The University of Iowa

22nd

Center for Biocatalysis and Bioprocessing Conference

Current Topics in Industrial Biotechnology

www.uiowa.edu/~biocat

The University of Iowa

October 21-22, 2013
Iowa Memorial Union
Iowa City, Iowa


ÄKTApilot
POWER
CONTINUE / RUN
PAUSE
Air trap
Flow Direct Valve
Mixer
Pressure Sensor
PUMP B

THE UNIVERSITY OF IOWA

22nd Annual
Biocatalysis and Bioprocessing
Conference

"Current Topics in Industrial Biotechnology"

Sponsored by:

THE UNIVERSITY OF IOWA
Center for Biocatalysis and Bioprocessing

October 21-22, 2013

www.uiowa.edu/~biocat

22nd Annual
Biocatalysis and Bioprocessing Conference

"Current Topics in Industrial Biotechnology"

Sponsored by:

The University of Iowa
Center for Biocatalysis and Bioprocessing

October 21-22, 2013

Conference Organizing Committee:

Mani Subramanian, Ph.D.
Sridhar Gopishetty, Ph.D.
Charles Brenner, Ph.D.
Alexander Horswill, Ph.D.
Robert Kerns, Ph.D.
Amnon Kohen, Ph.D.
Timothy Mattes, Ph.D.
Eric Nuxoll, Ph.D.
Tonya Peeples, Ph.D.
Daniel Quinn, Ph.D.
Mitchell Rotman

Director
Mani Subramanian, Ph.D.

Table of Contents

22nd Annual Center for Biocatalysis and Bioprocessing Conference
"Current Topics in Industrial Biotechnology"
Iowa Memorial Union, Iowa City, IA

MONDAY, OCTOBER 21, 2013

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/Buffet – Ballroom, 2nd Floor IMU

7:00 – 8:00 **Mark A. Behlke, M.D., Ph.D.** Chief Scientific Officer, Integrated DNA Technologies, Inc. (IDT)
"A new class of drugs: use of synthetic DNA and RNA to regulate gene expression"

TUESDAY, OCTOBER 22, 2013

7:00-8:00 am Registration – Iowa Theater (formerly Bijou) 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
Sridhar Gopishetty, Ph.D., Technical Director, Center for Biocatalysis and Bioprocessing, The University of Iowa

9:00 **Yong-Su Jin, Ph. D.**, Assistant Professor, Department of Food Science and Human Nutrition, Institute for Genomic Biology, and Energy Biosciences Institute, University of Illinois, Urbana, IL
"*Synthetic biological widgets: engineered yeasts for producing fuels and chemicals from renewable biomass"*

9:45 **Oliver Yu, Ph.D.,** Chief Science Officer, Blue California
"Fermentation production of natural BluCetin™-DHM by targeted biosynthesis"

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

10:45 **Thomas S. Vedvick, Ph.D.,** Vice President, Formulation and Process Development, Infectious Diseases Research Institute (IDRI)
"*Development of modern vaccines, recombinant protein plus adjuvant"*

11:30 Lunch – Ballroom, 2nd Floor IMU
Advisory Board Meeting, Penn State Room, 337 IMU

Afternoon Session – Iowa Theater, 1st Floor IMU

1:15 pm **Amnon Kohen, Ph.D.,** Professor, Department of Chemistry, University of Iowa
"Long-range effects of protein dynamics in enzyme catalysis"

2:00 – 3:00 **CBB/NIH Fellow Presentations**
Four selected graduate students will each have a 10 minute talk followed by a 5 minute Q&A session

Kyle Kloepping, Schultz Laboratory, Department of Radiation Oncology, Free Radical and Radiation Biology Program, The University of Iowa
"A new small molecule therapy for Metastatic Melanoma"

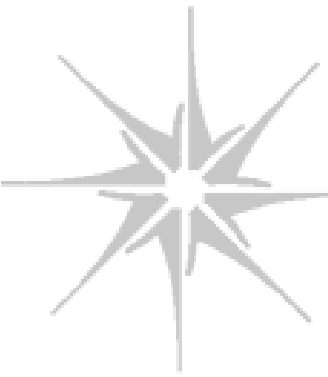
Yi Liang, Mattes Laboratory, Department of Civil and Environmental Engineering, The University of Iowa
"Enhanced polychlorinated biphenyl degradation by bioaugmentation with Burkholderia Xenovorans LB400 in a switchgrass planted Rhizosohere"

Duncan Mackie, Roman Laboratory, Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, The University of Iowa
"Development of a novel high-throughput screen targeting RGS1-17 & characterization of lead small molecule inhibitors for the treatment of metastatic lung and prostate cancers"

Cicily J. Ronhovde, Geng Laboratory, Department of Chemistry, The University of Iowa
"Modeling particle clustering using pair correlation function analysis"

3:00 – 5:00 **Poster Session** – Wine/hors d'oeuvres, Ballroom 2nd Floor IMU
Announcement of Usha Balakrishnan 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. **A NEW CLASS OF DRUGS: USE OF SYNTHETIC DNA AND RNA TO REGULATE GENE EXPRESSION**
   Mark A. Behlke, M.D., Ph.D.
   Chief Scientific Officer, Integrated DNA Technologies, Inc., Coralville, IA

2. **SYNTHETIC BIOLOGICAL WIDGETS: ENGINEERED YEASTS FOR PRODUCING FUELS AND CHEMICALS FROM RENEWABLE BIOMASS**
   Yong-Su Jin, Ph. D.
   Assistant Professor, Department of Food Science and Human Nutrition, Institute for Genomic Biology, and Energy Biosciences Institute, University of Illinois, Urbana, IL

3. **FERMENTATION PRODUCTION OF NATURAL BLUCETIN™-DHM BY TARGETED BIOSYNTHESIS**
   Oliver Yu, Ph.D.
   Chief Science Officer, Blue California, Rancho Santa Margarita, CA

4. **DEVELOPMENT OF MODERN VACCINES, RECOMBINANT PROTEIN PLUS ADJUVANT**
   Thomas S. Vedvick, Ph.D.
   Vice President, Formulation and Process Development, Infectious Diseases Research Institute, Seattle, WA

5. **LONG-RANGE EFFECTS OF PROTEIN DYNAMICS IN ENZYME CATALYSIS**
   Amnon Kohen, Ph.D.
   Professor, Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

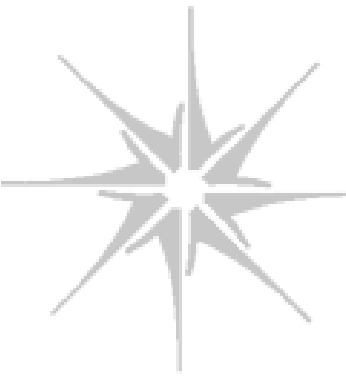
6. **A NEW SMALL MOLECULE THERAPY FOR METASTATIC MELANOMA**
   Kyle C. Kloepping, Mitchell C. Coleman, Brett A. Wagner, Melissa A. Fath, James A. Jacobus, Kranti A. Mapuskar, Garry R. Buettner, Douglas R. Spitz and Michael K. Schultz*
   Department of Radiation Oncology, Free Radical and Radiation Biology Program, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

7. **ENHANCED POLYCHLORINATED BIPHENYL DEGRADATION BY BIOAUGMENTATION WITH *BURKHOLDERIA XENOVORANS* LB400 IN A SWITCHGRASS PLANTED RHIZOSPHERE**
   Yi Liang, Richard Meggo, Dingfei Hu, Jerald L. Schnoor and Timothy E. Mattes*
   Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, IA

8. **DEVELOPMENT OF A NOVEL HIGH-THROUGHPUT SCREEN TARGETING RGS17 & CHARACTERIZATION OF LEAD SMALL MOLECULE INHIBITORS FOR THE TREATMENT OF METASTATIC LUNG AND PROSTATE CANCERS**
   Duncan I. Mackie and David L. Roman*
   Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

9. MODELING PARTICLE CLUSTERING USING PAIR CORRELATION FUNCTION ANALYSIS

Cicily J. Ronhovde and M. Lei Geng*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA



Speaker's Profiles

KEYNOTE SPEAKER

Mark A. Behlke, M.D., Ph.D.
Chief Scientific Officer
Integrated DNA Technologies, Inc. (IDT)

As the Chief Scientific Officer, Dr. Behlke directs activities at IDT across a variety of new product development and basic research areas including functional genomics (RNAi and antisense), DNA thermodynamics, probe chemistries, amplification methods, and next generation sequencing technologies. He has directed research activities at IDT since joining the company in 1995. Dr. Behlke (together with Dr. John Rossi, from the Beckman Research Institute at the City of Hope) is a scientific co-founder of Dicerna Pharmaceuticals, located in Boston, Massachusetts and is a member of the Dicerna Scientific Advisory Board.

Before joining IDT, Dr. Behlke was a Physician Postdoctoral Fellow of the Howard Hughes Medical Institute at the Whitehead Institute for Biomedical Research, MIT, where he studied human sex determination in the laboratory of Dr. David Page. He was a Resident Physician in Internal Medicine and Fellow in Endocrinology at Brigham and Women's Hospital, Boston. Dr. Behlke received his M.D. and Ph.D. degrees from Washington University, St. Louis in 1988, where he studied immunogenetics in the laboratory of Dr. Dennis Loh. He received his B.S. degree from the Massachusetts Institute of Technology in 1981.

Mark A. Behlke is an inventor on over 20 issued US patents, has numerous pending patent applications, and is author of over 90 scientific publications in peer-reviewed journals. He is an internationally recognized expert in nucleic acid technologies.

Yong-Su Jin, Ph.D.
Assistant Professor in the Department of Food Science and Human Nutrition
Faculty member of the Institute for Genomic Biology
Principal investigator of the Energy Biosciences Institute
Member of the Center for Advanced BioEnergy Research at the University of Illinois at Urbana-Champaign

Dr. Yong-Su Jin received B.S. and M. S. degrees in Food Science and Technology from Seoul National University and received Ph. D. degree in Food Science and Bacteriology (minor) from the University of Wisconsin-Madison. After completing a post-doctoral training in the Department of Chemical Engineering at the Massachusetts Institute of Technology, he served as an Assistant Professor at the Sungkyunkwan University in Korea.
Dr. Jin's research is centered on metabolic engineering of microorganisms to produce biofuels and chemicals from renewable biomass. His research aims to identify, characterize, and engineer beneficial genetic perturbations eliciting rapid and efficient production of target products, such as biofuels, nutraceuticals, and food ingredients. Dr. Jin was a Faculty Fellow of National Center for Supercomputing Applications for 2009-2010 and received the ACES College Faculty Awards for Excellence in Research.

Oliver Yu, Ph.D.
Adjunct professor at the Department of Biology, Washington University in St Louis and the Division of Plant Sciences at the University of Missouri-Columbia
Serves on the editorial board of JBC and GM Crop
Serves as the Chief Science Officer for Blue California since 2010

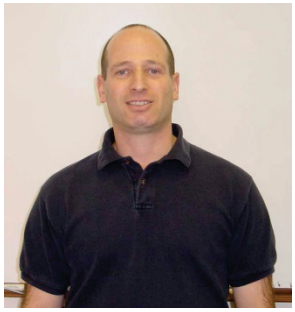
Dr. Yu received his B.Sc. from the Department of Biophysics at Fudan University in Shanghai, China, and his Ph.D. from the Department of Biology at the University of South Carolina, Columbia, S.C. He did his postdoctoral training at the DuPont Company in Wilmington, Delaware and joined the Donald Dansforth Plant Science Center in 2001, serving as an Assistant Member and later as an Associate Member and Principal Investigator. Dr. Yu is currently an adjunct professor at the Department of Biology, Washington University in St Louis and the Division of Plant Sciences at the University of Missouri-Columbia. He has published more than 60 manuscripts and patents and serves on the editorial board of JBC and GM Crop. Dr. Yu formally joined Blue California in 2010 and currently serves as its Chief Science Officer.

Thomas S. Vedvick, Ph.D.
Vice President of Formulation and Process Sciences
Infectious Disease Research Institute (IDRI)

Dr. Vedvick has been recognized both nationally and internationally in the fields of Infectious Disease vaccines, Protein chemistry and Adjuvant development. He is currently Vice President of Formulation and Process Sciences for the IDRI in Seattle, Washington. Dr. Vedvick is responsible for the development of recombinant protein production and for the formulation and development of adjuvants to stimulate immune responses. He has published approximately 90 peer reviewed articles and currently hold 36 issued US Patents.

Dr. Vedvick is recognized within IDRI for both project and team leadership. He has established a micro protein sequencing facility and has successfully produced vaccines for the developing world in the field of Infectious Disease. Dr. Vedvick is currently working on a vaccine for Leprosy to help in the complete eradication of this disease.

Amnon Kohen, Ph.D.
Professor, Department of Chemistry, The University of Iowa



Prof. Amnon Kohen is a Professor of Chemistry and of Molecular and Cellular Biology. Amnon was born and raised in Israel. He received his B.Sc. degree in Chemistry in 1989 from the Hebrew University in Jerusalem and D.Sc. degree in 1994 from Technion-Israel Institute of Technology. After training with Prof. Karen Anderson at Yale he was a postdoctorial fellow with Prof. Judith Klinman at the University of California at Berkeley. In 1999, he moved to the University of Iowa. His main interest is Enzymology, Bioorganic and Biophysical chemistry. His group studies the mechanisms by which enzymes activate C-H bonds, a research that focuses on the relationship between enzyme structure, dynamics, and catalysis. The group also pursues new mechanisms in DNA biosynthesis and medical and technological applications of biological catalysts.

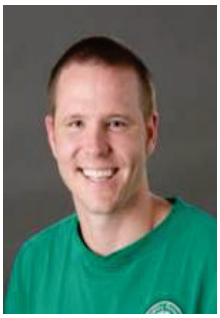
Kyle C. Kloepping
Ph.D. candidate
Schultz Research Group
Department of Radiation Oncology
Free Radical and Radiation Biology Program
The University of Iowa

Kyle is from Freeport, Illinois. He earned his Bachelor of Science in Biology with a minor in Biotechnology from the University of Wisconsin – Platteville in 2010. Kyle is currently a PhD student in the Department of Radiation Oncology (Free Radical and Radiation Biology Program) in the research laboratory of Michael K. Schultz, PhD. After earning his degree, Kyle plans to pursue a career in industry.

Yi Liang
Ph.D. candidate
Mattes Research Group
Department of Civil and Environmental Engineering
The University of Iowa

Yi Liang earned her Bachelor degree of Environmental Science from Nanjing University in China, and she gained a Master degree in Environmental Engineering at University of Iowa. She is currently a doctoral candidate in the Department of Civil and Environmental Engineering working with Dr. Mattes.

Duncan Mackie
Ph.D. candidate
Roman Research Group
Department of Pharmaceutical Sciences and Experimental Therapeutics
Division of Medicinal and Natural Products Chemistry
The University of Iowa



Duncan is from Fort Lauderdale, Florida. He earned his Bachelors of Science in Mathematics from The University of the Cumberlands in Williamsburg, Kentucky in 2006. He continued his studies in Biology and Chemistry in 2008, during which time he was a 14-Time All-American as a member of the swimming and diving team. Upon graduation he joined the University of South Florida as an Academic Coach to the football team. Duncan is currently a doctoral candidate in the Department of Pharmaceutical Sciences and Experimental Therapeutics as a member of the Division of Medicinal and Natural Products Chemistry, working for Dr. David L. Roman. He is also currently a member of the University of Iowa's, top 15 ranked, USAT triathlon team. Upon graduation he plans to pursue an academic or industrial post-doctoral position working on the development of novel pre-therapeutic leads for the treatment of cancer.

Cicily J Ronhovde
Ph.D. candidate
Geng Research Group
Department of Chemistry
The University of Iowa

Cicily is from a small town outside of Lincoln, Nebraska. She earned her Bachelor of Arts in Chemistry from Cornell College in Mount Vernon, Iowa, in 2010. Cicily is currently a doctoral candidate in the Department of Chemistry, working with Professor M. Lei Geng. After earning her degree, she plans to pursue a career in industry.

Oral Presentation Abstracts

Presenter 1

A NEW CLASS OF DRUGS: USE OF SYNTHETIC DNA AND RNA TO REGULATE GENE EXPRESSION

Mark Behlke, M.D., Ph.D.
Chief Scientific Officer, Integrated DNA Technologies, Inc., Coralville, IA

The first experiments using "antisense oligonucleotides" (ASOs) to suppress gene expression were performed in 1978. These early experiments used synthetic DNA to trigger endogenous RNase H to degrade a targeted mRNA. Through advancements in medicinal chemistry, modern ASOs have over 1000-fold higher potency than the original compounds studied. An injectable ASO drug (mipomersen) was recently approved by the FDA for treatment of familial hypercholesterolemia. More ASO drugs are in clinical trials and more approvals are expected in the future. Newer RNA-based approaches are even more promising.

