCB exposure (PCB-52, 77 and 153 mixture). Biphenyl dioxygenase gene (*bphA*), which codes for the key enzyme catalyzing the first step of aerobic PCB degradation, is selected as a biomarker gene. Preliminary PCR results have confirmed the presence of *bphA* genes and transcripts in a switchgrass planted rhizosphere soil sample, indicating the possible occurrence of aerobic PCB degradation. We are also using reverse transcription quantitative PCR (RT-qPCR) to monitor the change of *bphA* transcript and gene abundances in soil systems with or without switchgrass planting throughout a six-month incubation period. A higher ratio of *bphA* transcript abundance to gene abundance is expected in switchgrass planted system when compared to a system without switchgrass planting. Meanwhile the concentrations of PCB congeners in both systems will be measured to evaluate the degradation efficiency. Overall this study will help better understand microbial PCB degradation in the rhizosphere and how to achieve efficient phytoremediation for PCBs.
SPECIFICITY, STRUCTURE, DYNAMICS AND INHIBITION OF TIA1 PDZ DOMAIN/SYNDECAN1 COMPLEX

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The T-cell lymphoma invasion and metastasis 1 (Tiam1) is a guanine exchange factor (GEF) for the Rho-family GTPase Rac1 that is crucial for cell-cell adhesion and cell migration. Deregulation of Tiam1/Rac1 signaling leads to various malignancies, including cardiovascular disease and cancer. Tiam1 contains several protein–protein interaction domains, including a PDZ domain. We have previously shown that the cell adhesion receptor syndecan1 activates Rac1 signaling through an interaction with the Tiam1 PDZ domain, but little is known about the specificity with other syndecan family members. Here, we used equilibrium binding experiments to show that the Tiam1 PDZ domain specifically interacts with syndecan1, phosphorylated syndecan1 and syndecan3, but not syndecan2 and syndecan4. We determined the crystal structures of the Tiam1 PDZ domain in complex with syndecan1 peptide, which revealed the structural basis for this specificity. Remarkably, comparison of the PDZ/syndecan1 and PDZ/phosphorylated syndecan1 structures showed that a new specificity pocket was used to accommodate the phosphate group. Moreover, NMR methyl relaxation experiments of PDZ/SDC1 and PDZ/pSDC1 complexes reveal that internal protein dynamics are highly sensitive to ligand conformational change induced by phosphorylation. In particular, the phosphate moiety dampens the dynamic motions in distal regions of the PDZ domain by decoupling them from the ligand-binding site. Finally, we combined structure-based in silico docking screening and unbiased AlphaScreen-based high-throughput screening to discover inhibitors targeting Tiam1 PDZ/syndecan1 interactions. Collectively, our results provide insight into the specificity, structure and dynamics of the Tiam1 PDZ domain/syndecan1 complex and identify several primary small-molecule inhibitors that target this complex.
SYNTHESIS AND EVALUATION OF HUMAN ACETYLCHOLINESTERASE INHIBITORS

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The acetylcholinesterase (AChE) enzyme is a powerful molecule that catalyzes the hydrolysis of the neurotransmitter acetylcholine. Hydrolysis occurs at the catalytic triad of AChE which lies 20 Å from the enzyme’s surface. AChE inhibition is the most effective clinical target for the treatment of Alzheimer’s Disease (4 of the 5 current drugs target AChE), and AChE is inhibited by powerful organophosphate (OP) chemical warfare agents. When AChE is inhibited by some OPs, it undergoes an irreversible process called “aging”. Novel AChE inhibitors have been synthesized and evaluated along with a class of methyl pyridinium molecules. Dose response assays, using a modified Ellman’s method, was performed for the methyl pyridinium inhibitors and provided a range of IC$_{50}$ values. Fluorescent organophosphate inhibitors were synthesized to be used as assay probes for direct detection of AChE inhibition. These analogues featured 4-methylumbelliferyl, 4-trifluoromethylumbelliferyl and n-methylquinolinium motifs as a fluorophore. OP inhibited AChE is evaluated for its aging kinetics and subsequent “resurrection” of aged OP-AChE adducts.

Reference:
TARGETING THE Gαo: RGS17 PROTEIN: PROTEIN INTERACTION FOR THE TREATMENT OF LUNG AND PROSTATE CANCERS

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G-Protein Coupled Receptors (GPCR) are the most important target in disease treatment with over 60% of all drugs on the market targeting these receptors. Regulator of G-Protein Signaling (RGS) proteins are known to regulate the complex signaling pathways activated by GPCRs. Recent studies have implicated RGS-proteins in the development and progression of pathologies, including some cancers. RGS17, the most-recently identified family member of the RZ family of RGS proteins has been implicated in the growth, proliferation, metastasis and migration of prostate tumors as well as small-cell and non-small cell lung cancers. RGS17 is up-regulated in lung and prostate tumor tissues up to a 13 fold increase over patient-matched normal tissues. Studies have shown that RGS17 knockdown inhibits colony formation and decreases tumorigenesis in nude mice. Our studies implemented a high-throughput screen campaign to determine the first Gαo: RGS17 protein: protein interaction (ppi) inhibitors. Immediate goals include determining the effectiveness of these compounds on inhibiting GTPase acceleration activity of RGS17 in a steady-state GTPase assay and testing structurally related compounds for inhibition. The goal of this study is to establish lead compounds and develop a pharmacophore model for optimization of structure focused on activity for future avenues in cancer treatment.
HYPOXIA INDUCIBLE FACTOR DEPENDENT OXYGEN SENSING

