THE UNIVERSITY OF IOWA

23rd

Center for Biocatalysis and Bioprocessing Conference

Current Topics in Industrial Biotechnology

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THE UNIVERSITY OF IOWA

October 20-21, 2014
Iowa Memorial Union
Iowa City, Iowa

THE UNIVERSITY OF IOWA

23rd Annual
Biocatalysis and Bioprocessing
Conference

"Current Topics in Industrial Biotechnology"

Sponsored by:

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Biocatalysis and Bioprocessing Conference

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Center for Biocatalysis and Bioprocessing

October 20-21, 2014

Conference Organizing Committee:

Mani Subramanian, Ph.D.
Sridhar Gopishetty, Ph.D.
Charles Brenner, Ph.D.
Alexander Horswill, Ph.D.
Robert Kerns, Ph.D.
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Eric Nuxoll, Ph.D.
Tonya Peeples, Ph.D.
Daniel Quinn, Ph.D.
Mitchell Rotman, M.S., M.H.A., M.B.A.

Director
Mani Subramanian, Ph.D.

Special Thanks to Jo Dickens of the UI Center for Conferences

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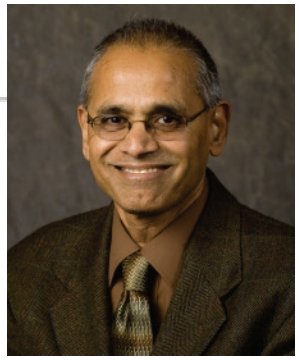
Message from the CBB Director



The Center for Biocatalysis and Bioprocessing (CBB), The University of Iowa, has been conducting an annual conference in October of every year, for the past 22 years. This year, the 23rd CBB Conference is scheduled for October 20 and 21. The conference will be held in the beautiful downtown campus of The University of Iowa with world-renowned speakers, representatives and attendees from industry and academia. In addition to major presentations from experts, the conference also features short presentations and posters from graduate students. The proceedings are conducted in a friendly, open atmosphere to promote interaction between experts, faculty from The University of Iowa, post-doctoral fellows and students. Several major industries have consistently provided 'gift in kind' to defray the cost of conference.

We thank them for continued support. Traditionally, the theme of the CBB Conference has been on cutting edge technology in the areas of "Biocatalysis and Biotechnology" in the broadest sense, including enzyme-mediated chemical transformations and mechanisms, metabolic engineering, directed evolution, genomics, agricultural feedstock utilization, protein expression/production, and bioprocessing.

I would like to welcome all the attendees for this conference and also thank the sponsors. I am looking forward to another enjoyable scientific meeting.

Sincerely,
Mani Subramanian
Director, Center for Biocatalysis and Bioprocessing
Professor, Chemical & Biochemical Engineering
The University of Iowa

The University of Iowa
Center for Biocatalysis and Bioprocessing

23rd Annual Center for Biocatalysis and Bioprocessing Conference
"Current Topics in Industrial Biotechnology"
Iowa Memorial Union, Iowa City, IA

MONDAY, OCTOBER 20, 2014

4:30 pm Registration – outside Ballroom, 2nd Floor IMU

4:30 – 6:00 Poster set up and display – Ballroom, 2nd Floor IMU

4:30 Tour of CBB

6:00 – 7:00 Welcome Dinner– Ballroom, 2nd Floor IMU

7:00 – 8:00 **Sonya J. Franklin, Ph.D.**, Director, Microbial Traits, Monsanto Company,
"Microbial Traits for Sustainable Agriculture"

TUESDAY, OCTOBER 21, 2014

7:00-8:00 am Registration – Iowa Theater Lobby, 1st floor IMU

7:30 Continental Breakfast – across from Iowa Theater, 1st floor IMU

8:30 **Program** – Iowa Theater, 1st floor IMU
Introduction and Welcome
Mani Subramanian, Ph.D., Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research and Economic Development, The University of Iowa
Professor, Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa

9:00 **J. Eric Lee,** Fermentation Market Manager, Roquette America
"*Advancing Industrial Applications in Green Chemistry, The View from 2015 and Beyond"*

9:45 **Basil Nikolau, Ph.D.**, Frances M. Craig Professor of Biochemistry, Biophysics and Molecular Biology, Iowa State University
"The Fundamental Metabolic Underpinning for Bioengineering Solutions"

10:30 Break – Iowa Theater Lobby, 1st floor IMU

10:45 **Dan Widmaier, Ph.D.,** CEO and Founder**,** Refactored Materials Inc.
"Producing Silk Polymers for Commercial Applications"

11:30 Lunch – Ballroom, 2nd Floor IMU

Afternoon Session – Iowa Theater, 1st Floor IMU

1:15 pm **Mark A. Arnold, Ph.D.,** Edwin B. Green Chair Professor in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa
"Insights and Optimization of Bioreactor Processes through Real-time, Noninvasive Monitoring of Cellular Nutrients"

2:00 – 3:00 **NIH/CBB Fellow Presentations**
Three selected graduate students will each have a 15 minute talk followed by a 5 minute Q&A session

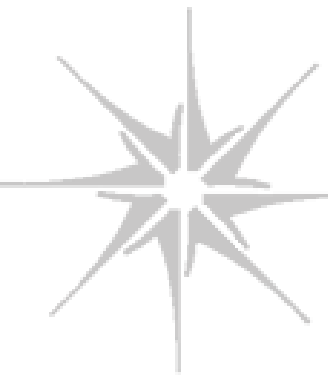
Brigitte C. Vanle, Doorn Research Group,
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa
"Protein Reactivity and Enzyme Inactivation by an Aldehyde Metabolite of Dopamine"

Aashay Shah, Jin Research Group,
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa
"Synthesis and Biological Evaluation of Truncated Superstolide A"

Zahidul Islam, Kohen Research Group,
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa
"Hydride Transfer Mechanism in Thymidylate Synthase"

3:00 – 5:00 **Poster Session** – Wine/hors d'oeuvres, Ballroom 2nd Floor IMU
Announcement of Usha Balakrishnan's Award Winner
Announcement of Director's Award Winner
Product Show presented by S3-Scientific Sales Solutions

5:00 **Adjourn**



List of Oral Presentations

ORAL PRESENTATIONS

1. MICROBIAL TRAITS FOR SUSTAINABLE AGRICULTURE
Sonya J. Franklin, Ph.D.
Director, Microbial Traits, Monsanto Company, Chesterfield, MO

2. ADVANCING INDUSTRIAL APPLICATIONS IN GREEN CHEMISTRY, THE VIEW FROM 2015 AND BEYOND
J. Eric Lee
Fermentation Market Manager, Roquette America, Geneva, IL

3. THE FUNDAMENTAL METABOLIC UNDERPINNING FOR BIOENGINEERING SOLUTIONS
Basil Nikolau, Ph.D**.**
Frances M. Craig Professor of Biochemistry, Department of Biophysics and Molecular Biology, Iowa State University, Ames, IA

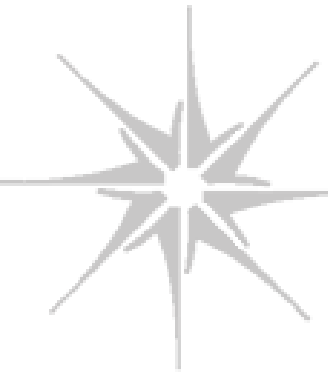
4. PRODUCING SILK POLYMERS FOR COMMERCIAL APPLICATIONS
Dan Widmaier, Ph.D.
CEO and Founder**,** Refactored Materials, Inc., San Francisco, CA

5. INSIGHTS AND OPTIMIZATION OF BIOREACTOR PROCESSES THROUGH REAL-TIME, NONINVASIVE MONITORING OF CELLULAR NUTRIENTS
Mark A. Arnold, Ph.D.
Edwin B. Green Chair Professor in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

6. PROTEIN REACTIVITY AND ENZYME INACTIVATION BY AN ALDEHYDE METABOLITE OF DOPAMINE
Brigitte C. Vanle, Virginia Florang, and Jonathan Doorn*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

7. SYNTHESIS AND BIOLOGICAL EVALUATION OF TRUNCATED SUPERSTOLIDE A
Aashay K Shah, Lei Chen, and Zhendong Jin*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

8. HYDRIDE TRANSFER MECHANISM IN THYMIDYLATE SYNTHASE
Zahidul Islam, Timothy S. Strutzenberg, Ilya Gurevic and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA



Speaker's Profiles

KEYNOTE SPEAKER

Sonya J. Franklin, Ph.D.
Director, Microbial Traits
Monsanto Company

Sonya Franklin joined Monsanto in January of 2007, after nine years on the faculty of the University of Iowa. She grew up in northwestern Iowa, and attended Carleton College in Northfield, MN, where she majored in Chemistry. She then moved to the "Left coast," where she received her Ph.D. degree in Inorganic Chemistry at the University of California, Berkeley, working on lanthanide MRI contrast agents. Her interests continued to drift more biological, so following graduate school, she took a NIH-funded postdoctoral position at the California Institute of Technology working on metallointercalators in DNA. In 1998, she returned to Iowa and began her independent career in the Chemistry Department at the University of Iowa, where she received tenure in 2004, and graduated seven Ph.D. students. Her research at Iowa comprised several interrelated projects in bioinorganic chemistry, with the major focus of the lab on de novo metalloprotein design. When she was approached with the opportunity to do structure, function, and design at Monsanto as the Protein Science Lead, she left her academic career and has had no regrets.

At Monsanto she has held various roles leading teams in the areas of Genomic and Protein Sciences, including sequencing, bioinformatics, cell biology, and protein characterization and design. She has served as the Cambridge Site Lead, Director of the Protein Technologies Program, and then the Enabling Technologies Program within Biotechnology. She has recently taken a new role as Biotechnology Microbial Traits Program Director, responsible for the discovery and development of Microbial-based foliar and seed treatment products, as well as for the development of our Biotechnology transgenic disease product pipeline.

Sonya lives in Chesterfield with her husband, a chemistry professor (Maryville) and Moto Guzzi aficionado, their daughter the rambunctious second grader, and two slightly unruly young cats.

J. Eric Lee
Fermentation Market Manager/ Commercial Sales
Roquette America

Eric joined Roquette America in 2011 as a Project Coordinator within the Industrial Business Unit. Eric currently provides technical support for all Roquette products used within the Fermentation/Bio-Industries market segment, as well as assisting in development of new products. Eric is also responsible for Fermentation Market Development as a part of the Roquette global team. Eric has nearly 30 years of experience in the fermentation industry, primarily related to fermentation optimization, strain selection, process scale up, and technology transfer. Eric has led several teams to commercialization of novel fermentation products over the course of his career. Eric received BS degrees in Biology and Chemistry from Manchester College. He is a member of the Society for Industrial Microbiology.

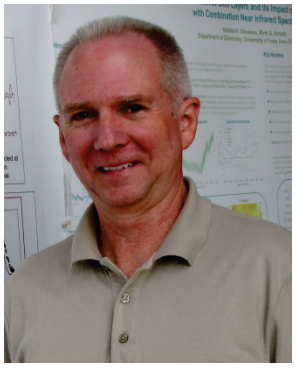
Basil Nikolau, Ph.D.
Frances M. Craig Professor, Department of Biochemistry, Biophysics and Molecular Biology
Center for Metabolic Biology (Director)
Engineering Research Center for Metabolic Biology (Deputy Director)
Iowa State University

Nikolau obtained his undergraduate degree and graduate training at Massey University, New Zealand. The PhD in chemistry and biochemistry was awarded in 1981, and he conducted post-doctoral training with Professor Paul K. Stumpf (University of California, Davis) and Dr. Daniel F. Klessig (University of Utah). In 1985 he joined a small plant biotechnology entity, Native Plants Inc. (Salt Lake City, UT) as a Senior Scientist. In 1988 he accepted an academic faculty position in the Department of Biochemistry and Biophysics at Iowa State University. Nikolau's research interests and expertise are focused on the functional genomics of metabolism. Integrating biochemical, molecular, genetic and computational approaches to decipher how complex metabolic networks are structured and regulated. He has published over 100 peer-reviewed manuscripts, 6 patents, and edited a book on metabolomics. He currently provides leadership in the area of metabolism at Iowa State University serving as the Director of the Center of Metabolic Biology, and the Deputy Director of the NSF-funded Engineering Research Center for Biorenewable Chemicals.

Dan Widmaier, Ph.D.
CEO and Founder
Refactored Materials, Inc.

