



Center for Biocatalysis and Bioprocessing Conference Defining the Future of Biocatalytic Science

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The University of Iowa

October 19-20, 2015 Iowa Memorial Union Iowa City, Iowa





24th Annual Biocatalysis and Bioprocessing Conference

"Defining the Future of Biocatalytic Science"

Sponsored by:



THE UNIVERSITY OF IOWA

Center for Biocatalysis and Bioprocessing

October 19-20, 2015

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"Defining the Future of Biocatalytic Science"

Sponsored by:

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Conference Organizing Committee:

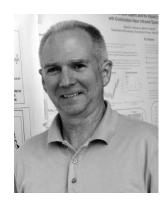
Mark Arnold, Ph.D.
Sridhar Gopishetty, Ph.D.
Bales Boles, Ph.D.
Charles Brenner, Ph.D.
Robert Kerns, Ph.D.
Amnon Kohen, Ph.D.
Timothy Mattes, Ph.D.
Eric Nuxoll, Ph.D.
Tonya Peeples, Ph.D.
Daniel Quinn, Ph.D.
Mitchell Rotman

Associate and Interim Director Mark Arnold, Ph.D.

Table of Contents	
Message from the Associate and Interim Director	 4
Conference Schedule	5
List of Oral Presentations	8
Speaker's Profiles	11
Oral Presentation Abstracts	25
List of Posters and Authors	39
Poster Abstracts	47
CBB/NIH Fellowships	90
2015 Conference Sponsors	91
2016 Conference Announcement	92
CBB Faculty, Research Group and Staff	93
Notes	

Message from the Associate and Interim Director

The Center for Biocatalysis and Bioprocessing (CBB) at the University of Iowa has been conducting an annual conference in October for the past 23 years. This year, the 24th CBB Conference is scheduled for October 19 and 20. The conference will be held in the beautiful downtown campus of The University of Iowa with world-renowned speakers, representatives and attendees from industry and academia. In addition to major presentations from experts, the conference also features oral and poster presentations from graduate students across scientific disciplines. The proceedings are conducted in a friendly, open atmosphere to promote interaction between experts, faculty from The University of Iowa, post-doctoral fellows and students. As in the past, several major industries have provided 'gifts in kind' to defray conference expenses and we thank them for their continued support.



Traditionally, the theme of the CBB Conference has been on the cutting edge technology in the areas of "Biocatalysis and Biotechnology" in the broadest sense. This year's program continues this general theme but with a twist toward defining a new term: Biocatalytic Science. As the field of biotechnology advances, we find the terms Biocatalysis and Bioprocessing to rather limited in the scope of practice within industrial processes and basic research. For this reason, I offer the term *Biocatalytic Science* to more broadly encompass research and processes that center on enzymes and their function, including enzyme discovery and mechanisms, drug discovery and biotherapeutics, enzyme-mediated chemical transformations and mechanisms, metabolic engineering, directed evolution, genomics, agricultural feedstock utilization, and protein expression, production and purification. This year's program has been designed to highlight a small fraction of the rich diversity of research ongoing within academia and industry in the area of *Biocatalytic Science*.

It is my pleasure to welcome all the attendees for this conference and also thank the sponsors. Mingling and heated – but healthy – scientific discussions are encouraged.

Sincerely, Mark Arnold, PhD

Associate and Interim Director

Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research and Economic Development, University of Iowa Research Park, Coralville, IA Professor and Edwin B. Green Chair in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA

24th Annual Center for Biocatalysis and Bioprocessing Conference "Defining the Future of Biocatalytic Science" Iowa Memorial Union, Iowa City, IA

MONDAY, OCTOBER 19, 2015

IOWA THEATER	(1 ST FLOOR)
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12:00 – 1:30 pm	Registration – Hubbard Lobby (outside Iowa Theater, 1 st floor IMU)	
1:30 – 1:45	Opening Remarks:	
	Mark Arnold, Ph.D., Associate and Interim Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research and Economic Development, University of Iowa Research Park, Coralville, IA Professor and Edwin B. Green Chair in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA	
	Dan Reed, Ph.D., Vice President for Research and Economic Development, Office of the Vice President for Research and Economic Development, University of Iowa, Iowa City, IA	
1:45 – 2:30	Jonathan Dordick, Ph.D., Howard P. Isermann Professor of Chemical and Biological Engineering, and Vice President for Research, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, Troy, NY "Molecular Bioprocessing as an Emerging Paradigm for Advancing Medicine"	
2:30 – 3:15	John Kirby, Ph.D., Professor, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA "Predator-Prey Dynamics are Regulated by Intercellular Signaling and Secondary Metabolites in Soil Bacteria"	
3:15 – 3:30	Break	
3:30 – 4:15	Mishtu Dey, Ph.D., Assistant Professor, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA "Structural Enzymology of Redox Active Metalloproteins"	
4:15 – 5:00	Timothy Mattes, Ph.D., Associate Professor, Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA "Bioremediation and Biocatalysis with Aerobic Microorganisms that Oxidize Chloroethenes"	
BALLROOM (2 ND FLOOR)		
5:00 – 6:30 pm	Social Gathering and Poster set up – Ballroom, 2 nd Floor IMU	
5:00 - 6:30	Fermentation facility tour—please register for tour online	

6:00-7:30	Welcome Dinner/Buffet – Ballroom, 2 nd Floor IMU
7:30 – 8:00	Sol Resnick Ph.D. , Director of Fermentation, CALYSTA, Inc. Menlo Park, CA "Methane-Based Biotechnology Platform for Higher-Value Products"
8:00 – 8:30	Gloria Borgstahl, Ph.D., Professor, The Eppley Institute, University of Nebraska Medical Center, Omaha, NE "How Does Superoxide Dismutase Work?"

TUESDAY, OCTOBER 20, 2015

IOWA THEATER (1ST FLOOR)

7:30 – 8:00 am	Registration – Hubbard Lobby (outside Iowa Theater, 1st floor IMU)
7:30 – 8:30	Continental Breakfast – across from Iowa Theater, 1st floor IMU
8:30 – 8:45	Program – Iowa Theater, 1 st floor IMU Introduction and Welcome Mark Arnold, Ph.D., Associate and Interim Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research and Economic Development, University of Iowa Research Park, Coralville, IA Professor and Edwin B. Green Chair in Laser Chemistry, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA
8:45 – 9:30	Todd Washington, Ph.D., Professor, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA "Assembly and Architecture of the Protein Complexes that Replicate Damaged DNA"
9:30 – 10:15	M. Ashley Spies, Ph.D., Associate Professor, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA "Promiscuity in Enzyme-Ligand Complexation Revealed at the Atomistic Level: Application to Glutamate Racemase"
10:15 – 10:30	Break – Iowa Theater Lobby, 1 st floor IMU
10:30 – 11:15	Anders Gram, Ph.D., SVP and Chief Production Officer, Pronutria Biosciences, Cambridge, MA "Process Design and Optimization in Biotech/Biopharma"
11:15 – 12:00	Tonya Peeples, PH.D. , Professor, Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA Associate Dean for Diversity and Outreach, University of Iowa, Iowa City, IA "Engineering Analysis of Adaptation in Microbial Systems for Sustainable Biocatalysis"

12:00 – 1:30 Lunch – Ballroom, 2nd Floor IMU Advisory Board-Penn State Room

Afternoon Session – Iowa Theater, 1st Floor IMU

1:30 – 2:45 **CBB/NIH Fellow Presentations**

Chaitanya Kulkarni, Ph.D. Candidate, Professor Kern's Research Group, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

"Novel N-1 Substituted Fluoroquinolones and Quinazoline-2,4-diones Designed to Target the Active Site Tyrosine of Bacterial Type-II Topoisomerases"

Kyle Powers, Ph.D. Candidate, Professor Washington's Research Group, Department of Biochemistry, Roy J. Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

"Probing Dynamics of Intrinsically Disordered Peptide Stretches in Translesion Synthesis"

Kalani Karunaratne, Ph.D. Candidate, Professor Kohen's Research Group, Department of Chemistry, College of Liberal Arts and Sciences, Iowa City, IA "Mechanistic Studies of Flavin-dependent Thymidylate Synthase"

2:45 – 3:00 Concluding Remarks

3:00 – 5:00 **Poster Session** – Wine/hors d'oeuvres, Ballroom 2nd Floor IMU Announcement of Usha Balakrishnan Award Winner

Announcement of Director's Award Winner

5:00 Adjourn



List of Oral Presentations

ORAL PRESENTATIONS

1. MOLECULAR BIOPROCESSING AS AN EMERGING PARADIGM FOR ADVANCING MEDICINE

Jonathan Dordick, Ph.D.

Howard P. Isermann Professor of Chemical and Biological Engineering, and Vice President for Research, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, Troy, NY

2. PREDATOR-PREY DYNAMICS ARE REGULATED BY INTERCELLULAR SIGNALING AND SECONDARY METABOLITES IN SOIL BACTERIA John Kirby, Ph.D.

Professor, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

3. STRUCTURAL ENZYMOLOGY OF REDOX ACTIVE METALLOPROTEINS Mishtu Dey, Ph.D.

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

4. BIOREMEDIATION AND BIOCATALYSIS WITH AEROBIC MICROORGANISMS THAT OXIDIZE CHLOROETHENES

Timothy Mattes, Ph.D.

Associate Professor, Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

5. METHANE-BASED BIOTECHNOLOGY PLATFORM FOR HIGHER-VALUE PRODUCTS

Sol Resnick Ph.D.

Director of Fermentation, CALYSTA, Inc. Menlo Park, CA

6. HOW DOES SUPEROXIDE DISMUTASE WORK

Gloria Borgstahl, Ph.D.

Professor, The Eppley Institute, University of Nebraska Medical Center, Omaha, NE

7. ASSEMBLY AND ARCHITECTURE OF THE PROTEIN COMPLEXES THAT REPLICATE DAMAGED DNA

Todd Washington, Ph.D.

Professor, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

8. PROMISCUITY IN ENZYME-LIGAND COMPLEXATION REVEALED AT THE ATOMISTIC LEVEL: APPLICATION TO GLUTAMATE RACEMASE M. Ashley Spies, Ph.D.

Associate Professor, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

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

9. PROCESS DESIGN AND OPTIMIZATION IN BIOTECH/BIOPHARMA

Anders Gram, Ph.D.

SVP and Chief Production Officer, Pronutria Biosciences, Cambridge, MA

10. ENGINEERING ANALYSIS OF ADAPTATION IN MICROBIAL SYSTEMS FOR SUSTAINABLE BIOCATALYSIS

Tonya Peeples, Ph.D.