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Hypoxia Inducible Factor (HIF-α), the master regulator of the eukaryotic cellular response to inadequate oxygen supply (hypoxia), is a transcriptional factor that plays an essential role in tumor angiogenesis, erythrocytosis, and pathophysiology associated with reduced blood supply to vital organs (ischemia). When cellular oxygen supply is normal (normoxia), HIF-α isoforms (HIF-1/2α) undergo post-translational modifications by metalloenzymes that use oxygen to hydroxylate specific proline and asparagine residues. The post-translational hydroxylation of HIF-α is required for recognition by the ubiquitin ligase, Von Hippel-Lindau protein (VHL). As a consequence of this recognition event, HIF-α is degraded and transcriptional activity is silenced. Inherited mutations in proteins found in the oxygen sensing pathway, HIF-2α, HIF-prolyl hydroxylase (PHD2), and VHL cause familial erythrocytosis, a disease characterized by increased red blood cell (RBC) volume and elevated hemoglobin concentrations leading to blood vessel obstruction. However, the molecular basis attributed to these hereditary mutations remains to be determined. We are using a combination of biochemical and structural approaches to study how clinical mutations perturb protein-protein interactions in the mammalian oxygen sensing pathway.
TOWARDS BETTER ANTIBIOTICS: STUDIES OF ALTERNATIVE THYMIDYLATE SYNTHASE

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The enzyme thymidylate synthase (TSase) is responsible for de novo production of thymidylate, one of DNA building blocks. To date, two different classes of TSase enzymes are known: the well-characterized classical TSase and a less understood flavin-dependent TSase (FDTS). Although the two enzymes catalyze the same net chemical conversion, they differ greatly in their structure, cofactor requirement and mechanism. Several mechanisms have been proposed for FDTS catalysis, but identification of any intermediate(s) that may support a specific mechanism has never been reported. In this presentation, I report the chemical trapping and identification of such intermediates in the FDTS-catalyzed reaction, using labeling of the substrates with radioactive isotopes and rapid-quenching techniques. The identity of the trapped intermediates underlines the uniqueness of FDTS chemistry and narrows down the possible chemical mechanisms. Furthermore, experiments conducted in deuterated solvent distinguish between the proposed mechanisms and elucidate the timing of events in FDTS catalysis. The described experimental approach provides an important tool for future studies of flavin-dependent thymidylate synthesis. Because many human pathogens depend on FDTS for thymidylate, knowledge of FDTS chemistry may assist the efforts to rationally design inhibitors as leads for antibiotics.
DEVELOPMENT OF A RAPID COLORIMETRIC ASSAY FOR DETECTION OF CAFFEINE IN BEVERAGES AND BODY FLUIDS USING CAFFEINE DEHYDROGENASE FROM PSEUDOMONAS sp. CBB1

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Background: Caffeine is a key component of widely consumed beverages like coffee, tea, and soft drinks. Various biological effects and symptoms caused by ingestion of caffeine include diuresis, headaches, insomnia, cardiac and respiratory stimulation. Restricted consumption of caffeine is highly recommended for infants and seniors, and is strictly restricted for pregnant women owing to its possible teratogenic effects. Although decaffeinated versions of various caffeine containing beverages are available, a suitable method needs to be developed to enable specific, rapid and semi-quantitative detection of caffeine in these beverages. Also detection of caffeine in nursing mother’s milk will be of value in terms of feeding the infants. Caffeine-degrading strain Pseudomonas sp. CBB1, metabolizes caffeine by direct oxidation at the ‘C-8’ position to form trimethyluric acid (TMU). This reaction is catalyzed by a novel heterotrimeric quinone-dependent caffeine dehydrogenase (Cdh)1. The enzyme is specific for caffeine1 and appears suitable for rapid detection of caffeine in fluids.

Methods: Cdh was partially purified from CBB1 by Fast Protein Liquid Chromatography using a Phenyl Sepharose HP column. A dye-based colorimetric assay was developed for detection of caffeine. A typical assay mixture contained partially purified Cdh in potassium phosphate buffer (pH 7.5), 0.5mM tetrazolium salt and variable caffeine concentration.

Results: The oxidation of caffeine with H2O in presence of Cdh, with concomitant reduction of the tetrazolium salt occurred in less than one minute to produce dark blue formazan. Sensitivity for detection of caffeine ranged from 1.4 mg L⁻¹ to 98 mg L⁻¹. Different tetrazolium salts were screened to optimize the Cdh-based caffeine detection, for sensitivity, speed of detection, and good visibility of color using various beverages, milk and breast-milk. This test is now being extended for caffeine detection at sub-ppm levels, in environmental samples, esp. river and ground water. Caffeine is regarded as a ‘universal indicator of pollution’ due to human activities2.

Conclusion: A rapid colorimetric assay has been developed using Cdh for semi-quantitative detection of caffeine in beverages and body fluids. This assay has the potential to detect low levels of caffeine in environmental samples such as rivers and ground water.

References:
Beauveria bassiana is an entomopathogenic fungus widely used as a biopesticide and biocatalyst. Research efforts have leaned towards virulence enhancement for better pest management; however, the effect of heightened virulence on oxidative biocatalysis has not been thoroughly investigated. The goal of this research is to explore B. bassiana’s oxidative proteome and biotransformation performance under virulence parameters. The expression of extracellular and intracellular enzymes has been quantified under virulence conditions with special interest in oxidative enzymes. Organic solvents serve as carbon sources during growth of B. bassiana and have been shown to induce the expression of oxidative enzymes. Efforts to categorize key catalytic enzymes under starvation and other environmental stress will be discussed. The long-term goal is to standardize and to control biocatalysis capacity of B. bassiana under different operational parameters. This research will provide new insight on B. bassiana’s catalytic performance.
Bacterial biofilm infection is a common (~2 to 4%) complication for surgically implanted medical devices. We are investigating the method of using thermal shock as an innovative treatment in order to deactivate *Pseudomonas aeruginosa* biofilms. The development of this treatment is hindered by the difficulty to quantify biofilm viability. Confocal fluorescent microscopy of the biofilms as well as direct enumeration of colony forming units from sonicated biofilms has been observed. Microscopy is beneficial because of the rapid, direct feedback, however, is limited by varying light intensities of the fluorescing dyes. Currently it is unclear whether the diverse intensities are attributed to the biomass in the plane-of-focus or to background light from out-of-plane extracellular material. This project researches and discusses the importance of comparing image-based quantification using both subjective and objective light thresholding methods and direct enumeration of the colony forming units.
CLOSTRIDIUM DIFFICILE VIRULENCE REQUIRES NOVEL CELL SHAPE FACTORS