Dan is passionate about turning biology into an engineering medium to solve pressing problems around the world. He holds a PhD in Chemistry and Chemical Biology, specializing in Synthetic Biology from UC San Francisco.

Mark Arnold, Ph.D.
Edwin B. Green Chair Professor in Laser Chemistry
Department of Chemistry
The University of Iowa



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

Brigitte C. Vanle
Ph.D. Candidate
Doorn Research Group
Division of Medicinal and Natural Products Chemistry
Department of Pharmaceutical Sciences and Experimental Therapeutics
College of Pharmacy
The University of Iowa

Brigitte Vanle is from Dana Point, California. She received her Bachelor of Science in Chemistry with a minor in Music Theory from Concordia University Irvine. She is currently a PhD student in the Medicinal & Natural Products Chemistry, a subdivision of the College of Pharmacy. Her main research interest revolves around neurological disorders, including neurotoxicology and neurodegeneration. Her past work experience has been in conducting research and writing technology patents at start-up biotechnology companies in Orange County. After graduation, she intends to pursue a career in pharmaceutical and/or biotechnology industries.

Aashay Shah
Ph.D. Candidate
Jin Research Group
Division of Medicinal and Natural Products Chemistry
Department of Pharmaceutical Sciences and Experimental Therapeutics
College of Pharmacy
The University of Iowa

Aashay is from Mumbai, India. After completing his Bachelors in Pharmacy in 2010, he decided to obtain his Ph.D. at The University of Iowa in the department of Medicinal and Natural Product Chemistry. He is currently a Ph.D. candidate in Dr. Zhendong Jin's laboratory and his research endeavors revolves around the development of novel anticancer agents derived from natural products. After obtaining his Ph.D., Aashay will pursue his career in Industry.

Zahidul Islam
Ph.D. Candidate
Kohen Research Group
Department of Chemistry
College of Liberal Arts and Sciences
The University of Iowa

Zahid graduated from University of Dhaka, Bangladesh, with a chemistry bachelor degree in 2008. Then he went to Japan and obtained his MS in 2010 in Materials Science and Engineering from Toyohashi University of Technology. Right after his graduation, he flew to the other side of the world and joined in the Department of Chemistry at the University of Iowa. Zahid has been working in Kohen lab since then and exploring mechanism of an enzyme required for DNA biosynthesis such as Thymidylate Synthase.

Oral Presentation Abstracts

Presenter 1

MICROBIAL TRAITS FOR SUSTAINABLE AGRICULTURE

Sonya J. Franklin, Ph.D.
Director, Microbial Traits, Monsanto Company, Chesterfield, MO

Agricultural biological products include microbial-based seed treatments and foliar sprays that protect crops from pests and diseases and enhance plant productivity and fertility. By harnessing the natural interactions between the soil microbiome and plants, Microbial treatments can increase plant health and productivity, and help farmers meet global demand for food and feed in a sustainable way. This talk will discuss the discovery and development process for Microbial products in Agriculture, and the business context for sustainable, next generation microbial technologies.

Presenter 2

ADVANCING INDUSTRIAL APPLICATIONS IN GREEN CHEMISTRY, THEVIEW FROM 2015 AND BEYOND

J. Eric Lee
Fermentation Market Manager, Roquette America, Geneva, IL

Applications for Biotechnology and Bio-Industry in the market space continue to evolve in an ever changing landscape. Due to advancements in molecular genetics, fermentation technologies, substrate conversion, and applied engineering we are beginning to observe developing commercial activities in market sectors at an incredible rate. This presentation will focus on the expanding arena of green chemistry at the industrial scale, and what we may expect to develop in the coming years. Of keen interest is sustainable bio-economy where plant based chemistries play a critical role. Discussion of commercial successes in this area, as well as those who have struggled will be reviewed, as well as events that are on the horizon.

Presenter 3

THE FUNDAMENTAL METABOLIC UNDERPINNING FOR BIOENGINEERING SOLUTIONS

Basil Nikolau, Ph.D.
Frances M. Craig Professor of Biochemistry, Department of Biophysics and Molecular Biology, Iowa State University, Ames, IA

Fatty acid biosynthesis is a metabolic process akin to polyketide biosynthesis, in that both processes utilize the properties of a carbonyl group to drive C-C bond formation, and chemically reduce carbon atoms. As such, these processes have been evolutionary adapted to the efficient storage of energy-rich metabolites. Products of these metabolic processes therefore offer considerable opportunities to bioengineer systems for the production of bio-based chemicals and fuels. Because of enzymatic substrate promiscuity and adaptability of the Type II enzyme systems that support fatty acid and polyketide biosynthesis our research has focused on fundamentally understanding these enzymes, and through directed enzyme engineering generate novel bio-based chemicals. The presentation will focus on two enzymes that determine the priming and termination of the Type II fatty acid synthase system, namely 3-ketoacyl-ACP synthase and acyl-ACP thioesterase. The former enzyme determines the chemical structure of the ω-end of the fatty acid products, and the latter enzyme determines the carbon-chain length of the products.

Presenter 4

PRODUCING SILK POLYMERS FOR COMMERCIAL APPLICATIONS

Dan Widmaier, Ph.D.
CEO and Founder**,** Refactored Materials, Inc., San Francisco, CA

Industrial biotechnology has the potential to create new products and technologies that improve the lives of consumers, delight them with new functionalities, while also being produced from renewable resources. The past decade has shown tremendous progress in our understanding of biology as an engineering science to support this vision, but the translation of this work into products has taken an excess of time and money from inception to product with only a few notable successes. As the field progresses, new ventures can intelligently leverage the knowledge, skills and expertise from previous ventures toward substantially reducing development time and costs. Refactored Materials has combined industry expertise and best practices to produce silk polymer products, and provides one example of how lessons learned from prior commercialization efforts can inform prudent technology development, scale up and manufacturing.

Presenter 5

INSIGHTS AND OPTIMIZATION OF BIOREACTOR PROCESSES THROUGH REAL-TIME, NONINVASIVE MONITORING OF CELLULAR NUTRIENTS

Mark A. Arnold, Ph.D.
Edwin B. Green Chair Professor in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Bioprocesses are used to produce a wide variety of biotechnology products across a range of industries, including pharmaceuticals, biofuels, food, agriculture, dietary supplements, specialty chemicals, polymers and others. Between-run consistency and optimal product yield are parameters that directly impact the commercial value of these products. Dissolved oxygen, pH, temperature, and agitation rate are typically measured in real-time during upstream processes and provide valuable information to control and optimize both fermentation and cell cultivation processes. Real-time control of cellular nutrients has been recognized for many years as a means to enhance production consistency and product yield, yet devices capable of continuous monitoring of cellular nutrients have been difficult to realize owing to complications associated with poor reliability of sensing elements under the demanding conditions used for upstream bioprocesses. The lack of accurate and robust sensing technology limits the process engineer to grab samples collected and analyzed periodically during the process. In general, the time delay and sporadic nature of grab samples renders this approach incompatible with real-time control and significantly increases labor costs associated with each bioprocess run.
We are interested in pursuing the utility of noninvasive monitoring for real-time measurements of nutrients within bioreactors as a means to enhance control and productivity. In this measurement, near infrared light passes through the reactor broth and the concentration of the targeted analyte is determined from an analysis of the resulting spectrum. ASL Analytical, Inc. has developed a commercial instrument for real-time bioreactor measurements where a small volume of the reactor broth is circulated externally through a closed-loop passing through the monitor and the concentration of the targeted analyte is determined and provided for control and documentation purposes. The utility of this approach has been demonstrated for measurements of glycerol, methanol, and relative cell density during *Pichia pastoris* fermentations. Accurate operation over many months without recalibration illustrates the robustness of this monitor for bioprocess applications. In this presentation, the analytical utility of this approach will be presented and the enabling capability of real-time monitoring will be discussed in the context of bioprocess control and optimization.

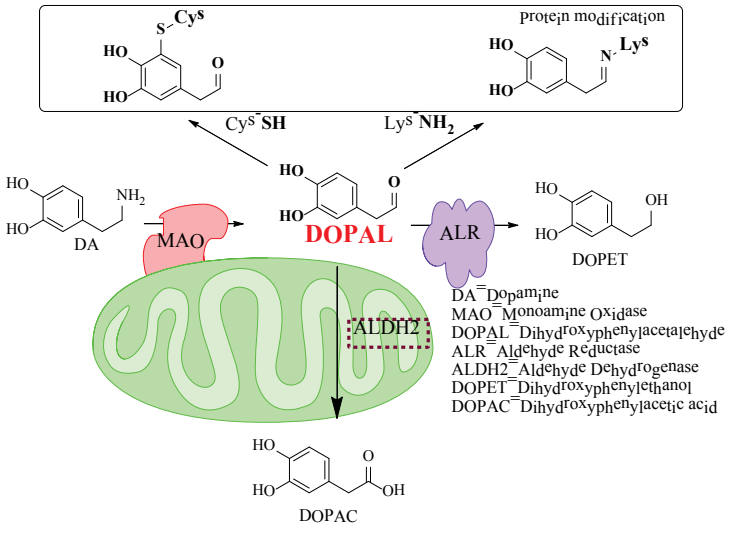
Presenter 6

PROTEIN REACTIVITY AND ENZYME INACTIVATION BY AN ALDEHYDE METABOLITE OF DOPAMINE

Brigitte C. Vanle, Virginia Florang, and Jonathan Doorn*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

Parkinson's 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 monoamine oxidase to form the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehye (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. 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. This work describes the interaction of GAPDH and DOPAL: 1). GAPDH is irreversibly inhibited by DOPAL, but not by analogues lacking the catechol or aldehyde moieties 2). DOPAL modifies thiols and amines on GAPDH 3). Protein adduction yields crosslinking and aggregation in purified and cellular GAPDH. Thus, GAPDH is a viable target of modification by DOPAL. In addition, the intracellular aggregate formation of GAPDH may serve as a cellular phenotype in neurodegenerative disorders.

DOPAMINE METABOLISM AND PROTEIN MODIFICATION OF DOPAL



Presenter 7

SYNTHESIS AND BIOLOGICAL EVALUATION OF TRUNCATED SUPERSTOLIDE A

Aashay K Shah, Lei Chen, and Zhendong Jin*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

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 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 the design and synthesis of truncated superstolide A, which contains the basic pharmacophore of a 16-member lactone ring attached to a cyclohexene ring. 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. Such a design is considered important to test our hypothesis on the interaction between the natural product and the receptor and provide important information regarding the structure-activity-relationship. In summary, truncated superstolide A was successfully synthesized in 15 steps from commercially available starting material in 6.2% overall yield.

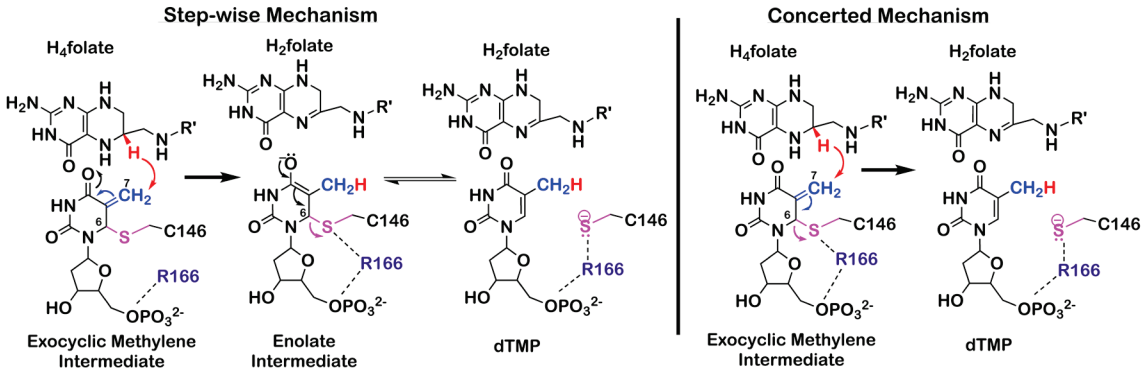
The antiproliferative effect of truncated Superstolide A was evaluated in eight cancer cell lines by using the MTT assay. Truncated Superstolide A is about seven times more potent in suppressing tumor cell proliferation than its parent natural product HT-29 cell. In addition, truncated Superstolide A is also potent in suppressing tumor cell proliferation in the other seven tested cell lines with IC_{50} values ranging from 12–77 nM. These results have confirmed our hypothesis that the 16-membered macrolactone is indeed the pharmacophore that interacts with its putative target in the cells, and the modification of the fuctionalized cis-decalin to a cyclohexene ring apparently does not affect its potent anticancer activity.