Professor, Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Associate Dean for Diversity and Outreach, University of Iowa, Iowa City, IA

11. NOVEL N-1 SUBSTITUTED FLUOROQUINOLONES AND QUINAZOLINE-2,4-DIONES DESIGNED TO TARGET THE ACTIVE SITE TYROSINE OF BACTERIAL TYPE-II TOPOISOMERASES

Chaitanya Kulkarni

Ph.D. Candidate

Professor Kern's Research Group, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

12. PROBING DYNAMICS OF INTRINSICALLY DISORDERED PEPTIDE STRETCHES IN TRANSLESION SYNTHESIS

Kyle Powers

Ph.D. Candidate

Professor Washington's Research Group, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

13. MECHANISTIC STUDIES OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

Kalani Karunaratne

Ph.D. Candidate

Professor Kohen's Research Group, Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City



Speaker's Profiles

Jonathan Dordick, Ph.D. Howard P. Isermann Professor of Chemical and Biological Engineering, and Vice President for Research

Rensselaer Polytechnic Institute Center for Biotechnology and Interdisciplinary Studies Troy, NY



Jonathan S. Dordick is the Howard P. Isermann Professor of Chemical and Biological Engineering at Rensselaer Polytechnic Institute where he is also the Vice President for Research. Prof. Dordick received his B.A. degree in Biochemistry and Chemistry from Brandeis University and his Ph.D. in Biochemical Engineering from the Massachusetts Institute of Technology. He has held chemical engineering faculty appointments at the University of Iowa (1987-1998), where he also served as the Associate Director of the Center for Biocatalysis and Bioprocessing, and Rensselaer Polytechnic Institute (1998-present) where he also holds joint appointments in the departments of Biomedical Engineering, Materials Science and Engineering, and Biology. Prof. Dordick's research group includes chemical engineers, bioengineers, materials scientists, biologists, chemists and microbiologists all focused on gaining a quantitative understanding of biological principles and applying them to advance bioengineering and biomanufacturing, drug discovery, nanoscale hierarchical assemblies.

He has received numerous awards, including the 2007 Marvin J. Johnson Award and the 2007 Elmer Gaden Award both of the American Chemical Society, the 2003 International Enzyme Engineering Award, the 1998 Iowa Section Award of the American Chemical Society, and an NSF Presidential Young Investigator Award in 1989. He was elected as a Fellow of the National Academy of Inventors in 2015, the American Chemical Society in 2010, the American Association for the Advancement of Science in 2004, and the American Institute of Medical and Biological Engineers in 1996. He presently serves on the Scientific Advisory Boards for several biotechnology companies and venture capital firms, and has cofounded several companies, including EnzyMed (now part of Albany Molecular Research, Inc.), Solidus Biosciences, Inc. and The Paper Battery Co. (an energy storage device company). Dr. Dordick has published 335 papers and is an inventor/co-inventor on nearly 40 patents and patent applications.

John Kirby, Ph.D. Professor

Department of Microbiology Roy J. and Lucille A. Carver College of Medicine University of Iowa Iowa City, IA



John Kirby obtained his undergraduate and graduate training at the University of Illinois at Urbana-Champaign in Biochemistry. His postdoctoral training was in Molecular and Cell Biology at the University of California, Berkeley. He became an Assistant Professor of Biology at the Georgia Institute of Technology in 2002 and moved to the University of Iowa as an Associate Professor of Microbiology in 2007. Kirby's research interests are focused on microbial communities, with a particular interest in intercellular signaling and signal transduction to regulate community architecture and composition. Kirby is participating in several ongoing multi-investigator projects to ascertain the role of small molecules in microbial communities. Recently, the Kirby lab has begun to investigate a role for xenobiotics affecting the composition of microbial communities in the human gut (i.e. the microbiome). In addition, Professor Kirby participates in several interdisciplinary, educational programs on campus. He taught the Advanced Bacterial Genetics Course at Cold Spring Harbor between 2006 and 2010 and is involved in the Sloan Center for Exemplary Mentoring here at Iowa.

Mishtu Dey, Ph.D. Assistant Professor

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



Mishtu Dey is an Assistant Professor in Chemistry and Molecular & Cellular Biology Graduate Program at the University of Iowa. She started as an assistant professor at the University in 2011 after completing her postdoctoral studies at Massachusetts Institute of Technology With Professor Catherine Drennan as a Howard Hughes Medical Institute Research Specialist. She conducted her first post-doctoral training with Professor Stephen Ragsdale at the University of Nebraska-Lincoln and University of Michigan Medical School. Her research program lies at the interface of chemistry and biology and Professor Dey is investigating the molecular mechanisms of metalloenzymes important for bioenergy conversion, human health and disease, or environmentally valuable. The Dey lab applies a combination of enzymology, biochemistry, microbiology, molecular biology, and protein crystallography tools to understand biological processes at a molecular level. Professor Dey's research is highly interdisciplinary, drawing from synthetic chemistry, protein biochemistry, biophysics, and microbial bioprocessing.

Timothy Mattes, Ph.D.Associate Professor

Department of Civil and Environmental Engineering College of Engineering University of Iowa Iowa City, IA



Prof. Mattes received his Bachelor's in Civil Engineering from The Johns Hopkins University in 1994, and his Master's in Geography and Environmental Engineering from The Johns Hopkins University in 1995. After working for 3 years as a practicing engineer designing water and wastewater facilities, he returned to graduate school in 1998. In 2004, he received a PhD in Civil and Environmental Engineering from Cornell University working with Prof. James Gossett. Since 2004, Tim has been a faculty member in the department of Civil and Environmental Engineering at The University of Iowa, and is currently an Associate Professor. His research focuses on bioremediation of toxic compounds such as vinyl chloride, PCBs, and explosives. He specializes in applying molecular biology tools and techniques to track the presence and activity of microorganisms mediating biodegradation of toxic compounds in the environment.

Sol Resnick Ph.D.Director of Fermentation

CALYSTA, Inc. Menlo Park, CA



Dr. Sol Resnick is Director of Fermentation at Calysta Inc. and is responsible for microbial fermentation and bioprocess development capability using gaseous feedstock for biobased chemicals. Previously Sol was an R&D Leader in Fermentation Development for The Dow Chemical Company in San Diego, CA (2000-2009) where he was involved in capability development and led multidiscipline projects to evaluate expression strains and improve fermentation processes for production of therapeutic proteins and enzymes using a *Pseudomonas fluorescens* expression platform (Pfēnex). This differentiated platform technology was the basis for establishing Pfenex Inc. in 2009 as a separate company focused on non-glycosylated biosimilars. Sol has experience in development of microbial fermentation processes for biocatalysis, enzyme-based bioprocesses, biocatalyst improvement and microbial biooxidation reactions. He is an author on more than 40 scientific publications, book chapters, patents and pending patents. Sol received his Ph.D. in Microbiology from The University of Iowa in the lab of Prof. David T. Gibson and conducted postdoctoral research at the Swiss Federal Institute for Environmental Science & Technology (EAWAG) and Swiss Federal Institute of Technology (ETH) in Zürich, Switzerland.

Gloria Borgstahl, Ph.D. Professor

The Eppley Institute University of Nebraska Medical Center Omaha, NE



Dr. Gloria Borgstahl is from Dubuque, Iowa and graduated from Hempstead High School in 1981. She then went all the way to Iowa City for her degree in Biomedical Engineering in 1985 and Ph.D. in Biochemistry in 1992. During graduate school she learned protein X-ray crystallography with Dr. Arthur Arnone and was one of the first recipients of the NIH BioCATs doctoral fellowship. She moved to the San Diego area to learn time-resolved crystallography and other structural biology methods during a postdoctoral fellowship at The Scripps Research Institute. After a brief postdoctoral fellowship at Los Alamos National Laboratory she became an Assistant Professor of Chemistry at the University of Toledo. She developed an independent research program involving protein crystal quality, defense mechanisms against reactive oxygen species and in double-strand break repair. In 2002 she was happily recruited to Omaha, Nebraska where she continues these research programs at the Eppley Institute for Research in Cancer and Allied Diseases on the campus of the University of Nebraska Medical Center. She is also a courtesy faculty member in the Department of Biochemistry and Molecular Biology and the Department of Pharmaceutical Sciences; as well as, a member of the NCI-designated Fred & Pamela Buffet Cancer Center.

Dr. Borgstahl uses the tools of X-ray crystallography to "see" what biological macromolecules look like and to understand the molecular basis of the chemistry of life. Many avenues of research are under exploration, with an emphasis on DNA repair, cellular motility, and collaborations within the Cancer Center to directly visualize cancer-related macromolecules. For example, her research on homologous recombination-based double-stranded DNA break repair has focused on understanding the structural basis for HR and searching for therapeutics for breast, ovarian and pancreatic cancer based on the synthetic lethality of the RAD52 repair pathway with the BRCA1/BRCA2/PALB2 pathway. Specifically her research has concentrated on human Replication protein A (RPA), the ubiquitous, single-stranded DNA-binding protein and complexes formed with the DNA repair factors RAD51 and RAD52. This research has led to three different crystal structures of the full-length RPA heterodimer, composed of the RPA14 and RPA32 subunits, several biophysical publications on the RAD52:RPA complex, along with research on regulation by posttranslational modifications and ongoing screening efforts to inhibit the RAD52:RPA complex. The laboratory has also had a long-standing interest in oxidative damage, and has determined several structures to contribute to the understanding of the enzymatic mechanism of superoxide dismutases. Another project includes an innovative research program in cellular motility that will provide new tools to macromolecular crystallographers for structure determination from modulated crystals employing the four-dimensional superspace approach, and will also provide unique structural information for the actin filament. This work is supported by ACS, DHHS, NRI, NIH, NSF MCB, NSF CNIC and NASA.

Todd Washington, Ph.D. Professor

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



After receiving my Ph.D. in biochemistry from Ohio State University in 1998, I was a post-doctoral researcher at the University of Texas Medical Branch in Galveston. I joined the faculty of the Biochemistry Department at the University of Iowa in 2003. My research focuses on the structures and mechanisms of proteins involved in DNA replication, repair, and recombination.