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Clostridium difficile is a Gram-positive, anaerobic, rod-shaped, opportunistic pathogen and is the primary causative agent of antibiotic-associated diarrhea in hospital and nursing home patients. To further understand C. difficile pathogenesis, we wanted to identify novel virulence factors. Previously, cell shape has been shown to affect virulence in other pathogens; however, cell shape determining factors remain largely uncharacterized in C. difficile. Here we describe the role of a C. difficile operon that contributes to cell shape. The operon contains three putative genes. The first gene appears to encode a protein with a SPOR domain, but the remaining ORFs have no significant homology to proteins of known function. SPOR domains bind peptidoglycan, and most characterized SPOR domain proteins are involved in cell division. Overexpression of some genes in the operon results in altered cell morphology. An insertion mutation in the putative SPOR gene results in morphological defects including curved-shaped cells and chaining. Additionally, the mutant exhibits increased sensitivity to a subset of antibiotics that target peptidoglycan synthesis. Furthermore, the mutant has a virulence defect in a Syrian hamster model. We conclude the operon is required for maintaining cellular morphology and is essential for C. difficile pathogenesis.
GENERATING A UNIFORM TISSUE PHANTOM WITH HYDROPHOBIC NANOPOROUS SILICA PARTICLES: TOWARD THE QUANTIFICATION OF PARTICLE DISPERSION

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Tissue phantoms are lab-constructed imitations of biological tissue that can be used to mimic cellular processes or to simulate disease formation. For example, the spectroscopic properties of tissue – scattering, absorption, and fluorescence – which change during the progression of a disease, such as cancer, can be modeled in these synthetic materials. These phantoms are of particular interest for the development of a noninvasive optical method of cancer detection and diagnosis.

Uniformity of the phantoms used to set instrumental parameters is an essential consideration for the development of a robust methodology. Two specific barriers to the generation of a uniform tissue phantom have been identified – dispersal of hydrophobic particles in agar and distribution of loaded molecules in the nanopores. Previously, uniform suspension of loaded hydrophobic nanoporous silica particles in agar has been difficult because of the tendency of the particles to aggregate in aqueous media. In this project, tissue phantoms were prepared utilizing various methods to suspend the silica particles. Qualitative analysis suggests that uniform dispersal of these particles in agar has been accomplished through surfactant coating in contrast with other attempts using sonication and vortexing. However, quantitative assessments of the particle dispersal in the phantom products of each method are necessary to clearly demonstrate that the proposed preparation method has achieved a homogeneity exceeding the other methods.

We explored a method for quantitative evaluation of particle dispersal with radial correlation function. We first established the correlation approach through simulated systems. Specific correlation peaks and signatures were examined through a number of simulated particle structures of increasing levels of complexity, from tight hexagonally packed aggregates and completely randomly dispersed particles with uniform particle size, to a mixture of particle aggregates and dispersed particles of various levels of size distribution. To generate the clusters, particles were created of different sizes following a normal distribution; a nonlinear least squares minimization process brought the particles into close contact with each other, yet allowing their random assembly through different minimization paths. The correlation signatures provide quantitative evaluation of particle dispersion in real samples. The “mixed” simulation most closely resembles the images obtained from phantoms prepared via the less successful suspension methods in terms of homogeneity. Images which contain clusters have a characteristic sharp peak corresponding to nearest-neighbor distances while randomly distributed particles are characterized by a broad correlation function. The nearest-neighbor correlation peak can be used to clearly distinguish between images of fully dispersed particles and images containing clusters.
STAPHYLOCOCCUS AUREUS HYALURONATE LYASE, A POTENT VIRULENCE FACTOR

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\textit{Staphylococcus aureus} is the causative agent of both acute and chronic bacterial infections ranging from mild skin and soft tissue infections to more serious conditions such as pneumonia, endocarditis, toxic shock syndrome, and sepsis. The ability to cause a wide range of infections in multiple tissue types is due in part to the vast array of virulence factors produced by this bacterial pathogen. Some of the most important factors are secreted proteins such as the hemolysins, superantigens, and exo-enzymes. One of these exo-enzymes, called hyaluronate lyase, cleaves the $\beta$-1,4 glycosidic bond of hyaluronic acid, a host matrix polymer composed of repeating disaccharide subunits of N-acetyl-glucosamine and D-glucuronic acid. Hyaluronate lyases have been implicated as virulence determinants in a number of bacterial pathogens, facilitating the dissemination of bacterial cells and other secreted factors during infection. Here we report the construction and characterization of a hyaluronate lyase ($\text{hys}A$) null mutant in a USA300 community-associated methicillin-resistant \textit{S. aureus} (CA-MRSA). Using quantitative enzyme assays, we confirmed the absence of hyaluronate lyase activity in the USA300 $\text{hys}A$ mutant and we observed that activity increased significantly in USA300 $\text{sig}B$ and $\text{sar}A$ global regulatory mutants. To assess hyaluronate lyase as a USA300 virulence factor, we performed a neutropenic murine model of pulmonary infection and determined that the USA300 $\text{hys}A$ mutant is significantly attenuated. Altogether, these results indicate that the \textit{S. aureus} hyaluronate lyase is a potent virulence factor and future studies will further elucidate the regulation and pathogenic role of this enzyme.
TWO COMPLEMENTARY APPROACHES ILLUMINATE THE HYDRIDE TRANSFER CATALYZED BY DIHYDROFOLATE REDUCTASE

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Dihydrofolate reductase is a small enzyme that serves as an excellent model for hydride transfer reactions. Experiments have found that mutations—both in the active site and quite distant from the active site—alter the nature of the chemical transformation: the reaction is slower and the kinetic isotope effects (KIEs) are changed in the mutants. In order to gain a molecular understanding of how these mutations give such results, we have employed two approaches. In one approach, we have developed a method to fit experimental KIE data to a Marcus-like model for hydrogen tunneling. Fits to this model show that the mutant enzymes have far more conformational flexibility, and that in some mutants, the reaction occurs from at least two distinct conformational substates. In the second approach, we have conducted hybrid quantum mechanics/molecular mechanics simulations of the enzyme and its mutants to gain a detailed molecular understanding of the catalyzed reaction and the effects of the mutations. Preliminary results show that the hindered catalytic effect of the mutants results from subtle but global changes to the enzyme. We will present progress along both approaches towards understanding this enzyme and the effects of mutations.