Presenter 8

HYDRIDE TRANSFER MECHANISM IN THYMIDYLATE SYNTHASE

Zahidul Islam, Timothy S. Strutzenberg, Ilya Gurevic, and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Thymidylate Synthase (TSase) is a crucial enzyme that maintains cellular dTMP (2'-deoxythymidine-5'-monophosphate) pool required for DNA biosynthesis in most living organisms. TSases utilizes methylene tetrahydrofolate (CH_2H_4folate) as a cofactor and catalyzes transfer of a methylene and subsequently a hydride to dUMP (2'-deoxyuridine-5'-monophosphate) to form dTMP. The hydride transfer, which is rate-limiting for the overall reaction, can undergo in two possible fashions: a step-wise process where the hydride transfer precedes the cleavage of the bond between C6 of dUMP and nucleophilic cysteine of TSase, and a concerted process where both events occur together. While, structurally and thermodynamically, the step-wise mechanism seems favorable, our QM/MM calculations predicted the concerted process being the minimum energetic pathway for the hydride transfer.[1] We tested these two possibilities by a combination of experiments including secondary (2^o) kinetic isotope effect (KIE), mutagenesis, and primary (1^o) KIEs. Our findings indicated that the hydride transfer and the cleavage of thioether occur concertedly,[2] thereby supporting the prediction from QM/MM calculations.



Scheme: A step-wise mechanism (left) where the breakage of C6-S in exocyclic methylene intermediate follows the hydride transfer from H_4folate, and a concerted mechanism (right) where the hydride transfer and the cleavage of C6-S happen together. Arginine 166 (R166) plays a critical role to stabilize the resulting thiolate in the transition state of the concerted mechanism, which promotes the scission of thioether bond and the concomitant hydride transfer.

References:

1. Kanaan, N.; Ferrer, S.; Marti, S.; Garcia-Viloca, M.; Kohen, A.; Moliner, V., *J. Am. Chem. Soc.* **2011,** *133* (17), 6692-6702.
2. Islam, Z.; Strutzenberg, T. S.; Gurevic, I.; Kohen, A., *J. Am. Chem. Soc.* **2014,** *136* (28), 9850-9853.

List of Posters and Authors

POSTERS

1. CHALLENGE OF PRODUCING PARAXANTHINE FROM CAFFEINE BY *E. COLI* METABOLICALLY ENGINEERED WITH N-DEMETHYLASE A/D AND B/D GENES

Khalid H R Al-Gharrawi[1,2], Ryan M. Summers[3], Sridhar R. Gopishetty[4], and Mani Subramanian[2,4]*
[1]Department of Chemical Engineering, University of Baghdad, Baghdad, Iraq
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA, USA
[3]Department of Chemical and Biochemical Engineering, The University of Alabama, Tuscaloosa, AL, USA
[4]Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research and Economic Development, The University of Iowa, Coralville, IA, USA

2. IN VITRO EVALUATION OF *N*-ARYLACYL *O*-SULFONATED AMINOGLYCOSIDES FOR THEIR INHIBITION OF HNE, CatG AND Pr3

Ioana Craciun and Robert J. Kerns*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

3. IN VIVO DELIVERY OF MESSENGER RNA TO MOUSE LIVER

Samuel T. Crowley and Kevin G. Rice*
Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

4. RETENTION AND SELECTIVITY OF POLYMORPHIC HYDROGEN-BONDING MOTIFS IN CO-CRYSTALS OF 5-FLUOROURACIL

Roxanne L. Dudovitz, Shawna J. Dudovitz, Andrew J. Duncan, and Leonard R. MacGillivray*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

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Poster Abstracts

Poster 1

CHALLENGE OF PRODUCING PARAXANTHINE FROM CAFFEINE BY *E. COLI* METABOLICALLY ENGINEERED WITH N-DEMETHYLASE A/D AND B/D GENES

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The goal of this research is to develop a pilot plan for the production of paraxanthine (PX, 1,3-dimethylxanthine) from caffeine (1,3,7-trimetrthylxanthine) by using metabolically engineered *E. coli* as a biocatalyst. PX is of interest to nutraceutical industries as a health supplement. Metabolically engineered *E. coli* were engineered with NdmA and NdmD genes. NdmA and NdmD are N-demethylases where NdmA catalyzes the N-demethylation of caffeine at N_1, and NdmD is a cofactor that transfers electrons from NADH to NdmA. Even though NdmA engineered in *E. coli* catalyzes N_1 demethylation of caffeine to theobromine (98.5%), the enzyme exhibits low activity of N_3 demethylation of caffeine to paraxanthine (1.5%). NdmB, in combination with NdmD is highly specific for N_3 demethylation; hence NdmB/D metabolically engineered in *E. coli* was also tested for conversion of caffeine to PX. *E. coli* pDdA strain, which has one copy of NdmA gene and one copy NdmD gene, and pBDdDB strain, which has two copies of each of Ndm B and D, were chosen for PX production using whole cells. pBDdDB strain had no activity on caffeine at the 3-position; hence direct conversion of caffeine to PX was not feasible. Reaction rate was found to increase by glucose addition; optimum ratio of glucose to caffeine was found to be 1:4 for caffeine disappearance from the reaction mixture. Biocatalyst reuse and caffeine spiking were studied to improve the yield of PX; the activities were found to be $5.2 * 10^{-6}$ and $11.2 * 10^{-6}$ mg PX/mg wet cells/min respectively. Four different fermentation growth media were used to develop a high cell density fermentation of the engineered *E. coli* strain. These media are modified minimal broth, 2X Luria broth, terrific broth, and super broth. Super broth grown cells gave the best activity of about $5.8 * 10^{-6}$ PX/mg wet cells/min. This media also yielded ~7.58 g of wet cells/500 mL media. However, the PX yield was very low, i.e, only 1.5% conversion; rest of the product was theobromine. Theobromine is not a high value compound.

Several attempts were made to "evolve" Ndm A for higher specificity for PX conversion, including (i) error prone PCR (ii) gene shuffling. None of these methods improved PX specificity of Ndm A. Thus it was concluded that the low yield of PX is not a viable process for production. The current focus is to produce 3-methylxanthine from theophylline (1,3-dimethylxanthine) using *E. coli* metabolically engineered with NdmA/D.

Poster 2

IN VITRO EVALUATION OF *N*-ARYLACYL *O*-SULFONATED AMINOGLYCOSIDES FOR THEIR INHIBITION OF HNE, CatG AND Pr3

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Neutrophil serine proteases (NSPs) play an important role in the innate immune system, however when the balance between NSPs and their endogenous protease inhibitors (PIs) is disrupted, they also play a critical role in the pathogenesis of chronic inflammatory lung diseases. Excessive release of NSPs such as human neutrophil elastase (HNE), proteinase 3 (Pr3) and cathepsin G (CatG) leads to destruction of the lung matrix and continued propagation of acute inflammation. Under normal conditions, PIs counteract these effects by inactivating NSPs. However, in chronic inflammatory lung diseases there are insufficient amounts of PIs to mitigate damage. Therapeutic strategies are needed to modulate excessive NSP activity in chronic inflammatory lung diseases. The Kerns laboratory previously demonstrated that heparin derivatives substituted with structurally unique aromatic residues bind with high affinity and selectivity to select Glycosaminoglycan-binding proteins, including NE and CatG. In the work presented here, using a chromogenic peptidolytic assay, we evaluated the members of a recently synthesized panel of *N*-arylacyl *O*-sulfonated aminoglycosides for their ability to inhibit NE, CatG and Pr3. We identified *O*-sulfonated *N*-carbobenzyloxy Kanamycin as a novel and structurally unique inhibitor of all three neutrophil serine proteases and characterized its type of inhibition with respect to CatG. Finally, we established a high-throughput cell based assay to assess the ability of these aminoglycoside derivatives to mitigate protease induced cell detachment. This assay can be used to screen multiple derivatives in a high-through put manner against each protease individually as well as protease cocktails.

Poster 3

IN VIVO DELIVERY OF MESSENGER RNA TO MOUSE LIVER

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In vivo delivery of either DNA or RNA has the potential to treat a wide variety of disease, including genetic disorders, cancers, viral infections, and others. Currently, gene delivery is categorized as viral or nonviral. While viral gene delivery has been more efficient in vivo, potential side effects such as immune response or insertional mutagenesis has driven interest in nonviral methods. Nonviral vectors include cationic lipids or polymers that condense DNA or RNA and protect it against degradation. Previously, our lab has developed a series of PEGylated Polyacridine Peptides, using a combination of lysines to bind to DNA by electrostatic interaction and Acridines to bind by intercalation. These peptides form DNA polyplexes that are stable against nucleases and can protect DNA in the bloodstream of a mouse for up to 12 hours[1]. DNA and RNA can be efficiently delivered to mouse hepatocytes by hydrodynamic tail vein injection. The polyplex is suspended in a large volume of normal saline (0.09mL per g body mass) and injected into the tail vein within 5-7 seconds.

In this study, luciferase mRNA was produced by in vitro transcription. The mRNAs were bound to PEGylated polyacridine peptide at 0.8nmol peptide per 1μg RNA to form RNA polyplexes. RNA polyplex formation was confirmed by dynamic light scatting particle sizing, with diameter of 104nm and zeta potential of 15mV.

RNA polyplexes were delivered to mice by tail vein hydrodynamic injection at 1ug RNA per mouse, and bioluminescence was measured 24 hours later. mRNA bound to PEGylated Polyacridine Peptides produced a bioluminescence of 10^8 to 10^9 photons/sec/cm^2/steradian, comparable to luciferase expressing pGL3 plasmid DNA. When a non-acridine PEGylated Peptide was used, or when no peptide was used, bioluminescence was approximately ten fold lower.

RNA polyplexes were incubated in mouse serum for 5 and 30 minutes, then delivered to mice by hydrodynamic tail vein injection. These incubated polyplexes produced signal equivalent to non-incubated controls, demonstrating protection against serum nucleases.

This demonstrates that the PEGylated Polyacridine Peptide binds to and protects mRNA. The RNA polyplexes also show improved activity by hydrodynamic dosing than mRNA alone. Delivery of mRNA may have advantages over DNA, since mRNA does not need to enter the nucleus of a cell to produce protein. Therefore, mRNA may be simpler than DNA to deliver in vivo without use of hydrodynamic dosing.

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Poster 4

RETENTION AND SELECTIVITY OF POLYMORPHIC HYDROGEN-BONDING MOTIFS IN CO-CRYSTALS OF 5-FLUOROURACIL

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The efficacy of active pharmaceutical ingredients (APIs) is known to be influenced by formulation methods. Among the formulation strategies being studied for solid form delivery of APIs, co-crystallization has been shown a promising means of improving physicochemical properties relative to effective drug delivery. 5-Dluorouracil (5-FU) has been marketed commercially as intravenous and topical formulations for the treatment of numerous forms of cancer. However, the behavior of 5-FU has been poorly studied in co-crystal systems, and currently no solid formulation of the drug is available commercially.

Our studies of co-crystals of 5-FU and symmetrical bipyridines have revealed a unique habit, whereby the hydrogen bonding patterns of pure 5-FU remain largely intact within the co-crystals. Co-crystals were obtained exhibiting hydrogen-bonding motifs similar to both Form I and Form II polymorphs of 5-FU. Furthermore, it was observed that the substitution pattern of the bipyridines imparts some degree of selectivity for these polymorphic hydrogen-bonding patterns. Additionally, molecular packing in the co-crystals conformed to the geometric requirements for a [2+2] photocycloaddition and solid-state reactivities were studied.

Poster 5

DEVELOPMENT OF NOVEL INHALATION FORMULATIONS FOR ERADICATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS

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Cystic Fibrosis (CF) is a genetic disorder characterized by mucus buildup in the lungs of the patients, affecting lung function. *Pseudomonas aeruginosa* are Gram-negative bacteria that inhabit the lungs of CF patients and produce biofilms, which protect the bacteria from hostile environment and antibiotics. These biofilms, thus, render any antibiotic treatment ineffective, resulting in chronic bacterial infection. However, it has been observed that bacteria dispersed out of a biofilm are susceptible to conventional antibiotic treatment. To this end, we have proposed that the incorporation of a nutrient dispersion compound in the antibiotic formulation will induce bacterial dispersion from the biofilm and enhance the efficacy of the antibiotic. The purpose of this research is to develop a novel inhalation formulation consisting of an antibiotic and a nutrient dispersion compound for *in vitro* eradication of *P. aeruginosa* biofilms.