RNA interference (RNAi) is an evolutionarily conserved pathway present in organisms ranging from plants to humans, in which double-stranded RNA (dsRNA) triggers a series of biochemical events culminating in sequence-specific suppression of gene expression. Artificial RNA duplexes can be designed to engage the natural RNAi machinery. This is an extremely potent process and synthetic small interfering RNAs (siRNAs) can have an EC50 of below 1 pM *in vitro*. Synthetic siRNAs can be used as an experimental tool to selectively reduce expression of targeted genes. Chemical modification of the siRNA can confer nuclease stability and improve pharmacodynamic properties. Building upon historical work done in the area of antisense therapeutics, the medicinal chemistry of therapeutic siRNAs is already advanced and a number of effective chemical modification strategies have been described. Several siRNA drugs are already in clinical trials for a variety of indications.

Presenter 2

SYNTHETIC BIOLOGICAL WIDGETS: ENGINEERED YEASTS FORPRODUCING FUELS AND CHEMICALS FROM RENEWABLE BIOMASS

Yong-Su Jin, Ph.D.
Assistant Professor, Department of Food Science and Human Nutrition, Institute for Genomic Biology, and Energy Biosciences Institute, University of Illinois, Urbana, IL

Synthetic biological widgets, i.e. engineered biological systems executing desirable functions, can be constructed for efficient and rapid production of fuels and chemicals from cellulosic biomass. While cellulosic hydrolyzates consist of mixed sugars (glucose and xylose) and acetate, naturally existing or engineered yeasts use glucose and xylose sequentially. The preferential utilization of glucose to xylose and inhibition of yeast metabolism by acetate results in lower conversion yields and productivities of target fuels and chemicals. Therefore, simultaneous utilization of glucose and xylose and *in situ* detoxification of acetate are necessary to achieve economic conversion of cellulosic hydrolyzates into fuels and chemicals. To this end, we engineered yeasts to co-ferment mixtures of cellobiose and xylose. After constructing an efficient xylose-fermenting strain of *Saccharomyces cerevisiae* through rational and combinatorial strategies, we introduced intracellular cellobiose utilizing pathways. Therefore, degradation of cellobiose takes place inside yeast cells through the action of an intracellular β-glucosidase or cellobiose phosphorylase following import by wild type or engineered cellodextrin transporters. The resulting yeasts not only co-fermented cellobiose and xylose simultaneously, but also exhibited improved ethanol yields and productivities as compared to when either cellobiose or xylose was used as a sole carbon source. Additionally, we used a redox balancing strategy to convert cellulosic sugars and toxic levels of acetate together into ethanol. These engineered yeasts are synthetic biological widgets which enable efficient and rapid utilization of carbon compounds in cellulosic biomass as well as demonstrate innovative strategies for metabolic engineering.

Presenter 3

FERMENTATION PRODUCTION OF NATURAL BLUCETIN™-DHM BY TARGETED BIOSYNTHESIS

Oliver Yu, Ph.D.
Chief Science Officer, Blue California, Rancho Santa Margarita, CA

Targeted biosynthesis, or synthetic biology, is a powerful fermentation technology that relies on metabolic pathway engineering to produce natural products found in plants, animals, and other sources. Targeted biosynthesis can complement traditional extraction methods to produce large quantities of specific natural products. For natural products that have a long growing season, difficult to obtain sources, and low natural abundance, targeted biosynthesis can be an essential method of production. Here, we outlined the biosynthesis approaches for producing natural BluCetin™, an all-natural anti-hangover dietary supplement. BluCetin™ allow users to remain more alert and in control while drinking alcohol in moderation. We were able to establish a de novo biosynthetic pathway using several plant enzymes. Initially, the reconstituted pathway produced very low levels of BluCetin™. Using various synthetic biology methods, we were able to down-regulate the competing pathway that consumes the key intermediate pathway, over-express a transcription factor that coordinately up-regulate the upstream pathway, and engineered a mutant version of a key enzyme that significantly boosted the catalytic activity. In addition, we improved the production platform by directed evolution. Together, these engineering efforts allowed us to generate a strain that has a much higher production yield than initial test strains.

Presenter 4

DEVELOPMENT OF MODERN VACCINES, RECOMBINANT PROTEIN PLUS ADJUVANT

Thomas S. Vedvick, Ph.D.
Vice President, Formulation and Process Development, Infectious Diseases Research Institute (IDRI), Seattle, WA

The Infectious Disease Research Institute in Seattle, USA, is a non-profit biotechnology institute dedicated to developing vaccines, diagnostics, and drugs for diseases that affect developing countries. IDRI has built a portfolio of recombinant vaccine antigens and adjuvant formulations that have shown promising stability, preclinical, and clinical biological activity in an array of disease models, including Leishmaniasis, Tuberculosis, Malaria, and Leprosy. Modern vaccines based on recombinant antigens generally require adjuvant help to generate adequate immune responses. Even live attenuated or inactivated vaccines contain intrinsic adjuvant structures. Thus, vaccines can be considered to consist of two principal components: antigen and adjuvant. In this presentation we will discuss the transformation from crude vaccines to today's fully defined safe and effective vaccines.

Presenter 5

LONG-RANGE EFFECTS OF PROTEIN DYNAMICS IN ENZYME CATALYSIS

Amnon Kohen, Ph.D.
Professor, Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Enzymes are biological catalysts of critical importance in biology, chemistry, medicine, and biotechnology. Despite immense work throughout the last century and the availability of many protein structures, the role of protein motions and dynamics in its function is not yet clear. Better understanding of that role in catalyzing chemical conversions may have significant impact in rational drug design, biomimetic catalyst design, and other related fields. Using two enzymes involved in *de novo* thymine Biocatalysis as model systems we examined the role of residues remote from the active site in the enzyme catalyzed C-H activation reactions. Studies with thymidylate synthase (TSase) and dihydrofolate reductase (DHFR) and several of their mutants will be discussed in the context of their structure-dynamics-function relationship.

Presenter 6

A NEW SMALL MOLECULE THERAPY FOR METASTATIC MELANOMA

Kyle C. Kloepping, Mitchell C. Coleman, Brett A. Wagner, Melissa A. Fath, James A. Jacobus, Kranti A. Mapuskar, Garry R. Buettner, Douglas R. Spitz and Michael K. Schultz*
Department of Radiation Oncology, Free Radical and Radiation Biology Program, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Incidence of melanoma is increasing faster than any other form of cancer worldwide. Although disease identified early can be cured by surgery, metastatic melanoma is lethal. Despite advances in chemotherapeutic, biological, and targeted therapies, no treatment affords durable benefit to melanoma patients. The precise mechanism of resistance is debated, but is thought to be related to innate immune surveillance avoidance and multidrug resistance machinery that is upregulated in melanoma cells. The current study explores a small molecule triphenylphosphonium (TPP) platform technology that targets fundamental differences in oxidative metabolism and response to oxidative stress in melanoma cells relative to non-malignant cells. TPP derivatives have long been used to study mitochondrial biophysics and bioenergetics, which take advantage of mitochondrial targeting properties of TPP. However, few studies have examined how rational molecular modifications to the side chain of TPP derivatives can be used to selectively kill cancer cells, including melanoma, relative to non-malignant cells. The goal of the current study was to evaluate the potential of TPP designed to inhibit melanoma mitochondrial oxidative metabolism, and induce melanoma-specific cytotoxicity via mechanisms involving oxidative stress. To test the working hypothesis that rationally designed TPP derivatives can selectively kill melanoma cells via oxidative stress, preliminary studies were performed with a small library of TPP derivatives. *In vitro* measurements of cell viability; mitochondrial membrane potential; electron transport chain complex activity; oxidative stress; and oxygen consumption rates were conducted. *In vivo* studies were performed in mice bearing melanoma tumor xenografts to evaluate the potential for the use of TPP for melanoma therapy. Further, in order to test a larger range of chemical space, cell viability screens were performed with a compound library containing ~50 TPP derivatives, each with an unique molecular side chain composition. Results indicate that TPP derivatives can be designed to disrupt oxidative metabolism and lead to melanoma cell death via increased oxidative stress. Further, melanoma tumor bearing mice treated with TPP exhibited decreased melanoma tumor growth rates compared to untreated mice. Importantly, TPP treatment resulted in no adverse effects and tumor lysates showed increased oxidative stress markers, thus providing evidence that TPP derivatives can be designed to disrupt oxidative metabolism and selectively kill melanoma cells via increased oxidative stress. Further, screens have identified 10 lead TPP compounds that decrease viability in melanoma versus non-malignant cells. Collectively, these results support the continued development of a TPP-based therapy for the treatment of metastatic melanoma.

Presenter 7

ENHANCED POLYCHLORINATED BIPHENYL DEGRADATION BY BIOAUGMENTATION WITH *BURKHOLDERIA XENOVORANS* LB400 IN A SWITCHGRASS PLANTED RHIZOSPHERE

Yi Liang, Richard Meggo, Dingfei Hu, Jerald L. Schnoor and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Phytoremediation represents a cost-effective and sustainable approach for polychlorinated biphenyl (PCB) removal, in which plants and associated microbes remove and detoxify PCBs. Previous results have shown significantly higher reductions of PCB-52, 77 and 153 in switchgrass planted systems compared to the unplanted systems. In this study, we are analyzing microbial PCB biodegradation in a switchgrass planted system with PCB exposure (PCB-52, 77 and 153 mixtures). After 12 weeks incubation in switchgrass-planted reactor, 18.4% of PCB parent compounds were transformed into less chlorinated congeners, compared with 8.6% of transformation in unplanted reactor, indicating enhanced dechlorination in switchgrass-planted reactor. However, the total PCB removal was not improved. Furthermore, although biphenyl dioxygenase gene (*bph*A), the gene codes for the key enzyme catalyzing the first step of aerobic PCB degradation, was more abundant in switchgrass planted reactor, *bph*A transcripts in both planted and unplanted reactors were below our quantification limit of RT-qPCR, indicating inactive aerobic PCB biodegradation. To improve aerobic PCB degradation and total PCB removal, *Burkholderia xenovorans* LB400, one of the most effective aerobic PCB degrader, was introduced into soil reactors. The addition of LB400 helped to increase total PCB removal from 1% to 6.3%. And the enhanced total PCB removal in bioaugmented reactors was associated with elevated abundance of *bph*A and its transcripts, indicating the enhanced aerobic degradation after bioaugmentation. Overall this study helps better understand microbial PCB degradation and provide valuable information about how to optimize phytoremediation strategies to achieve efficient PCB removal.

Presenter 8

DEVELOPMENT OF A NOVEL HIGH-THROUGHPUT SCREEN TARGETING RGS17 & CHARACTERIZATION OF LEAD SMALL MOLECULE INHIBITORS FOR THE TREATMENT OF METASTATIC LUNG AND PROSTATE CANCERS

Duncan I. Mackie and David L. Roman*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

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

Presenter 9

MODELING PARTICLE CLUSTERING USING PAIR CORRELATION FUNCTION ANALYSIS

Cicily J. Ronhovde and M. Lei Geng*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

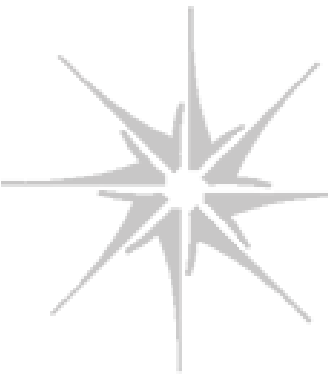
Tissue phantoms are synthetic imitations of biological tissue that can be used to mimic many characteristics of tissue including optical, acoustic, thermal, and mechanical properties. These properties can be adjusted in the synthetic material to simulate disease progression for the development of diagnostic or treatment techniques. For example, the spectroscopic properties of tissue – scattering, absorbance, and fluorescence – can be modeled in a synthetic material by incorporating a range of concentrations of cellular components with optical properties. These phantoms are of particular interest for the development of an optical method of cancer detection and diagnosis that could be applied non-invasively and provide instantaneous and quantitative results with high sensitivity.

An essential requirement for validating such a diagnostic technique is the ability to clearly distinguish boundaries between adjoining regions that have different spectral properties. Mesoporous silica particles functionalized with octadecylsilane were selected as the loading platform for fluorophores because these particles provide biochemically well-defined micrometer and nanometer domains due to hydrophobic trapping of loaded molecules.[1] A second requirement for developing a robust optical methodology is uniformity of the tissue phantom samples that are used to calibrate an optical device. One barrier to the generation of a uniform tissue phantom is the dispersal of hydrophobic particles in agar.

Previously, uniform suspension of particles in agar has been difficult because of the tendency of the particles to aggregate in aqueous media.[1] Current efforts to disperse particles include surfactant coating and various methods of physical dispersion. An analysis method utilizing the pair correlation function has been developed to quantify particle clustering as a means of evaluating the particle dispersal in the resulting tissue phantoms. Different particle dispersal patterns – clustered, uniform, and random – are distinguishable based on characteristic features produced in the pair correlation function. The robustness of the mathematical analysis of the phantom images is established using simulated images with various dispersal patterns.

References

1. Skvortsova, Y. A.; Freeney, R. M.; Zhong, Z.; Geng, M. L. *Anal. Chem*. **2010**, *82*, 6712-6716.



List of Posters and Authors

Posters

1. **EFFECTS OF A REMOTE MUTATION ON DIFFERENT CHEMICAL STEPS CATALYZED BY THYMIDYLATE SYNTHASE**
   Thelma Abeysinghe and Amnon Kohen*
   Department of Chemistry, College of Liberal Arts and Sciences,The University of Iowa, Iowa City, IA

2. **ASSEMBLY AND CHARACTERIZATION OF A CELL-PARTICLE HYBRID SYSTEM AS A POTENTIAL CANCER VACCINE**
   Kawther K. Ahmed, Sean M. Geary and Aliasger K. Salem*
   Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Pharmaceutics and Translational Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

3. **PRODUCTION OF PARAXANTHINE FROM CAFFEINE USING METABOLICALLY ENGINEERED *E. COLI* AS A CATALYST**
   Khalid H R Al-Gharrawi[2,3], Ryan M. Summers[1], Sridhar R. Gopishetty[1] and Mani Subramanian[1, 2]*
   [1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
   [2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
   [3]Department of Chemical Engineering, The University of Baghdad, Aljadria, Baghdad, Iraq

4. **BIOFILM DEACTIVATION VIA MAGNETIC HYPERTHERMIA**
   Erica Bader, Ann O'Toole and Eric Nuxoll*
   Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

5. **ROLE OF HOMOLOG CuZnSOD IN BACULOVIRUS INFECTION**
   Bhakti Bapat and David Murhammer*
   Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

6. **BIOCHEMICAL, KINETIC, AND SPECTROSCOPIC CHARACTERIZATION OF A MONONUCLEAR IRON-DEPENDENT DMSP-LYASE**
   Adam E. Brummett, Nicholas J. Schnicker and Mishtu Dey*
   Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

7. **STRUCUTURALLY UNIQUE *N*-ARYLACYL *O*-SULFONATED AMINOGLYCOSIDES AS POTENTIAL LEADS TO MODULATE INFLAMMATORY LUNG DISEASES**
   Ioana Craciun, Amanda M. Fenner and Robert J. Kerns*
   Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

8. **MINIATURIZATION OF TRANSFECTION ASSAYS FOR IMMORTAL AND PRIMARY CELLS**
Samuel T. Crowley, Jing Li and Kevin Rice*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

9. **NICOTINAMIDE RIBOSIDE IN HIGH FAT DIET FED MICE: PHENOTYPES AND MECHANISMS**
Sirisha Ghanta, Samuel Trammell, Ruth Grossmann and Charles Brenner*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

10. **EFFECTS OF Y94F IN THE PROTON ABSTRACTION STEP IN THYMIDYLATE SYNTHASE**
Ananda Ghosh and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

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

12. **STRUCTURAL AND KINETIC STUDIES OF FORMATE DEHYDROGENASE**
Qi Guo, Lokesh Gakhar, Kyle Wickersham, Kevin Francis, Chris Cheatum and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

13. **CHARACTERIZING *PSEUDOMONS* SP. STRAIN ADP BIOFILM WITH RAMAN SPECTROSCOPY**
Victoria A. Henry, Jose D. Guizar, Julie L. Jessop* and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

14. **KINETIC ISOTOPE EFFECTS TO PROBE HYDRIDE-TRANSFER MECHANISM IN *E.COLI* THYMYDYLATE SYNTHASE**
Zahidul Islam and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

15. **RLPA IS A PEPTIDOGLYCAN HYDROLASE THAT FACILITATES DAUGHTER CELL SEPARATION IN *PSEUDOMONAS AERUGINOSA***
Matthew A. Jorgenson[1,2], David L. Popham[3] and David S. Weiss[1,2]*
[1]Interdisciplinary Program in Genetics, The University of Iowa, Iowa City, IA
[2]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[3]Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, VA