References:
ENZYMEOLOGY OF DIFFERENT DIMETHYLSULFONIOPROPIONATE BIODEGRADATION PATHWAYS

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Dimethylsulfoniopropionate (DMSP) produced by marine phytoplankton is degraded by marine bacteria to form methanethiol (MeSH) and the climatically active gas dimethylsulfide (DMS). DMS emissions from marine-oceanic environments contribute to about 90% of natural sulfur emissions and about 60% of global biogenic sulfur flux to the atmosphere and has a significant impact on global climate. There are two known pathways of DMSP biodegradation. The lyase pathway where DMSP is cleaved to DMS and acrylic acid; and an alternate demethylation pathway by which DMSP is metabolized to liberate MeSH, a bacterial carbon and sulfur source. Despite the implication of DMSP catabolism in the global sulfur and carbon cycles, the biochemical pathways, genes, and regulating enzymes are poorly studied. In this study, we have identified enzymes containing surprising metallocofactors involved in DMSP dissimilation. We have used biochemical, spectroscopic, and X-ray crystallographic tools to elucidate the mechanism of key enzymes in the DMSP biodegradation pathway. Revealing the mechanistic and structural relevance of these novel metalloenzymes is the first step towards understanding the enzymatic mechanism of DMSP degradation, which will further our understanding on how marine microbes use metals to assimilate organosulfur compounds.
STUDIES TOWARDS SYNTHESIS OF TRUNCATED SUPERSTOLIDE A

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Marine natural products are of considerable interest because of their structural novelty, functional diversity and potent biological activity. Superstolides A and B are marine macrolides, isolated in minute amounts from the deep-water marine sponge *Neosiphonia superstes*, collected off New Caledonia, an island in the Pacific Ocean. The structural novelty of these two molecules is characterized by a unique 16-membered macrolactone attached to a functionalized cis-decalin. Both superstolides A and B exhibit potent antiproliferative effects against several tumor cell lines with IC$_{50}$ values ranging from 4.8 to 64 nM. Their structures are novel and unprecedented, suggesting that they might have a unique cellular target(s) and a novel mechanism of action. However, due to the scarcity of these marine natural products, there has not been enough material for further biological investigation and the cellular target of Superstolides have not been identified.

The lack of sufficient amount of natural products coupled with the overwhelming difficulty in the development of a practical total synthesis approach entails designing of simplified superstolide A. Herein, we report for the first time the design and studies towards the synthesis of a truncated superstolide A, which contains the basic pharmacophore of a 16-member lactone ring attached to a cyclohexene ring which can be easily synthesized in a shorter reaction sequence. The design of truncated Superstolide A is based on the hypothesis that the 16-membered macrolactone may be the key pharmacophore that interacts with cellular target(s) while the cis-fused decalin may lock the macrolide into a certain conformation. These modifications would simplify the synthesis substantially and at the same time maintain the basic template of the parent molecule. Such a design is considered important so that it could test our hypothesis on the interaction between the natural product and the receptor and provide important information regarding the structure-activity-relationship and pharmacophore identification. In addition, the synthesis of truncated Superstolide A would also serve as an important model study that would provide some critically important information on the feasibility of key coupling reactions.
GLOBAL NETWORKS IN ENZYMATIC CATALYSIS: STUDIES OF DIHYDROFOLATE REDUCTASE

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Enzymes display vast catalytic power and substrate specificities that preclude possible side-reactions. Although classical enzymology together with structural biology has offered many insights into the chemical mechanisms of enzymes, enzyme dynamics and their relation to catalytic function remain poorly understood. In our study, we have used dihydrofolate reductase from *E. coli* (EcDHFR) as a model to address whether enzymes have networks of coupled motions across the protein which may enhance their catalyzed chemical transformation. Previous studies of the distal mutants G121V and M42W suggested that G121 and M42 are part of a network of coupled motions that enhance catalyzed hydride transfer. In this work, temperature-dependence of intrinsic KIEs for W133F, F125M, G121V-F125M and M42W-F125M mutants have been studied. Based on the results, W133F does not appear to affect the chemical step or be important for hydrophobic stabilization of the protein. However, F125M exhibits inflated KIEs and substantial temperature-dependence, indicative of residues that affect the chemical step. To examine the possibility that F125 is also a part of global dynamic network in EcDHFRs, temperature dependence of intrinsic KIEs for a double mutants M42W-F125M and G121V-F125M were studied and found to be steeply temperature dependent, indicative of synergistic effect between these residues. These findings suggest that F125M is part of the global dynamic network in EcDHFRs.
Tamoxifen is a Selective Estrogen Receptor Modulator (SERM) and works by blocking the actions of estrogen to inhibit cell proliferation that results from estrogen binding. While tamoxifen has been successfully utilized for many years in treatment of estrogen-dependent breast cancer, cases of endometrial cancer and cardiovascular disease have been reported in some women following treatment. Human hydroxysteroid sulfotransferase (hSULT2A1) is a drug metabolizing enzyme that catalyzes the formation of a carcinogenic α-sulfonoxy derivative of tamoxifen capable of binding to DNA. On the other hand, the enzyme has a critical role in the formation of pregnenolone sulfate, an endogenous neurosteroid that has been implicated in the prevention of heart disease. Our research addresses the extent to which the various metabolites of tamoxifen can modulate the activities of sulfotransferases to gain insight into the mechanisms of tamoxifen toxicity in breast cancer patients. We hypothesize that major metabolites of tamoxifen regulate the catalytic activity of hSULT2A1 either through direct inhibition or through serving as alternate substrates for the enzyme. Our studies show endoxifen is the most potent inhibitor of hSULT2A1-catalyzed sulfation of pregnenolone and dehydroepiandrosterone when compared to N-desmethyltamoxifen and 4-hydroxytamoxifen. Subsequent assays indicate that only N-desmethyltamoxifen is a substrate for the enzyme. These results may be useful in interpreting clinical trials of endoxifen and in improving the design of related molecules. [Supported by NIH grant CA038683]
GAPDH IS A TARGET OF THE ENDOGENOUS 3,4-DIHYDROXYPHENYLACETALDEHYDE: ENZYME INHIBITION AND COVALENT MODIFICATION