Clinical isolates of *Pseudomonas aeruginosa* were obtained. Growth curves of *P. aeruginosa* clinical isolates were generated in Mueller Hinton Broth (MHB). The isolates were grown in MHB and samples were withdrawn at various time intervals and plated. After incubating the plates for 24 hours, the number of bacterial colonies were counted. Minimum biofilm eradication concentration (MBEC^{TM}) assays were performed on biofilms of both the laboratory strain PAO1 and the clinical isolates, grown for 24 hours, using antibiotics alone and combinations of antibiotics and nutrient dispersion compounds. Biofilms were grown for 24 hours on peg lids in MBEC^{TM} troughs at 37°C and then treated with either the antibiotic alone, or combinations of antibiotic and nutrient dispersion compound in a 96-well plate for another 24 hours at 37°C. Residual biofilms on the pegs were then dispersed in sterile growth medium using sonication, and the UV absorbance was measured after 24 hours of incubation.

Mueller Hinton Broth (MHB) gave distinct growth curves for *P. aeruginosa* clinical isolates. The growth curves allowed for understanding the various phases of bacterial growth in the MHB with respect to time. MBEC^{TM} assays performed on the biofilms of PAO1 and clinical isolates demonstrated enhanced biofilm eradication by certain combinations of antibiotic and nutrient dispersion compound compared to antibiotics alone. Biofilm eradication was observed at lower antibiotic concentrations in the combination treatments compared to those in antibiotics alone.

However, more studies need to be carried out using more such combinations of antibiotics and nutrient dispersion compounds to determine the most potent combination for eradicating *P. aeruginosa* biofilms *in vitro.* The selected combination can then be used in *in vivo* studies to further validate the efficacy of the combination.

Poster 6

INVESTIGATING THE ROLE OF TYROSINE 94 ON PROTON ABSTRACTION 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), which is a precursor of one of the DNA bases. The cofactor methylene tetrahydrofolate (CH_2H_4folate) is used by this enzyme as both the methylene and hydride donor. Thymidylate synthase is one of the targets of chemotherapeutic drugs as the enzyme constitutes the last committed step of the *de novo* biosynthesis of DNA. The catalyzed reaction involves breaking and formation of several covalent bonds, which includes a hydride transfer and a proton abstraction. For the wild type enzyme proton abstraction is followed by a rate limiting hydride transfer. X-ray crystallography study suggested that a nearby and conserved tyrosine residue (Y94 in TSase from *E. coli*) could be the general base for this proton abstraction step.[1] Several mutation studies on the corresponding tyrosine residue (Y146) of *L. casei* shows about 100 to 1000 fold decrease on k_{cat}, but the specific role of this residue on the proton abstraction has not been studied yet.[2] A study by Hong et al., suggested that the whole network of Hydrogen bonds rather than Y94 alone, could serves as general base for this proton abstraction step.[3] But their study did not examined the temperature dependence of intrinsic KIE, and thus did not expose effects on the C-H bond cleavage per se.

Consequently, the current studies focus on exploiting temperature-dependence of intrinsic KIEs of the Y94F mutant of ecTSase. Such investigation could detect even minor changes in reaction potential energy surface of the bond cleavage. If Y94 catalyzes the proton abstraction, it is predicted that the mutation will cause a change in the reaction potential energy surface, which will bring about a relative change in temperature dependence of intrinsic KIEs of the mutant relative to the WT. The findings should unravel the role of this specific residue on proton abstraction step.

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Poster 7

OPTIMIZATION OF VALUABLE INTERMEDIATES SYNTHESIS BY 11 ALPHA-HYDROXYLATION OF STEROID DHEA BY SOLVENT-ENHANCED BIOCATALYST

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This research evaluates the oxidative capacity of fungus *Beauveria bassiana*; a versatile whole-cell biocatalyst used in the biotransformation of chemicals. The goal of this research is to standardize, control and optimize the biocatalysis capacity of *B. bassiana* in the biotransformation of DHEA. To extend use of *B. bassiana* to commercial applications, the optimization of reaction conditions and accurate prediction of biotransformation products are necessary. This work enhances the selective hydroxylation capacity of Strain ATCC 7159, resulting in a cost effective and eco-friendly process for the synthesis of valuable 11α-hydroxy steroids. Our work establishes the metabolic pathway of DHEA and defines the optimum reactor arrangement, substrate concentration, reaction temperature and pH. NMR and LC-MS analysis were performed to characterize reaction products. Higher substrate conversion, selectivity and yield of desired product was achieved in a "Resting Cells" arrangement. As well, higher volumes of growing medium compared to reaction buffer, increase the activity of the biocatalyst. The apparent rate of reaction fits a Michaelis-Menten kinetics model with a maximum reaction rate of 4.45mM-day. Interestingly, when a diluted amount of substrate is used, a higher yield of 11α-hydroxy steroids is achieved. Also, reactions at 26°C with pH ranges between 6.0 and 7.0 resulted in the highest conversion (70%) and the higher product yield (45.8%). *B. bassiana* has the capacity to metabolize DHEA and similar steroids in different set-ups and has a promising future as biocatalyst to be used in the production of drug metabolites.

Poster 8

STRUCTURAL AND KINETIC STUDIES ON FORMATE DEHYDROGNEASE FROM *CANDIDA BOIDINII*

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Whether and how the fast protein structural fluctuations affect the catalyzed chemistry in enzymes' active site is the subject of an open debate. Formate dehydrogenase from *Candida boidinii* (CbFDH) was found to be a unique model system to address this question. Our previous kinetic and two-dimensional infrared spectroscopy (2DIR) studies on commercial CbFDH identified probable relations between femtosecond-picosecond dynamics and the catalyzed H-tunneling step. However, in a search for a correlation between protein dynamics and the chemical step kinetics we attempted mutagenesis studies. For design of site-directed mutagenesis at the active site and for the corresponding computational studies, three-dimensional structural information has been required. The X-ray structures of the apo form (FDH) and the holo form (the ternary FDH-NAD^+-azide complex) of CbFDH were solved at 1.75 and 1.5 Å resolution. Surprisingly the apo-enzyme was crystallized under saturation of NAD^+ and azide, which means the majority of complexes in solution are excluded during the crystallization step. In addition, the intrinsic kinetic isotope effects (KIEs) and their temperature dependency for wild type and three active site mutants (I175A, V123A, and I175A/V123A) were measured to reveal the nature of hydride-transfer step. The increasing temperature dependency of KIEs suggested that more flexible active site in the mutants result in poorly organized transition state, and required more thermally activated motions for effective H-tunneling. This structure-function correlation together with the 2DIR measurements accordingly shall provide insight into coupling between enzyme function and fast dynamic motions.

Poster 9

ALTERNATIVE QUENCHER AND SUBSTRATE CANDIDATES IN MECHANISTIC STUDIES OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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Flavin-dependent thymidylate synthase (FDTS) catalyzes the production of 2'-deoxythymidine-5'-monophosphate (dTMP) from 2'-deoxyuridine-5'-monophosphate (dUMP)[1]. In contrast to classical thymidylate synthase, the methyl group of dTMP is composed of a methylene unit derived from methylene tetrahydrofolate (MTHF) and a hydride derived from reduced flavin adenine dinucleotide[2]. This enzyme is present in many pathogens, but the human enzyme that catalyzes the same conversion of dUMP to dTMP is structurally, genetically, and mechanistically orthogonal[3]. This hints at a reasonable chance of developing FDTS-specific inhibitors as candidates for antibiotic drug-leads. The mechanism of the reaction is yet unknown, and identification of reaction intermediates could greatly assist in revealing that mechanism. The identification of reaction intermediates could be aided by use of a quencher to terminate the reaction rapidly in a flow-quench experiment. Acids and bases have been used, but those chemically alter the intermediates, while an agent that stops catalysis at neutral pH might preserve some of the intermediates. Progress in the search for such quenchers will be presented.

Another avenue of mechanistic investigation involves the synthesis of 6-iodo-dUMP as an alternative substrate or inhibitor candidate for FDTS. There is precedent for 6-iodo-UMP serving as a covalent enzyme inhibitor[4] and for iodide release from the C6 position of a deoxyuridine derivative[5]. If 6-iodo-dUMP serves as an alternative substrate, iodine retention or iodide release could help distinguish between mechanistic hypotheses. Alternatively, inhibition and binding studies could report on active site flexibility in the vicinity of dUMP's C6.

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Poster 10

HYDRIDE TRANSFER MECHANISM IN THYMIDYLATE SYNTHASE

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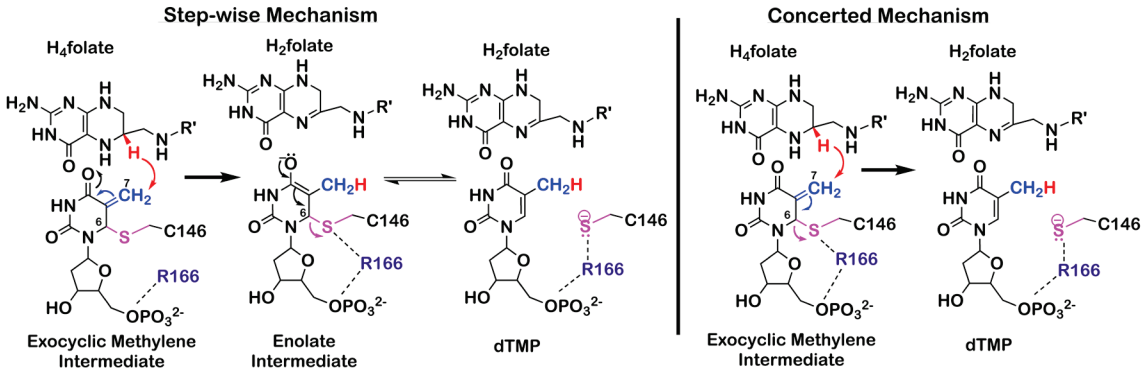
Thymidylate Synthase (TSase) is a crucial enzyme that maintains cellular dTMP (2'-deoxythymidine-5'-monophosphate) pool required for DNA biosynthesis in most living organisms. TSases utilizes methylene tetrahydrofolate (CH_2H_4folate) as a cofactor and catalyzes transfer of a methylene and subsequently a hydride to dUMP (2'-deoxyuridine-5'-monophosphate) to form dTMP. The hydride transfer, which is rate-limiting for the overall reaction, can undergo in two possible fashions: a step-wise process where the hydride transfer precedes the cleavage of the bond between C6 of dUMP and nucleophilic cysteine of TSase, and a concerted process where both events occur together. While, structurally and thermodynamically, the step-wise mechanism seems favorable, our QM/MM calculations predicted the concerted process being the minimum energetic pathway for the hydride transfer.[1] We tested these two possibilities by a combination of experiments including secondary (2^o) kinetic isotope effect (KIE), mutagenesis, and primary (1^o) KIEs. Our findings indicated that the hydride transfer and the cleavage of thioether occur concertedly,[2] thereby supporting the prediction from QM/MM calculations.



Scheme: A step-wise mechanism (left) where the breakage of C6-S in exocyclic methylene intermediate follows the hydride transfer from H_4folate, and a concerted mechanism (right) where the hydride transfer and the cleavage of C6-S happen together. Arginine 166 (R166) plays a critical role to stabilize the resulting thiolate in the transition state of the concerted mechanism, which promotes the scission of thioether bond and the concomitant hydride transfer.