16. IMMUNE STIMULATING CHEMO LOADED BIODEGRADABLE PARTICLES AS A NEW THERAPY FOR TUMORS

Vijaya B. Joshi, Caitlin Lemke, Amaraporn Wongrakpanich, Sean M. Geary and Aliasger K. Salem*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

17. EXPLORING THE MECHANISM OF FDTS FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

Kalani U. Karunaratne[1], Tatiana V. Mishanina[1], Frank W. Foss Jr.[2] and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]Department of Chemistry and Biochemistry, College of Science, The University of Texas at Arlington, Arlington, TX

18. A NEW SMALL MOLECULE THERAPY FOR METASTATIC MELANOMA

Kyle C. Kloepping, Mitchell C. Coleman, Brett A. Wagner, Melissa A. Fath, James A. Jacobus, Kranti A. Mapuskar, Garry R. Buettner, Douglas R. Spitz and Michael K. Schultz*
Department of Radiation Oncology, Free Radical and Radiation Biology Program, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

19. NOVEL *N*-1 FLUOROQUINOLONES DESIGNED TO COMBAT BACTERIAL RESISTANCE DUE TO MUTANT TYPE II TOPOISOMERASES

Chaitanya A. Kulkarni, Tyrell R. Towle and Robert J. Kerns*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

20. AN INVESTIGATION INTO THE OXIDATIVE COUPLING OF NAPTHOLS AND ANTHRACENONES

Alvin LeGall and Horacio F. Olivo*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

21. ENHANCED POLYCHLORINATED BIPHENYL DEGRADATION BY BIOAUGMENTATION WITH *BURKHOLDERIA XENOVORANS* LB400 IN A SWITCHGRASS PLANTED RHIZOSPHERE

Yi Liang, Richard Meggo, Dingfei Hu, Jerald L. Schnoor and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, IA

22. NOVAL STRUCTURAL AND DYNAMIC PROPERTIES REWIRE THE LIGAND BINDING SPECIFICITY IN A Tiam1 PDZ MUTANT

Xu Liu[1], David C. Speckhard[2], Tyson R. Shepherd[1] and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Department of Chemistry, Loras College, Dubuque, IA

23. DEVELOPMENT OF A NOVEL HIGH-THROUGHPUT SCREEN TARGETING RGS17 & CHARACTERIZATION OF LEAD SMALL MOLECULE INHIBITORS FOR THE TREATMENT OF METASTATIC LUNG AND PROSTATE CANCERS

Duncan I. Mackie and David L. Roman*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

24. CALMODULIN RECOGNITION OF ANTI-PARALLEL NESTED BINDING SITES IN THE NEURONAL VOLTAGE-GATED SODIUM CHANNEL $Na_V 1.2$

Mark S. Miller and Madeline A. Shea*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

25. MECHANISM OF OXIDATIVE HALF-REACTION OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

Tatiana V. Mishanina[1], John M. Corcoran[1], Eric M. Koehn[1], Scott A. Lesley[2] and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]The Joint Center for Structural Genomics at the Genomics Institute of Novartis Research Foundation, San Diego, CA

26. *BEAUVARIA BASSIANA* METABOLISM OF ORGANOSULFUR COMPOUNDS

Felipe Nicolau-Manterola and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

27. DEVELOPMENT OF AN OBJECTIVE METHOD TO QUANTIFY DEACTIVATION USING CONFOCAL FLUORESCENT MICROSCOPY

Ann O'Toole, Bryce Hundley and Eric Nuxoll*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

28. INCORPORATION OF THIOCYANATE AS AN INFRARED PROBE FOR FAST DYNAMICS AT THE ACTIVE-SITE OF *E. COLI* DIHYDROFOLATE REDUCTASE

Philip L. Pagano, Christopher M. Cheatum and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

29. ARCHITECTURE OF THE MACROMOLECULAR COMPLEXS FORMED IN TLS

Kyle T Powers, M. Todd Washington*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

30. MODELING PARTICLE CLUSTERING USING PAIR CORRELATION FUNCTION ANALYSIS

Cicily J. Ronhovde and M. Lei Geng*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

31. THE REGULATION AND PATHOGENIC FUNCTION OF *STAPHYLOCOCCUS AUREUS* HYALURONATE LYASE

Carolyn B. Rosenthal[1], Crystal L. Jones[2], Mary J. Mantz[3], Shweta Singh[2], Matthew C. Wise[2], Christopher A. Squier[3], Daniel V. Zurawski[2] and Alexander R. Horswill[1]*
[1]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Walter Reed Army Institute of Research (WRAIR), Department of Wound Infections, Silver Spring, MD
[3]Department of Oral Pathology, Radiology and Medicine, and Dows, College of Dentistry, The University of Iowa, Iowa City, IA

32. THE ROLE OF REACTIVE OXYGEN SPECIES AND A TOXIC DOPAMINE METABOLITE IN NEURODEGENERATION

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

33. STUDIES TOWARDS SYNTHESIS OF TRUNCATED SUPERSTOLIDE A

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

34. DIHYDROFOLATE REDUCTASE ROLE IN CATALYSIS

Priyanka Singh, Zhen Wang, Vern L. Schramm and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

35. ASYMMETRIC SYNTHESIS OF PYRROLIDINES VIA Mn-MEDIATED RADICAL ADDITION

Kara A. Slater and Gregory K. Friestad*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

36. PROBING CASPASE-7 ALLOSTERY WITH ATOMISTIC MOLECULAR DYNAMICS SIMULATIONS AND FRAGMENT SCREENING

Nick R. Vance and M. Ashley Spies*.
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

37. THE DOPAMINE METABOLITE 3,4-DIHYDROXYPHENYLACETALDEHYDE TARGETS PROTEINS AND IS TOXIC TO DOPAMINERGIC CELLS

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

38. TRIAZOLE–BASED INHIBITORS OF GERANYLGERANYL TRANSFERASE II

Veronica S. Wills[1], Xiang Zhou[1], Ella J. Born[2], Sarah A. Holstein[2] and David F. Wiemer[1]*

[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

[2]Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

39. TOWARD A MOLECULAR MECHANISM FOR TIAM1 REGULATION

Zhen Xu[1], Lokesh Gakhar[2] and Ernesto J. Fuentes[1]*

[1]Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

[2]X-ray Crystallography Core Facility, The University of Iowa, Iowa City, IA

40. KINETIC ASSESMENT OF "BAIT AND SWITCH" ANALOGS OF 2-PAM AS METHYL TRANSFER AGENTS AND RESURRECTING AGENTS FOR AGED HUMAN ACETYLCHOLINESTERASE

Sumana N. Yasapala and Daniel M. Quinn*

Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

41. EVIDENCE AND QUANTITATION OF AROMATIC ORGANOSULFATES IN AMBIENT AEROSOLS IN LAHORE, PAKISTAN

Shuvashish Kundu[1], Tauseef A. Quraishi[2], Ge Yu[3], Catherine Suarez[3], Frank. N. Keutsch[3] and Elizabeth A. Stone[1]*

[1] Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

[2]University of Engineering and Technology, Lahore, Pakistan

[3]Department of Chemistry, University of Wisconsin-Madison, Madison, WI

42. VALIDATION OF CAFFEINE DEHYDROGENASE FROM *PSEUDOMONAS* SP. CBB1 AS A SUIABLE ENZYME FOR A RAPID CAFFEINE DIAGNOSTIC TEST

Sujit K. Mohanty[2], Chi-Li Yu[3], Sridhar Gopishetty[1] and Mani Subramanian[1,2]*

[1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA

[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

[3]Proteomics Facility, The University of Iowa, Iowa City, IA

43. REGULATION OF CAFFEINE-INDUCED ENZYMES IN *PSEUDOMONAS PUTIDA* CBB5Δ*ndmE*

Ryan M. Summers[1], Venkat S. Iyer[1], Sujit K. Mohanty[2], Chi Li Yu[3], and Mani Subramanian[1,2]*

[1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA

[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

[3]Proteomics Facility, The University of Iowa, Iowa City, IA

44. FUNCTIONAL ANALYSES OF THE SPOR DOMAIN FROM FtsN, A CELL DIVISION PROTEIN FROM *ESCHERICHIA COLI*

Atsushi Yahashiri[1], Tammi R. Duncan[1], David L. Popham[2] and David S. Weiss[1]*
[1]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, VA

45. RAPID IDENTIFICATION OF METABOLITES USING STABILIZED HUMAN CYP2D6 AND PRODUCTION OF DEXTRORPHAN FROM DEXTROMETHORPHAN USING STABILIZED HUMAN CYP2D6

Shuvendu Das[1] and Mani Subramanian[1,2,3] *
[1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[3]Redox Biocat LLC, The University of Iowa Research Park, BioVentures Center, Coralville, IA

46. IDENTIFICATION AND QUANTITATIVE PROFILING OF CAFFEINE INDUCED ENZYMES IN A CAFFEINE-DEGRADING STRAIN *PSEUDOMONAS* SP. CES USING HIGH-THROUGHPUT MULTIPLEXED MASS SPECTROMETRY

Chi Li Yu[1], Ryan Summers[2], Yalan Li[1], Sujit Mohanty[3], Mani Subramanian[2,3]* and Marshall Pope[1]*
[1]Proteomics Facility, The University of Iowa, Iowa City, IA
[2]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
[3]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

47. CONTINUOUS, REAL-TIME MONITORING OF KEY ANALYTES FOR *PICHIA PASTORIS* BIOPROCESSES

Elizabeth R. Gibson[1], Kaylee Lanz[1], Edwin Koerperick[1], Jonathon T. Olesberg[1], Chris Evans[1], Gary W. Small[1,2,3] and Mark A. Arnold[1,2,3]
[1]ASL Analytical, Inc., Coralville, IA
[2]Optical Science and Technology Center, The University of Iowa, Iowa City, IA
[3]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Poster Abstracts

Poster 1

EFFECTS OF A REMOTE MUTATION ON DIFFERENT CHEMICAL STEPS CATALYZED BY THYMIDYLATE SYNTHASE

Thelma Abeysinghe and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Thymidylate synthase (TSase) catalyzes the *de novo* biosynthesis of the DNA building block thymidine, 2'–deoxythymidine 5'–monophosphate (dTMP) in most organisms. The essential role of TSase makes it a common target for chemotherapeutic and antibiotic drugs. Recent studies with *Escherichia Coli* (*ec*TSase), showed that a highly conserved residue, Tyr-209 (9 Å away from the reaction site), plays a key role in the catalytic cycle and protein dynamics. Although the crystal structures of wild type (WT) *ec*TSase and its Y209W mutant are nearly identical at a 1.3 Å resolution, the anisotropic B factors of several loops across the WT protein are all oriented in the same direction. However, in Y209W these anisotropic B factors are randomly oriented, indicating a disruption of the correlated atomic vibrations of protein residues in the mutant. To assess which of the many kinetic and mechanistic steps catalyzed by TSase we have studied various structural and kinetic properties of different chemical steps and compared the WT and mutant.

In contrast to WT, experiments with Y209W suggested that thiols from the media can trap an intermediate indicating extended life time of the ternary complex prior to the hydride transfer, i.e., slower reorganization of the enzyme toward the last chemical step (hydride transfer).[1] Intrinsic KIEs for the hydride and proton transfer steps were measured at temperature range of 5 ˚C to 35 ˚C, and the outcome indicated that none of the chemical steps was altered significantly. However, a comparison of observed KIEs to their intrinsic values indicated that other kinetic steps were dramatically affected. These findings emphasize the importance of the relationship between long-range dynamics of the enzymatic complex and the enzyme's catalytic cycle.

Poster 2

ASSEMBLY AND CHARACTERIZATION OF A CELL-PARTICLE HYBRID SYSTEM AS A POTENTIAL CANCER VACCINE

Kawther K. Ahmed, Sean M. Geary and Aliasger K. Salem*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Pharmaceutics and Translational Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA

Purpose
Cancer vaccines represent a promising treatment modality for a world-wide health problem. Cancer vaccines involve the administration of a tumor antigen along with an adjuvant. Whole cell tumor vaccines have shown promise in preclinical and clinical settings. This study involves the use of the avidin-biotin linkage to manufacture an irradiated tumor cell-particle hybrid as a prospective cancer vaccine where the tumor cell can be transduced to secrete immunostimulatory cytokine (e.g. GM-CSF) and the particles can be loaded with an adjuvant (e.g. CpG). The study represents a "proof of concept" of the possibility of manufacturing the proposed cancer vaccine.

Method
Polymeric poly(lactide-co-glycolide) (PLGA) particles were prepared using the double-emulsion solvent evaporation method with and without rhodamine loading. Particles were characterized for morphology, size and zetapotential. Prepared particles were coated with streptavidin using EDC/NHS chemistry. Murine melanoma cells were biotinylated indirectly using biotin-antibodies. Particles were assembled to cells under specific incubation conditions using two particle:cell ratios and subsequently irradiated. Successful cell-particle binding was confirmed by flow cytometry, laser scanning confocal microscopy, and scanning electron microscopy. Cell-particle hybrids were also assembled using the murine prostatic cancer cell line to demonstrate the generality of the method adopted in this study for manufacturing cell-particle hybrids.

Results
Prepared particles showed smooth surface and narrow size distribution. Streptavidin-coated particles were successfully bound to biotinylated murine melanoma cells with the extent of binding increasing as the particle:cell ratio increased. The assembled hybrid was intact subsequent to irradiation. Hybrid assembly was successful for the murine prostatic cancer cells. The particles were localized on the cell surface as shown by microscopy images.

Conclusion
Stable cell-particle hybrids were successfully assembled using avidin-biotin linkages. The assembled hybrids have the potential of a prospective cancer vaccine that delivers tumor antigens (endogenous to the tumor cell) and an immunoadjuvant (encapsulated in the particles) to the same antigen presenting cell.

Poster 3

PRODUCTION OF PARAXANTHINE FROM CAFFEINE USING METABOLICALLY ENGINEERED *E. COLI* AS A CATALYST

Khalid H R Al-Gharrawi[2,3], Ryan M. Summers[1], Sridhar R. Gopishetty[1] and Mani Subramanian[1, 2]*
[1]Center for Biocatalysis and Bioprocessing , The University of Iowa Research Park, Coralville, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[3]Department of Chemical Engineering, The University of Baghdad, Aljadria, Baghdad, Iraq

Methylxanthines are naturally occurring purine alkaloids that are used as pharmaceutical products for the treatment of asthma, diuretics, and cardiac, pulmonary, and neurological stimulants. Paraxanthine (1,7-dimethylxanthine) is the most valuable methylxanthine, with a cost of approximately $1,000 per gram. This research is aimed at developing a bioprocess for the production of paraxanthine from caffeine (1,3,7-trimethylxanthine) by using metabolically engineered *E. coli* as a catalyst.

Five important steps are required to develop the bioprocess for the production of paraxanthine:

1. Screening three different *E. coli* strains for the maximum production of paraxanthine,
2. Optimization of reaction parameters,
3. Scale up,
4. Production, and
5. Separation and purification of products.

Three *E. coli* BL21(DE3) strains have been metabolically engineered to produce paraxanthine and theobromine from caffeine. These strains, pAD1dDD, pDdA, and pDdAA contain different copy numbers of plasmids containing *ndmA* and *ndmD*, a caffeine N_1-demethylase gene and its partner reductase gene, respectively.

Strains pDdA and pDdAA completely consumed 4 mM of caffeine in 40 minutes, while strain pAD1dDD consumed the same amount of caffeine in 50 minutes. Conversion of caffeine to paraxanthine was 1.75% in strains pDdA and pAD1dDD and 1.70% in strain pDdAA. The remaining caffeine was converted to theobromine (3,7-dimethylxanthine) in all strains. Thus, strain pDdA was chosen to optimize the reaction parameters such as initial caffeine concentration, catalyst reuse, temperature, and pH.

Future work will involve scale up of the reaction mixture, separation of products, and finally production of paraxanthine.

Poster 4

BIOFILM DEACTIVATION VIA MAGNETIC HYPERTHERMIA

Erica Bader, Ann O'Toole and Eric Nuxoll*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Each year in the United States thousands of people have implants removed due to biofilm infections obtained during the implantation of the device, ultimately requiring invasive surgeries and long-term antibiotic treatments. These biofilms form on the surfaces of the implants and are difficult for doctors to treat and for the immune system to eradicate due to an extracellular matrix the bacteria form. Researchers are attempting to find a solution to the problem of increased resistance to antibiotics in biofilms. A promising new approach is heat application. By coating an implant with a polymer encasing iron oxide nanoparticles, an alternating magnetic field induces remote heating to the biofilm infection. Heat shock experiments have been run to verify that heating decreases the biofilm viability. Temperatures applied for different time sets have been studied and an overall trend of a decrease in biofilm viability has been seen with the increase in temperature and an increase in the time amount the biofilms are exposed to that temperature. It has been found that even a 10ºC change in temperature has a greater effect on deactivating the biofilms than increasing the time by a factor of five. These studies will evolve the understanding of biofilms and how to efficiently deactivate them on implant surfaces. Future work will study the synergistic effects of heating with antibiotics and with local bodily shear stress on the implant's surface. The introduction of such a novel coating in conjunction with antibiotics and the knowledge of local shear will obviate thousands of surgeries and save billions of dollars spent on explantation, recovery, and re-implantation on many individuals.