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Parkinsons disease is a slow-progressive neurodegenerative disorder affecting 5-6 million people around the globe. The disease is manifested by the rapid deterioration of dopaminergic cells in the substantia nigra portion of the brain; however, the pathological mechanism of selective dopaminergic neuronal death is unknown. Dopamine is oxidatively deaminated and catalyzed by monamine oxidase to form the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehyde (DOPAL). The reduction in levels of DOPAL is biologically critical as this aldehyde has been shown to be toxic to dopaminergic cells and is a highly reactive electrophile. Investigating neuronal protein targets of the DOPAL electrophile is essential in determining the cause of dopaminergic cell toxicity. An essential protein, Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) is an abundantly expressed enzyme known for its glycolytic activity and recent research has directly implicated its role in oxidative stress-mediated neuronal death. GAPDH has been shown to be highly susceptible to covalent modification and inactivation by DOPAL. Upon treatment with DOPAL (5-25 µM), enzyme activity was significantly inhibited compared to control. In order to assess the mechanism of enzyme inhibition by DOPAL, enzyme was digested with trypsin and analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). The resulting tryptic peptides were mapped for possible sites with DOPAL modification. A total of eight adduction sites with Lys and Arg residues were discovered. Of these seven, five are within the Rossman Fold of the enzyme. This NAD⁺ binding domain is crucial in maintaining normal enzyme function. Disruption of cofactor binding by small molecules such as DOPAL can be detrimental for enzyme activity. In addition, extensive GAPDH crosslinking by DOPAL was observed using Western blotting. Given GAPDH’s intracellular abundance and its pivotal role in multiple metabolic and apoptotic pathways, compromise on protein structure and enzymatic activity may have devastating effects on cellular homeostasis. Thus, GAPDH is a viable target of modification by DOPAL. In addition, the intracellular formation of GAPDH may serve as a cellular phenotype in neurodegenerative disorders.
ACCOUNTING FOR INTERSTITIAL WATERS IN INHIBITOR DESIGN: EVIDENCE OF AN ACTIVE ROLE OF WATER IN GLUTAMATE RACEMASE

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Interstitial waters in enzyme-receptor active sites represent a significant challenge in structure-based drug design. The mutagenesis of active site residue, asparagine 75, in glutamate racemase from Bacillus subtilis (RacE) to alanine, or leucine, does not hinder catalytic function, but yields a cryptic effect on inhibitor binding affinities, which is readily explained by examining the role of interstitial water. MD simulations show the N75A mutant enzyme's active site to be joined with a water channel, which is not accessible in the native enzyme or the bulkier N75L mutant enzyme. The nexus of the RacE-N75A active site and water channel results in a concomitant introduction of an interstitial water molecule within the active site. MD studies on the native and mutant enzymes show that the active site-water channel nexus only exists in the N75A enzyme. Importantly, this N75A mutation allows for tight control over the presence or absence of an interstitial water molecule in the experimental system.

In addition to altering the KM of one of the natural substrates (D-glutamate), the interstitial water associated with RacE-N75A has striking effects on inhibitor binding. Two competitive inhibitors, previously characterized by our research group, were assayed against wild-type, RacE-N75A and RacE-N75L. In the case of croconic acid, the N75A mutation results in a mild, 2-fold increase in Ki (inhibitor binding constant). In the case of 1H-benimidazole-2-sulfonic acid, the mutation results in 26-fold increase in Ki, an approximately +1.97 kcal/mol loss in binding energy. Docking and steepest-descent minimization show this inhibitor molecule competing with the introduced interstitial water molecule for binding to the back of the active site. Thus, the ΔAG for inhibitor binding is hypothesized to be the energetic cost for ejecting an interstitial water, which is in good agreement with MD studies on the free energy of moving a single water molecule from a favorable interstitial location to bulk solvent (+2.77 kcal/mol; Helms and Wade, Biophysical Journal, 1995, 69: 810). These results imply an active role of water in ligand binding where interstitial waters, if correctly considered, can substantially dampen or enhance the potency of inhibitors.

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COMPUTATIONAL STUDIES SUGGEST A NEW REACTION INTERMEDIATE FOR THYMIDYLATE SYNTHASE

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The cleavage of covalent C-H bonds is one of the most energetically demanding yet biologically essential chemical transformations. To interpret our recent experimental observations,1 we used QM/MM potentials to examine the C-H→B (B: general base) proton abstraction catalyzed by thymidylate synthase (TSase). Since TSase catalyzes the last step of de novo synthesis of thymidylate (i.e. the DNA base T), it is a common target for antibiotic and chemotherapeutic drugs. Our previous calculations suggested a new reaction intermediate that does not form a covalent bond with TSase, providing a new direction for drug designs that target DNA synthesis. The current work employs higher-level simulations to verify this new mechanism, and reveals that the cleavage and formation of a very labile C-S bond are concerted with the proton abstraction and the following C-N cleavage, respectively. Collective protein motions assist an arginine residue to approach and polarize the C-S bond, thereby promoting the proton abstraction step. These promoting vibrations are similar to those reported for the C-H→C hydride transfer that is rate limiting for the turnover of TSase.2 Our simulations also revealed different features of these two C-H bond activations, suggesting a more variable transition state for the proton abstraction than the hydride transfer. These observations agree with our experimentally measured temperature dependence of kinetic isotope effects on both C-H cleavages, and implicate an indispensable role of protein motions in enzymatic reactions.