M. Ashley Spies, Ph.D. Associate Professor

Division of Medicinal & Natural Products
Department of Pharmaceutical Sciences and Experimental
Therapeutics
College of Pharmacy
University of Iowa
Iowa City, IA

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



Michael Ashley Spies is an Associate Professor in the Division of Medicinal and Natural Products Chemistry (College of Pharmacy), and in the Department of Biochemistry (Carver College of Medicine), at the University of Iowa. He began his carrier as an independent investigator at the University of Illinois, Urbana-Champaign in 2006, before relocating to the University of Iowa in 2012. A major focus of Dr. Spies' research group is on novel approaches that combine molecular dynamics simulations with high throughput *in silico* screening, including cheminformatics and docking, in order to gain better insight into protein-ligand interactions, and provide new platforms for drug-discovery. Dr. Spies' Ph.D. thesis work was done under the supervision of Prof. Richard Schowen, at the University of Kansas, after which he performed postdoctoral work at Osaka University (Katsuyuki Tanizawa's group), where he was a JSPS fellow and at the University of California, Davis (Michael Toney's group).

Anders Gram, Ph.D.SVP and Chief Production Officer

Pronutria Bioscience Cambridge, MA



Dr. Gram joined Pronutria Biosciences in March 2015 and brings with him more than 25 years of experience in industrial biotechnology, pharmaceutical and the medical device industry. Throughout his career, Dr. Gram has focused on process and product development, operational improvements, supply chain and manufacturing in the biotech space.

Before Pronutria Biosciences, Dr. Gram was VP of Operations at Ferrosan Medical Devices where he was responsible for the full manufacturing and supply chain of the company's gelatin based advanced hemostatic products. Prior to joining Ferrosan, Dr. Gram directed operations for the Human Health and Nutrition division at Chr. Hansen. At Chr. Hansen he was in charge of streamlining and improving the production and finished goods packaging of the company's world leading portfolio of probiotic products. At Bavarian Nordic, Anders was EVP, Chief Technology Officer and during his tenure matured the manufacture of the company's live virus vaccine product, including the external supply chain from development stage to industrial output. He was also the host of the company's successful FDA preapproval inspection for the smallpox vaccine, IMVAMUNE®.

Dr. Gram's first position in industry was with Novozymes (previously Novo Industri and Novo Nordisk), where he worked for more than 20 years in process and product development, and was instrumental in improving procedures to enable rapid and reliable scale up of enzyme products. At Novozymes he spearheaded the company's move into developing and manufacturing biotech ingredients for the pharmaceutical industry. Dr. Gram received his PhD from the Danish Technical University, and completed the Advanced Management Program at Harvard Business School.

Tonya Peeples, Ph.D. Professor

Department of Chemical and Biochemical Engineering College of Engineering University of Iowa Iowa City, IA

Associate Dean for Diversity and Outreach University of Iowa Iowa City, IA



Tonya Peeples is Associate Dean for Diversity and Outreach in the UI College of Engineering and Professor of Chemical and Biochemical Engineering. Dr. Peeples researches biochemical engineering and is a member of the UI Center for Biocatalysis and Bioprocessing coordinating committee for the National Institutes of Health (NIH) training grant in biotechnology. Her group applies an understanding of biological systems to engineer stability in enzyme and cell systems in green chemical process applications. Current activities involve the characterization of adaptation of fungal and archaeal systems to extreme conditions and the elucidation of three dimensional architecture and gene expression in environmental biofilms using fluorescence and RAMAN spectroscopy. The Peeples group is adept at designing reactor systems for the cultivation of fastidious organisms. Specific areas of expertise are in the application of biological systems in interfacial catalysis for oxidative conversions in complex molecules.

Dr. Peeples obtained her B. S. in Chemical Engineering from North Carolina State University and earned her Ph.D. in Engineering from Johns Hopkins University. After post-doctoral study at the California Institute of Technology, she joined the UI as an assistant professor in 1995. In her 20 years of advancing as an academician she has served to advance diversity and to develop faculty skills in mentoring diverse students in Science Technology Engineering and Mathematics (STEM) fields. Peeples is a member of the Advisory Board for the SE Regional STEM Hub and works within that board to increase STEM access and awareness in Eastern Iowa. She has received several awards for research, teaching, and service to the local state and national STEM communities, including the Lloyd N. Ferguson Young Scientist Award for the National Association for the Professional Advancement of Black Chemists and Chemical Engineers, Outstanding Service Award from the American Institute of Chemical Engineers (AIChE) Minority Affairs Committee, the Collegiate Service Award from the UI CoE, the Michael J. Brody Award for Faculty Excellence in Service, and the UI Diversity Catalyst Award. She is currently an Executive Leadership in Academic Technology and Engineering fellow. In 2015, she is the recipient of the Diversity Pioneers Award from the AIChE. She and her husband are balancing life and work as they raise two children in Coralville, IA.

Chaitanya Kulkarni Ph.D. Candidate

Professor Kern's Research Group
Division of Medicinal and Natural Products Chemistry
Department of Pharmaceutical Sciences and Experimental
Therapeutics
College of Pharmacy
University of Iowa
Iowa City, IA



Chaitanya is originally from a small village called Lavel, 200 kilometers south of Mumbai, India. He received his Bachelor of Pharmacy from the University of Mumbai in 2011 after which, he decided to join the University of Iowa in the pursuit of graduate education. Currently he is a fifth year Ph.D. student in Dr. Robert Kerns' Group in the Division of Medicinal and Natural Products Chemistry. His graduate research focuses on design, synthesis and evaluation of two distinct sets of molecules as a) anti-infective and anti-cancer agents and b) anti-obesity and anti-diabetic agents. After obtaining his Ph.D., Chaitanya intends to do post-doctoral research before eventually pursuing a career in the pharmaceutical industry.

Kyle Powers Ph.D. Candidate

Professor Washington's Research Group Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine University of Iowa Iowa City, IA



Kyle comes from a northern suburb of Chicago named Antioch. He obtained a Bachelor of Science in Biochemistry and minor in Chemistry from the University of Illinois at Urbana-Champaign in 2012. Post-graduation he began a Ph.D. program at the University of Iowa in the Carver College of Medicine pursuing a degree in Biochemistry. There he joined the Washington research group where he has been exploring the dynamics of intrinsically disorder regions in proteins which function in the DNA damage bypass pathway known as translesion synthesis. After obtaining a Ph.D., Kyle intends to seek employment in industry.

Kalani Karunaratne Ph.D. Candidate

Professor Kohen's Research Group Department of Chemistry College of Liberal Arts and Sciences University of Iowa Iowa City, IA



I obtained my B.Sc. Special degree in Chemistry from University of Peradeniya Sri Lanka in year 2011. In 2012 I moved to Iowa and joined Kohen group in December. I joined FDTS (Flavin-dependant thymidylate synthase project) project and started working with Quench Flow instrument. I enjoy swimming and watching movies in my free time.

Oral Presentation Abstracts

MOLECULAR BIOPROCESSING AS AN EMERGING PARADIGM FOR ADVANCING MEDICINE

Jonathan Dordick, Ph.D.

Howard P. Isermann Professor of Chemical and Biological Engineering, and Vice President for Research, Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies, Troy, NY

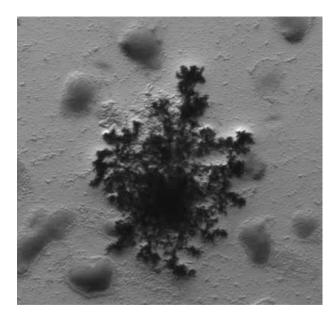
Nature is unparalleled in its structural and functional diversity. In many cases, nature has provided us with a blueprint to design and assemble both natural and synthetic building blocks to create a new generation of functional, organized, and responsive materials. We have taken cues from nature to design materials with unique structural and functional properties, along with new process technologies with the ability to produce a wide range of biomimetic structures. In this talk I will highlight our recent efforts to exploit the interface of biology with materials science to address clinical applications. In particular, we have identified and engineered cell-lytic enzymes. and generated hybrid enzyme-containing surfaces with tailored activity against hospital-acquired infections (e.g., MRSA), food-borne illnesses (e.g., Listeria), and bacillus spores. Such activity provides a safe and potentially broadly applicable route to eliminating toxic compounds and pathogenic microorganisms from common surfaces. We have also developed a genetically encoded protein-based nanoparticle-generating system for remote regulation of gene expression by low-frequency radio waves (RFs) or a magnetic field. In mice with stem cell or viral expression of these genetically encoded components, remote stimulation of insulin transgene expression with RF or a magnet lowers blood glucose. We have also demonstrated a similar RF/magnetic field platform that can inhibit hypothalamic glucose-sensing neurons to regulate metabolism and behavior in mice. This robust, repeatable method for remote regulation in vivo may ultimately have applications in basic science, technology and therapeutics.

PREDATOR-PREY DYNAMICS ARE REGULATED BY INTERCELLULAR SIGNALING AND SECONDARY METABOLITES IN SOIL BACTERIA

John Kirby, Ph.D.

Professor, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Predatory Myxococcus xanthus and endospore forming Bacillus subtilis are common soildwelling bacteria that produce a wide range of secondary metabolites, form highly organized biofilms, and sporulate under nutrient-limiting conditions. Both organisms are thought to affect the composition and dynamics of microbial communities within the soil. While laboratory strains of B. subtilis are susceptible to predation by M. xanthus, a wild strain of B. subtilis, NCIB3610, is resistant to predation by M. xanthus. We have determined that the pks gene cluster, required for production of bacillaene, is the major factor allowing B. subtilis NCIB3610 cells to resist predation by M. xanthus. In addition, M. xanthus is incapable of consuming B. subtilis spores even from laboratory strains, indicating the evolutionary fitness of sporulation as a survival strategy. Prolonged predator-prey interactions were found to induce the formation of a new type of B. subtilis biofilm, termed megastructures, which are tree-like brachiations as large as 500 μm in diameter, and raised above the agar surface between 150 and 200 µm. The megastructures are filled with viable endospores embedded within a dense matrix, and their formation was found to be genetically distinguishable from colony biofilm formation. Because B. subtilis endospores are not susceptible to predation by M. xanthus, megastructures appear to provide an alternative mechanism for long-term survival. Lastly, a transposon mutant library was used to screen for defects in predation and revealed that M. xanthus produces the secondary metabolite, myxoprincomide, to facilitate predation of B. subtilis NCIB3610 cells. Thus, production of secondary metabolites regulates complex interactions within this predator-prey system, which ultimately culminates in complex biofilm formation to facilitate escape into dormancy via sporulation.



STRUCTURAL ENZYMOLOGY OF REDOX ACTIVE METALLOPROTEINS

Mishtu Dev. Ph.D.