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Poster 11

GRP78 TARGETED PEPTIDES FOR MOLECULAR IMAGING OF METASTATIC MELANOMA

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Melanoma incidence is increasing rapidly and is now the 6th most diagnosed cancer in the United States. Surgery can be curative for disease detected early, but metastatic melanoma is lethal (5 year survival <5%). Recent discoveries of genetic mutations (*e.g.* BRAF/NRAS) in melanoma have led to promising new therapies, but these therapies extend life expectancy by only months[1]. Targeted radionuclide therapy has long been considered a promising alternative that has the potential to circumvent melanoma drug resistance pathways by directing radiation selectively to the tumor microenvironment. However, a suitable cell-surface target has yet to be identified for melanoma treatment. GRP78 is a molecular chaperone that has long been considered to be confined to the endoplasmic reticulum (ER), where it plays a major role in mediating appropriate protein folding and the unfolded protein response (UPR) in the cell. On the other hand, emerging evidence demonstrates that GRP78 is exported from the internal organelle to the surface of the cell under conditions of cellular stress. Although the mechanism and reasons for the protein to be present on the surface of cells is not well understood, it is increasingly recognized as related to intracellular and extra cellular stresses that are associated with the tumor microenvironment. Furthermore, clinical evidence correlates high cell-surface expression of GRP78 with progression of metastatic melanoma[6]. These new findings have led to the recent development of targeted antibody-based therapies currently in clinical trials for melanoma treatment. However, the potential for GRP78 as a target for radionuclide-based imaging and therapy has yet to be explored. A phage display study identified peptide-based ligands that bind specifically to extracellular GRP78, however the molecular design that leads to sufficient binding affinity and specificity for a radiopharmaceutical has yet to be determined. We hypothesize that GRP78 cell surface expression is a result of chronic elevated levels of oxidative stress in metastatic melanoma tumors and that peptide targeting cell surface GRP78 can be developed for targeted image-guided radionuclide therapy for metastatic melanoma. We will test our hypothesis by: (1) synthesizing a library of GRP78 targeted peptides and analyze their specificity and binding affinity via in-vitro binding assays; and (2) subjecting the cells to oxidative stress induced by hypoxia, glucose deprivation; ER stress inducers; and electron transport chain inhibitors known to increase levels of oxidative stress (*e.g.* triphenylphosphonium derivatives used in our lab) and analyzing their effect on extracellular expression of GRP78 via flow cytometry. Our *preliminary data* shows remarkably higher expression of GRP78 in melanoma tumor tissue vs. surrounding tissue. Further, our initial peptides design has established that a structure activity relationship can be identified that can lead to a molecular peptide design that binds specifically to cell surface GRP78 with high affinity. Further, early designs could be radiolabeled with PET radionuclide gallium-68 (^{68}Ga) for molecular imaging. These positive findings strongly support our hypothesis.

Poster 12

FLAVIN ANALOGUE (5-DEAZA-FAD) AS A MECHANISTIC PROBE TO STUDY THE ROLE OF FAD IN FDTS FLAVIN-DEPEDENT THYMIDYLATE SYNTHESE

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Thymidylate (2'-deoxythymidine 5'-monophosphate or dTMP) is a unique DNA nucleotide because unlike other deoxyribonucleotides, it cannot be directly synthesized by ribonucleotide reductase. Thymidylate has to be synthesized in a cell *de novo*. This *de novo* biosynthesis of dTMP requires the enzyme thymidylate synthase (TSase), thus making TSase crucial to all living organisms. In all eukaryotes including humans, TSase encoded by *thy*A gene (Classical TSase) catalyzes the reductive methylation of dUMP (2'-deoxyuridine 5'-monophosphate) to form dTMP. (1,2) A new class of thymidylate synthases was discovered that is encoded by the *thy*X gene (3). This new class of TSases makes use of a noncovalently bound flavin adenine dinucleotide (FAD) to catalyze the reduction of dUMP (hence named as flavin-dependent thymidylate synthases, or FDTSs). Several prokaryotes including disease causing bacteria such as typhus-causing *Rickettsia prowazekii*, *Mycobacterium tuberculosis*, and *Bacillus anthracis* rely on FDTS (3). In this work we have replaced FAD with a flavin analogue, 5-deaza-FAD where the N5 of FAD is replaced with a carbon atom. This will help to understand the mechanism of FDTS and the role of flavin in catalysis, e.g., establish direct hydride transfer to the substrate, and revealing the stereochemistry of both the oxidative and reductive half reactions. Classical TSase and FDTS are substantially different in structure and chemical mechanisms and share no common inhibitors, hence understanding the mechanism by which FDTS catalyzes the synthesis of thymidylate will allow the development of antibiotics with minimal toxicity to humans.

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Poster 13

USING iTC TO CHARACTERIZE PROTEIN CORONA FORMATION AROUND NANOPARTICLES

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The formation of a protein corona around material introduced into biological environments occurs rapidly, and present understanding of this phenomenon indicates that the corona plays a significant role in the material's interactions with biological systems. Specific proteins can induce cellular uptake, and current research reveals that surface properties of materials determine which proteins have an affinity for adsorption. Because of these affinities, varying material surface composition results in unique protein corona composition. In the case of nanoparticulate drug and gene delivery, the affinity of proteins for the material used is a factor which should be considered in the selection of carriers. Novel drug and gene carriers can be found by identifying materials which have a high affinity for proteins known to select for uptake by specific cell types.

Isothermal Titration Calorimetry (iTC) is a calorimetric technique designed to measure the energy evolved or consumed when interactions occur between two substances. iTC accomplishes this by measuring the energy required to maintain a constant temperature during an interaction. By comparing the adsorption energies of various proteins with a given biomaterial, this technique reveals which proteins adsorb strongly to the biomaterial surface. The time component of the measurement makes iTC capable of offering information regarding the rate at which these adsorption processes occur.

Polymeric nanoparticles are of particular interest in drug and gene delivery because of the ease of synthesis and the capacity to imbue them with degradation and load-release properties by varying composition. Polymer particles are also showing potential as adjuvants in studies of vaccine delivery. Understanding how polymer chemistry affects protein adsorption allows us to add targeted delivery to the growing list of uses for polymeric gene and drug delivery. This technique was used to measure the interaction between protein and carboxylate-modified polystyrene particles 200nm in diameter. The iTC data was used to measure the number of a given protein which could be adsorbed to each particle, as well as the time over which these adsorptions occurred. Comparing the data from a series of protein exposures to identical particles reveals the protein components which are likely to form a stable corona.

Poster 14

A NEW SMALL MOLECULE THERAPY FOR METASTATIC MELANOMA

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Metastatic melanoma is a lethal disease for which there are no durable treatment options. Emerging evidence suggests that fundamental differences in oxidative metabolism and response to oxidative stress in cancer cells (including melanoma) versus normal cells may provide an avenue for new therapies designed to exploit these differences. The research presented here investigates the use of triphenylphosphonium (TPP) derivatives that can target these differences in oxidative metabolism to selectively kill melanoma cells relative to normal cells. We hypothesize that TPP derivatives can be designed to preferentially accumulate in melanoma cell mitochondria and increase cytotoxic hydroperoxide species production.

In this study, we examined a small library of TPP variants to examine the effect of structural variation in TPP design on melanoma cell oxidative metabolism and survival. Measurements of clonogenic viability, oxidative stress (DHE oxidation), electron transport chain complex activity, and oxygen consumption rates were conducted in vitro. The potential for TPP therapy for melanoma was examined by treating tumor-bearing mice with the TPP derivative 10-TPP.

Results demonstrate that TPP derivatives modified with molecular side chains disrupt mitochondrial oxidative metabolism and induce melanoma cell cytotoxicity via a mechanism involving oxidative stress. Further, there is a structure-activity relationship between the molecular side chain composition and oxidative metabolism disruption and cytotoxicity. 10-TPP treatment of tumor bearing mice led to a reduction in melanoma tumor growth rate, thus highlighting the potential of TPP-drugs as a therapy for metastatic melanoma. Results demonstrate that TPP derivatives can be designed to disrupt mitochondrial oxidative metabolism and induce melanoma-cell specific cytotoxicity via a mechanism of oxidative stress and highlight the potential of TPP derivatives as a new small molecule therapy for metastatic melanoma.

Poster 15

ENSEMBLE REFINEMENT OF PROTEIN CRYSTAL STRUCTURES

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X-ray crystallography is the most commonly used experimental method for determination of protein structures at atomic resolution, allowing insights about protein function and the molecular causes of disease. While proteins naturally exist as an ensemble of conformations, current x-ray crystallography techniques represent them as a single model. We use rotamer optimization to optimally repack side chains and generate an ensemble of structures within an energy cutoff of the optimum. This ensemble is clustered into microstates, and random representative structures are locally minimized to produce a final ensemble of structures, all of which are independently fit to crystal data. We plan to reweight the ensemble to better match crystal data; preliminary hybrid electron density maps were generated by assuming all ensemble members were equally important. A calmodulin ensemble shows altered hydrogen bonding networks and improved R compared to best single-structure refinement, but R_{free} indicates no improvement of our representation. Reweighting and other improvements are necessary to show validated improvement of protein representation; fortunately, the improved R value suggests this is possible. This approach has the potential to reveal insights about naturally heterogeneous protein regions such as enzyme active sites and better represent poorly packed regions.

Poster 16

PRELIMINARY WORK TOWARDS DEVELOPING DETAILED MODELS OF THE GRAM-NEGATIVE BACTERIUM *ESCHERICHIA COLI*

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Computational molecular modeling and dynamic simulations provide valuable insight into biological processes. Previous work in our laboratory has modeled the crowded environment of the cytoplasm of the bacterium *Escherichia coli*. We next begin the process of modeling and simulating other cellular compartments. Just as we did for the cytoplasmic model, we began by analyzing recent quantitative proteomics data. We have queried how well these data recapitulate the expected values for the bacterial proteome, taking into account the biases intrinsic to their analytical techniques. In addition to analyzing the identification of and expression levels for the bacterial proteome, we are working to characterize the reported proteomes by cellular localization and net charge. The use of protein structures will also be necessary for molecular modeling, so we have begun to sift through structural data in the Protein Data Bank to identify and utilize available models. With these analyses in hand, the groundwork will be set to model different environments of the model Gram-negative bacterium *Escherichia coli*.

Poster 17

APPLICATION OF AN ALDEHYDE AMINO ACID TAG FOR ION CHANNEL STUDY

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Orthogonal protein epitopes provide a myriad of avenues for drug design, fluorescent modification and specific covalent attachment(s) for downstream protein study and purification. Simply put, such epitopes present the opportunity to chemically label or modify a unique sequence within an expressed protein. To this end, we are utilizing the formyl-glycine generating enzyme necessary in sulfatase activation whereby it cotranslationally converts the cysteine in the amino-acid recognition sequence LCTPSR into an aldehyde containing formyl-glycine sequence L(FGly)TPSR. This endogenous process provides an opportunity for the generation of an orthogonal epitope as aldehydes are particularly sparse in proteins, other than sulfatases which bury the aldehyde in their catalytic site on folding. This technique allows for a selective protein attachment through a variety of chemical modification strategies, including the Hydrazino-Pictet-Spengler ligation reaction for covalently coupling a desired target molecule to the protein at the formyl-glycine residue. In collaboration with Redwood Biosciences, whom utilizes this system in their SMARTag antibody-drug conjugates, we are testing this method as a bioorthogonal tag to specifically label extracellular domains of ion channels in living cells. This technique will ultimately be used to attach an environmentally sensitive fluorophores to ion channel voltage sensing domains which could provide visualization of domain movement through fluorophore intensity and spectral shift.

Poster 18

STRATEGIES TO ENHANCE THE CONVERSION OF PHENOTHIAZINE IN *BEAUVARIA BASSIANA*

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Beauveria bassiana is a filamentous fungus widely used as a bio pesticide and biocatalyst. However one of its main catalytic constrains is low substrate conversions, so any mean to increase it can further impact its' use. *B. bassiana* can enhance the yield and conversion of oxidative biotransformations when it is grown in presence of chemical and/or environmental stresses. The purpose of this project is to determine the ability of *B. bassiana* ATCC 7159 to metabolize sulfur containing azaarene, phenothiazine, in presence of inducers such as hydrocarbon and insecticides. Expression of oxidases can increased up to 3 fold when *B. bassiana* is grown in presence of n-hydrocarbons or exposed to insecticides, neonicotinoids and carbamates. Results include metabolites characterization and oxidative activity assay. Oxidative activity was evaluated via the 7-Ethoxycoumarin O-deethylation assay since it is a prototypic substrate to monitor CYP450 activity. The metabolites were identified by mass spectrometry and proton nuclear magnetic resonance spectroscopy. The fungus oxidized phenothiazine to phenothiazine sulfoxides, 3-hydroxyphenothiazine sulfoxides, and phenothiazine-3-one. The success of these efforts will provide a new insight on *B. bassiana's* oxidative performance.