References:
NOVEL FLUOROQUINOLONE ANALOGS WITH ANTIMUTANT ACTIVITY

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Antibiotic resistance eventually emerges in the treatment of most bacterial infections because current pharmaceutical practices place antibiotic dosing levels directly within the mutant selection window (MSW). The MSW is a dosing range between the minimum inhibitory concentration (MIC) of wild-type bacteria and the mutant prevention concentration (MPC) of the least susceptible mutant populations. Dose levels below the MIC do not effectively lower bacterial populations to combat disease, and dose levels above the MPC are possibly toxic. Though doses within the MSW effectively treat infection, the resultant selection of mutant strains has given rise to highly resistant bacteria such as multi-drug resistant (MDR) and extensive drug resistant (XDR) M. tuberculosis. The programmatic goal of the work presented here is to develop novel approaches for the development of new antibiotics that restrict the emergence of resistant strains of M. tuberculosis by having a very narrow MSW. In the studies presented here, computer modeling was employed to generate further understanding of the drug binding sites in topoisomerase IV and DNA gyrase A, the protein targets of ciprofloxacin and other antibiotics, to design new antimutant fluoroquinolones. The synthesis of aryl-substituted C-7 fluoroquinolone analogues will be described and their activity with wild-type and fluoroquinolone-resistant bacteria will be presented.
The stellettins (e.g. 1–4), a small family of natural products isolated from marine sponges, have been of interest because they exhibit potent and differential cytotoxicity in the National Cancer Institute’s (NCI) 60 cell–line assay. Furthermore their pattern of activity does not correlate to any known mechanism of action. For some years our group has pursued synthesis of the schweinfurthins, and many of the goals of that program have been met. While there is little similarity of structure between the schweinfurthins and the stellettins, there is a strong correlation between their profiles of biological activity, with the most prominent activity against CNS, leukemia, and renal cancer cell lines. This strong correlation suggests that both families of compounds affect the same target or at least the same biological pathway, but their target remains unknown. Moreover, the stellettins are both more potent and more selective than the schweinfurthins. Because the stellettins are not readily available from their natural source, we have decided to pursue a chemical synthesis of these complex natural products.

Synthesis of stellettin A has been divided into a left and right half and poses two main challenges: stereochemistry of the left half ring system and the polyene tail in the right half. The stellettins feature a \textit{trans–syn–trans} tricyclic core that causes the B ring to be in a strained twisted boat conformation. Therefore, care must be taken to control the stereochemistry around this ring so that the \textit{trans–anti–trans} system is not formed. Installation of the polyene tail late in the synthesis of stellettin A is imperative because it isomerizes and decomposes when exposed to light, so our initial efforts have focused on preparation of the tricyclic core. Key steps in the formation of the left half include oxidation of farnesol, Wittig olefination, hydroboration-oxidation to provide homofarnesol, bromination of the alcohol, addition of trimethyl(propargyl)silane via an alkylation reaction of the bromide, and an asymmetric epoxidation to introduce a stereogenic center. The epoxidation will be followed by a cascade cyclization initiated by a Lewis acid, which opens the epoxide to afford the desired carbocycle. Our synthesis of the stellettin family will allow a more in-depth exploration of the biological activity of these rare, but very potent compounds.

1 \text{R=R’}=\text{O}, 13-\text{E}= \text{stellettin A}
2 \text{R=R’}=\text{O}, 13-\text{Z}= \text{stellettin B}
3 \text{R=H, R’}=\text{OAc}, 13-\text{E}= \text{stellettin C}
4 \text{R=H, R’}=\text{OAc}, 13-\text{Z}= \text{stellettin D}
METABOLIC ENGINEERING OF E. COLI FOR PRODUCTION OF HIGH-VALUE METHYLXANTHINES FROM CAFFEINE, THEOPHYLLINE, AND THEOBROMINE

Ryan M. Summers2, Sridhar Gopishetty2 and Mani Subramanian1,2*
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Alkylxanthines are naturally occurring compounds that are used in pharmaceutical preparations as diuretics, asthma treatments, and cardiac, pulmonary, and neurological stimulants. The most common of these compounds, caffeine, is also found in many common food and beverage products such as coffee, tea, soft drinks, and chocolates. Because of their wide use, caffeine and related alkylxanthines enter the environment through human waste water and both solid and liquid wastes from coffee and tea processing facilities. Not surprisingly, bacteria capable of growing on caffeine have been isolated previously. However, the mechanism of bacterial caffeine degradation has not been elucidated until now.

We have isolated a caffeine-degrading bacterium, Pseudomonas putida CBB5, capable of growing on caffeine as the sole source of carbon and nitrogen. CBB5 degrades caffeine via sequential N-demethylation to theobromine or paraxanthine, then to 7-methylxanthine, and finally to xanthine. CBB5 also sequentially N-demethylates theophylline, which has not been previously reported to be degraded by bacteria, to xanthine. CBB5 contains five novel N-demethylase genes, ndmA-BCDE, which are responsible for caffeine degradation. These genes have been isolated, cloned, heterologously expressed, and their biochemical properties have been confirmed. NdmA and NdmB are Rieske monooxygenases that catalyze positional-specific N1- and N3-demethylations, respectively. Both enzymes receive reducing equivalents from NADH via a redox-center-dense Rieske reductase, NdmD. NdmC is an N7-demethylase with activity specific for 7-methylxanthine and is active only with NdmD and NdmE, a glutathione-S-transferase homolog.

Using ndmABD, we have metabolically engineered 31 different strains of E. coli for production of the high-value methylxanthines paraxanthine, 1-methylxanthine, 3-methylxanthine, and 7-methylxanthine. These derivatives of E. coli strain BL21(DE3) were engineered with five pET32a and six pACYCDuet-1 expression vectors. The pET32a vectors contain a polycistronic insert comprised of ndmA plus ndmD or ndmB plus ndmD with different ribosomal binding sites between the two genes. The pACYCDuet-1 vectors contain two genes, one in each multiple cloning site, and provide each possible combination of ndmABD genes. These new strains have the potential to rapidly produce high value methylxanthines from inexpensive natural substrates, such as caffeine, theophylline, and theobromine.
POTENTIAL RESURRECTION OF AGED AChE-OP ADDUCTS