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Metalloproteins play an important role in biology; from global cycling of carbon, nitrogen, sulfur to biosynthesis of antibiotics, natural products, vitamins, collagen. Redox active transition metal ions perform challenging chemistry at the metal-protein interface in the generation of radical and carbon intermediates. While mononuclear iron containing dimethyl sulfoniopropionate (DMSP) lyases from marine microbes are responsible for generating a biogenic volatile sulfur gas (dimethyl sulfide) and by-products (acrylate and 3-hydroxypropoinate) of immense industrial values, a unique mononuclear iron enzyme utilizes dioxygen for catalyzing the rate-limiting step in collagen biosynthesis. Two different catalytic mechanisms have been proposed for DMSP lysis: one includes a metal-tyrosyl intermediate and tyrosine initiated catalysis, while the other involves water or histidine acting as the base. Using structural enzymology and site-directed mutagenesis, metal-tyrosyl intermediate and the catalytic residues have been identified. The Xray crystal structure of the metal-tyrosyl intermediate has been determined to 1.9 Å resolution. A prolyl 4-hydroxylase from Bacillus anthracis (BaP4H) is an unusual enzyme in that it was believed to target collagen-like peptidyl prolyl for hydroxylation rather than free L-proline like other bacterial P4Hs. We examined cofactor and substrate binding affinities, specificity, and hydroxylation activity of BaP4H employing biochemical, mass-spectrometry, and X-ray crystallographic methods. Here we present the first biochemical and structural analysis of the cofactor-bound forms of BaP4H and identified collagen-derived peptides as targets, with hydroxylation localized towards the C-terminus end. The crystal structures identifying metal and cofactor binding sites illustrate conformational changes required for substrate peptide access to the active site. Thus, the present work provides important insights into the structure and dynamics of metalloenzymes. A molecular picture of enzyme structural dynamics and their relationship to function can serve as a basis for rational approaches to drug design and industrial biocatalysis.

BIOREMEDIATION AND BIOCATALYSIS WITH AEROBIC MICROORGANISMS THAT OXIDIZE CHLOROETHENES

Timothy Mattes, Ph.D.

Associate Professor, Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

Developments in the field of environmental biotechnology are primarily aimed at protecting ecological and human health via sustainable clean-up and pollution prevention of our air, water, and soil resources. Bacteria in particular are powerful agents of environmental remediation and pollution prevention. For instance, in situ bioremediation of hazardous pollutants in groundwater is often the most cost-effective means of achieving clean-up goals.

This presentation will focus on aerobic bacteria that are capable of degrading chloroethenes, particularly vinyl chloride (VC), in groundwater environments. VC is a known human carcinogen and common groundwater pollutant. VC can enter groundwater directly via inadvertent spills of VC monomer, but is more commonly generated by anaerobic transformation of chloroethenes and chloroethanes. Once the VC migrates into a region where oxygen is present, certain aerobic bacteria can fortuitously oxidize (i.e. co-metabolize) VC in the presence of oxygen and a growth supporting compound (e.g. methane). A potentially important group of VC-oxidizers is the ethene-oxidizing bacteria (also called "etheneotrophs"). These obligate aerobes can co-metabolize VC in the presence of ethene, and under the appropriate conditions can also utilize VC as a sole source of carbon and energy.

Functional genes involved in VC biodegradation by etheneotrophs include *etnC*, which encodes the alkene monooxygenase (AkMO) alpha subunit. AKMO initiates the initial enzymatic attack on VC generating an epoxide intermediate. The second enzyme in the pathway is epoxyalkane:coenzyme M transferase, encoded by *etnE*, which further metabolizes the epoxide. In recent efforts, we have developed a quantitative, real-time PCR (qPCR) method that targets these key functional genes and their transcripts. We are currently applying these methods to study the abundance and activity of VC-oxidizing bacteria at contaminated sites. We have also employed metagenomics, qPCR, and stable isotope probing to study the transition of VC cometabolizers to growth-coupled VC-oxidizers in an aerobic mixed culture derived from a contaminated site in Alaska. Continued research in both the laboratory and in the field will shed new light on the ecology and evolution VC-oxidizing etheneotrophs in contaminated groundwater environments.

Interestingly, bacterial enzymes such as AkMO employed in the breakdown of toxic compounds may also be useful in biocatalytic chemical production strategies. Our work exploring the use of AkMO as an environmentally beneficial approach to chiral epoxide production will also be summarized.

METHANE-BASED BIOTECHNOLOGY PLATFORM FOR HIGHER-VALUE RODUCTS

Sol Resnick Ph.D.

Director of Fermentation, CALYSTA, Inc., Menlo Park, CA

Methane is the main component of both natural gas and biogas. The recent rise in domestic production of methane has driven the cost of natural gas to record lows. Calysta, Inc. has developed a platform for host organisms (methanotrophs) capable of metabolizing this abundant domestic feedstock to a variety of products including higher value biochemicals. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if 'green' methane-based technologies are developed.

Calysta's proprietary biological Gas-to-Chemicals (Bio-GTC) platform utilizes genetically engineered methanotrophs - prokaryotes that utilize methane as their sole source of carbon and energy. Methanotrophs have been observed in a wide range of environments, both aerobic and anaerobic, typically in association with natural methane sources such as degrading biomass or petroleum off-gas. While methanotrophs are a logical starting point for the development of a biological methane conversion platform, a critical requirement for the development of a biotechnology platform is the availability of tools for the directed manipulation and modification of the host cell's metabolism. Although such tools are commonplace for model organisms (e.g., *E. coli* or *S. cerevisiae*), relatively little effort has been expended to develop similar capabilities in methanotrophs. Calysta has therefore developed a suite of tools for the expression of heterologous proteins in methanotrophs, as well as tools for the efficient targeted manipulation of the methanotroph genome. The genetic tools, together with innovative fermentation and bioprocess approaches, enable the rapid implementation of well-characterized pathways to utilize natural gas as a biological feedstock instead of sugar. This presentation will focus on the development of the platform, the tools and their application to target products

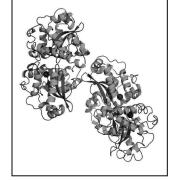
HOW DOES SUPEROXIDE DISMUTASE WORK

Gloria Borgstahl, Ph.D.

Professor, The Eppley Institute, University of Nebraska Medical Center, Omaha, NE

Superoxide dismutases (SODs) are important antioxidant enzymes that protect all living cells

against toxic oxygen metabolites, also known as reactive oxygen species (ROS). SODs are one of the fastest known enzymes with a $k_{\text{cat}}/K_{\text{m}}$ of $10^9~M^{-1}\text{s}^{-1}$ and are rate-limited only by the diffusion of the substrate and products. SODs are the first line of defense to protect organisms against metabolically generated and/or ionizing radiation-induced ROS. SOD protects cells by dismuting two molecules of superoxide anions to form hydrogen peroxide and molecular oxygen via a cyclic oxidation-reduction reaction. SODs contain metal ions in their active sites. Humans have Cu/ZnSOD in the cytosol and extracellular spaces and



MnSOD in their mitochondria. Mutations in SOD lead to aging and degenerative diseases such as *amyotrophic lateral sclerosis* (ALS), diabetes, and cancer. This brief talk will discuss (1) how SODs work with in the body to protect cells from ROS, (2) what is known of the enzymatic mechanism, and (3) our future efforts to understand the enzyme using large volume crystal growth on the international space station for complete structure determination by neutron macromolecular crystallography.

ASSEMBLY AND ARCHITECTURE OF THE PROTEIN COMPLEXES THAT REPLICATE DAMAGED DNA

Todd Washington, Ph.D.

Professor, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Dynamic, multi-protein complexes carry out many fundamental biological processes, such as signal transduction and cell cycle regulation. Understanding the high-resolution structures of these complexes is one of the major challenges facing molecular and cellular biologists. Our laboratory is studying the protein complexes responsible for the replication of damaged DNA. This is important because proper replication of damaged DNA is essential for maintaining genome stability and minimizing the occurrence of cancer. We are using a combination of approaches including X-ray crystallography, small-angle X-ray scattering, single-molecule fluorescence microscopy, and Brownian dynamics simulations to study the structure and dynamics of these multi-protein complexes.

PROMISCUITY IN ENZYME-LIGAND COMPLEXATION REVEALED AT THE ATOMISTIC LEVEL: APPLICATION TO GLUTAMATE RACEMASE

M. Ashley Spies, Ph.D.

Associate Professor, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

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

Glutamate racemase (GR) catalyzes the cofactor independent stereoinversion of L- to Dglutamate for biosynthesis of bacterial cell walls. Due to its essential nature, this enzyme is under intense scrutiny as a drug target for the design of novel antimicrobial agents. However, the flexibility of the enzyme has made inhibitor design challenging. Previous MD simulations, docking, and experimental studies have suggested that the enzyme is forming highly varied complexes with different competitive inhibitor scaffolds. The current study focuses on addressing the vexing questions surrounding how to obtain meaningful structural and dynamic information about an enzyme drug-target, which is so flexible that it radically changes shape and binding kinetics when binding to distinct competitive inhibitor scaffolds. A wide range of integrated experimental biophysical and computational approaches have been employed in order to provide a quantifiable and atomistic model for explaining these strange idiosyncratic ligandassociated phenomena in glutamate racemases. The experimental studies include the biosynthesis of an active and novel glutamate racemase, $GR^{Y53/7HC}$, containing a site specific 7hydroxycoumarin (7HC) amino acid incorporated into a region (remote from the active site) identified by molecular dynamics simulations to be associated with ligand-dependent allostery. The 7HC moiety has a very large quantum yield, making it an exquisitely sensitive probe for studying ligand-associated allostery. Ligand binding studies to GR^{Y53/7HČ} indicate radically different signatures for different competitive inhibitor scaffolds. Additional biophysical studies, including thermal melt (TM) and surface plasmon resonance (SPR), further confirm the radically different allosteric consequences of different small molecule scaffolds binding to the active site of GR. SPR studies on GR have revealed large and significant differences in the association and dissociation rates between various small molecule scaffolds. The grand challenge to rational structure based drug design efforts is to produce predictive and quantitative models that accurately report on these phenomena. The use of steered molecular dynamics (SMD) simulations in order to estimate an unbinding trajectory for each ligand, as well as the average work needed to unbind from the enzyme active site provides deep insight into the physical determinants that lead to large differences in off rates between ligands. The advantages and challenges to the application of SMD-based approaches to addressing the GR-ligand binding phenomena are discussed.

PROCESS DESIGN AND OPTIMIZATION IN BIOTECH/BIOPHARMA

<u>Anders Gram, Ph.D.</u> SVP and Chief Production Officer, Pronutria Biosciences, Cambridge, MA

Having worked for almost 30 years in Biotech and Biopharma process and product development, the presenter will share his insights in process development and optimization from industrial enzyme to live-virus vaccine manufacturing.