Poster 5

ROLE OF HOMOLOG CuZnSOD IN BACULOVIRUS INFECTION

Bhakti Bapat and David Murhammer*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Baculoviruses are widely used with insect cells to express recombinant proteins. While the rate of recombinant protein expression can be very high in this system, the protein expression ceases within 2-3 days due to cell death resulting from the virus infection. Oxidative stress is regarded as a major contributor to this cell death. A baculovirus-encoded Copper-Zinc Superoxide Dismutate (CuZnSOD) homolog gene contributes to this oxidative stress. This homolog has a high affinity towards Cu^{2+} that results in inactivation of the CuZnSOD enzyme through the removal of copper, thereby leading to increased oxidative stress and presumably more rapid cell death. Cell longevity potentially can be extended by increasing cellular antioxidant defenses, e.g., by removing the CuZnSOD homolog and over expressing active CuZnSOD. This presentation tests this idea through studies with two modified baculoviruses: (i) one in which the CuZnSOD homolog gene has been removed and Human CuZnSOD is over expressed and (ii) one in which the homolog CuZnSOD gene is disrupted, thereby inactivating its production during infection.

Poster 6

BIOCHEMICAL, KINETIC, AND SPECTROSCOPIC CHARACTERIZATION OF A MONONUCLEAR IRON-DEPENDENT DMSP-LYASE

Adam E. Brummett, Nicholas J. Schnicker and Mishtu Dey*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Dimethylsulfoniopropionate (DMSP) produced by marine phytoplankton is degraded by marine bacteria to form methanethiol (MeSH) and the climatically active gas dimethylsulfide (DMS). DMS emissions from marine-oceanic environments contribute to about 90% of natural sulfur emissions and about 60% of global biogenic sulfur flux to the atmosphere and has a significant impact on global climate. Production of DMS arises from the lyase pathway of DMSP degradation in which acrylic acid is the other catabolite.[1] Despite the implication of DMSP catabolism in the global sulfur and carbon cycles, the biochemical pathways, genes, and regulating enzymes are poorly studied. In this study, we have identified an enzyme involved in DMSP dissimilation that can utilize several metal ions. Even though different metals were observed to affect the activity a preferred metallocofactor could be discerned. We have used biochemical, kinetic, and spectroscopic tools to understand the mechanism of a key enzyme in the DMSP lyase pathway. Revealing the mechanistic and structural relevance of this novel DMSP lyase is the first step towards understanding the enzymatic mechanism of DMSP degradation, which will further our understanding on how marine microbes use metals to degrade organosulfur compounds.

References

1. Curson, A.R.J., Todd, J.D., Sullivan, M.J., Johnston, W.B. Catabolism of dimethylsulphonionpionate: microorganisms, enzymes and genes. Nature Reviews. **2011**, 9, 849-859.

Poster 7

STRUCUTURALLY UNIQUE *N*-ARYLACYL *O*-SULFONATED AMINOGLYCOSIDES AS POTENTIAL LEADS TO MODULATE INFLAMMATORY LUNG DISEASES

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

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 obstructive pulmonary disease (COPD). 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 COPD. 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, we evaluated the members of our recently synthesized panel of *N*-arylacyl *O*-sulfonated aminoglycosides for their ability to inhibit NE, CatG and Pr3.

Poster 8

MINIATURIZATION OF TRANSFECTION ASSAYS FOR IMMORTAL AND PRIMARY CELLS

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

High throughput screening, HTS, has become a popular method for drug discovery. Microwell plates, liquid handling robotics, plate readers, and chemical libraries allow researchers to study tens of thousands of compounds at a time. HTS techniques are most commonly used to study small molecules, but they can be applied to large molecules as well. To allow HTS of transfection reagents, we have developed assays to measure in vitro nonviral gene delivery in 384 and 1536 well plate formats. Luciferase or Green Fluorescent Protein genes were delivered and luminescence or fluorescence was measured on a plate reader. HepG2 cells were transfected with polyethyleneimine and calcium phosphate nanoparticles as DNA condensing agents in both 384 and 1536 well plates. Primary hepatocytes were harvested from mouse liver and transfected with polyethyleneimine and calcium phosphate nanoparticles in 384 well plates. Optimal conditions for HepG2 cells in 384 microwell plates were 5000 cells per well, 250ng of DNA per well, with PEI N:P ratio of 6. In 1536 well plates, 1200 cells per well with 75ng of DNA per well with PEI N:P ratio of 9 is optimal. When using luciferase, the 384 well assay has a Z' score of 0.53, while the 1536 well assay has a Z' score of 0.42, most likely due to evaporation from wells around the edge of the plate. GFP assays had lower signal to noise ratios and greater variability than luciferase assays. Optimal conditions for primary hepatocytes in 384 well format PEI transfection agent were 250 cells per well, with 250ng DNA per well at N:P ratio of 7. When using calcium phosphate nanoparticles, optimal conditions were 250 cells per well, with 50ng DNA per well at a Ca:P ratio of 200. These assays will help to more rapidly develop new DNA transfection agents. The high throughput format will save both time and materials, with smaller wells requiring fewer cells, less DNA, and less reagent.

Poster 9

NICOTINAMIDE RIBOSIDE IN HIGH FAT DIET FED MICE: PHENOTYPES ANDMECHANISMS

Sirisha Ghanta, Samuel Trammell, Ruth Grossmann and Charles Brenner*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Nicotinamide riboside (NR) is a recently discovered NAD^+ precursor vitamin found in milk that uses different biosynthetic routes to NAD^+ with respect to nicotinic acid and nicotinamide. In yeast, NR extends replicative life span despite the presence of high glucose. Recently, it was shown that NR protects against diet-induced obesity in high fat diet (HFD) fed mice, through mechanisms that are not understood. We aim to test the hypothesis that HFD reduces liver mitochondrial function by promoting inhibitory protein acetylation and that NR increases mitochondrial function by promoting NAD^+ and Sirt3-dependent protein deacetylation. By combining metabolomics, proteomics and mitochondrial functional analysis, we aim to probe the molecular basis for modulation of energy expenditure by macronutrients and micronutrients in vertebrate diets.

Poster 10

EFFECTS OF Y94F IN THE PROTON ABSTRACTION STEP IN THYMIDYLATE SYNTHASE

Ananda Ghosh and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Thymidylate synthase (TSase) catalyzes the reductive methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to form 2'-deoxythymidine-5'-monophosphate (dTMP), using methylene tetrahydrofolate (CH_2H_4folate) as both the methylene and hydride donor. The reaction constitutes the last committed step of the *de novo* biosynthesis of DNA and is the target of antibiotic and chemotherapeutic drugs. The catalyzed reaction involves several covalent bonds making and breaking, including a proton abstraction from the pyrimidine ring. The general base in that abstraction appears to be a nearby (via X-ray crystallography)[1] and strictly conserved tyrosine residue (Y94 in TSase from *E. coli*).[2] Studies of Y94F indicated a 400-fold decrease on k_{cat} but the effect on the proton abstraction has not been studied. A kinetic study by Hong et al.,[3] suggested that the whole network of H-bonds, rather than Y94, serves as general base, thereby assists proton abstraction, but fail short of testing the temperature dependence on intrinsic KIEs for that step.
The current study focuses on the role of Y94 in the proton transfer by exploiting temperature-dependence of intrinsic KIEs. This tool can detect even minor changes in reaction potential energy surface. Experiments are underway to measure the KIEs on the proton transfer at a range of temperatures. If Y94 is involved in proton abstraction, we predict that the mutation will cause a change in the reaction potential energy surface, which will eventually bring about a relative change in temperature dependence of KIEs in Y94F. In general, the results should aid in unraveling the nature of enzyme catalysis and could explore hidden chemical steps, thus providing new targets for inhibitors and drug design.

References

1. Roberts, S. A., Hyatt, D. C., Honts, J. E., Changchien, L., Maley, G. F., Maley, F., and Montfort, W. R. *Acta Crystallogr.* **2006,** *F62*, 840−843.
2. Hyatt, D. C., Maley, F., and Monfort, W. R. Biochemistry **1997**, 36, 4585−4594
3. Hong, B.; Maley, F.; Kohen, A. Role of y94 in proton and hydride transfers catalyzed by thymidylate synthase. *Biochemistry* **2007**, *46*, 14188–14197.

Poster 11

SOLVENT-ENHANCED BIOTRANSFORMATIONS OF STEROIDS BY *BEAUVERIA BASSIANA* AS BIOCATALYST

Richard Gonzalez and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa. Iowa City, IA

This research enhances the oxidative capacity of fungal *Beauveria bassiana,* a versatile whole-cell *biocatalyst* used in the biotransformation of steroids and in the production of valuable organic intermediates in pharmaceuticals. n-Alkane solvents such as dodecane, and hexadecane serve as carbon sources during growth of *B. bassiana.* These hydrocarbons induce the expression of oxidative enzymes and enhance the growth rate of biomass synthesis in a glycerol culture media. The objective of this research is to optimize yield and selectivity in the hydroxylation of unfunctionalized 11-carbon on DHEA (Dehydroepiandrosterone, 3β-hydroxyandrost-5-en-17-one), as result of virulence enhancement conditions. DHEA is an essential endogenous steroid male-hormone that provides the opportunity to study the optimization in the activation of unfunctionalized carbons due to its chemical structure, and inexpensive cost. *B. bassiana* was adapted to hydrocarbon solvents since June 2011 in a repetitive 15 days process to develop specific protein machinery to target the degradation of hydrocarbons. Resting cells reacted with DHEA, and extracted products were analyzed with TLC and HPLC. Results include growth curve, and biomass yield analysis of *B. bassiana*. During the course of a 5 days biotransformation, 3 metabolites were produced with n-alkane-induced biocatalyst and 2 metabolites with non-adapted cells. An analysis of the n-alkane effect on biocatalyst performance, including impact on reaction selectivity as well as correlations between biomass and reaction yields will be presented.

Poster 12

STRUCTURAL AND KINETIC STUDIES OF FORMATE DEHYDROGENASE

Qi Guo, Lokesh Gakhar, Kyle Wickersham, Kevin Francis, Chris Cheatum and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

One hotly debated topic in enzymology is whether and how the fast protein structural fluctuations affect the catalyzed chemistry in enzymes' active site. Formate dehydrogenase from *Candida boidinii* (CbFDH) was found to be a unique model system to address this question. Our previous kinetic and 2DIR studies[1] identified probable relations between femtosecond-picosecond dynamics and the catalyzed H-tunneling step. Structural information is critical for mutational studies that may reveal direct correlation between dynamics and the catalyzed hydride transfer. Here, we report crystallization and structural solution of both holo-CbFDH (with NAD^+ and azide) and the apo-CbFDH using the recombinant enzyme (Fig.1). The activity of this recombinant enzyme is identical to that of the commercial mixture of CbFDH isozymes used in ref 1. Surprisingly the apo-enzyme has been crystallized under saturation of NAD^+ and azide, which means the majority (>99.99999%) of complexes in solution are excluded during the crystallization step. The new CbFDH structures were compared to those of *Pseudomonas* FDH (PsFDH)[2], which share 90% identity in the active site residues overlap. The special alignment between CbFDH and PsFDH was excellent for both closed and open conformations for holo- and apo-enzymes, relatively. Finally, the intrinsic kinetic isotope effects (KIEs) and their temperature dependency for the recombinant and the commercial mixture was found to be identical, which opens the door to mutational studies of the recombinant enzyme in pursuit of correlation between the fast dynamics (from 2DIR) and the nature of hydride transfer (from intrinsic KIEs and their temperature dependence).

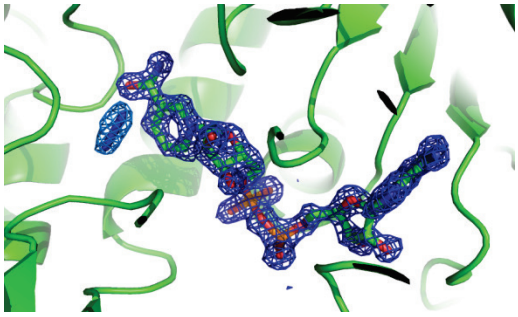


Figure 1. Active site crystal structure of CbFDH with NAD, azide and the corresponding electron density.

Poster 13

CHARACTERIZING *PSEUDOMONS* SP. STRAIN ADP BIOFILM WITH RAMAN SPECTROSCOPY

Victoria A. Henry, Jose D. Guizar, Julie L. Jessop* and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Within a biofilm, organisms are protected by a complex matrix and are able to survive longer than free cells against environmental challenges. This persistence may have both positive and negative implications. The purpose of this research is to grow *Pseudomonas* sp. strain ADP as a biofilm and examine the chemical and physical characteristics the microbe undertakes in a sticky extracellular matrix. ADP is the organism of choice because it has not been greatly explored as a biofilm and because of its ability to degrade atrazine. Cells are grown in a drip reactor and in flow cells under varying shear stress and growth times to gain insight to biofilm formation while using atrazine as the sole nitrogen source. As a positive control, *Escherichia coli* are grown in a similar manner. Chemical analysis is performed using Raman spectroscopy and confocal laser scanning microscopy to examine cellular distribution. The first Raman spectrum of ADP is presented. Success of this research will aid in the building of the microbial Raman library and development of non-invasive techniques to examine both internal composition and spatial distribution of compounds present in biofilms.

Poster 14

KINETIC ISOTOPE EFFECTS TO PROBE HYDRIDE-TRANSFER MECHANISM IN *E.COLI* THYMYDYLATE SYNTHASE

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

Thymidylate synthase (TSase) catalyzes the reductive methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to form 2'-deoxythymidine-5'-monophosphate (dTMP) using N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate (CH_2H_4folate) as both methylene and hydride donor. Its activity is crucial for DNA biosynthesis and it is a common target for chemotherapeutic and antibiotic drugs. The TSase in *E. coli* (ecTSase) catalyzes a series of bond cleavages and formations including a rate-limiting hydride transfer. The hydride transfer can occur in two principle ways: a concerted process in which the hydride transfer and the cleavage of thioether bond are concurrent, analogues to 1,3-S_N2 substitution, or a stepwise addition of the hydride followed by the elimination of thiolate (C146). Quantum mechanics/molecular mechanics (QM/MM) calculations suggested that the hydride transfer follows the concerted, but asynchronized, mechanism.[1] The calculations also identified a nearby residue (R166) that facilitates the hydride transfer, by polarizing the thioether bond at the transition state (TS).

Temperature dependence of intrinsic kinetic isotope effects (KIEs) has been used to diagnose perturbations to the H-transfer coordinate by an altered protein's environment[2]. In this study, we aim to exploit this method to test the effect of R166 mutation to Lys (R166K) on the hydride transfer. This mutant has reduced ability to stabilize the thiol leaving group, which, by the concerted mechanism, would also affect the concerted hydride transfer. We competitively measured H/T KIEs on the hydride transfer in R166K at a range of temperatures (5-35 ˚C). The observed H/T KIEs in R166K were found to be lower than that of wild-type. Experiments are underway to measure D/T KIEs. Intrinsic KIEs will be extracted from the observed H/T and D/T KIEs.[2] The intrinsic KIEs and its temperature dependence will then be compared to that of wild-type that showed a temperature-independent KIEs. The prediction of the concerted mechanism is that the intrinsic KIEs will become temperature dependent. In the step-wise mechanism, on the other hand, the thiol cleavage occurs after the hydride transfer and is not part of its reaction coordinate, and the mutation is not expected to affect the measured intrinsic KIEs.

References

1. Kanaan, N.; Marti, S.; Moliner, V.; Kohen, A., *J. Phys. Chem. A* **2009,** *113* (10), 2176-2182; Kanaan, N.; Ferrer, S.; Marti, S.; Garcia-Viloca, M.; Kohen, A.; Moliner, V., *J. Am. Chem. Soc.* **2011,** *133* (17), 6692-6702.
2. Roston, D.; Islam, Z.; Kohen, A., *Molecules* **2013,** 18, 5543-5567; Hong, B.; Haddad, M.; Maley, F.; Jensen, J. H.; Kohen, A., *J. Am. Chem. Soc.* **2006,** *128* (17), 5636-5637.