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Organophosphates (OP) are exquisite transition state analogue inhibitors of the cholinergic system. The potency of organophosphates makes them both effective pesticides and horrible chemical warfare agents. The development of antidotes to organophosphates from both deliberate and accidental exposure is of high priority due to the millions of cases of mortality or morbidity caused by these agents each year. The classical antidote, 2-PAM, has several shortcomings; most significantly it is unable to reactivate “aged” AChE-OP adducts. To overcome this limitation, we have developed 2-PAM analogues which should be capable of “resurrecting” an “aged” acetylcholinesterase (AChE) complex by selective methyl transfer to the AChE-OP adduct. Presented here are our initial results utilizing 2-methoxypyridinium species as methyl transfer agents and a detailed kinetic analysis of a model system.
STRUCTURE DETERMINATION AND PEPTIDOGLYCAN BINDING ASSAYS OF SPOR DOMAIN FROM THE *Escherichia coli* CELL DIVISION PROTEIN DamX

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*Sporulation*-related (SPOR) domains are found in many bacteria with over 5000 proteins with a SPOR domain annotated in the Pfam database. SPOR domains are ~75 a.a. long, localize to the division site, and are thought to bind to some form of peptidoglycan (PG) that accumulates transiently at the midcell during cell division. In *E. coli*, there are four SPOR domain proteins: FtsN, DamX, DedD, and RlpA, all of which are involved in cell division. Interestingly, DamX has multiple functions and its SPOR domain (DamX<sub>SPOR</sub>) has low similarity to other SPOR proteins. To understand the molecular mechanism and role of DamX in cell division, we determined the solution structure of the SPOR domain from DamX by NMR. DamX<sub>SPOR</sub> comprises a $\beta_1\alpha_1\beta_2\alpha_2\beta_3\alpha_3$ secondary structure and forms a 4-stranded antiparallel $\beta$-sheet; $\alpha_1$ and $\alpha_2$ associate with the top of the sheet, while $\alpha_3$ associates with the bottom. Site-directed mutagenesis revealed that a single mutation of Q351, S354, or W416 impairs localization to the midcell and binding to PG. These amino acids are in the $\beta$-sheet, so we propose that the $\beta$-sheet is important for targeting SPOR domains to septal PG. Deletions of $\alpha_3$-helix destabilized the domain, but there was still considerable septal localization when most of $\alpha_3$ was removed. To understand how DamX<sub>SPOR</sub> binds to PG, we use HSQC NMR to look for interactions between DamX<sub>SPOR</sub> and analogs of the glycan backbone of PG. Titration with four different PG analogs elicited chemical shifts for several residues. Some of these residues may contribute to PG binding, while others may be in parts of the domain that undergo a conformational change.
Optically active 2-hydroxy acids are important building blocks for asymmetric synthesis of numerous bioactive molecules (e.g., ACE inhibitors, A2 antagonists, Ca-channel blockers, beta-blockers) including glycols, halo esters and epoxides. Glycolate oxidase (GO, EC 1.1.3.15) is a flavin mononucleotide-dependent enzyme, which catalyzes the oxidation of (S)-2-hydroxy carboxylic acids such as (S)-lactate to the corresponding 2-keto acids. Spray-dried whole cells of Pichia pastoris expressing GO from spinach were used for the dynamic resolution of 2-hydroxy acids. As GO is absolutely specific on (S)-enantiomer, GO catalyzed oxidation of racemic (RS)-2–hydroxy acids produced 2-keto acids, only from (S)-enantiomer, keeping (R)-isomer intact. Non-selective reduction of 2-ketoacids using sodium borohydride produced (RS)-2–hydroxy acids to facilitate a one-pot dynamic process for production of (R)-2–hydroxy acids with high yield (> 95%). GO could be recycled twice in this dynamic process with > 92% yield of the (R)-acids. The entire “one pot – two step” process was carried out in water and at room temperature in order to make the process economical. Finally, (R)-2-hydroxy acids were purified from the crude post-reaction mixtures by cation exchange using a Semba Octave™ simulated moving bed chromatography (SMBC) system. The initial SMBC conditions were determined using triangle theory based separation parameters, and optimized by adjusting those parameters based on the analysis of the purified fractions. Continuous separation of the hydroxy acids from their 2-keto acids and other reaction components was achieved with yields >80% and purities >99%.
MODULAR AND EXPANDABLE DETECTION PLATFORM FOR CURRENT AND POTENTIAL FOOD TOXINS AND ADULTERANTS: IDENTIFICATION AND ISOLATION OF RICININE NITRILASE FROM MICROBIAL SCREENING AND SOIL ISOLATES

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**Background:** The castor seed contains about 40% oil, 1-5% ricin, and 0.3-0.8% ricinine. Castor plant is of industrial importance because many products including oils, lubricants, pharmaceuticals, cosmetics, and engineering plastics (e.g., Nylon 11) are made from castor oil or its derivatives. Unfortunately, ricin is also one of the most potent plant toxins found in castor plant. Lethal doses (LD) for this protein are estimated to be 0.70 mg to 70 mg for a 70-kg adult. Ricinine itself is a toxic compound that is also a marker for ricin. It has a typical LD\textsubscript{50} in mice of 25 mg/kg body weight in subcutaneous administration. The Department of Homeland Security has recognized detection of Ricinine/Ricin as high priority. Analytical methods have been published for determination of ricinine in castor bean plant material, beverages, and urine by HPLC, LC-MS, etc. However, there are no reports of a rapid enzymatic method for the analysis of ricinine. Previously, we identified two strains, *Acinetobacter* AP1200, and *Burkholderia cepacia* R34 from CBB/UI culture collection, and two soil enrichment strains, SE No.21 and SE No.28 that showed ricinine nitrilase activity. All four strains were submitted for sequencing in order to annotate nitrilases, and clone them to confirm ricinine nitrilase.

**Methods:** Six putative nitrilase genes were annotated from the genomic DNA sequences of four strains and cloned into *E.coli* BL21(DE3) by the University of Minnesota group. Recombinant His-tagged nitrilases in *E. coli* BL21(DE3) were purified by immobilized-metal affinity chromatography (IMAC). A rapid colorimetric assay, indophenol blue method (Berthelot assay), was used for the analysis of nitrilase activity against ricinine via quantitative detection of ammonia/ammonium formation.