The vantage point is the biotech industry – producers of hundreds of metric tons of enzymes, millions of doses of vaccines, more than 10^{24} probiotic cells pr. year. How do we develop production strains, processes and products that are economically sound, safe and efficacious for the consumer? What is the science base and what is just plain common sense?

Examples:

Application of Genetic techniques in optimizing host strain and process design concurrently.

Engineering, Bioscience and Analytics – the value of a common platform.

Raw materials for large scale fermentation – key considerations and learning points.

Process development/CMC in Biopharma, comparison to Biotech and a couple of practical

Regulatory and customer understanding – why is this at all an issue for the biochemist, the analyst and the process engineer?

ENGINEERING ANALYSIS OF ADAPTATION IN MICROBIAL SYSTEMS FOR SUSTAINABLE BIOCATALYSIS

Tonya Peeples, Ph.D.

Professor, Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

Associate Dean for Diversity and Outreach, University of Iowa, Iowa City, IA

Understanding how organisms respond to environmental changes can enable researchers and industrial biotechnologists to develop more robust efficient, sustainable bio-based conversions as economically viable alternatives to traditional catalysis. Gaining and understanding of adaptation and stability of natural systems that thrive under extreme conditions guides our development of biocatalysts for specialty chemicals and pharmaceutical synthesis and aids in the understanding of natural processes for environmental remediation. Current activities involve the characterization of adaptation of fungal, bacterial, and archaeal systems. Combining bioreactor design and analysis for the cultivation of fastidious organisms with parametric analysis of bioconversions, we are able to enhance yields and rates in oxidative reactions. This includes the conversion of steroids and oxidation of sulfur-containing substrates. We are also applying this approach to expand our understanding of biofilm systems that may be significant in atrazine degradation. Key methods include Raman spectroscopy, SEM imaging, and traditional reaction engineering analysis. Efforts to elucidate reaction environment changes, which leverage cellular adaptations of *Pseudomonas* sp. ADP and *Beauveria bassiana* and lead to improved biocatalysis, will be presented.

NOVEL N-1 SUBSTITUTED FLUOROQUINOLONES AND QUINAZOLINE-2,4-DIONES DESIGNED TO TARGET THE ACTIVE SITE TYROSINE OF BACTERIAL TYPE-II TOPOISOMERASES

Chaitanya Kulkarni

Ph.D. Candidate

Professor Kern's Research Group, Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

Fluoroguinolones are broad spectrum antibiotics that are widely used to treat bacterial infections. These antibiotics target bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) and inhibit these enzymes by forming a 'cleaved complex' (also called ternary complex) consisting of the drug, nicked DNA, and the enzyme. In cleaved complex, the C-3, C-4 dicarbonyl moiety of fluoroquinolones is complexed with a magnesium ion and a critical binding contact is formed with serine and aspartate/glutamate residues through a magnesium-water bridge. Mutation of either of these residues prevents bridge formation, thereby decreasing the binding of fluoroguinolones in the cleaved complex and leads to resistance. In recent years there has been a rise in the number of bacterial strains that are resistant to fluoroquinolones, thereby emphasizing the need to develop new molecules active against these resistant mutants. One way to do so is by forming new drug-topoisomerase binding contacts in ternary complex. Evaluation of crystal structures of ternary complexes for clinically established fluoroguinolones, like moxifloxacin, showed that modifications to the N-1 position on the fluoroquinolone scaffold might provide analogues capable of forming non-conventional hydrophobic, ionic or pi-stacking interactions in the cleaved complex. In particular, it was envisioned that select analogues could be designed to target a non-mutable, active-site tyrosine of the enzyme making it difficult to develop resistance against these analogues. Quinazoline-2,4-diones are structurally analogous to the fluoroguinolones. The N-3 amine and C-2 carbonyl groups of the quinazolinediones form a binding contact with a conserved arginine, orienting the diones in a position similar to that of fluoroguinolones. However, unlike fluoroguinolones, the guinazoline-2,4-diones do not form a magnesium water bridge, and therefore these molecules are active with fluoroquinolone resistant DNA gyrase and/or topoisomerase IV. The quinazoline-2,4-diones, however, have limited potency against wild type enzyme and thus a novel binding contact to another, non-mutable amino acid would greatly benefit this class of compounds as well. The goal of identifying novel binding contacts between fluoroquinolones and type-II topoisomerases is to eliminate the dependence on the magnesium-water bridge, thereby enhancing activity against current fluoroquinolone resistant mutants. This talk outlines the design and synthesis of N-1 modified fluoroguinolones and quinazoline-2,4-diones and their in vitro evaluation against purified enzymes.

PROBING DYNAMICS OF INTRINSICALLY DISORDERED PEPTIDE STRETCHES IN TRANSLESION SYNTHESIS

Kyle Powers

Ph.D. Candidate

Professor Washington's Research Group, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Regulation of several cellular pathways including cell cycle control, signaling, transcription, DNA replication, and DNA repair rely upon dynamic protein complexes which often contain intrinsically disordered regions. Obtaining high resolution structural information of such complexes and their disordered regions is one of the greatest difficulties in structural biology. To overcome this challenge, we are using a combination of Brownian dynamic (**BD**) simulations and single molecule Förster resonance energy transfer (sm-FRET). Our studies focus on the protein complexes formed in translesion synthesis (TLS), the replicative bypass of damaged forms of DNA. Particular focus is directed on polymerase eta (η) , specifically its intrinsically disordered c-terminal region (CTR) which has no determined secondary structure. This region has been implicated in recruitment to proliferating cell nuclear antigen (PCNA) which plays the role of the central hub in several DNA processing pathways. Simulations of polymerase n alone and in complex with PCNA are being used to predict the behaviors of the intrinsically disordered CTR. These models then are being compared to sm-FRET measurements to experimentally validate and improve their accuracy. This combination of in *silico* and in *vitro* approaches developed here can be applied to other biologically important systems with intrinsic disorder including other DNA metabolic pathways.

MECHANISTIC STUDIES OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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Ph.D. Candidate

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The Biosynthesis of thymidylate (dTMP, one of the four building blocks of DNA) is different than that of other deoxyribonucleotides, because in addition to the reduction by ribonucleotide reductase, a methylation of dU to dT is required. In most eukaryotes, including humans, the enzyme thymidylate synthase (TSase) encoded by *thy*A gene catalyzes the reductive methylation of dUMP (2'-deoxyuridine 5'-monophosphate) to form dTMP^{1,2}. However, not all organisms have *thy*A in their genome, and a new class of thymidylate synthases was discovered that is encoded by the *thy*X gene³. This new class of TSases makes use of a noncovalently bound flavin adenine dinucleotide (FAD), hence named as flavin-dependent thymidylate synthases (FDTSs). Several prokaryotes including disease causing bacteria such as *Rickettsia prowazekii* (typhuscausing), *Mycobacterium tuberculosis*, and *Bacillus anthracis* rely on FDTS³. Classical TSase and FDTS are substantially different in structure⁴ and chemical mechanism^{5,6,7} and share no common inhibitors. Hence,

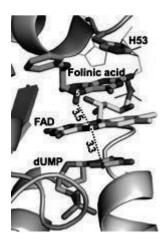


Figure 1- Active site of TmFDTS⁴

understanding the FDTS mechanism may allow the development of mechanism based antibiotics with minimal toxicity to humans. Two related lines of investigation will be presented: 1) the cofactor FAD was replaced with the analogue 5-deaza-FAD, where the N5 of FAD is replaced with a carbon atom. This will help establish several aspects of the mechanism, including the stereochemistry of both the oxidative and reductive half reactions, the role of N5 of the flavin as a methylene carrier, and more; and 2) several reaction intermediate were trapped using quench-flow technique, and identified via MS and NMR analysis. Those intermediates and their time course along the oxidative half reaction are key to the elucidation of the complex reaction mechanism catalyzed by FDTS.

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List of Posters and Authors

Posters

1. NOVEL QUINAZOLINE-2,4-DIONE DERIVATIVES: NEW BINDING CONTACTS IN TERNARY COMPLEX WITH DNA AND BACTERIAL TYPE II TOPOISOMERASES

Arturo L. Aguirre and Robert J. Kerns*

Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

2. PRODUCTION OF 3-METHYLXANTHINES BY METABOLICALLY ENGINEERED E. COLI

<u>Khalid Al-Gharrawi</u>^{1, 2}, Ryan M. Summers³, Sridhar Gopishetty⁴, and Mani Subramanian^{1*}

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³Department of Chemical and Biological Engineering, University of Alabama, Tuscaloosa, AL

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3. INVESTIGATION OF GENE TRANSFECTION EFFICIENCY OF PEGYLATED POLYLYSINE PEPTIDE SCAVENGER RECEPTOR INHIBITORS

Rondine Allen and Kevin G. Rice*

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4. EXPRESSION OF HYDRAZINE SYNTHASE BY ANAEROBIC AMMONIUM OXIDIZING (ANAMMOX) BACTERIA IN SUSPENDED BIOMASS AND BIOFILM AT COLD TEMPERATURES

Ellen Black and Craig Just*

Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

5. A HIGH THROUGHPUT CAMPAIGN INTERROGATING RGS6 AND RGS17: IDENTIFICATION OF NOVEL, UNIQUE LEAD COMPOUNDS FOR RGS INHIBITION AND THERAPEUTIC DEVELOPMENT

Christopher Bodle¹, Rory Fisher ² and David Roman ^{1*}

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6. ANALYSIS OF CORNEA HEALTH USING NEAR-INFRARED SPECTROSCOPY

<u>Ariel O. Bohman</u>¹, Natalie R. Slach¹, Mark A. Arnold, PhD^{1*}, Benjamin T. Aldrich, PhD^{2,3}, Kimberlee A. Burckart, PhD³, Gregory A. Schmidt, BS, CEBT³, Cynthia R. Reed, RN, PhD³, and Mark A. Greiner, MD^{2,3,5}

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7. UNDERSTANDING THE MECHANISM OF FLAVIN DEPENDENT THYMIDYLATE SYNTHASE

Michael Choi^{1,2}, Kalani Karunaratne¹ and Amnon Kohen¹*

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

²Medical Scientist Training Program, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

8. DETERMINING RATES OF ATRAZINE DEGRADATION IN *PSEUDOMONAS* SP. ADP BIOFILMS AND PLANKTONIC CELLS BY CHEMICAL ANALYSIS

Michael A. Delcau and Tonya L. Peeples*

Department of Chemical and Biochemical Engineering, College of Engineering, University of Iowa, Iowa City, IA