Poster 15

RLPA IS A PEPTIDOGLYCAN HYDROLASE THAT FACILITATES DAUGHTER CELL SEPARATION IN *PSEUDOMONAS AERUGINOSA*

Matthew A. Jorgenson[1,2], David L. Popham[3] and David S. Weiss[1,2]*
[1]Interdisciplinary Program in Genetics, The University of Iowa, Iowa City, IA
[2]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[3]Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, VA

RlpA is a protein of heretofore unknown function found in many Gram-negative bacteria. In *Escherichia coli* RlpA localizes to the septal ring, suggesting the protein is involved in cell division, but mutants of *rlpA* have no obvious phenotype (Gerding *et al*, *J. Bacteriol*. 2009; Arends *et al*, *J. Bacteriol*. 2010). The sequence of RlpA indicates it is an outer membrane lipoprotein with a double-psi beta barrel domain and a peptidoglycan-binding SPOR domain. We report here that *E. coli* RlpA is present at about 600 molecules per cell and traffics to the outer membrane, as predicted. Because we could not find an *rlpA*-related phenotype in *E. coli*, we turned to *Pseudomonas aeruginosa,* for which we had access to an ordered library of transposon mutants (Liberati *et al*, *PNAS* 2006). Interestingly, an *rlpA*::Tn mutant formed chains of 4-8 cells when grown in media of low osmotic strength, suggesting RlpA is needed for efficient separation of daughter cells at the end of cell division. We confirmed the chaining phenotype by constructing an in-frame deletion (Δ*rlpA*). Both mutants could be rescued by complementation with *rlpA* or an *rlpA-mCherry* fusion. The RlpA-mCherry fusion protein localized to the midcell during cell division and localization required the SPOR domain. Analysis of purified peptidoglycan from the Δ*rlpA* mutant by HPLC revealed an increase in a muropeptide whose structure was determined to be a tetrasaccharide (GlcNAc-MurNAc-GlcNAc-MurNAc) by amino acid/amino sugar analysis and mass spectrometry. Purified RlpA protein hydrolyzed the tetrasaccharide moiety from peptidoglycan isolated from the Δ*rlpA* mutant. RlpA proteins with amino acid substitutions in the double-psi beta barrel domain abrogated cell wall hydrolysis activity and failed to support daughter cell separation *in vivo*. We conclude that RlpA cleaves the glycosidic linkage between GlcNAc and MurNAc in the peptidoglycan strands to facilitate daughter cell separation.

Poster 16

IMMUNE STIMULATING CHEMO LOADED BIODEGRADABLE PARTICLES AS A NEW THERAPY FOR TUMORS

Vijaya B. Joshi, Caitlin Lemke, Amaraporn Wongrakpanich, Sean M. Geary and Aliasger K. Salem*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

Purpose
This study focuses on development of PLGA particles encapsulating doxorubicin (Dox) and CpG oligonucleotides (CpG) followed by in-vivo evaluation of their anticancer activity in tumor bearing mice.

Methods
Three different preparation of poly(lactide-co-glycolide) (PLGA) particles: 1) admixture of PLGA 50:50 encapsulating Dox and PLGA 50:50 encapsulating CpG; 2) admixture of PLGA 50:50 encapsulating Dox and PLGA 75:25 encapsulating CpG; 3) PLGA 50:50 co-loaded with Dox and CpG, were fabricated using a double emulsion solvent evaporation method. Particle morphology and size were examined using scanning electron microscopy. Loading of Dox and CpG was evaluated by fluorescence spectroscopy.
A therapeutic C57BL/6 mouse model was used to evaluate antitumor activity of the microparticle formulations. Mice (n = 4) were challenged subcutaneously with 106 EL 4 cells. EL 4 is a cell line derived from T-cell lymphoma of C57BL/6 mouse. An intratumoral (i.t.) injection of different treatment groups were given on day 3 post tumor challenge. Each mouse was treated with 100 μg of Dox and 50 μg of CpG. Tumor volumes were calculated using the formula: $(\pi/3)$ x length x breath x height. Mice with tumors greater than 20 mm in any dimension were sacrificed.

Results
PLGA particles loaded with Dox and CpG were successfully prepared. Scanning electron microscopy showed that the particles had smooth morphology and narrow size distributions. Spectrophotometric analysis showed that the use of 0.1 M ammonium acetate buffer (pH 8.2) in the external aqueous phase of particle fabrication significantly increases the loading of Dox. Opposite charges of Dox and CpG leads to the aggregation and precipitation of Dox and CpG during the fabrication of the PLGA particles. This was prevented by emulsification of Dox or CpG independently in polymer solutions. In-vivo murine tumor studies showed that treatment with PLGA particles co-loaded with Dox and CpG showed enhanced protection and improved survival in mice.

Conclusion
A successful method for co-delivering Dox and CpG in PLGA particles to tumors was developed. This therapeutic strategy resulted in enhanced protection and survival in EL 4 tumor challenged mice.

Poster 17

EXPLORING THE MECHANISM OF FDTS FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

Kalani U. Karunaratne[1], Tatiana V. Mishanina[1], Frank W. Foss Jr.[2] and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]Department of Chemistry and Biochemistry, College of Science, The University of Texas at Arlington, Arlington, TX

Thymidylate (2'-deoxythymidine 5'-monophosphate or dTMP) is an essential DNA nucleotide, which unlike other deoxyribonucleotides, cannot be synthesized from RNA base by ribonucleotide reductase. The *de novo* biosynthesis of dTMP requires the enzyme thymidylate synthase (TSase). 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.

References

1. Carreras, C.W.; Santi, .D.V. Annu. Rev. Biochem. **1995**, 64, 721-762.
2. Finner-Moore, J.S.; Santi, .D.V.; Stroud, R.M. *Biochemistry* **2003**, *42*, 248-256.
3. Myllykallio, H.; Lipowski, G.; Leduc, D.; Filee, J.; Forterre, P.; Liebl, U. *Science* **2002**, *297*, 105-107.

Poster 18

A NEW SMALL MOLECULE THERAPY FOR METASTATIC MELANOMA

Kyle C. Kloepping, Mitchell C. Coleman, Brett A. Wagner, Melissa A. Fath, James A. Jacobus, Kranti A. Mapuskar, Garry R. Buettner, Douglas R. Spitz and Michael K. Schultz*
Department of Radiation Oncology, Free Radical and Radiation Biology Program, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Incidence of melanoma is increasing faster than any other form of cancer worldwide. Although disease identified early can be cured by surgery, metastatic melanoma is lethal. Despite advances in chemotherapeutic, biological, and targeted therapies, no treatment affords durable benefit to melanoma patients. The precise mechanism of resistance is debated, but is thought to be related to innate immune surveillance avoidance and multidrug resistance machinery that is upregulated in melanoma cells. The current study explores a small molecule triphenylphosphonium (TPP) platform technology that targets fundamental differences in oxidative metabolism and response to oxidative stress in melanoma cells relative to non-malignant cells. TPP derivatives have long been used to study mitochondrial biophysics and bioenergetics, which take advantage of mitochondrial targeting properties of TPP. However, few studies have examined how rational molecular modifications to the side chain of TPP derivatives can be used to selectively kill cancer cells, including melanoma, relative to non-malignant cells. The goal of the current study was to evaluate the potential of TPP designed to inhibit melanoma mitochondrial oxidative metabolism, and induce melanoma-specific cytotoxicity via mechanisms involving oxidative stress. To test the working hypothesis that rationally designed TPP derivatives can selectively kill melanoma cells via oxidative stress, preliminary studies were performed with a small library of TPP derivatives. *In vitro* measurements of cell viability; mitochondrial membrane potential; electron transport chain complex activity; oxidative stress; and oxygen consumption rates were conducted. *In vivo* studies were performed in mice bearing melanoma tumor xenografts to evaluate the potential for the use of TPP for melanoma therapy. Further, in order to test a larger range of chemical space, cell viability screens were performed with a compound library containing ~50 TPP derivatives, each with an unique molecular side chain composition. Results indicate that TPP derivatives can be designed to disrupt oxidative metabolism and lead to melanoma cell death via increased oxidative stress. Further, melanoma tumor bearing mice treated with TPP exhibited decreased melanoma tumor growth rates compared to untreated mice. Importantly, TPP treatment resulted in no adverse effects and tumor lysates showed increased oxidative stress markers, thus providing evidence that TPP derivatives can be designed to disrupt oxidative metabolism and selectively kill melanoma cells via increased oxidative stress. Further, screens have identified 10 lead TPP compounds that decrease viability in melanoma versus non-malignant cells. Collectively, these results support the continued development of a TPP-based therapy for the treatment of metastatic melanoma.

Poster 19

NOVEL *N*-1 FLUOROQUINOLONES DESIGNED TO COMBAT BACTERIAL RESISTANCE DUE TO MUTANT TYPE II TOPOISOMERASES

Chaitanya A. Kulkarni, Tyrell R. Towle and Robert J. Kerns*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

Fluoroquinolones are broad spectrum antibiotics that are widely used to treat bacterial infections that are resistant to other antibiotic classes. These antibiotics target the bacterial type-II topoisomerases (DNA gyrase and topoisomerase IV) which are responsible for maintaining the DNA topology during replication and transcription. These enzymes perform their function through an ATP driven process that involves introducing double strand nicks in the DNA, relaxing the double helix, introducing supercoils and relieving torsional strain and finally religating the nicked DNA. Fluoroquinolones inhibit these processes by poisoning the enzymes, i.e. forming a ternary complex with DNA and topoisomerase, thereby blocking religation of the nicked DNA. Resistance to fluoroquinolones is occurring more frequently and thus underlines the need for new antibiotics, preferably with a different mechanism of action. Recent biochemical research has demonstrated that the *C*-3, *C*-4 diketo moiety of fluoroquinolones requires a magnesium-water bridge through which a binding contact is made in the ternary complex to a serine residue and an aspartate or glutamate residue (depending on the enzyme). Mutation of these amino acids confers resistance against fluoroquinolones by preventing bridge formation and therefore decreasing binding of fluoroquinolones to form the ternary complex. Members of a structurally analogous chemical class, quinazoline-2,4-diones, do not require this magnesium-water bridge and are active against such mutated enzymes. The work presented here shows the design, synthesis, and evaluation of a panel of previously unreported *N*-1 variant fluoroquinolones that are hypothesized to make novel binding contacts with bacterial type II topoisomerases. The goal of identifying novel binding contacts between a fluoroquinolone and DNA gyrase or topoisomerase IV is to reduce or eliminate the need for the magnesium-water bridge, thus enhancing activity against current fluoroquinolone resistant mutants. Initial results will be discussed, and indicate that these novel fluoroquinolones may indeed be acting by a different mechanism from traditional fluoroquinolones, probably by inhibiting the catalytic activity rather than poisoning the enzyme.

Poster 20

AN INVESTIGATION INTO THE OXIDATIVE COUPLING OF NAPTHOLS AND ANTHRACENONES

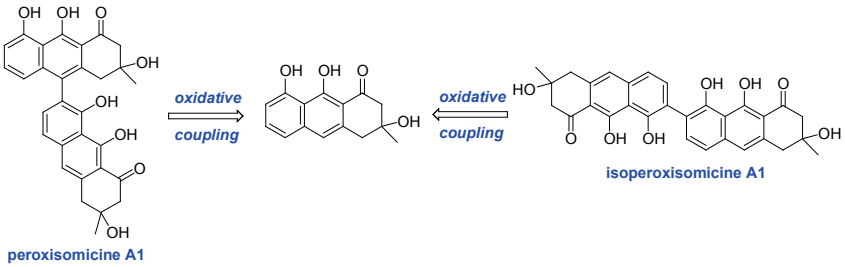
Alvin LeGall and Horacio F. Olivo*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

The seeds found in the fruit growing in Southwestern Texas known as 'coyotillo' or 'tullidora' possess toxic natural products which cause toxicity in both humans and animals. The scientific name of this plant is *Karwinskia humboldtiana*. Interestingly a dimeric dihydroxyanthracenone was found to have unique cytotoxicity for liver, lung, and colon cancer cells, and much less selectivity for these types of normal cells.

Several natural products were isolated and characterized from the fruit and the roots of the plant. These compounds were investigated in laboratory studies using mice and found that the each compound targeted different organs. T-544 is the toxin that goes into the brain and causes reversible-paralysis of the limbs (in animals and humans). T-514 damaged the lungs and the liver, and T-496 produces intense diarrhea.

An interesting point is made when these natural products are compared with the selectivity of common chemotherapeutic agents. Though these chemotherapeutic agents are very potent and efficient at attacking cancerous cell lines, healthy cell lines are sometimes attacked before cancerous cell lines are effected. We are very interested in the selective cytotoxicity of these natural products found in *K.Humboldtiana*, as they may offer an alternative to common chemotherapeutic agents. Specifically we are very interested in the T-514 compound, as it demonstrates a high characteristic of selectivity in which it will attack the cancerous cell lines before the healthy cell lines are effected.

Due to the relatively low yield of the isolated T-514 compound we have turned to the total synthisis of the T-514 in order to obtain greater yields and to conduct more studies on the compund. We envision a synthetic strategy that consists of the oxidative coupling of two dihydroxyanthracenones to prepare T-514. These naphthols bear structural resemblance to the compound of interest and they should couple the same way. This synthetic strategy will also allow us to prepare not only this natural product but also other *Karwinskia*-like natural products.



Poster 21

ENHANCED POLYCHLORINATED BIPHENYL DEGRADATION BY BIOAUGMENTATION WITH *BURKHOLDERIA XENOVORANS* LB400 IN A SWITCHGRASS PLANTED RHIZOSPHERE

Yi Liang, Richard Meggo, Dingfei Hu, Jerald L. Schnoor and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Phytoremediation represents a cost-effective and sustainable approach for polychlorinated biphenyl (PCB) removal, in which plants and associated microbes remove and detoxify PCBs. Previous results have shown significantly higher reductions of PCB-52, 77 and 153 in switchgrass planted systems compared to the unplanted systems. In this study, we are analyzing microbial PCB biodegradation in a switchgrass planted system with PCB exposure (PCB-52, 77 and 153 mixtures). After 12 weeks incubation in switchgrass-planted reactor, 18.4% of PCB parent compounds were transformed into less chlorinated congeners, compared with 8.6% of transformation in unplanted reactor, indicating enhanced dechlorination in switchgrass-planted reactor. However, the total PCB removal was not improved. Furthermore, although biphenyl dioxygenase gene (*bph*A), the gene codes for the key enzyme catalyzing the first step of aerobic PCB degradation, was more abundant in switchgrass planted reactor, *bph*A transcripts in both planted and unplanted reactors were below our quantification limit of RT-qPCR, indicating inactive aerobic PCB biodegradation. To improve aerobic PCB degradation and total PCB removal, *Burkholderia xenovorans* LB400, one of the most effective aerobic PCB degrader, was introduced into soil reactors. The addition of LB400 helped to increase total PCB removal from 1% to 6.3%. And the enhanced total PCB removal in bioaugmented reactors was associated with elevated abundance of *bph*A and its transcripts, indicating the enhanced aerobic degradation after bioaugmentation. Overall this study helps better understand microbial PCB degradation and provide valuable information about how to optimize phytoremediation strategies to achieve efficient PCB removal.

Poster 22

NOVAL STRUCTURAL AND DYNAMIC PROPERTIES REWIRE THE LIGAND BINDING SPECIFICITY IN A Tiam1 PDZ MUTANT

Xu Liu[1], David C. Speckhard[2], Tyson R. Shepherd[1] and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Department of Chemistry, Loras College, Dubuque, IA

The T-cell lymphoma invasion and metastasis (Tiam) family of proteins are guanine exchange factors (GEFs) for the Rho-family GTPase Rac1 crucial for cell-cell adhesion and cell migration. Deregulation of Tiam1/Rac1 signaling leads to various malignancies, including cardiovascular disease and cancer. Tiam proteins contain several protein–protein interaction domains, in particular a PDZ domain. Previously we found that the Tiam1 and Tiam2 PDZ domains had distinct binding specificities. Intriguingly, four residues in the ligand binding pocket were not conserved between the Tiam1 and Tiam2 PDZ domains. To test their importance in specificity, we engineered a quadruple mutant of the Tiam1 PDZ domain (PDZ-QM), where four residues in the Tiam1 PDZ domain were substituted for those in Tiam2. Remarkably, the Tiam1 PDZ-QM binding preference was changed to that of Tiam2. Here, we used equilibrium binding experiments and structural analyses to investigate the origins for this altered specificity. Ligand-free and -bound PDZ-QM crystal structures showed that enlarged P_0 and P_{-2} ligand binding pockets and a favorable electrostatic interaction at the P_{-4} sub-pocket were critical for the changed specificity. Biochemical studies indicated that Tiam1 PDZ-QM was less thermally stable than the WT, while NMR studies showed that a set of residues explored multiple conformations. Backbone (^{15}N) and side chain methyl (^{13}C) NMR relaxation studies confirmed the dynamic features of the ligand free Tiam1 PDZ-QM domain. In the presence of ligand, however, PDZ-QM dynamics were dampened. These studies provide novel insights into the structural and dynamic basis for Tiam1 and Tiam2 PDZ domain specificity.