Poster 19

HEAVY ENZYME EFFECTS ON PROTEIN DYNAMICS OF FORMATE DEHYDRONGENASE

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The role of protein dynamics in the chemical step of enzyme-catalyzed reactions has been one of the hotly debated topics in enzymology. Studies with isotopically substituted "heavy enzymes", in which C, N and non-exchangeable H are substituted with C-13, N-15 and H-2 respectively, have been developed to address the role of femtosecond (fs) to picosecond (ps) timescale dynamics in the catalytic mechanism of enzymes. The heavy enzyme explored in this work is formate dehydrogenase (FDH), which catalyze the hydride transfer from formate to nicotinamide adenine dinucleotide (NAD^+) producing NADH and carbon dioxide. As a model system, FDH can practically be used to study the protein mass-modulated effects and primary kinetic isotope effects (KIEs) on catalysis. Moreover this particular reaction has gained much importance since its transition state analogue could be potentially used for 2D-IR experiments to directly probe the dynamic timescales at the transition state besides studying the effect of heavier mass on catalysis. Heavy FDH (*h*-FDH) has been expressed and purified and has been determined to have an 11.2% increase in mass compared to the light FDH (*l*-FDH). Initial velocity parameters between *h*-FDH and *l*-FDH show significant difference. Thus the observations indicate that the steady state kinetic parameters, i.e. rates of catalysis are affected by the vibrational changes due to increased mass of the whole entity. It is from intrinsic KIE measurements that in depth analysis about the chemical step of the reaction can be obtained and those experiments are underway.

Poster 20

NOVEL APPROACH TO STUDY SUBCELLULAR PROTEIN LOCALIZATION AND GENE EXPRESSION IN *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile is linked to >14,000 deaths annually in the United States and is the most common hospital-acquired infection in Europe. While concern has caused vast interest in studying *C. difficile*, research is still hindered due to the lack of genetic tools available in *C. difficile*. For example, oxygen-dependent reporters such as GFP have not been used because *C. difficile* is an especially oxygen-sensitive microbe. However we recently demonstrated the use of an oxygen-dependent fluorescent protein (cyan fluorescent protein) in *C. difficile*. Here, we expand upon our initial findings with a newly synthesized red fluorescent protein that was codon-optimized for AT-rich bacteria. Using this variant, gene expression and subcellular protein localization can be determined in *C. difficile*. For example, we found three *C. difficile*-specific proteins (MldABC) of unknown function localized to the mid-cell, suggesting a role in cell division. This was confirmed when mutants lacking these proteins exhibited morphological defects: loss of rod shape (a curved cell phenotype) and inefficient separation of daughter cells (a chaining phenotype). Furthermore, these mutants were attenuated in the Syrian hamster model of *C. difficile* pathogenesis. Since these proteins are unique to *C. difficile* and play a role in pathogenicity, these newly identified cell division proteins could serve as therapeutic targets. In sum, we believe studying subcellular protein localization and gene expression with oxygen-dependent fluorescent proteins will prove invaluable for research in strict anaerobes such as *C. difficile*.

Poster 21

IDENTIFYING REMARKABLE DIFFERENCES IN MALIGNANT MELANOMA CELL-SURFACE GLYCOPROTEIN EXPRESSION USING A NEW FLUORESCENT-LABELED MONOFLUORINATED CYCLOOCTYNE

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We have synthesized a monofluoro-substituted cyclooctyne moiety (MFCO) coupled with a fluorescent label (MFCO-FITC) to examine differences in cell-surface glycoprotein expression in metastatic melanoma and non-malignant melanocyte cells by fluorescence microscopy. Our results demonstrate that our new MFCO-FITC is sufficiently stable to undergo the site-selective copper-free cycloaddition (click) reaction with azide-modified sugar epitopes on the surface of malignant and non-malignant cells in culture. The MFCO-FITC catalyst-free click chemistry approach identified remarkable differences in the cellular uptake and cell-surface expression of azide-modified hexoses (glucosamine, mannosamine, and galactosamine) in metastatic melanoma cells relative to melanocytes, which is directly dependent on a mole per cell dose and the activity of glucose transporter I. The MFCO-FITC reagent was compared to the more lipophilic, commercially-available Alexa Fluor 488 DIBO. Significantly, no differences were observed between the performance of the MFCO-FITC and the Alexa Fluor DIBO reagents. These findings suggest the MFCO-FITC is a promising reagent for examining differences in cell-surface glycosylation.

Poster 22

EVALUATING MESOPOROUS SILICA NANOPARTICLES FOR DRUG DELIVERY OF DOXORUBICIN

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Mesoporous silica nanoparticles (MSNs) are of significant interest as a drug delivery vehicle due to a variety of advantageous properties such as synthetic tunability in structure and surface functionalization, high surface to volume ratio due to porosity, and apparent biocompatibility compared to many other possible solid matrices such as quantum dots or zeolites. Additionally, a magnetic core may be incorporated into the MSNs to direct their location or, possibly, promote drug release at the target site. Using MSNs for delivery of doxorubicin, an anti-cancer drug used to treat many different types of cancer, is of particular interest because the current method of administration, intravenous therapy, carries a high risk of damage to the heart as well as lowering white blood cell and platelet production.

Doxorubicin is easily detected by fluorescence and can be quantified down to nanomolar concentrations. Loading is determined by fluorescence measurement of the doxorubicin remaining in the loading solution after separating and washing the particles. The release of doxorubicin into phosphate buffered saline is determined by taking aliquots from the release media. The aliquots are centrifuged to remove particles; the particles are resuspended and returned to the release media; and fluorescence measurements of the supernatant of these aliquots are used to determine the amount of drug released.

Initial studies using wormhole-type MSNs with magnetic cores show a drug loading of 25-30 milligrams of doxorubicin per gram of particle. The release profile follows a first-order kinetic exponential decay as described by the equation:

$$Q = Q_{max}(1 - e^{-k_1 t})$$

where Q is the percent of drug released at time t, Q_{max} is the maximum percent of drug released, and k_1 is the first-order release constant. In these studies, Q_{max} was reached over the course of about 24 hours, though maximum release only reached 10-12 percent of the total drug load for the time period studied. Future studies will elucidate how pore structure, presence of a magnetic core, and surface functionality affect both loading and release of doxorubicin.

Poster 23

THE ROLE OF DOPAMINE METABOLISM IN OXIDATIVE STRESS-INDUCED NEUROTOXICITY

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Parkinson's disease (PD) is a chronic and progressive neurological disorder caused by selective degeneration of the substantia nigra region of the brain, specifically the dopaminergic nuclei. This work explores the roles of endogenous neurotoxins and oxidative stress (OxS), especially toxic dopamine metabolites and reactive oxygen species (ROS), as contributors to the neurotoxicity. The neurotransmitter dopamine (DA) is metabolized by monoamine oxidase (MAO) to produce an aldehyde species, 3, 4-dihydroxyphenylactaldehyde (DOPAL). Previous work has shown that DOPAL, in comparison with the parent compound DA and other DA metabolites, is more toxic to neuronal cells both *in vitro* and *in vivo*. DOPAL contributes to cell death through a variety of mechanisms including: protein modification, alteration of oxidative stress (produces ROS), as well as auto-oxidation (produces ROS). DOPAL is able to interact with proteins at Lysine or Arginine resides to form protein adducts, which contribute to cell dysfunction and eventual cell death. Additionally, DOPAL, as well as DA, under physiological conditions undergoes autooxidation to a semiquinone radical and then to an orthoquinone. These quinone products react with proteins and lead to protein modification and aggregation. In both of these mechanisms DOPAL damages the cell as well as produces toxic ROS. In this work along with the focus of protein modification and ROS production by DOPAL, antioxidant status is being investigated. Modulation of antioxidant levels through addition of N-acetylcysteine (NAC) and ascorbate, and depletion using agents such as diamide and buthionine sulfoxide (BSO) will be utilized. Evidence from these studies suggests that antioxidants are able to regulate DOPAL-protein interactions. For example, NAC is able to attenuate modification of proteins by DOPAL. This investigation of protein modification and oxidative stress levels, specifically the roles of toxic dopamine metabolites, ROS, and antioxidants will help in exploring the DOPAL-mediated neurotoxicity.

Poster 24

SYNTHESIS AND BIOLOGICAL EVALUATION 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 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 the design and synthesis of truncated superstolide A, which contains the basic pharmacophore of a 16-member lactone ring attached to a cyclohexene ring. 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. Such a design is considered important to test our hypothesis on the interaction between the natural product and the receptor and provide important information regarding the structure-activity-relationship. In summary, truncated superstolide A was successfully synthesized in 15 steps from commercially available starting material in 6.2% overall yield.

The antiproliferative effect of truncated Superstolide A was evaluated in eight cancer cell lines by using the MTT assay. Truncated Superstolide A is about seven times more potent in suppressing tumor cell proliferation than its parent natural product HT-29 cell. In addition, truncated Superstolide A is also potent in suppressing tumor cell proliferation in the other seven tested cell lines with IC_{50} values ranging from 12–77 nM. These results have confirmed our hypothesis that the 16-membered macrolactone is indeed the pharmacophore that interacts with its putative target in the cells, and the modification of the fuctionalized cis-decalin to a cyclohexene ring apparently does not affect its potent anticancer activity.

Poster 25

LINKING PROTEIN MOTIONS TO ENZYME CATALYSIS: EXPLORATIONS IN ENZYMOLOGY

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Enzyme motions on a broad range of time scales can play an important role in various intermolecular events including substrate binding and product release in achieving the catalyzed chemical conversion. Therefore, the relationship between protein motions and the catalytic activity is of contemporary interest in enzymology. To understand the factors influencing the rates of enzyme catalyzed reactions, it has become apparent that dynamics of protein as well as its environment must be considered. The current work presents the role of protein motions in catalyzed reaction in an enzyme dihydrofolate reductase (DHFR). We have used kinetic isotope effect as an effective tool in probing such a role in the enzyme-catalyzed reactions.[1,2]

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IRIDIUM CATALYZED ENANTIOSELECTIVE ALLYLIC FLUORINATION AND [^{18}F] FLUORINATION FOR LATE STAGE POSITRON EMISSION TOMOGRAPHY IMAGING RADIOTRACERS

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Synthetic methodologies that introduce fluorine into an organic framework have seen a great deal of interest recently due to the many favorable attributes that carbon-fluorine bonds can impart upon a molecular entity. It has been shown that the incorporation of fluorine at a relevant position on a target drug molecule can improve that drugs metabolic stability and bioavailability.[1] Also, [^{18}F] Fluorine is widely used in the field of radiochemistry as a radiotracer in positron-emission tomography (PET) imaging. Once incorporated into a molecular framework, fluorine's similar size and reactivity to that of hydrogen make it an ideal bioisostere that does not interfere significantly with metabolic imaging process *in vivo*.[2]

The attributes that are obtained from incorporating fluorine into a molecular framework has led many chemists to look for novel methods of generating C-F bonds that could be potentially useful to the pharmaceutical industry as well as the PET community. Transition metal catalysis is one of the more recent avenues for accessing different C-F motifs that has proven to be useful.

In 2011, our group introduced an iridium catalyzed method for the high-yielding regioselective synthesis of branched allylic fluorides from secondary and tertiary allylic trichloroacetimidates using $Et_3N{\cdot}3HF$ as the soft nucleophilic fluorinating reagent.[3] Our recent studies have expanded upon this work which has led to two different methodologies using iridium catalysis to incorporate fluorine into an allylic system (Figure 1).

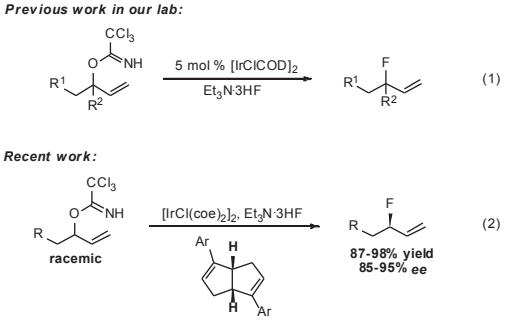
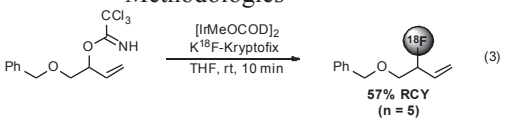


Figure 1 – Nyugen's Allylic Fluorination Methodologies

A ligation procedure done on the commercially available $[IrCl(coe)_2]_2$ metal complex with a c_2-symmetric [3.3.0] bicyclic chiral diene ligand allows us to generate a chiral catalyst to impart a high yielding allylic fluorination with high levels of asymmetric induction (Figure 1-2).

The original methodology was modified even further so that the widely used radioactive complex, $K^{18}F$-Krptofix, could be used as the nucleophilic fluorinating agent (Figure 1-3). The bridging methoxy iridium dimer was found to be the most suitable catalyst for the radiofluorination. Fluorination of the substrate scope has shown that this catalyst system generates radiolabeled allylic fluorides with relatively high radiochemical yields (RCY) in 10 minutes. The bench top stability of the reagents and ease of reaction make this methodology potentially suitable for late stage [^{18}F] fluorination of PET radiotracers.