9. DESIGN, SYNTHESIS, AND EVALUATION OF NOVEL FLUOROQUINOLONE N-1 ARYL SUBSTITUENTS ON BACTERIAL TYPE-II TOPOISOMERASE INHIBITION AND DNA BINDING

<u>Justine Delgado</u>, Chaitanya Kulkarni, Tyrell R. Towle, and Robert J. Kerns* Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

10. 3D PRINTED ALGINATE-PLGA TUBES FOR CONTROLLED SEQUENTIAL RUG RELEASE

Anh-Vu Do¹, Adil Akkouch², Brian Green¹, Ibrahim Ozbolat², and Aliasger K. Salem¹*

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University of Iowa, Iowa City, IA

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11. [2+2] PHOTOCYCLOADDITION OF A COMMON ANTI-CANCER DRUG IN THE SOLID STATE

Andrew J. E. Duncan, Roxanne L. Dudovitz, Shawna J. Dudovitz, Jelena Stojaković, and Leonard R. MacGillivray*

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

12. SYNTHESIS OF ISOPRENOID BISPHOSPHONATES AS INHIBITORS OF GERANYL GERANYL DIPHOSPHATE SYNTHASE

Benjamin J. Foust and David F. Wiemer*

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

13. TRICLOSAN INDUCED BIOFILM FORMATION IN OPPORTUNISTIC PATHOGENS

Sudeshna Ghosh¹, Adnan Syed², Nancy G. Love³, and Blaise R. Boles¹*

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²Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI

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14. EFFECTS OF THE DONOR-ACCEPTOR DISTANCE AND DYNAMICS IN FORMATE DEHYDROGENASE CATALYZED REACTIONS

Qi Guo, Philip Pagano, Hepeng Ye, Christopher Cheatum, and Amnon Kohen* Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA

15. MUTATIONAL STUDY OF AN ACTIVE SITE ASPARAGINE IN THYMIDYLATE SYNTHASE TO GAIN INSIGHT INTO A NEWLY PROPOSED REACTION INTERMEDIATE

<u>Ilya Gurevic</u>, Zahidul Islam, Tasnia Iqbal, Ananda Ghosh, and Amnon Kohen* Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, IA

16. PROTON AND HYDRIDE TRANSFER MECHANISMS IN THE THYMIDYLATE SYNTHASE CATALYZED REACTION

Zahidul Islam, Timothy S. Strutzenberg, Ananda K. Ghosh, and Amnon Kohen* Department of Chemistry, College of Liberal Arts and Sciences, University of Iowa. Iowa City, IA

17. SYSTEM-SPECIFIC RESPONSE OF POLYSTYRENE PARTICLES IN THE BODY

Benjamin King and Jennifer Fiegel*

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18. NOVEL *N*-1 SUBSTITUTED FLUOROQUINOLONES AND QUINAZOLINE-2,4-DIONES DESIGNED TO TARGET THE ACTIVE SITE TYROSINE OF BACTERIAL TYPE-II TOPOISOMERASES

Chaitanya A. Kulkarni, Tyrell R. Towle and Robert J. Kerns*

Division of Medicinal and Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA

19. ESTABLISHING THE ROLE OF GLOBAL NETWORKS IN HUMAN DIHYDROFOLATE REDUCTASE THROUGH EXAMINATION OF INTRINSIC ISOTOPE EFFECTS

Jiayue Li and Amnon Kohen*

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

20. ABUNDANCE AND ACTIVITY OF AEROBIC VINYL CHLORIDE DEGRADERS IN CONTAMINATED GROUNDWATER

Yi Liang and Timothy E. Mattes*

Department of Civil and Environmental Engineering, College of Engineering, University of Iowa, Iowa City, IA

21. PROTEIN SEQUENCE OPTIMIZATION WITH A POLARIZABLE FORCE FIELD

<u>Jacob M. Litman</u>¹, Young Joo Sun¹, Titus Hou¹, Stephen D. LuCore², Thomas Simonson³, Ernesto Fuentes¹, and Michael J. Schnieders^{1,2}*

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22. MICROBIAL ADAPTATION TO VINYL CHLORIDE IN GROUNDWATER MICROCOSMS AS REVEALED BY METAGENOMICS

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23. MODELING THE FORMATE DEHYDROGENASE TERNARY COMPLEX: A MOLECULAR DYNAMICS APPROACH TO 2DIR SPECTROSCOPY

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24. VALIDATION OF A PROTEIN FORCE FIELD WITH OSMOTIC PRESSURE MEASUREMENTS OF ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS Mark S. Miller and Adrian H. Elcock*

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25. PHOTO-CROSSLINKABLE UNNATURAL AMINO ACID WITH "CLICK" FUNCTIONALITY FOR PROTEIN INTERACTION STUDIES

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26. SYNTHESIS OF SMALL-MOLECULES AS MECHANISTIC PROBE OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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27. [2+2] PHOTOCYCLOADDITIONS DIRECTED BY 2-BUTYNE-1,4-DIOL

Shalisa M. Oburn and Leonard R. MacGillivray*

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28. STEROID HORMONE SULFATION CATALYZED BY HUMAN SULFOTRANSFERASES IS INHIBITED BY METABOLITES OF COMMONLY OCCURRING AIRBORNE POLYCHLORINATED BIPHENYLS

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29. DEVELOPMENT OF STABILIZED mRNA NANOPARTICLES FOR IN VIVO GENE DELIVERY

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30. PROBING THE DYNAMICS OF INTRINSICALLY DISORDERED PEPTIDE STRETCHES

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31. MASS MODULATED EFFECTS ON FUNCTIONAL DYNAMICS OF FORMATE DEHYDROGENASE

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32. THERMAL MITIGATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS

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33. RHIZOBIUM SP. PRODUCES NOVEL METABOLITES OF 2,4-DINITROANISOLE IDENTIFIED USING STABLE ISOTOPES

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34. ARGENTOPHILIC AND PERFLUOROPHENYL-PERFLUOROPHENYL INTERACTIONS SUPPORT A HEAD-TO-HEAD [2+2] PHOTODIMERIZATION IN THE SOLID STATE

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- ²Department of Chemical and Biomolecular Engineering, Lehigh University, Bethlehem, PA
- 35. PHOTOCATALYTIC ADDITION OF A-BROMOPHOSPHONOACETATE ESTERS INTO INDOLE RING SYSTEMS TO ACCESS POTENTIAL THERAPEUTIC TARGETS AS WELL AS SYNTHONS FOR HORNER-WADSWORTH-EMMONS OLEFINATION

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36. BIOPHYSICAL STUDIES OF THE S. AUREUS SRRB-SRRA TWO-COMPONENT SYSTEM INVOLVED IN TOXIC SHOCK SYNDROME

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- 37. NEURONAL TOXICITY DUE TO REACTIVE DOPAMINE METABOLITES AND ALDEHYDE DEHYDROGENASE INHIBITION

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38. HEPATOCYTE TARGETED ENDOSOMAL ESCAPE AGENTS

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39. SULFONIUM SPECIES AS REVERSIBLE INHIBITORS OF HUMAN ACETYLCHOLINESTERASE

Sumana N. Yasapala, Joseph J. Topczewski, Sarah J. Gross, Alexander M. Lodge, Jason A. Morrill, and Daniel M. Quin*

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40. RAPID IDENTIFICATION AND PRODUCTION OF METABOLITES USING NOVEL STABILIZED DRIED POWDER (SDP) OF HUMAN CYTOCHROME P450S (CYPS) ENGINEERED IN YEAST

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41. EXTENTDING CAFFEINE DRHYDROGENASE-BASED RAPID CAFFEINE DETECTION TECHNOLOGY TO HEALTH-CARE APPLICATIONS

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42. COORDINATED EFECT OF DISTAL MUTATIONS ON ACTIVE SITE IN DIHYDROFOLATE REDUCTASE

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Poster Abstracts

NOVEL QUINAZOLINE-2,4-DIONE DERIVATIVES: NEW BINDING CONTACTS IN TERNARY COMPLEX WITH DNA AND BACTERIAL TYPE II TOPOISOMERASES

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Fluoroquinolones are a class of antibiotics used to treat a wide variety of bacterial infections. These small molecules target bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) through the formation of a ternary complex, which is comprised of the fluoroquinolone, nicked DNA and the enzyme (DNA gyrase or topoisomerase IV), and prevents religation of the nicked DNA. The primary mechanism of resistance to fluoroquinolones lies in its mode of binding to the topoisomerase enzymes, where the keto acid moiety of the fluoroquinolone is complexed with a magnesium ion and a magnesium-water bridge is formed with a serine and an aspartate, or glutamate residue, on helix-4 of the enzyme. Mutation-mediated resistance arises through substitution of the serine or glutamate/aspartate residues, thus preventing formation of the magnesium-water bridge. Quinazolinediones are fluoroquinolone-like structures that lack the keto acid moiety, thus forming ternary complex in a similar way and maintaining equipotent activity with wild-type and fluoroquinolone-resistant type-II topoisomerases, which contain helix-4 mutations that prevent formation of the magnesium-water bridge. These findings indicate that the quinazolinedione skeleton can be used to facilitate drug-enzyme binding in a more direct manner, rather than its fluoroquinolone counterpart. The C2 carbonyl of a quinazolinedione is believed to form a hydrogen bond with a conserved arginine residue in GyrA; however, this binding interaction is weaker than the magnesium-water bridge, and additional binding contacts are needed to increase quinazolinedione binding contact, in ternary complex, to increase overall potency. In this presentation the design of novel quinazoline-2,4-dione derivatives expected to have additional binding contact in ternary complex will be discussed, and progress toward the synthesis of a panel of these agents is presented.