Poster 23

DEVELOPMENT OF A NOVEL HIGH-THROUGHPUT SCREEN TARGETING RGS17 & CHARACTERIZATION OF LEAD SMALL MOLECULE INHIBITORS FOR THE TREATMENT OF METASTATIC LUNG AND PROSTATE CANCERS

Duncan I. Mackie and David L. Roman*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

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

Poster 24

CALMODULIN RECOGNITION OF ANTI-PARALLEL NESTED BINDING SITES IN THE NEURONAL VOLTAGE-GATED SODIUM CHANNEL Na_V 1.2

Mark S. Miller and Madeline A. Shea*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Calmodulin (CaM) mediates intracellular signal transduction by responding to calcium influxes, triggering a conformational change that exposes a binding cleft capable of binding numerous protein targets over a vast range of affinities, from pM to μM. Understanding the specificity and the energy in molecular recognition is paramount to understanding CaM-mediated cell signalling. CaM regulates the neuronal voltage-gated sodium channel (Na_V) 1.2, an intermembrane protein responsible for the rising phase of the action potential in the central nervous system. CaM binds to Na_V 1.2 at two positions: to a conserved isoleucine glutamine (IQ) motif found in the c-terminal tail, and to an inactivation gate found in one of the intracellular loops. CaM binds tightly to the IQ motif under both calcium-free (apo, resting cell) and calcium-saturated (excited cell) conditions. We recently determined a structure of apo CaM bound to the IQ motif of Na_V1.2, and from the insight into the binding interface, we could infer residues of the IQ motif that contribute to the specificity and the energy of CaM binding.
We measured the binding affinity of CaM for the IQ motif using a fluorescent biosensor probe consisting of the IQ motif sandwiched between the FRET-pair CFP and YFP. For the first time we resolved the nM-scale affinities of apo CaM and calcium-saturated CaM for the IQ motif. Earlier methods had set a limit of 10 nM while under stoichiometric conditions; using this biosensor probe, we could now determine binding under equilibrium conditions. To explore the specificity of the CaM-IQ interaction, we perturbed both individually and as pairs those residues making many contacts with apo CaM. Mutagenesis demonstrated that IQ residues that made multiple contacts with apo CaM greatly disrupted apo CaM binding (50-10,000-fold) yet only mildly disrupted calcium-saturated CaM binding (5-25-fold), suggesting a different binding interface between the IQ motif and the two states of CaM. In parallel, we determined the solution NMR structure of calcium-saturated CaM bound to the IQ motif. In agreement with our thermodynamic data, this structure revealed that calcium-saturated CaM binds in a reverse orientation on the IQ motif compared to apo CaM. For such a change in binding orientation to occur, apo CaM would first have to detach from the IQ motif, which would allow calcium-saturated CaM to bind to other target sequences, potentially the nearby inactivation gate of the Na_V. Nature has engineered nested, antiparallel binding sites housed in the same amino acid sequence of the Na_V 1.2 IQ motif, and each site specifically and tightly binds to one state of CaM. We will next investigate whether this is true for the other 8 Na_V isoforms expressed throughout the body, and we will understand how CaM-binding specificity correlates to the localization and function of these other channels.

Poster 25

MECHANISM OF OXIDATIVE HALF-REACTION OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

Tatiana V. Mishanina[1], John M. Corcoran[1], Eric M. Koehn[1], Scott A. Lesley[2] and Amnon Kohen[1]*
[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]The Joint Center for Structural Genomics at the Genomics Institute of Novartis Research Foundation, San Diego, CA

Thymidylate is a DNA nucleotide that is essential to all organisms and is synthesized *de novo* by the enzyme thymidylate synthase (TSase). Several human pathogens, including *M. tuberculosis*, *B. anthracis* and others, rely on an alternative FAD-dependent thymidylate synthase (FDTS), which differs from the human TSase in nucleotide sequence, structure and molecular mechanism. In light of mechanistic and structural differences between human and pathogens' enzymes, FDTS presents a promising new target for antibiotics with low toxicity. FDTS catalysis can be separated into two half-reactions: the reductive half-reaction, where the flavin cofactor is reduced by a reducing agent (e.g. nicotinamide, dithionite, ferredoxin, etc.), and the more interesting oxidative half-reaction, in which reduced FAD is oxidized by the substrates to form thymidylate. In the past, we have shown that the oxidative half-reaction of FDTS does not rely on an enzymatic nucleophile and that the proposed reaction intermediates are not covalently bound to the enzyme during catalysis, an important distinction from the human TSase. In this presentation, I will describe the chemical trapping, isolation, and identification of derivatives of such intermediates in the FDTS-catalyzed synthesis of thymidylate. The identity of the trapped intermediates underlines the uniqueness of FDTS chemistry and narrows down the possible chemical mechanisms. Substrate activation and other mechanistic aspects of flavin-dependent thymidylate biosynthesis will also be discussed.

Poster 26

BEAUVARIA BASSIANA METABOLISM OF ORGANOSULFUR COMPOUNDS

Felipe Nicolau-Manterola and Tonya L. Peeples*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Beauveria bassiana is a filamentous fungus capable of increasing the yield and selectivity of oxidative biotransformations through the induction of key catalytic enzymes. The expression of these oxidases can increased up to 3 fold when *B. bassiana* is grown in presence of n-hydrocarbons or exposed to insecticides, neonicotinoids and carbamates. The purpose for this project is to evaluate and enhance the conversion of thioethers into sulfoxides by the induction of oxidases with n-alkanes and insecticides. We addressed the physiological quality of cells grown in in presence of sub lethal doses insecticides by measuring radial growth. We followed conversion of phenothiazine and thioanisole into their respective sulfoxides via reverse phase HPLC and MS. The major metabolite identified were sulfoxides with minor hydroxylated products. The oxidation capacity increased by 20% in presence of 50 ppm of insecticides. The synthesis of optical sulfoxides is of importance in pharmaceutical and fine chemistry industry; especially as ligands for chiral catalyst. The final outcome will be a robust active catalyst with tunable oxidative capacities.

Poster 27

DEVELOPMENT OF AN OBJECTIVE METHOD TO QUANTIFY DEACTIVATION USING CONFOCAL FLUORESCENT MICROSCOPY

Ann O'Toole, Bryce Hundley and Eric Nuxoll*
Department of Chemical and Biochemical Engineering, College of Engineering The University of Iowa, Iowa City, IA

We are investigating the thermal mitigation of *Pseudomonas aeruginosa* bacterial biofilms at a variety of time and temperature combinations as a method to eradicate biofilms on infected medical devices. Quantification of deactivation has been observed through both confocal fluorescent microscopy using membrane permeability dyes and through direct enumeration of colony forming units form post-treated biofilms. Microscopy provides direct, rapid feedback but is limited by the ability to objectively threshold images because of variable light intensities in the confocal plane. Here we discuss the development of a objective threshold method called the iterative selection method. This is designed to choose a threshold intensity exactly half way between the foreground and background intensities. This value is similar when compared to subjective thresholding values but is preferred since it is objectively calculated. This threshold value can then be used to be quantify live and dead bacteria count in a biofilm. This can then possibly lead to a relationship between microscopy and direct enumeration.

Poster 28

INCORPORATION OF THIOCYANATE AS AN INFRARED PROBE FOR FAST DYNAMICS AT THE ACTIVE-SITE OF *E. COLI* DIHYDROFOLATE REDUCTASE

Philip L. Pagano, Christopher M. Cheatum and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Dihydrofolate reductase is known to catalyze the transfer of a hydride to dihydrofolate (DHF) from an NADPH cofactor. The kinetic isotope effect (KIE) on this reaction is temperature independent for the wild-type enzyme, yet becomes increasingly temperature dependent when residue I14 is mutated from valine to alanine to glycine.[1,2] A model that accounts for this observation is that the wild type enzyme may exhibit a rigid structure at the transition state that fluctuates at the same time scale as the hydride transfer itself (fs to ps) and that this system loses this rigidity upon mutation to smaller and smaller residues.[3] While KIE's suggest a rigid active site structure, these kinetic experiments do not directly examine the enzyme's dynamics. A recent technique that directly measures such fast dynamics in condensed phase systems is 2-dimensional infrared spectroscopy (2D IR), where the vibrational relaxation of an infrared reporter group is followed in a time-resolved fashion in order to extract information about that reporter molecule's environment.

While 2D IR is a powerful tool to access dynamic information at such fast time scales, measuring infrared spectra of a protein in water can be tricky, as water and amino acid functionalities saturate nearly all parts of the spectrum. A relatively silent portion of this spectrum exists in these systems in the region of ~2000-2300 cm^{-1} region where triple bonded molecules absorb (azides, nitriles, etc.) In this study, we incorporate a nitrile functionality to an engineered cysteine residue at the active site of DHFR in hopes of introducing a unique infrared reporter group that minimally perturbs this enzymatic system.

References

1. Stojkovic, V.; Perissinotti, L. L.; Lee, J.; Benkovic, S. J.; Kohen, A. The Effect of Active-site Isoleucine to Alanine Mutation on the DHFR Catalyzed Hydride-transfer. *Chem. Commun.* **2010**, *46*, 8974-8976.
2. Stojkovic, V.; Perissinotti, L. L.; Willmer, D.; Bnkovic, S. J.; Kohen, A. Effects of the Donor-Acceptor Distance and Dynamics on Hydride Tunneling in the Dihydrofolate Reductase Catalyzed Reaction. *J. Am. Chem. Soc.* **2012**, *134*, 1738-1745.
3. Roston, D.; Cheatum, C. M.; Kohen, A. Hydrogen Donor-Acceptor Fluctuations from Kinetic Isotope Effects: A Phenomenological Model. *Biochemistry (N. Y.)* **2012**, *51*, 6860-6870.

Poster 29

ARCHITECTURE OF THE MACROMOLECULAR COMPLEXS FORMED IN TLS

Kyle T Powers and M. Todd Washington*
Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Large, dynamic protein complexes with whose members have a high degree of intrinsic disorder play critical roles in cell cycle control, signaling, transcription, DNA replication, repair, and many other essential biological processes. One of the greatest challenges in molecular and cellular biology is obtaining high resolution structural information about such macromolecular complexes. Use of computational force fields, whose behavior relies upon crystallographic, NMR, and distant constraint data, will be a unique and unprecedented way to obtain this information. Our model system to develop this methodology is the complex of proteins that carryout translesion synthesis (TLS), the replication bypass of damaged DNA. The complexes modeled include proteins such as proliferating cell nuclear antigen (PCNA), multiple TLS specific DNA polymerases, and several ubiquitin ligases. The approaches developed here can be applied to other biologically relevant systems with similar large protein complexes providing otherwise unobtainable structural information.

Poster 30

MODELING PARTICLE CLUSTERING USING PAIR CORRELATION FUNCTION ANALYSIS

Cicily J. Ronhovde and M. Lei Geng*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Tissue phantoms are synthetic imitations of biological tissue that can be used to mimic many characteristics of tissue including optical, acoustic, thermal, and mechanical properties. These properties can be adjusted in the synthetic material to simulate disease progression for the development of diagnostic or treatment techniques. For example, the spectroscopic properties of tissue – scattering, absorbance, and fluorescence – can be modeled in a synthetic material by incorporating a range of concentrations of cellular components with optical properties. These phantoms are of particular interest for the development of an optical method of cancer detection and diagnosis that could be applied non-invasively and provide instantaneous and quantitative results with high sensitivity.

An essential requirement for validating such a diagnostic technique is the ability to clearly distinguish boundaries between adjoining regions that have different spectral properties. Mesoporous silica particles functionalized with octadecylsilane were selected as the loading platform for fluorophores because these particles provide biochemically well-defined micrometer and nanometer domains due to hydrophobic trapping of loaded molecules.[1] A second requirement for developing a robust optical methodology is uniformity of the tissue phantom samples that are used to calibrate an optical device. One barrier to the generation of a uniform tissue phantom is the dispersal of hydrophobic particles in agar.

Previously, uniform suspension of particles in agar has been difficult because of the tendency of the particles to aggregate in aqueous media.[1] Current efforts to disperse particles include surfactant coating and various methods of physical dispersion. An analysis method utilizing the pair correlation function has been developed to quantify particle clustering as a means of evaluating the particle dispersal in the resulting tissue phantoms. Different particle dispersal patterns – clustered, uniform, and random – are distinguishable based on characteristic features produced in the pair correlation function. The robustness of the mathematical analysis of the phantom images is established using simulated images with various dispersal patterns.

Reference

1. Skvortsova, Y. A.; Freeney, R. M.; Zhong, Z.; Geng, M. L. *Anal. Chem*. **2010**, *82*, 6712-6716.

Poster 31

THE REGULATION AND PATHOGENIC FUNCTION OF *STAPHYLOCOCCUS AUREUS* HYALURONATE LYASE

Carolyn B. Rosenthal[1], Crystal L. Jones[2], Mary J. Mantz[3], Shweta Singh[2], Matthew C. Wise[2], Christopher A. Squier[3], Daniel V. Zurawski[2] and Alexander R. Horswill[1]*
[1]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Walter Reed Army Institute of Research (WRAIR), Department of Wound Infections, Silver Spring, MD
[3]Department of Oral Pathology, Radiology and Medicine, and Dows, College of Dentistry, The University of Iowa, Iowa City, IA

Staphylococcus aureus is a Gram-positive human pathogen and is extremely problematic because of its ability to cause a diverse range of bacterial infections. The pathogenic ability of *S. aureus* is due to an extensive arsenal of virulence factors that includes surface adhesins, secreted hemolysins, superantigens, and exoenzymes. Our studies have focused on the exoenzyme hyaluronate lyase, which cleaves hyaluronic acid polymer at the β-1,4 glycosidic bond. Hyaluronic acid is a large glycosaminoglycan composed of repeating disaccharide units of N-acetylglucosamine and D-glucuronic acid and is a main component of the mammalian extracellular matrix. Hyaluronate lyases have been implicated as virulence determinants in a number of bacterial pathogens, facilitating the dissemination of bacterial cells and other secreted factors during infection. Here we report on the hyaluronate lyase of *S. aureus*, HysA, and its role in the pathogenesis of the organism. We found that a Δ*hysA* mutant of a community-associated methicillin-resistant *S. aureus* was attenuated in a murine neutropenic pneumonia model of infection when compared to the wild-type strain. Mice infected with this mutant also exhibited reduced lung pathology and increased hyaluronic acid staining compared to wild-type infected mice. To address the HysA pathogenic mechanism, HysA protein was purified and found to significantly increase the permeability of porcine vaginal mucosa in an *ex vivo* model. Finally, through the use of an ordered transposon mutagenesis library, we identified 8 insertions that modulate *S. aureus* HysA activity. The genes affected include several from the *sigB* operon, as well as in the global regulators *sarA* and *codY*. Further examination of the interaction of CodY with HysA activity identified a CodY binding box in the promoter region of *hysA*, and we have evidence that CodY acts to repress *hysA* where HysA activity was increased 12-fold in a *codY* mutant. Together, these results indicate that the *S. aureus* hyaluronate lyase is a potent virulence factor and future studies will further elucidate the regulation and pathogenic role of this enzyme.

Poster 32

THE ROLE OF REACTIVE OXYGEN SPECIES AND A TOXIC DOPAMINE METABOLITE IN NEURODEGENERATION

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

Parkinson's disease (PD), a common neurological disorder, is characterized by the selective degeneration of dopaminergic-neuronal cells in the substantia nigra of the brain. This neurodegeneration is believed to be related to toxic dopamine metabolites, oxidative stress, and reactive oxygen species (ROS). Dopamine is metabolized by monoamine oxidase to produce 3, 4-dihydroxyphenylactaldehyde (DOPAL), a highly reactive aldehyde species. DOPAL has been shown to modify proteins and cause protein aggregation, which leads to cell dysfunction. DOPAL has also been shown to be toxic to neuronal cells both *in vitro* and *in vivo*. ROS is produced when DOPAL undergoes autooxidation to a semiquinone radical and then to an orthoquinone, under normal physiological conditions. Furthermore, ROS is generated upon reaction of DOPAL with proteins via an unknown mechanism. It has been shown that antioxidants, such as N-acetylcysteine and ascorbate, can block protein modification by DOPAL. The objective of this research is to determine the role of ROS in DOPAL-mediated neurodegeneration. We have found that superoxide anion and hydrogen peroxide are produced during DOPAL-mediated protein modification. Evidence suggests the presence of oxygen catalyzes protein modification by DOPAL and yields ROS. Finally, we have demonstrated using lysate of dopaminergic SH-SY5Y cells that decreasing free glutathione level with diamide yields an increase in DOPAL-protein adducts; conversely, addition of N-acetylcysteine results in a decrease of protein modification by DOPAL. This information will facilitate determination of the role of ROS production in DOPAL-mediated neurodegeneration observed in PD.