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Poster 27

ENDOCYTIC PATHWAYS OF FUNCTIONALIZED POLYMERIC PARTICLES FOR TARGETED DELIVERY TO THE LUNG EPITHELIUM

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The ability to target drugs directly to lung cells is an important, though challenging, strategy for the treatment of diseases that affect the mucosal tissues, including infections caused by cell-penetrating pathogens, gene therapy for cystic fibrosis, and asthma. Targeted drug delivery to the lung epithelium is particularly difficult due to the mucosal fluids, which limit transport to the tissues and to the limited uptake of drugs into the lung epithelium. The use of particulate carriers that have been surface modified to overcome these limitations is a promising strategy for targeted delivery in the lungs. We propose a strategy where drug-containing particles will be coated with poly (ethylene glycol) (PEG) to limit mucus adhesion and enhance particle penetration through mucus, as well as bacterial ligands known as lipooligosaccharides (LOS) to facilitate the attachment and uptake of the particles into lung epithelial cells. The goal of this research was to develop functionalized particles that mimic the targeting and uptake behavior of pathogenic bacteria to the lung epithelium.

Polystyrene particles (0.2 and 1 μm) were either left bare (no coating), or coated with gelatin, PEG, or 0.005-50 μg/mL of the isolated ligand LOS from non-typeable *Haemophilus influenzae* 3198. LOS was isolated and its activity was verified using dot immunoblot and ELISA. The LOS and PEG coating was verified using ELISA and trinitrobenzene sulfonic acid assay, respectively. The fate of coated particles was investigated using the bronchial epithelial cell models 16HBE14o- and Calu-3 at different particle concentrations from 0.5 to 24 hours. The particle uptake pathways were determined by eliminating one pathway at a time. The cells were treated with incubation at 4°C or chemical inhibitors: sodium azide, cytochalasin D, Dynasore, genistein, chlorpromazine, methyl β-cyclodextrin, and N-acetyl-D-lactosamine (NAL). Flow cytometry, scanning electron microscopy (SEM), and confocal microscopy techniques were used to quantify particle association with the cells.

Particle adherence and uptake into lung epithelial cells was observed through SEM and confocal micrographs, and then quantified via flow cytometry. The fate of the functionalized particles was dependent on particle size, concentration, and type of ligand coating. As the concentration of the particles increased, the percentage of cells associated with these particles increased until saturation. Uptake of the bare particles after 2 hours of incubation at 4ºC was only about 50% inhibited, showing the importance of both active and passive uptake mechanisms. However, uptake of LOS-coated particles was primarily inhibited at 4ºC, suggesting a stronger dependence on active endocytic pathways including caveolar-mediated and clathrin-mediated endocytosis. Particles coated with 5 μg/mL were most responsive to inhibition by the PAF receptor inhibitor, NAL, which is likely due to better particle coating at this concentration. Uptake of LOS-coated particles was dependent on ATP, actin and dynamin activities, which followed the bacterial uptake mechanism.

Poster 28

FRAGMENT SCREENING AGAINST CASPASE-7 USING A COMPLEMENT OF COMPUTATIONAL AND BIOPHYSICAL TECHNIQUES

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Caspases are a family of endopeptidases that play critical roles in apoptosis and inflammatory processes. Caspase is an acronym for cysteine-aspartic specific protease, named for their catalytic cysteine and preference to hydrolyze peptide bonds adjacent to aspartate residues. The function of these proteases in apoptosis has divided the family into initiator (-8, -9) and executioner (-3, -6, -7) caspases. Initiator caspases are activated through extrinsic (caspase-8) and intrinsic (caspase-9) pathways, involving mitochondrial cytochrome *c* release and death receptor signaling, respectively.[1] Initiator caspases then activate the executioner caspases to carry out dismantling of the cell by cleaving hundreds of intracellular targets. Inhibition of apoptosis via caspases has been the target of a number of ongoing clinical trials for the treatment of hepatitis C virus infection, acute liver failure, and ischemia reperfusion injury during organ transplant.[2]

Fragment-based drug discovery has been successfully established as a method for improving hit rates in drug discovery campaigns. This methodology has been developed to improve physicochemical properties of lead compounds and decrease attrition in the drug development pipeline.[3] Low complexity molecules shrink the chemical space, allowing for greater library diversity without the need to screen hundreds of thousands of compounds. A library of 1000 fragments (<300 M.W.) will be screened against caspase-7 with differential scanning fluorimetry. Hits will be validated with surface plasmon resonance to elicit dissociation constants and binding kinetics. Molecular dynamics simulations and docking using VinaMPI on the Helium Cluster at the University of Iowa High Performance Computing Center will be used to guide fragment growth, merging or linking strategies than can be used to develop clinically relevant caspase inhibitors.

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Poster 29

CELLULAR CONSEQUENCE OF FUNGICIDE EXPOSURE AND REACTIVE DOPAMINE METABOLITES ON DOPAMINERGIC NEURONS

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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 doparminergic 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-dihydroxyphenylacetaldehye (DOPAL). A 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. Exposure to environmental toxins such as pesticides and fungicides has been recently linked to DOPAL. The fungicide, benomyl is a potent ALDH inhibitor which is postulated to increase DOPAL levels and is positively associated with PD risk. The role of fungicide exposure and ALDH inhibition will be explored as it relates to selective neuronal toxicity.

Poster 30

SYNTHESIS OF A PEGYLATED GLYCOPEPTIDE FOR GENE DELIVERY

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Mannose receptors expressed on the surface of liver Kupffer cells specifically bind and internalize nanoparticles externally decorated with mannose, providing a target for the delivery of ligand-bearing DNA polyplexes. Effective delivery and expression of Kupffer cell-targeted DNA polyplexes could provide a potential therapy for lysosomal storage disorders, such as Gauche's disease.

Soy flour contains 0.3 wt% soybean agglutinin (SBA), a 31 kDa lectin possessing a single high-mannose N-glycan (Man_9, shown below). In the present study, we improved the purification of Man_9 to prepare a gene delivery glycopeptide[1]. Affinity purification of SBA using a galactose-Sepharose resin was greatly improved by batch-processing of 800 g of soy flour, allowing isolation of 2.1 g of pure SBA in a single step. Pronase digestion followed by low-pressure gel filtration and cationic exchange chromatography yielded 24.5 μmol (49 mg) of purified Man_9-Asn (36% yield).

Man_9-Asn was further modified by reaction with NHS-Tyr-Boc resulting in a quantitative yield of Man_9-Asn-Tyr-Boc that was characterized by LC-MS and 1H-NMR. Boc-removal with TFA allowed installation of a sulfhydryl moiety by conjugation to N-succinimidyl S-acetylthioacetate (SATA) (92.7% yield). Deacylation with hydroxylamine followed by reaction with maleimide-glycine-PEG_{5kDa}-NH-Boc, yielded the PEGylated N-glycan (Boc-PEG-Man_9) in 72.5% yield. Another Boc-deprotection followed by reaction with NHS-glycine-maleimide gave Mal-Gly-PEG-Man_9 (91.2% yield). A polyacridine peptide, $(Acr\text{-}K_4)_3Acr\text{-}KC$ (below), was synthesized by SPPS using FMOC-Lys-Acr (Acr) to introduce acridine on lysine side chains, thereby, allowing for poly-intercalation with plasmid DNA. Completion of the PEGylated glycopeptide synthesis was accomplished by the reaction of $(Acr\text{-}K_4)_3Acr\text{-}KC$ with Mal-Gly-PEG-Man_9. Following preparative RP-HPLC purification and 1H-NMR characterization, 6.9 μmol of the PEGylated glycopeptide (shown below) was recovered at a 10.1% overall yield starting from 800 g of soy flour.

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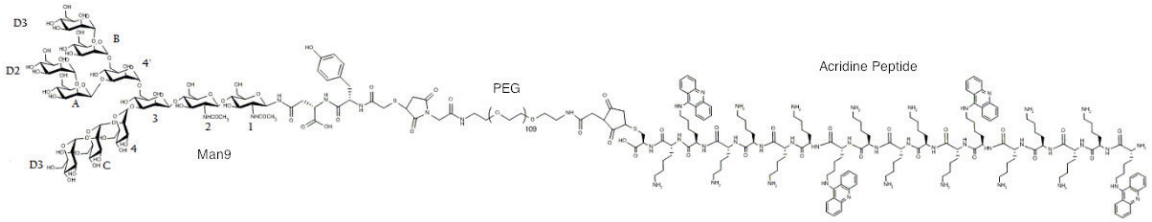

Poster 31

INHIBITION OF GERANYLGERANYL DIPHOSPHATE SYNTHASE VIA HOMOISOPRENOID TRIAZOLES

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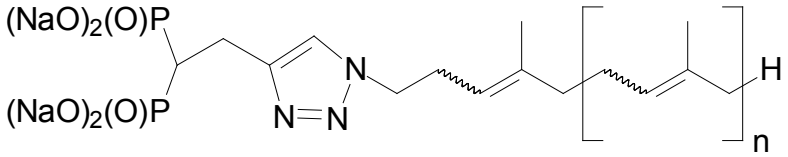
Addition of the 5-carbon unit, isopentenyl pyrophosphate, to farnesyl pyrophosphate in the isoprenoid biosynthetic pathway is catalyzed by the enzyme geranylgeranyl diphosphate synthase (GGDPS) and provides formation of geranylgeranyl pyrophosphate (GGPP). The intermediate GGPP is essential for protein prenylation for cellular signaling and trafficking. There has been interest in GGDPS inhibitors due to their anti-proliferative effects. In an effort to make new inhibitors of this enzyme, we have developed a number of triazole (e.g. **1**–**4**) derivatives bearing a geminal bisphosphonate. The triazoles are substituted with homoisoprenoid chains of varying lengths and have been prepared. The synthesis of these compounds and their activity as inhibitors of GGDPS will be presented.



1 n = 0
2 n = 1
3 n = 2
4 n = 3

Poster 32

EFFECT OF N-1 ARYL SUBSTITUENTS ON DNA BINDING BY FLUOROQUINOLONES

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Fluoroquinolones are broad spectrum antibiotics used to treat a variety of bacterial infections. Due to the emergence of bacterial resistance to current fluoroquinolones, the need for novel fluoroquinolones active against resistant mutants continues to grow. Fluoroquinolones exert their antibacterial activity by poisoning DNA gyrase and/or topoisomerase IV, through the formation of a ternary complex with the enzyme and DNA. Mutations to genes encoding these two target enzymes are the main cause of fluoroquinolone resistance. Through the use of crystallographic investigation, the N-1 and C-7 positions of fluoroquinolones were identified as possible positions for structural modification to generate novel fluoroquinolones that would retain activity with resistant mutants. During this study it was found that incorporation of certain aryl groups at the N-1 position of the quinolone core resulted in loss of activity toward the bacterial enzymes. However, these compounds were found to have potent activity against human topoisomerases. This crossover to inhibit human topoisomerases, by a mechanism different than that observed for the inhibition of bacterial enzyme, indicated this new type of fluoroquinolone might have therapeutic application through the inhibition of human topoisomerases. Thus further investigation into the mechanism of action of these novel fluoroquinolones was desired.

We hypothesized that the planar nature of the N-1 groups on the new fluoroquinolone derivatives would allow them to intercalate DNA, thus causing disruption of enzyme function. Based on work by Hiasa and collaborators, the N-1 derivatives were shown to possess a novel mechanism of action based on DNA gyrase poisoning assays, although their activity with the bacterial enzymes is quite poor. Presented here are studies aimed to determine if direct DNA binding plays a role in the different mechanisms of topoisomerase inhibition observed for the individual N-1 derivatives, as well as efforts to correlate structural features and DNA binding properties with inhibition of topoisomerase function. To investigate the DNA binding affinities of the N-1 derivatives, a thiazole orange displacement assay was employed. Thiazole orange is an intercalating dye that is optically active when bound to DNA and upon displacement a loss of fluorescence is observed. The N-1 fluoroquinolone derivatives, 1-aminopyrene (control intercalator) and commercially available fluoroquinolones, Moxifloxacin and Ciprofloxacin, were tested for their ability to displace thiazole orange from DNA. Interestingly, only select compounds display high intercalative properties.