PRODUCTION OF 3-METHYLXANTHINES BY METABOLICALLY ENGINEERED E. COLI

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Methylxanthines are naturally occurring purine alkaloids that are used in pharmaceutical preparations as diuretics, for asthma treatment, and as cardiac, pulmonary, and neurological stimulants. By using *E. coli*, metabolically engineered with N-demethylase genes as a biocatalyst, we were able to produce 100 mg of pure 3-methylxanthine by N-demethylation of theophylline at 30 °C and ambient atmospheric pressure. The conversion of theophylline was 100%, 80% of which was to 3-methylxanthine and 20% 1-methylxanthine. It was previously reported that Pseudomonas putida CBB5 degrades caffeine via sequential N-demethylation to theobromine or paraxanthine, then to 7-methylxanthine, and further to xanthine. CBB5 contains five novel N-demethylase genes, ndmA, ndmB, ndmC, ndmD, and ndmE, which are responsible for caffeine degradation. Three genes, ndmA, B and D have been cloned, and expressed in *E. coli*. N-demethylase A (NdmA) and N-demethylase B (NdmB) are Rieske monooxygenases that catalyze position-specific N₁- and N₃- demethylations, respectively in the presence of NdmD. Both enzymes receive reducing equivalents from NADH via a redoxcenter-dense Rieske reductase, NdmD

Three *E. coli* strains were metabolically engineered using different combinations of ndmA, B and D genes. Strain pDdA which has one copy of each of NdmA and NdmD genes was grown overnight in 4 L super broth to produce 20 g wet cell paste. That cell paste was washed with potassium phosphate buffer pH 7.0 and used as a biocatalyst in 1.3 L reaction volume that contained 1 mM theophylline. The temperature and shaker speed used were 30 °C and 250 rpm respectively. Theophylline completely disappeared after two hours with 80% conversion to 3-methylxanthine as the major product. 3-methylxanthine was separated from post-reaction mixture by preparative chromatography by using BDS Hypersil C18 column (250 x 21.2 mm) as the stationary phase and 5% methanol as the mobile phase. The optimized solvent flow rate and the amount of post reaction mixture injected were found to be 2.5 mL/min and 25 mL respectively. 3-methylxanthine solution was concentrated by evaporation under vacuum at ~70 °C and the resulting concentrated solution was freeze-dried. The purified product, 100 mg 3-methylxanthine was confirmed by LC-MS and NMR with respect to authenticity and purity.

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INVESTIGATION OF GENE TRANSFECTION EFFICIENCY OF PEGYLATED POLYLYSINE PEPTIDE SCAVENGER RECEPTOR INHIBITORS

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Scavenger receptors (SR) found on Kupffer and fenestrated endothelial cells of the recticuloendothelial system (RES) in liver are responsible for the capture and degradation of both viral¹ and non viral gene delivery systems². SR inhibition using polyinosinic acid (Poly-I) has been shown to improve the gene transfer efficiency of AdV, AAV, and measles virus by blocking rapid metabolism in the liver. While Poly-I is reportedly not toxic to mice, coadministration of Poly-I with AdV decreases the lethal threshold for AdV in mice by an unknown mechanism¹, making Poly-I inhibition of SR a clinically unacceptable approach to inhibit viral uptake by the RES in the liver. We have recently reported a new class of SR inhibitors PEGylated(30kDa)-Cys-Trp-Lys(N) that block liver uptake and metabolism of DNA for 1 hour in circulation. These PEGylated polylysine-peptides are proposed to function by in situ formation of 30 nm albumin nanoparticles in the blood that bind to SRs. In the present study, we investigate the ability of these peptides where N = 10, 15, 20, 25, and 30 to improve gene transfection efficiency of PEGylated (5kDa) polyacridine peptides which bind to DNA with high affinity. In order to assay gene expression delayed hydrodynamic stimulation is applied. Without SR inhibition, administration of a 1 nmol of PEG- polyacridine peptide with 1 µg of ¹²⁵I-pGL3 remains active in circulation for up to 4 hours. However, preliminary results show that coadministration of a saturating dose of PEG-peptide improves the transfection efficiency for up to 9 hours in circulation.

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EXPRESSION OF HYDRAZINE SYNTHASE BY ANAEROBIC AMMONIUM OXIDIZING (ANAMMOX) BACTERIA IN SUSPENDED BIOMASS AND BIOFILM AT COLD TEMPERATURES

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Anaerobic ammonium oxidizing bacteria (anammox) remove nitrogen from wastewater by oxidizing ammonium (NH₄⁺) to produce nitrogen gas and are commonly used in sidestream treatment of high strength NH₄⁺ water at thermophilic conditions. However, the effectiveness of anammox-nitrogen removal is limited by their slow growth, especially at temperatures less than 35°C. A functional biomarker, hydrazine synthase (*hzs*A), was used to quantify anammox activity in suspended biomass and biofilm at cold temperatures. The goal of this experiment was to determine whether suspended biomass or biofilm anammox processes showed greater *hzs*A expression and therefore contributed most to nitrogen removal at non-ideal temperatures.

A HIGH THROUGHPUT CAMPAIGN INTERROGATING RGS6 AND RGS17: IDENTIFICATION OF NOVEL, UNIQUE LEAD COMPOUNDS FOR RGS INHIBITION AND THERAPEUTIC DEVELOPMENT

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G Protein Coupled Receptors (GPCRs) are highly targeted proteins for drug discovery, with over 50% of all drugs on the market targeting these receptors. Regulator of G Protein Signaling (RGS) Proteins regulate the signaling cascade of GPCRs by accelerating the intrinsic GTPase activity of G protein alpha subunits and returning the heterotrimeric G protein to its inactive state. Recently RGS proteins have been implicated as being important in a variety of disease states. RGS17 has been identified as being inappropriately upregulated in both lung and prostate cancer. Knockdown of RGS17 resulted in decreased cell proliferation in vitro, and reduced tumor mass and volume when compared to control when cell lines were injected subcutaneously in nude mice. RGS6 has been shown to play an essential role in doxorubicin mediated cardio toxicity, has been shown to have an important role in anxiolytic and depression phenotypes in mice, and has been implicated as a critical mediator of behavioral and pathological responses to alcohol. With significant shared homology in the 120 amino acid RGS domain, and with significant overlap in G protein specificity between RGS family members, selective targeting of a particular RGS can be challenging. Here we present the screening of two RGS proteins (RGS6 and RGS17) using Alpha Screen technology against the NCI Next Diversity Library. Roughly 20,000 compounds were screened against RGS6 resulting in identification of 15 possible inhibitors and 5 possible activators. Roughly 60,000 compounds (including the 20K also screened against RGS6) were screened against RGS17 resulting in the identification of 131 potential inhibitors. Potential inhibitors are defined as those compounds that resulted in a 50% decrease in signal compared to positive control, and potential activators (RGS6 only) are defined as those compounds that increased the signal over positive control by at least an additional 50%. Post hoc analysis supports the robustness of the Alpha Screen assay for both proteins, with RGS6 Z-factors routinely being above 0.7 and RGS17 Z-factors routinely above 0.6. Additionally, filtration of the potential lead compounds against known substructures of assay interfering compounds only resulted in one (1) problematic substructure in the RGS6 screen and only 21 in the RGS17 screen. Finally, of the 20,000 compounds that were screened against both proteins we found no overlapping hits, which suggests that the hits identified (at least in that 20,000 subset) are true hits rather than assay interfering compounds. The lack of overlapping hits is also important because it suggest that small molecule selectivity between RGS family members is possible, and that development of RGS specific small molecules for therapeutic intervention is potentially obtainable.

ANALYSIS OF CORNEA HEALTH USING NEAR-INFRARED SPECTROSCOPY

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Procured donor cornea tissues undergo extensive testing to determine their suitability for transplantation. These tests are both costly and labor intensive and can only be performed once the tissue has been excised. Oftentimes, significant resources are invested in tissues which are later deemed unsuitable for transplantation. A device capable of assessing tissue health in the field prior to tissue procurement would improve efficiency and reduce costs. Near-infrared spectroscopy is an excellent technique for studying tissues as it is nondestructive and can be used to probe protein and water structures within a sample. The cornea is composed of five layers; the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium. Corneas labelled unsuitable for transplantation frequently have a low endothelial cell count. The endothelium, consisting of a monolayer of cells, is responsible for controlling the flux of water into and out of the stroma, a protein layer comprised primarily of collagen. Tissue health depends on biocatalytic activity within the endothelial layer to control water transport within the stroma. It is hypothesized that poor quality corneas will have lower enzyme-based control of water transport into the stromal protein matrix, thereby creating different protein/water structures compared to cornea tissues of higher quality. In this work, near-infrared spectra collected from cornea tissues are paired with a corresponding tissue quality assessment performed at the Iowa Lion's Eye bank. The resulting spectral dataset, consisting of near-infrared spectra collected from corneas of poor and high quality, enable an accurate comparison of spectral features related to the viability of the cornea for transplantation. Multivariate calibration techniques, such as principal component regression and partial least squares regression are used to identify clinically meaningful correlations between spectral information and donor cornea tissues.

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UNDERSTANDING THE MECHANISM OF FLAVIN DEPENDENT THYMIDYLATE SYNTHASE

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2-Deoxythymidine monophosphate, dTMP, is used as one of four building blocks of DNA. The human enzyme thymidylate synthase catalyzes the synthesis of dTMP from 2'-deoxyuridine-5'monophosphate, dUMP, using N5,N10-methylene-5,6,7,8-tetrahydrofolate (MTHF) as a cofactor. MTHF is converted to dihydrofolate in the reaction, which needs to be recycled to tetrahydrofolate by the enzyme dihydrofolate reductase (DHFR). However, a different enzyme is used to synthesize dTMP in many pathogens such as *H. pylori*, *B. anthracis*, *M. tuberculosis*, and *Rickettsia* species: flavin-dependent thymidylate synthase (FDTS). In addition to dUMP and MTHF, FDTS uses NADPH to produce dTMP and tetrahydrofolate. Because tetrahydrofolate is produced instead of dihydrofolate, DHFR activity is not necessary to recycle the cofactor. Preliminary results indicate that the enzyme mechanism of FDTS differs from human thymidylate synthase. In order to better understand the mechanism of FDTS we will investigate reaction intermediates; we will accumulate intermediates of the reaction through quenching single turnover reactions. By understanding the chemical mechanism of FDTS, we can design inhibitors for the enzyme. Because dTMP is vital to the replication of these pathogens, inhibitors of FDTS could potentially serve as a novel class of antibiotics.

DETERMINING RATES OF ATRAZINE DEGRADATION IN *PSEUDOMONAS* SP. ADP BIOFILMS AND PLANKTONIC CELLS BY CHEMICAL ANALYSIS

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Atrazine, a pervasive herbicide used in the United States, frequently contaminates drinking water, rising well above the maximum contaminant level (MCL) of 3 ppb set by the EPA. To decrease the concentration of atrazine below the MCL, bioremediation is explored as an efficient and economical alternative to traditional physio-chemical remediation techniques. Bioremediation exploits the natural metabolic properties of microbes to degrade recalcitrant compounds such as atrazine. The bacterial strain Pseudomonas sp. ADP contains all the genes on a single catabolic plasmid, pADP-1, to degrade the herbicide atrazine via a multistep catabolic pathway. It is hypothesized the use of biofilms in bioremediation is more efficient in comparison to the use of free-cells due to 1) increased immobilization of contaminants and 2) a higher frequency of horizontal gene transfer between cells. In this research, we report the use of high performance liquid chromatography and gas chromatography – mass spectrometry to analyze the degradation of atrazine by mode of growth and identification of pathway metabolites, respectively.