**Results:** All six putative nitrilases-His\textsubscript{6} were expressed as soluble form and purified from Ni-column. However, no enzyme activity was observed on ricinine from any of purified preparations. Since chaperones, GroEL and GroES have been reported to help proper protein-folding, all six putative nitrilase genes in pET28b were co-expressed with the chaperone in the plasmid pAG. The nitrilases were then purified by IMAC chromatography and assayed with ricinine. Interestingly, two nitrilases, Nit1-SE 21 from strain SE No.21 and Nit1-R34 from *Burkholderia cepacia* R34 with chaperones were found have activity with ricinine. Time course analysis of ammonia formation from ricinine showed that recombinant nitrilase, Nit1-R34 expressed in the presence of the chaperone produced ammonia over a 2-hour period. No ricinine activity was detected from other four putative nitrilases, also cloned with chaperones. Protein fractions from three control *E.coli* strains containing only plasmids had no activity on ricinine.
SCALABLE HIGH THROUGHPUT SCREENING AND ITS APPLICATIONS

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The University of Iowa High Throughput Screening Facility (UIHTS) is a high throughput platform that integrates robotics, detection systems and chemical/biologics libraries. UIHTS enables scalable screening approaches, not only to foster hit and lead generation for drug discovery and development through screening of large chemical/biologics libraries; but also to facilitate molecular probe discovery for mechanism of action (MOA) studies of chemical biology through screening of focused intellectually-designed compound collections. As a university core facility, UIHTS provides highly flexible screening services, project management, and assay/technology development for investigators across campus as well as off-campus. Multiple examples of scalable HTS approaches will be presented.
CONTINUOUS, REAL-TIME MONITORS FOR PICHIA PASTORIS BIOREACTORS

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ASL Analytical is currently developing real-time monitors for controlling and optimizing the production of bio-therapeutics and industrial enzymes. Initial product offerings target the versatile, protein expression bioreactors of Pichia pastoris. The ASL Pichia Monitor continuously and noninvasively tracks the concentrations of the key metabolites, glycerol and methanol, as well as cell biomass density in real time during the fermentation process. Glycerol and methanol are the principal carbon sources for Pichia pastoris and the ability to monitor, control, and optimize the concentrations of these substrates is critical for the efficient production of the targeted protein product. Currently, key metabolites are monitored using off-line analyses that are time consuming and labor intensive, rendering any real-time feedback during bioreactor operation virtually impossible. The ASL Pichia Monitor offers the first, non-invasive, real-time monitoring of Pichia bioreactors, independent of reactor size and operating condition. In another first for the industry, the ASL Pichia Monitor has also been designed to provide closed-loop feedback control for both glycerol and methanol concentrations during the fermentation process. ASL’s Bioreactor Monitors are currently undergoing tests at the CBB.
Bioprocess development currently begins with screening in simple and uncontrolled cultivation systems such as shake flasks or microtiterplates, allowing researchers to gain only limited information. This situation changes with the application of microbioreactors. Microbioreactor platforms such as the BioLector® use small arrays of micro vessels for culturing cells. The BioLector® is based on microplates with the potential for high oxygen transfer rates (0.15 mol/L/h), allowing for improved consistency at large scale. The BioLector® measures highly relevant fermentation parameters such as biomass, fluorescence, pH and DO values online in up to 48 parallel wells. Recently, this system was integrated into standard liquid-handling systems for automated media preparation and high-throughput experimentation. The RoboLector® systems provide high-information content and automation to study bioprocesses more precisely and to dramatically reduce efforts and costs.

Here we present data on the application of Design of Experiments (DoE) in fed-batch fermentation and media optimization. The whole factorial design was imported into the RoboLector® software and applied for automated experimental set-up and execution. RoboLector® results are shown with *Pichia pastoris* expressing a lipase under control of AOX1-Promotor, comparing automated pulsed feeding of glycerol, nitrogen and methanol for growth and induction. The results give detailed understanding into the bioprocess from microbioreactors that are difficult to achieve in bench scale laboratory fermenters.
REGULATION OF CALCINEURIN BY DOMAIN-SPECIFIC INTERACTIONS WITH CALMODULIN

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Calcineurin (CaN) is a heterodimeric Ser/Thr phosphatase. Calcineurin regulates diverse biological functions, from T-cell activation to critical events in early heart development. The enzymatic activity of the A subunit (CaNA) is regulated by calcium in two ways: direct binding of Ca2+ to its B subunit (CaNB), and binding of the two-domain calcium-binding protein calmodulin (CaM) which is required for full activity. CaNA contains an auto-inhibitory domain that prevents full activation of CaN even when CaNB is fully saturated by Ca2+.

Interactions between CaM and CaN occur through a short CaM-binding domain (CaMBD). This sequence (aa 391-414 of CaNA) is a BAA-motif (basic amphipathic alpha-helix) in a region that becomes disordered when CaNB binds calcium (see figure). (Ca2+)4-CaM has very high affinity for CaN. The dissociation constant is near pM, as determined by thermodynamic linkage (O’Donnell et al, Proteins 2011). Creamer and coworkers (Rumi-Masante et al, JMB 2012) have shown that the effect of CaM on the ordered secondary structure of the full regulatory region of CaNA extends beyond this CaMBD.

We have also determined that calcium binding to CaM bound to the CaMBD is sequential, with the C-domain sites (III and IV) saturating at lower calcium than the N-domain sites (I and II). Thus, we are investigating a hypothesis that the mechanism of calcium-induced activation of CaN requires at least three steps that utilize the two domains of CaM (N and C) differently. We are using “knockout” mutants of CaM with modifications in one or more of the calcium-binding sites, and monitoring their binding to biosensor proteins that contain the CaMBD embedded between auto-fluorescent proteins YFP and CFP. Without CaM binding the fluorescent proteins are free to undergo FRET, while CaM binding to the BAA motif reduces FRET efficiency and allows calculations to be made on CaM-biosensor interaction kinetics. We will report domain-specific effects of these mutations on recognition of this region.

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## CBB/NIH Fellowships

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