Poster 33

IN VITRO EVALUATION OF N-METHYL-2-METHOXY-PYRIDINIUM SPECIES AS POTENTIAL AGING DELAY AGENTS FOR PHOSPHYLATED HUMAN ACETYLCHOLINESTERSE

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Acetylcholinesterase (AChE) is an essential enzyme in the human neuronal system. The essential role of AChE makes it a main target for the development of organophosphorus compounds (OP) such as pesticides and extremely toxic chemical warfare agents (CWAs). After inhibition of AChE by OP, the inhibited enzyme can undergo aging where the enzyme is irreversibly inhibited after a de-alkylation process. The reversibly inhibited enzyme can be reactivated by various oxime nucleophiles. However, aged enzyme, irreversibly inhibited, is resistant towards the current treatments, which at sufficient doses of CWAs leads to death. It is suggested that if the phosphonate anion of the aged adduct is alkylated, known oximes could then be used to reactivate the OP-inhibited AChE. Therefore, we synthesized potential methylating agents and studied kinetic parameters to determine the ability of reactivation of aged-AChE. Our preliminary studies showed some N-methyl-2-methoxy-pyridinium species as potential methylating agents for an enzyme analog. However, in vitro evaluation indicated that none of the species were able to resurrect the aged AChEwith known oximes such as 2-pyridine aldoxime methyl chloride (2-PAM) and monoisonitrosoacetone (MINA). However, recent mutagenesis studies indicated that replacement of some negatively charged amino acids residues with neutral residues results in significant reduction of the rate of the aging process. Therefore, further kinetic assessment of these potential methylating agents for the alkylation of those amino acids residues is important.

Poster 34

ENHANCED POLYCHLORINATED BIPHENYL REMOVAL IN A SWITCHGRASS RHIZOSPHERE

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Phytoremediation, the use of plants and associated microorganisms to clean up contaminated soils and sediments, is cost-effective and simple. In this study, switchgrass (*Panicumvirgatum*) was employed to improve the degradation of PCB 52, 77 and 153 in soil microcosms. After 24 weeks of incubation, loss of 39.9 ± 0.41% of total PCB molar mass was observed in switchgrass treated soil, significantly higher than in unplanted soil (29.5 ± 3.4%) ($p<0.05$). The improved PCB removal in switchgrass treated soils could be explained by phytoextraction processes and enhanced microbial activity in the rhizosphere. Redox cycling (sequential flooding and non-flooding) was performed to promote the PCB dechlorination. In redox cycled and unplanted soil, PCB 153 removal was increased by 5.3% after 24 weeks of incubation as compared to unplanted soil ($p<0.05$). Transformation products were detected in all soils, consistent with PCB dechlorination. Flooding successfully enriched the putative dechlorinating *Chloroflexi* populations, which were confirmed to be *Dehalogenimonas*, a potential PCB-dechlorinating species. Clone library analysis shows the dominance of *Proteobacteria* and *Acidobacteria.* Other dehalorespiring bacteria such as *Geobacter* and *Clostridium* were also detected, suggesting enrichment of the dechlorination potential of the indigenous microbial community and their possible role in PCB dechlorination.

Poster 35

CAFFEINE DEHYDROGENASE: A SUITABLE ENZYME FOR RAPID CAFFEINE DETECTION AND DEVELOPMENT OF AN "IN-HOME" DIAGNOSTIC TEST

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Excess and long-term consumption of caffeine (>400 mg/day/adult) can lead to adverse health-effects. Recent introduction of caffeinated products (gums, jelly beans, energy-drinks) might lead to excessive consumption, especially among children and nursing mothers; hence attracted FDA attention and product withdrawals. Currently, no method is available commercially for detection of caffeine, esp. in nursing mother's milk. An "in-home" test will aid vigilant consumers in detecting caffeine in beverages and milk easily and quickly, thereby restricting its consumption. Known diagnostic methods lack speed and sensitivity. Previously, caffeine dehydrogenase (Cdh), a novel heterotrimeric quinone-dependent enzyme was isolated from a caffeine-degrading strain *Pseudomonas* sp. CBB1 which oxidized caffeine in presence of tetrazolium dyes as electron acceptors[1]. In this work, we report the development of a Cdh-based caffeine diagnostic test, which is highly sensitive (1-5 ppm) and detects caffeine in beverages and mother's milk in one minute[3]. Other components in these complex test samples do not interfere with the detection. Caffeine-dependent reduction of the dye Iodonitrotetrazolium chloride (INT) results in shades of pink proportional to the levels in test samples. This test also estimates caffeine levels in pharmaceuticals, comparable to HPLC. The Cdh-based test is the first with the desired attributes for rapid and robust caffeine diagnostic kit[3]. In future, a dip-stick test will be developed for public and industrial applications like in pharmaceutical quality control for caffeine detection and quantification. Furthermore, this assay has the potential to detect low levels of caffeine in environment such as rivers and ground water; indicative of pollution due to human activities[4].

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Poster 36

RAPID IDENTIFICATION AND PRODUCTION OF METABOLITES USING STABILIZED DRIED POWDER (SDP) OF HUMAN CYTOCHROME P450S (CYPS) 2D6, 3A4, AND 2C9 ENGINEERED IN YEAST

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Our larger vision is to metabolically engineer a microbe to "mimic" drug metabolism of human liver. Also, the engineered microbes must be scalable to obtain tens of kilograms of cells within 2-4 days via high cell density fermentation (OD_{600} is around 500 ODU). This will enable biocatalytic prep-scale synthesis of drug metabolites. This innovation is "game-changing" in terms of (i) large scale preparation of drug metabolites for Metabolite in Safety Test (MIST) studies and use as reference standards (ii) drug-drug interaction analysis, and (iii) easy identification of human metabolites of drugs for registration. The innovation also significantly reduces usage of animal-derived materials. Every drug in development needs to be fully characterized with respect to all the metabolites produced in humans. Any metabolite >10% of the drug, must be subject to MIST. This requires prep-scale synthesis of metabolites, which is usually done by classical synthesis or using animal/human microsomes. This technology is old, cumbersome, difficult, low yielding and time consuming due to the complexity of the metabolite-structure. The biocatalytic tools available to generate mg amount of metabolites are extremely expensive. The present innovation changes this game due to availability of large amounts of "microbial human liver" for prep-scale synthesis of specific metabolites. The process is extremely simple; add SDP to drug-candidate of interest. Incubate for 1 to 4 h and analyze metabolites. There is no need for cumbersome microsomal preparation and addition of external NADPH. The present innovation has significantly reduced the hassle, time, and cost of the metabolite identification and preparation. This will help pharmaceutical companies make critical decision in terms of rapid identification of the winners in the drug pipeline.

We have completed the engineering of CYP2D6, CYP3A4, CYP2C9, & CYP2C19 in yeast in our first phase of experiments and made SDP of these CYPS successfully. These SDP preparations mimic "human liver" reactions of corresponding CYPs based on recommended standard substrates for each CYPs. Here, we describe (i) hCYP2C9-SDP catalyzed conversion of diclofenac (DN) to 4-hydroxydiclofenac in microtiter plate, and in 400 mL scale to generate 53.6 mg of HDN from 59.2 mg of DN in only 1.5 hours, and (ii) hCYP3A4-SDP catalyzed conversion of testosterone (TE) to 6β-Hydroxytestosterone (HTE) in microtiter plate and in 200 mL scale. Two cycles of reaction produced 7.01 mg of HTE from 28.8 mg of TE in 3.0 hours. We are currently scaling up other hCYP-SDPs to demonstrate facile synthesis of metabolites in large scale.

Poster 37

QUANTITATIVE FUNCTIONAL ANALYSIS OF CAFFEINE INDUCED ENZYMES IN WILD TYPE AND GENE KNOCKOUT STUDIES OF CAFFEINE-DEGRADING STRAIN CBB5

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Background: Deploying bacteria with the capacity to metabolize persistent xenobiotic compounds may convert environmental burdens into desirable feedstock for organic and pharmaceutical syntheses. However, the challenge of characterizing the enzymology and molecular genetics of biodegrading bacteria under different conditions remains a principle impediment to their application. For example, four years of intensive work in protein purification, genomic DNA library construction, gene cloning and functional expression studies were required metabolic enzymes, methylxanthine N-demethylases (NdmABCDE), induced in a caffeine-degrading strain of *Pseudomonas putida*, CBB5. Functional analyses of Ndm genes has been conducted by knockout (KO) of each gene (A-E) individually. However, their multi-component nature, especially the heterotrimeric complex NdmCDE, adds ambiguity to selective KO studies. Proteomics mass spectrometry makes global expression studies more precise and has greatly impacted our understanding of gene function in the post-genomic period. To address the issue mentioned above, a proteomics workflow, including in-solution tryptic digestion and stable isotope labeling coupled to LC-MS/MS analyses was developed to reliably quantify affected proteins in the caffeine-degrading pseudomonas putida strain CBB5.

Method: Bacteria cells from three cultures, *NdmE* KO *P. putida* CBB5 grown in soytone medium containing caffeine, and wild type strain CBB5 grown in soytone medium with caffeine and without caffeine were harvested and analyzed by proteomic methods described elsewhere. Briefly, protein comparable fractions were enriched based on Ndm activity (*Ndm active*) by DEAE Sepharose and then digested by trypsin, labeled with distinct stable isotope compositions using reductive amination, combined and subjected to simultaneous LS-MS/MS analyses. MS/MS spectra acquired on an Agilent 6530 Accurate-Mass Q-TOF LC-MS platform were searched using the Mascot server (version 2.4) against a composite database of all *Pseudomonas* strains listed in UniProtKB with its reversed complement appended for evaluation of the false discovery rate (FDR).

Results: Quantitative proteomic analysis indicated that NdmC was abrogated by KO of NdmE while expression of NdmD was substantially diminished though detectable relative to CST. Interestingly, the abundance of NdmA and NdmB in NdmE KO *P.putida* CBB5 are 1.2 and 1.3 times higher than that of wild type CBB5 grown on soytone with caffeine. This suggests that in NdmE KO and CST, NdmA and NdmB were up regulated to metabolize caffeine to methylaxanthines. Meanwhile, Xanthine dehydrogenase in NdmE KO was also suppressed substantially relative to CST. Consistent with our earlier report, the activity of XDH correlates with xanthine from caffeine metabolism in wild type *P. putida* CBB5.

Poster 38

CONTINUOUS, REAL-TIME CHEMICAL MONITOR FOR ON-LINE MEASUREMENT AND CONTROL OF *PICHIA PASTORIS* BIOPROCESSES

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Analytical sensing technologies that can measure key chemicals in real-time during cell expansion and protein expression phases of upstream processes has been of interest to the biotechnology community for some time. The driving goal for the development of these technologies, in addition to enhanced process efficiency, is consistent product quality through discovery, process development, scale up, and manufacturing. Real-time chemical monitoring is recognized as being particularly important during these complex processes, where multiple parameters including media composition, dissolved oxygen levels, and reactor scale are known to impact product quality.

A novel on-line bioprocess monitor is presented for the simultaneous, real-time measurement of glycerol and methanol and the tracking of cell density during production of recombinant protein from *Pichia pastoris*. This automated monitor uses a completely sealed and sterilized closed loop to continuously circulate a small sample from the bioreactor through the monitor and back to the bioreactor. There is zero sample lost from monitoring and the measurement method is completely nondestructive. In contrast with some previous approaches, very little operator expertise is required. The monitor is calibrated prior to installation and then tuned to the user's specific process. Operation is a simple process of injecting and briefly collecting data for a set of standard solutions, connecting the sterilized process loop from the bioreactor, and then collecting quantitative data. The monitor's solid-state construction is rugged and designed for industrial use. Results will be presented showing robust operation and accurate, monitoring of glycerol and methanol during multiple *Pichia* fermentation runs for more than 3 months post-calibration

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YEAR	CBB Fellowship	*NIH Trainee Fellowship
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1992-93	12	6
1993-94	12	6
1994-95	13	10
1995-96	13	6
1996-97	12	6
1997-98	12	6
1998-99	12	6
1999-00	13	7
2000-01	14	7
2001-02	12	7
2002-03	11	8
2003-04	14	8
2004-05	12	7
2005-06	11	7
2006-07	10	7
2007-08	10	7
2008-09	10	8
2009-10	6	8
2010-11	7	8
2011-12	6	8
2012-13	5	6
2013-14	4	5
2014-15	4	6
TOTAL	257	172

*Partially Sponsored by CBB

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Gratefully Acknowledges
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