Poster 33

STUDIES TOWARDS SYNTHESIS OF TRUNCATED SUPERSTOLIDE A

Aashay. K. Shah, Lei Chen and Zhendong Jin*
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, 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 a unique cellular target(s) and a novel mechanism of action. However, due to the scarcity of these marine natural products, there has not been enough material for further biological investigation and the cellular target of Superstolides have not been identified.

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

Poster 34

DIHYDROFOLATE REDUCTASE ROLE IN CATALYSIS

Priyanka Singh, Zhen Wang, Vern L. Schramm and Amnon Kohen*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

We are using dihydrofolate reductase (DHFR) as a model system to study the protein dynamics because of its small size, ubiquity, and well-established structural, kinetic and mechanistic characteristics. For a small and flexible enzyme like DHFR, various computational and evolutionary studies have proposed that there is a network of coupled motion across the whole protein along the reaction coordinates affecting chemical catalysis. The distal residues, which appear to be part of the network in DHFR, are G121, M42W and F125M. I14 is one of the active site residues that directly affect the catalyzed chemistry. In this work we want to understand whether and how the active site residue is coupled to distal residues. To facilitate this we made a double mutant of active site residue I14A with G121V and studied the temperature dependence of intrinsic kinetic isotope effects (KIE). While both single mutants i.e. I14A and G121V have weakly temperature dependent kinetic isotope effects we found that the double mutant I14A-G121V has steeply temperature dependent KIEs. This finding suggests that both the active site residue (I14) and the distal residue (G121) are part of the same dynamic network. Moreover it suggests a path by which G121 could be coupled to the chemistry at the active site (20 Å away). In a parallel study, we are using so called "Born-Oppenheimer enzyme" or "heavy enzyme", i.e., DHFR labeled with heavy isotopes (^{13}C, ^{15}N, and ^{2}H (only non-exchangeable hydrogens)) to investigate whether fast dynamics (fs-ps) are coupled to the C-H$\rightarrow$C transfer. Compared to native enzyme, the KIE for heavy enzyme is same at higher temperature while steeply temperature dependent at lower temperature suggesting a change in transition state structure from the native enzyme. Further studies are needed to reveal the enzyme dynamics for the observed kinetic effects.

Poster 35

ASYMMETRIC SYNTHESIS OF PYRROLIDINES VIA Mn-MEDIATED RADICAL ADDITION

Kara A. Slater and Gregory K. Friestad*
Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

Chiral 2-alkylpyrrolidines are prevalent in biologically active natural products. We report a manganese-mediated carbon-carbon bond construction approach to the asymmetric synthesis of pyrrolidines through a radical-ionic annulation pathway. The method expands upon the advantages of Mn-mediated photolysis for radical additions to chiral N-acylhydrazones, which include efficient use of various primary radicals and predictable control of stereochemistry. Mechanistic evidence suggests radical addition followed by S_N2-type cyclization.

Poster 36

PROBING CASPASE-7 ALLOSTERY WITH ATOMISTIC MOLECULAR DYNAMICS SIMULATIONS AND FRAGMENT SCREENING

Nick R. Vance and M. Ashley Spies*.
Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

Caspases have been the subject of intense study in the field of drug discovery. This family of cysteine-aspartic specific proteases facilitates a tightly regulated apoptotic event by judiciously cleaving hundreds of substrates. Overstimulation of this apoptotic executioner has been associated with a number of neurodegenerative disorders such as retinal degradation, Huntington's and Alzheimer's disease.[1] Inhibition of caspase mediated cell death has also been implicated in some cancers proliferation. Attempts by J. Wells et al have yielded some reactive thiol containing inhibitors that covalently bind the dimer interface, as well as a caspase-3 activator that was efficacious in accelerating cancer cell death.[2] Feldman et al also discovered a novel class of copper complex reversible inhibitors targeting the same allosteric pocket. For caspase-7, classes of small molecule activators or inhibitors that lie in ideal, drug-like space have yet to be identified.[3]
Atomistic molecular dynamics simulations (50-100ns) with knowledge based force fields were employed to model a catalytically competent caspase-7 dimer bound to a substrate. The models of the caspase-7 dimer in a near-attack conformation will be used for virtual screening, lead development and understanding of allosteric regulation. Using these models for docking studies, a chemically diverse, highly soluble library of fragments (150-300MW) will be screened against caspase-7. Fragment based drug discovery has garnered widespread attention in academia and industry in recent years. The process of growing a fragment into a bona fide lead compound allows the medicinal chemist to optimize functional groups to control log (P), prevent incorporation of metabolically labile groups, and maintain high ligand efficiency during lead development. NMR, crystallography and site-directed mutagenesis will provide essential protein-ligand structural information necessary for successful fragment development.

References

1. H.Y. Chang and X. Yang. Proteases for Cell Suicide: Functions and Regulation of Caspases. *Microbiol. Mol. Biol. Rev*. **64**, (4)821-46.
2. Wells et al. Small-Molecule Activators of a Proenzyme. *Science*. **326**, (6)853-58.
3. Feldman et al. A Class of Allosteric Caspase Inhibitors Identified by High-Throughput Screening. *Molecular Cell*. **47**, (4)585-595.

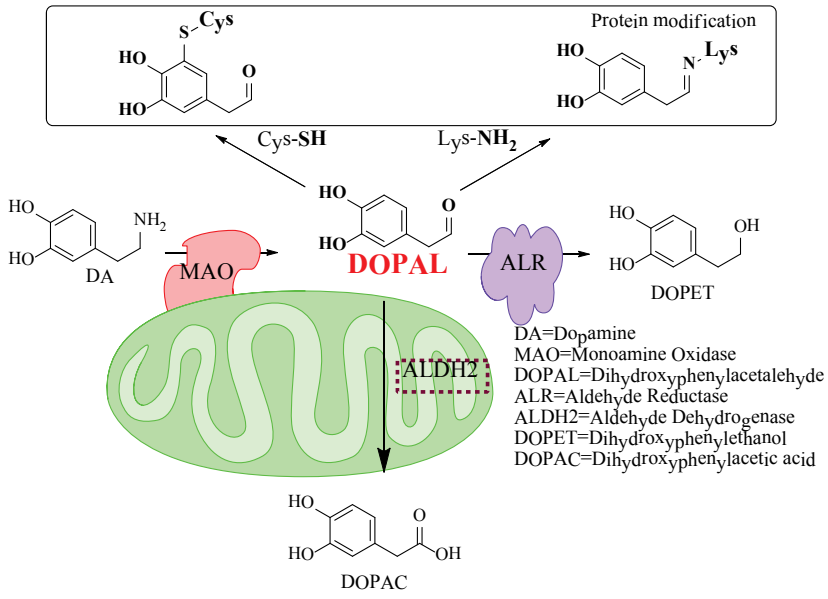
Poster 37

THE DOPAMINE METABOLITE 3,4-DIHYDROXYPHENYLACETALDEHYDE TARGETS PROTEINS AND IS TOXIC TO DOPAMINERGIC CELLS

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

Parkinsons disease is a slow-progressive neurodegenerative disorder affecting 5-6 million people around the globe. The disease is manifested by the rapid deterioration of dopaminergic cells in the substantia nigra portion of the brain; however, the pathological mechanism of selective dopaminergic neuronal death is unknown. Dopamine is oxidatively deaminated and catalyzed by monamine oxidase to form the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehye (DOPAL). A primary aim of this work is to measure the functional consequence of DOPAL in the SH-SY5Y cell line, a model for dopaminergic neurons. 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. 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

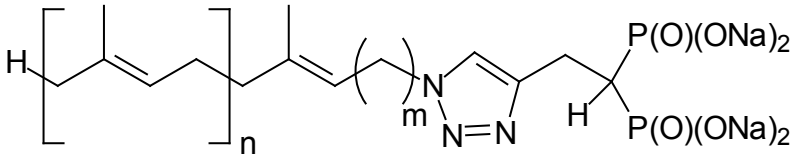


Poster 38

TRIAZOLE–BASED INHIBITORS OF GERANYLGERANYL TRANSFERASE II

Veronica S. Wills[1], Xiang Zhou[1], Ella J. Born[2], Sarah A. Holstein[2] and David F. Wiemer[1]*
[1]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Addition of the 20-carbon geranylgeranyl chain to Rab protein family members is catalyzed by the enzyme geranylgeranyl transferase II (GGTase II or RabGTase), and is essential for proper cellular localization of these important proteins. In an effort to develop new inhibitors of this enzyme, we have prepared a number of triazole derivatives bearing a geminal bisphosphonate to mimic the pyrophosphate of geranylgeranyl diphosphate, a triazole moiety that may complex with an active site zinc, and an isoprenoid chain that might occupy the distal hydrophobic pocket. While triazoles substituted with true isoprenoids (e.g. **1**) have been obtained as mixtures of olefin isomers, the homoisoprenoid series (e.g. **2**) can be prepared to favor specific olefin isomers. The synthesis of these compounds and their activity as inhibitors of GGTase II will be presented.



1 m = 1, n = 2
2 m = 2, n = 2

Poster 39

TOWARD A MOLECULAR MECHANISM FOR TIAM1 REGULATION

Zhen Xu[1], Lokesh Gakhar[2] and Ernesto J. Fuentes[1]*
[1]Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]X-ray Crystallography Core Facility, The University of Iowa, Iowa City, IA

T-cell lymphoma invasion and metastasis 1 (Tiam1) is a guanine nucleotide exchange factor protein (GEF) that is specific for the Rho-family GTPase Rac1. Mutations or aberrant regulation of Tiam1 have been implicated in oncogenic transformation of cells and linked to several kinds of invasive and metastatic forms of cancer. Tiam1 contains several protein-protein interactions domains (PH_n-CC-Ex-RBD-PDZ-DH-PH_c) that are thought to contribute to the regulation of its GEF activity. Moreover, activation of Tiam1 occurs by phosphorylation and through protein-protein interactions with the PH_n-CC-Ex domain. Here, we test the hypothesis that Tiam1 protein-protein interaction domains inhibit Tiam1 GEF activity through inter-domain interactions. Using biochemical GEF assays, we showed that the PH_n-CC-Ex, RBD and PDZ domains inhibit the GEF activity of the catalytic DH-PH_c bi-domain up to ~3-fold. In addition, small angle X-ray scattering (SAXS) data of the DH-PH_c, PDZ-PH_c and PH_n-PH_c fragments indicated that the DH-PH_c bi-domain adopts a compact globular structure, while the PDZ-PH_c and PH_n-PH_c regions present partially elongated structures. These data began to elucidate the biochemical and structural mechanisms of Tiam1 regulation.

Poster 40

KINETIC ASSESMENT OF "BAIT AND SWITCH" ANALOGS OF 2-PAM AS METHYL TRANSFER AGENTS AND RESURRECTING AGENTS FOR AGED HUMAN ACETYLCHOLINESTERASE

Sumana N. Yasapala and Daniel M. Quinn*
The Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

The enzyme acetylcholinesterase (AChE) is an essential enzyme in the human neuronal system. The enzyme hydrolyzes the neurotransmitter acetylcholine (ACh) with an amazing catalytic power (turnover number is over 10^4 s^{-1}). This essential role of AChE makes it a target for the drug development for Alzeimer's disease and the main target for the development of extremely toxic organophosphorus compounds (OP) such as chemical warfare agents (CWAs) and pesticides. The current OP poisoning treatments include the administration of a muscarinic antagonist, e.g.atropine, an agent to treat seizers that arise from inhibition of AChE in the central nervous system and certain oximes such as pralidoxime chloride (2-PAM) which serves as an antidote by nucleophilically displacing the phosphyl moiety from the active site. The AChE reacts with ACh and OP in a similar way but later undergoes a de-alkylation process called "aging". There is no known oxime that is able to displace the phosphyl moiety from the active site of aged-AChE. However, if the phosphonate anion of the aged adduct is alkylated, known oximes could then be used to reactivate the OP-inhibited acetylcholinesterase. Therefore, we synthesized several methyl analogs of 2-PAM and studied kinetic parameters to determine the abilities of methyl transferring and reactivation of aged-AChE. Although few compounds showed methyl transferring ability none of them showed reactivation of aged –AChE adduct. However, some of the methyl analogs of 2-PAM were reversible inhibitors and half maximal inhibitory (IC_{50}) values were low compared to that of 2-PAM.

Poster 41

EVIDENCE AND QUANTITATION OF AROMATIC ORGANOSULFATES IN AMBIENT AEROSOLS IN LAHORE, PAKISTAN

Shuvashish Kundu[1], Tauseef A. Quraishi[2], Ge Yu[3], Catherine Suarez[3], Frank. N. Keutsch[3] and Elizabeth A. Stone[1]*
[1] Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA
[2]University of Engineering and Technology, Lahore, Pakistan
[3]Department of Chemistry, University of Wisconsin-Madison, Madison, WI

Atmospheric aerosols absorb and scatter solar radiation and can act as cloud condensation nuclei (CCN). Aerosols also exert indirect effects on climate by changing biogeochemical cycles and have negative health effects on various morbidity and mortality. The health and climate effects of aerosols depend, in part, on their chemical composition. Organic matter is a significant component of ambient aerosol mass, but remains the most poorly characterized fraction due to its chemical complexity and multitude of sources. Organosulfates are important components of atmospheric organic aerosols, yet their structures, abundances, sources and formation processes are not adequately understood.

This study presents the identification and quantitation of benzyl sulfate in atmospheric aerosols, which is the first confirmed atmospheric organosulfate with aromatic carbon backbone. Benzyl sulfate was identified and quantified in fine particulate matter ($PM_{2.5}$) collected in Lahore, Pakistan during 2007-2008. An authentic standard of benzyl sulfate was synthesized, standardized, and identified in atmospheric aerosols with quadrupole time-of-flight (Q-ToF) mass spectrometry (MS). Benzyl sulfate was quantified in aerosol samples using ultra-performance liquid chromatography (UPLC) coupled to negative electrospray ionization triple quadrupole (TQ) MS. The highest benzyl sulfate concentrations were recorded in November and January 2007 (0.50 ± 0.11 ng m^{-3}) whereas the lowest concentration was observed in July (0.05 ± 0.02 ng m^{-3}). To evaluate matrix effects, benzyl sulfate concentrations were determined using external calibration and the method of standard addition; comparable concentrations were detected by the two methods, which ruled out significant matrix effects in benzyl sulfate quantitation. Three additional organosulfates with *m/z* 187, 201 and 215 were qualitatively identified as aromatic organosulfates with additional methyl substituents by high-resolution mass measurements and tandem MS. The observed aromatic organosulfates form a homologous series analogous to toluene, xylene, and trimethylbenzene, which are abundant anthropogenic volatile organic compounds (VOC), suggesting that aromatic organosulfates may be formed by secondary reactions. However, stronger statistical correlations of benzyl sulphate with combustion tracers (EC and levoglucosan) than with secondary tracers (SO_4^{2-} and α-pinene derived nitrooxy organosulfates) suggest that aromatic organosulfates may be emitted from the combustion sources or their subsequent atmospheric processing. Further studies are needed to elucidate the sources and formation pathways of aromatic organosulfates in the atmosphere.

Poster 42

VALIDATION OF CAFFEINE DEHYDROGENASE FROM *PSEUDOMONAS* SP. CBB1 AS A SUIABLE ENZYME FOR A RAPID CAFFEINE DIAGNOSTIC TEST

Sujit K. Mohanty[1], Chi-Li Yu[3], Sridhar Gopishetty[2] and Mani Subramanian[1,2]*
[1]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[2]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
[3]Proteomics Facility, The University of Iowa, Iowa City, IA

Introduction

Excessive and long-term caffeine consumption is associated with various neurological and cardio-vascular disorders. Also, caffeine is highly restricted for infants, seniors, nursing mothers and pregnant women owing to its possible teratogenic effects. Currently, no method is available commercially for detection of caffeine, esp. in nursing mother's milk. A suitable method is desirable to enable specific, rapid and semi-quantitative detection of caffeine in decaffeinated versions of various caffeine containing beverages and mother's milk.

Test Development

In caffeine-degrading strain *Pseudomonas* sp. CBB1, caffeine is oxidized by a novel heterotrimeric quinone-dependent caffeine dehydrogenase (Cdh)[1]. Cdh is caffeine specific and appears suitable for rapid detection of caffeine in fluids. Partially purified Cdh was used to develop a dye-based colorimetric/visual assay for detection of caffeine. Different tetrazolium salts were screened and the assay conditions optimized, for sensitivity (1 ppm), speed of detection (1 min), and distinguishable visual color. Typically, 20 units of Cdh with 0.5mM INT met the diagnostic attributes for caffeine detection including visual light pink color (1-10 ppm of caffeine) to shades of dark red (20-100 ppm) within a minute.

Enzyme Validation

Suitability of Cdh for caffeine diagnostic test was validated by rapid detection of caffeine in various commonly used caffeinated beverages (like coffee, soft drinks) and semi-quantitative estimation of caffeine in pharmaceuticals. For the first time, detection of caffeine in nursing mother's milk (spiked with caffeine) was made possible using this Cdh-based diagnostic test. 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[3].

References

1. Yu, C. L., Kale, Y., Gopishetty, S., Louie, T. M., Subramanian, M. 2008. A novel caffeine dehydrogenase in *Pseudomonas* sp. strain CBB1 oxidizes caffeine to trimethyluric acid. J. Bacteriol. 190:772–776.
2. Mohanty, S. K., Yu, C. L., Das, S., Louie, T. M., Gakhar, L., Subramanian, M. 2012. Delineation of the Caffeine C-8 Oxidation Pathway in Pseudomonas sp. Strain CBB1 via Characterization of a New Trimethyluric Acid Monooxygenase and Genes Involved in Trimethyluric Acid Metabolism. J. Bacteriol. 194(15): 3872-3882.
3. Buerge, I. J., Poiger, T., Muller, M. D., Buser, H. R. 2003. Caffeine, an Anthropogenic Marker for Wastewater Contamination of Surface Waters. Environ. Sci. Technol. 37(4) : 691–700.

Poster 43

REGULATION OF CAFFEINE-INDUCED ENZYMES IN *PSEUDOMONAS PUTIDA* CBB5Δ*ndmE*

Ryan M. Summers[1], Venkat S. Iyer[1], Sujit K. Mohanty[2], Chi Li Yu[3], and Mani Subramanian[1,2]*
[1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[3]Proteomics Facility, The University of Iowa, Iowa City, IA

We have isolated a caffeine-degrading bacterium, *Pseudomonas putida* CBB5, capable of growing on caffeine as the sole source of carbon and nitrogen. CBB5 degrades caffeine *via* sequential *N*-demethylation to theobromine or paraxanthine, then to 7-methylxanthine, and finally to xanthine. CBB5 also sequentially *N*-demethylates theophylline, which has not been previously reported to be degraded by bacteria, to xanthine. CBB5 contains five novel *N*-demethylase genes, *ndmA*BCDE, which are responsible for caffeine degradation. These genes have been isolated, cloned, heterologously expressed, and their biochemical properties have been confirmed. NdmA and NdmB are Rieske monooxygenases that catalyze positional-specific N_1- and N_3-demethylations, respectively. Both enzymes receive reducing equivalents from NADH *via* a redox-center-dense Rieske reductase, NdmD. NdmC is an N_7-demethylase with activity specific for 7-methylxanthine and is active only with NdmD and NdmE, a glutathione-*S*-transferase homolog. The NdmCDE proteins form a large multi-subunit enzyme complex ($\alpha_3\beta_3\gamma_3$). Neither *ndmC* nor *ndmE* are expressed as soluble protein in *E. coli* or CBB5 if any of the *ndmCDE* genes are absent. Similarly, knocking out the *ndmC* or *ndmE* genes results in less NdmA and NdmB activity in CBB5 resting cells. The purpose of this work was to determine the effects of *ndmE* deletion in CBB5 on expression of NdmABCD proteins.

CBB5Δ*ndmE* cells were grown in M9 mineral salts medium containing caffeine and soytone (M9CS medium). As controls, CBB5 (w.t.) cells were grown in M9CS medium (positive control) and M9CS medium without the caffeine (M9S medium, negative control). The cells were lysed and crude cell extract separated on a DEAE Sepharose gel. The fractions containing NdmABCDE proteins were combined and submitted to the University of Iowa Proteomics Facility for analysis.

Proteomic analysis confirmed that expression of *ndmABCDE* genes in CBB5 is almost zero without caffeine present to induce expression. The NdmC and NdmE proteins were not detected in the CBB5Δ*ndmE* cells grown in M9CS medium, while the expression of NdmD was significantly reduced. Interestingly, the expression of NdmA and NdmB increased in the knock-out strain, indicating that reduction in NdmA and NdmB activity in the knock-out strain is due to the decreased expression of the reductase protein, NdmD.

Poster 44

FUNCTIONAL ANALYSES OF THE SPOR DOMAIN FROM FtsN, A CELL DIVISION PROTEIN FROM *ESCHERICHIA COLI*

Atsushi Yahashiri[1], Tammi R. Duncan[1], David L. Popham[2] and David S. Weiss[1]*
[1]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA
[2]Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, VA

SPOR domains consist of approximately 75 amino acid residues and occur in over 2000 bacterial species and >7000 proteins in the Pfam database. SPOR domain proteins are involved in cell division in many bacteria, and isolated SPOR domains have been shown to localize to the midcell and bind peptidoglycan (PG). Here we report findings from structure-function studies of the SPOR domain from an *E. coli* division protein named FtsN ($FtsN^{SPOR}$). A thorough mutagenesis of surface-exposed residues in $FtsN^{SPOR}$ revealed that amino acids important for septal localization and binding to PG sacculi map to a ß-sheet. The correlation of a localization defect with a PG binding defect argues the ß-sheet is the septal PG-binding site. We noticed that the ß-sheet contains two adjacent conserved cysteines, and provide genetic evidence these residues form a disulfide bond that is important for FtsN stability and function. Finally, we explored the question of whether SPOR domains from different proteins are functionally interchangeable by replacing the SPOR domain in the *E. coli* cell division protein DamX (428 a.a,) with the SPOR domain from FtsN. The SPOR domain swap protein (i.e., DamX(1-337)-$FtsN^{SPOR}$) was stable as judged by Western blotting and localized to the midcell. *E. coli* cells that produced the swap protein instead of authentic DamX were normal in some assays, but defective in others. We infer that SPOR domains have both unique and overlapping functions, presumably because they bind PG with differing affinity and/or specificity (e.g. recognize different chemical features of the PG).

References

1. Ursinus, A., van den Ent, F., Brechtel, S., de Pedro, M., Holtje, J. V., Lowe, J., and Vollmer, W. (2004) *J Bacteriol 186*, 6728-6737.
2. Möll, A., and Thanbichler, M. (2009) *Mol Microbiol 72*, 1037-1053.
3. Gerding, M. A., Liu, B., Bendezu, F. O., Hale, C. A., Bernhardt, T. G., and de Boer, P. A. (2009) *J Bacteriol 191*, 7383-7401.
4. Williams K.B., Yahashiri A., Arends S.J.R., Popham D.L., Fowler C.A., and Weiss D.S. (2013) Biochemistry 52:627-639.

Poster 45

RAPID IDENTIFICATION OF METABOLITES USING STABILIZED HUMAN CYP2D6 AND PRODUCTION OF DEXTRORPHAN FROM DEXTROMETHORPHAN USING STABILIZED HUMAN CYP2D6

Shuvendu Das[1] and Mani Subramanian[1,2,3] *
[1]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[3]Redox Biocat LLC, The University of Iowa Research Park, BioVentures Center, Coralville, IA

Drug leads and drugs in development must be completely characterized with respect to metabolites produced, preferably with human liver enzymes. Any metabolite >10% of the drug, must be subject to "Metabolite in Safety Test" (MIST). Key metabolites are also needed as reference compounds. Prep-scale synthesis of metabolites in 2-10+ mg is mostly accomplished by use of microsomes from various sources as well as cloned human CYPs in *E. coli*. This is cumbersome, difficult, low yielding, and time-consuming, expensive. Use of microsomes results in multiple metabolites, including conjugated metabolites. We have undertaken a game-changing approach for prep-scale synthesis of metabolites via highly stabilized dry powder (SDP) of hCYPs. As an example, (i) miniaturization of dextromethorphan (DOM) to dextrorphan (DOH) conversion and rapid identification of DOH and (ii) scale up of DOH production has been demonstrated with SDP-CYP2D6. No external NADPH is required for this reaction. The reaction is conducted in the simplest form, i.e., mixing the drug solution with SDP-CYP2D6. The reaction is scalable to hundreds of mL for metabolite production in 1-2 days.

Poster 46

IDENTIFICATION AND QUANTITATIVE PROFILING of CAFFEINE INDUCED ENZYMES IN A CAFFEINE-DEGRADING STRAIN *PSEUDOMONAS* SP. CES USING HIGH-THROUGHPUT MULTIPLEXED MASS SPECTROMETRY

Chi Li Yu[1], Ryan Summers[2], Yalan Li[1], Sujit Mohanty[2], Mani Subramanian[2,3]* and Marshall Pope[1]*
[1]Proteomics Facility, The University of Iowa, Iowa City, IA
[2]Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA
[3]Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA

Background: An understanding of caffeine metabolism and its applications in biosynthesis and environmental waste remediation is a pressing concern from both health and general environmental perspectives. Caffeine metabolic pathways in bacteria, fungi, and yeast *via* N-demethylation or C-8-oxidation have been investigated since the 1970s. Only recently were the metabolic pathways, enzymology and molecular genetics of C-8 oxidation in *Pseudomonas* sp. CBB1 and N-demethylation in *Pseudomonas putida* CBB5 elucidated. Another caffeine-degrading bacterium, *Pseudomonas* sp. CES is especially interesting because it is able to thrive on concentrations of caffeine that are four times higher (11.0g L^{-1}) than the maximum tolerable levels of strain CBB5 and CBB1. Indeed, complete *N*-demethylation of caffeine to xanthine was observed in cell lysate from CES strains grown on caffeine but not in soytone medium alone. This suggests that a novel, highly efficient caffeine metabolic enzyme may be induced in CES upon caffeine exposure.

Previously, caffeine metabolic enzymes in strain CBB5 and CBB1 were identified only after intensive work and many delays required to; optimize protein purification, construct the genomic DNA library, perfect gene cloning and verify functional expression. Modern proteomics has provided a rapid, unbiased alternative to identify proteins in genetically characterized organisms. Here we present a new, fast and accurate approach to identify novel caffeine degrading enzymes in bacterium strain CES using minimal starting samples and novel multiplexed MS assays.

Methods: Protein fractions from *Pseudomonas* sp. CES grown in the presence of caffeine or absence of caffeine were enriched based on N-demethylation activity using DEAE Sepharose Liquid Chromatography and pooled together to create essentially identical matrices save their relative activity to caffeine. These two sera were digested by trypsin, labeled with distinct stable isotope compositions using reductive amination, pooled together and subjected to MS/MS analysis using Accurate-Mass Q-TOF LC/MS systems (Agilent 6530). Relative scales of global expression were established by tracking the signature patterns created with Stable Isotope Dimethyl Labeling. In the assay sequence-identical peptides appear as satellite clusters separated by 4 or 8 amu for each free amine in the sequence. Enzymes responding to the presence of caffeine may be recognized globally by a distinct ratio of labeled components within each set of surrogate peptides whereas proteolytic peptides from unaffected proteins appear equally abundant.

Results: From two protein samples by using four MS analyses, all components of the N-demethylase system and the response of several downstream metabolic enzymes affected by caffeine were quantified in this new approach. Caffeine-induced proteins identified from active fraction were also confirmed by in-gel tryptic digestion and LC/MS/MS analysis without prior labeling and also verified by PCR and gene sequence analyses. The role of these proteins involved in caffeine degradation in strain CES are discussed.

Poster 47

CONTINUOUS, REAL-TIME MONITORING OF KEY ANALYTES FOR *PICHIA PASTORIS* BIOPROCESSES

Elizabeth R. Gibson[1], Kaylee Lanz[1], Edwin Koerperick[1], Jonathon T. Olesberg[1], Chris Evans[1], Gary W. Small[1,2,3] and Mark A. Arnold[1,2,3]
[1]ASL Analytical, Inc., Coralville, IA
[2]Optical Science and Technology Center, The University of Iowa, Iowa City, IA
[3]Department of Chemistry, College of Liberal Arts and Sciences, The University of Iowa, Iowa City, IA

ASL Analytical is developing automated, on-line monitors to track and document key chemicals during the development and production of biopharmaceuticals. Currently critical analytes consumed and produced during these processes are monitored by analyzing intermittent grab samples with off-line methods. This approach is not only labor intensive and poses a contamination risk, but does not allow for continuous knowledge of critical chemicals and severely limits the implementation of process control strategies. ASL's chemical monitoring technology is applicable to a wide range of bio-industrial platforms (e.g. biofuels, feed supplements, and industrial enzymes), however applications in the development and production of biopharmaceuticals are particularly valuable. These life-changing biologic medications are very expensive to develop and produce, leading to a very high value-add for monitoring technologies that can increase the efficiency of the development and production processes. ASL's first product, the Pichia Bioprocess Monitor, targets fermentations using the *Pichia pastoris* protein expression platform by simultaneously measuring the concentrations of glycerol and methanol as well as tracking the cell density – *all on-line and in real-time*. The ASL Bioprocess Monitor will enable a fast-growing industry to make game-changing advances in the development and production of critically important drugs by reducing the risk of bioreactor contamination, improving process efficiency, and reducing run costs, ultimately lead to a faster time to market for drugs in development.

CBB/NIH Fellowships

YEAR	CBB Fellowship	*NIH Trainee Fellowship
1990-91	10	4
1991-92	12	8
1992-93	12	6
1993-94	12	6
1994-95	13	10
1995-96	13	6
1996-97	12	6
1997-98	12	6
1998-99	12	6
1999-00	13	7
2000-01	14	7
2001-02	12	7
2002-03	11	8
2003-04	14	8
2004-05	12	7
2005-06	11	7
2006-07	10	7
2007-08	10	7
2008-09	10	8
2009-10	6	8
2010-11	7	8
2011-12	6	8
2012-13	5	6
2013-14	4	5
TOTAL	253	166

*Partially Sponsored by CBB

The Center for Biocatalysis and Bioprocessing
Gratefully Acknowledges
The following contributors for their
support of the
22nd Biocatalysis and Bioprocessing Conference
"Current Topics in Industrial Biotechnology "
October 21-22, 2013

AJINOMOTO®

enzymatics® the new standard in enzyme production

IOWA economic development

IDT® INTEGRATED DNA TECHNOLOGIES

KEMIN INSPIRED MOLECULAR SOLUTIONS™

novozymes® Rethink Tomorrow

S3 Scientific Sales Solutions

and

Usha Balakrishnan

Scientific Sales Solutions

The Best Solutions for Buyers, Suppliers & End-Users

S3 Research Product Show

Oct 22nd 3:00 pm to 5:00 pm

Located with the Poster Session

"The Best Solutions for Buyers, Suppliers & End-Users"
Come visit these companies' representatives to see:
What's new…
What works…
What can aid your research!

Come enjoy refreshments and snacks.

The Best Solutions Deliver the Best Results For Your Research

Please join us!

The 23rd Biocatalysis and Bioprocessing Conference

will be held

October 20-21, 2014

CBB FACULTY, RESEARCH GROUP AND STAFF

BIOCHEMISTRY

♦Brenner, Charles	4-403 BSB	5-7934
Fuentes, Ernesto	4-362 BSB	3-4244
Khademi, Shahram	4-610 BSB	5-6515
Montgomery, Rex Emeritus	4-772 BSB	5-7897
Plapp, Bryce, Emeritus	4-550 BSB	5-7909
Shea, Madeline	4-450 BSB	5-7885
Washington, M. Todd	4-516 BSB	5-7518

BIOLOGY

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

CHEMICAL & BIOCHEM ENG

Aurand, Gary	3324SC	4-0970
Carmichael, Greg	4140SC	5-5191
Murhammer, David	4132SC	5-1228
♦Nuxoll, Eric	4140SC	3-2377
♦Peeples, Tonya	4128SC	5-2251
Rethwisch, David	4139SC	5-1413
◊Subramanian, Mani	C100 MTF	5-4900
Salem, Aliasger	S228 PHAR	5-8810

CHEMISTRY

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

CIVIL&ENV ENG

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

PHARMACY

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

MICROBIOLOGY

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

RADIOLOGY

Schultz, Michael	B180 ML	5-8017

CBB STAFF

Main	C100MTF	5-4900
Main	300 MTP2	5-4457
Subramanian, Mani		Director
Gopishetty, Sridhar		Technical Director
Rotman, Mitchell		Administrator
Spidle, Kay		Secretary
McCarthy, Troy		QA Assistant
Das, Shuvendu		Research Leader
Summers, Ryan		Staff Scientist
Coeur, Melissa		Research Assistant
Dostal, Larry		Research Assistant
Ehler, Jolene		Research Associate
Garner, Mary		Research Associate
Kasperbauer, Sarah		Research Associate
Lashmit, Philip		Research Associate
Lettington, Deanna		Research Assistant
Liu, Wensheng		Research Assistant
Railsback, Michelle		Research Associate
Xu, Jingying		Research Assistant
Bay, Katlyn		Lab Assistant
Brill, Randy		Lab Assistant
Wiedow, Ellayna		Lab Assistant

◊Director, ♦Coordinating Committee
Updated 09/18/13

Notes

The University of Iowa
Center for Biocatalysis
and Bioprocessing