Design, Synthesis, and Evaluation of Novel Fluoroquinolone N-1 Aryl Substituents on Bacterial Type-II Topoisomerase Inhibition and DNA Binding

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Fluoroquinolones are small molecule broad spectrum antibiotics that target bacterial type-II topoisomerases, DNA gyrase and topoisomerase IV. These enzymes are responsible for maintaining the topology of DNA during replication and transcription. This is accomplished through an ATP driven process that involves relaxing the double helix, introducing supercoils and relieving torsional strain. Fluoroquinolones exert their activity through formation of a ternary complex with DNA and topoisomerase IV, blocking religation of DNA.

The issue of fluoroquinolone resistance is on the rise as the number of resistant bacterial strains continues to grow every year. One of the main observed mechanisms of resistance is target-mediated resistance. An essential interaction between the fluoroquinolone and the enzyme is through formation of a magnesium-water bridge. A single key mutation in the enzyme can disrupt this interaction resulting in loss of fluoroquinolone activity. In order to overcome this mechanism of resistance, our lab is designing novel fluoroquinolone derivatives to establish novel binding contacts to alleviate the need of the magnesium-water bridge interaction. Novel fluoroquinolones have been developed to have potential new binding interactions within the active site of the enzyme. The ability of these fluoroquinolones to bind DNA, inhibit gyrase and topoisomerase IV activity are described here.

3D PRINTED ALGINATE-PLGA TUBES FOR CONTROLLED SEQUENTIAL DRUG RELEASE

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Controlled drug delivery systems, that include sequential and/or sustained drug delivery, have been utilized to enhance the therapeutic effects of many current drugs by effectively delivering drugs in a time-dependent and repeatable manner. In this study, with the aid of 3D printing technology, a novel drug delivery device was fabricated and tested to appraise functionality. With an alginate shell and a poly(lactic-co-glycolic acid) PLGA core, the fabricated tubes displayed sequential release of distinct fluorescent dyes and showed no cytotoxicity when incubated with HEK293 cell line or primary stem cells. This delivery system could be used in tissue engineering to allow for higher levels on ossification in bone regeneration, as the release of different growth factors at different times would yield higher bone growth. The sequential delivery system could also be used in cancer vaccination to provide an enhanced immunogenic response to tumor cells.

[2+2] PHOTOCYCLOADDITION OF A COMMON ANTI-CANCER DRUG IN THE SOLID STATE

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Pyrimidine derivatives are known to participate in [2+2] photocycloadditions in solution and have been studied to elucidate the mechanisms of DNA damage by UV irradiation. Among these derivatives, 5-Fluorouracil (5-FU) has been used almost ubiquitously as a chemotherapeutic agent in the treatment of many forms of cancer (*e.g.* colon, pancreatic, breast). Solution phase [2+2] photocycloadditions have been exploited in order to synthesize prodrugs and drug conjugates for controlled delivery of 5-FU. However, the crystallographic landscape of 5-FU is underdeveloped and, as yet, there are no known examples of its photoreactivity in the solid state. Recent reports have described the behavior of 5-FU as a building block for cocrystals involving N-based aromatic heterocycles, providing a basis for its continued investigation in the organic solid state.

Here we explore the solid-state behavior of 5-FU in a series of cocrystals of 5-FU formed with bipyridine cocrystal formers. The hydrogen-bonding motifs of 5-FU within the cocrystals are similar to those of the pure 5-FU polymorphs. Additionally, the photoreactivity of the cocrystals was studied and a [2+2] cross-photodimerization observed.

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SYNTHESIS OF ISOPRENOID BISPHOSPHONATES AS INHIBITORS OF GERANYLGERANYL DIPHOSPHATE SYNTHASE

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Geranylgeranyl diphosphate synthase (GGDPS) is a key enzyme in a later stage of the human mevalonate pathway. The product of this enzyme, geranylgeranyl diphosphate (GGDP), is used for the isoprenylation of certain signaling and transport proteins in the Ras superfamily. There are instances where it is desirable to inhibit GGDPS, for example, to deter the resorption of bone or to diminish the proliferation of cancerous cells. Through synthesis of analogues of the natural substrate that show selective inhibition of GGDPS, we hope to maximize these potential therapeutic benefits.

One compound that has been found to inhibit GGDPS selectively is digeranyl bisphosphonate, which is active in enzyme assays as the sodium salt and shows significant cellular activity as the POM prodrug (1). Early investigation into the binding of this compound with the enzyme elucidated the importance of the highly polar bisphosphonate head group that can coordinate with magnesium cations in the active site as well as the two nonpolar side chains that can occupy hydrophobic channels within the enzyme making a 'V-shaped' inhibitor.

The most recent family of bisphosphonates (2) that we have synthesized to assay for potential GGDPS inhibition contain these important structural features arranged in new and interesting ways. The geranyl, prenyl, and citronellol chains provide the nonpolar character to occupy the necessary channels within the enzyme and the charged bisphosphonate can be liberated intracellularly through esterase-mediated removal of the prodrug functionality. Hopefully this strategy will yield more potent and selective inhibitors of GGDPS.

TRICLOSAN INDUCED BIOFILM FORMATION IN OPPORTUNISTIC PATHOGENS

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Triclosan is a widely used antimicrobial and antiseptic. It is commonly used in hand soap and toothpaste. It also has prophylactic uses in health-care. Excessive triclosan use has led to widespread and persistent contamination of the environment with low levels of triclosan. In fact, now triclosan can be detected in human urine and serum, even in the absence of active triclosan use. Our research shows that triclosan, at environmentally relevant concentrations, induces biofilm formation in diverse opportunistic pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Furthermore, we have preliminary evidence that triclosan in human nasal secretion corresponds with *S. aureus* nasal carriage.

EFFECTS OF THE DONOR-ACCEPTOR DISTANCE AND DYNAMICS IN FORMATE DEHYDROGENASE CATALYZED REACTIONS

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How the fast (femtosecond-picosecond, fs-ps) protein dynamics contribute to enzymatic function has gained popularity in modern enzymology. With a lot of experimental and theoretical studies developed, the most challenging part is to assess both the chemical step kinetics and the relevant motions at the transition state (TS) on the fast time scale. Formate dehydrogenase (FDH), which catalyzes a single hydride transfer reaction from formate to NAD⁺, is a model system to address this specific issue¹. We have crystallized and solved the structure of FDH from Candida boidinii (CbFDH) in complex with NAD⁺ and azide ² (Figure 1). We identified two active site residues. V123 and I175, which hold the H-acceptor (C4 position of the nicotinamide ring of the NAD⁺) close to the H-donor (formate). These two residues could be responsible for the narrow donoracceptor-distance (DAD) distribution observed in the wild type CbFDH¹. Kinetic isotope effects (KIEs) and their temperature dependence were measured for the recombinant CbFDH and its V123 and I175 mutants. Those mutants were design to systematically reduce the size of their side chain (I175V, I175A, I175G, V123A and double mutant I175V/V123A), leading to broader distribution of DADs. The kinetic experiments identified a correlation between the DAD distribution and the intrinsic KIEs. The contribution of the fs-ps dynamics was examined via two-dimensional infrared spectroscopy (2D IR) by measuring the vibrational relaxation of TS analog inhibitor, aizde, reflecting the TS environmental motions. Our results provide a test of models for the kinetics of the enzyme-catalyzed reaction that invokes motions of the enzyme at the fs-ps time scale to explain the temperature dependence of intrinsic KIEs.

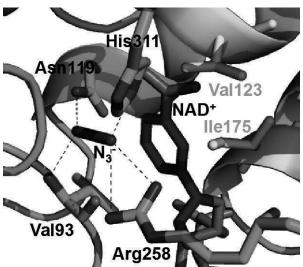


Figure 1. Active site structure of CbFDH-azide-NAD⁺ complex ². Dashed lines represent the hydrogen bonds forms between azide and the active residues. Val123 and Ile175 discussed in the text are highlighted in yellow.

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MUTATIONAL STUDY OF AN ACTIVE SITE ASPARAGINE IN THYMIDYLATE SYNTHASE TO GAIN INSIGHT INTO A NEWLY PROPOSED REACTION INTERMEDIATE

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Essentially all organisms require a thymidylate-synthesizing enzyme to maintain a sufficient pool of thymidylate¹. Thymidylate synthase (TSase) catalyzes the production of 2'deoxythymidine-5'-monophosphate (dTMP) from 2'-deoxyuridine-5'-monophosphate (dUMP). Although TSase has been studied for many decades, just recently a new reaction intermediate composed of a covalently bridged combination of both substrates been predicted on the basis of both computational² and experimental³ studies. The newly proposed mechanism obviates the formation of enolates along the reaction pathway, and the proposed intermediate is not covalently bonded to the enzyme, implying the potential for its structural mimics to serve as inhibitors. It has been proposed that an asparagine residue making contact with the carbonyl oxygen of dUMP⁴ plays a role in the traditional mechanism, but a much lesser role in the newly proposed one. That asparagine has been mutated to aspartate and to serine, and these mutants have been expressed and purified. Steady-state kinetic studies and kinetic isotopic effects (KIEs) of these mutants will be brought to bear on this system. Those studies will reveal the effect of the mutation on different steps along the catalytic path and the substrate binding pattern in the reaction. Intrinsic (chemical step-specific) isotope effects will be used to explore the necessity of this asparagine for proper atomic positioning and distances as the system approaches transition states for different chemical bond breaking and making steps. This in turn would test the validity of the recently proposed mechanism.

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PROTON AND HYDRIDE TRANSFER MECHANISMS IN THE THYMIDYLATE SYNTHASE CATALYZED REACTION

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Thymidylate Synthase (TSase) is an enzyme that plays a key role in DNA biosynthesis and is a target for several anticancer drugs. TSase catalyzes reductive methylation of deoxyuridylate (dUMP) to thymidylate, one of the four DNA building blocks. In this reaction, TSase exploits a cofactor (methylene tetrahydrofolate) that serves first as a methylene donor and subsequently a reductant. Various pyrimidine and folate derivatives (e.g., 5-fluorourcacil and raltitrexed, respectively) have long been used as chemotherapeutic drugs. However, development of resistance as well as toxicity warrants a more careful investigation structures and mechanism and the interplay between them.

TSase catalyzes two sequential C-H bond cleavages: a reversible proton abstraction from dUMP and an irreversible hydride transfer. QM/MM calculations on these H-transfers predicted mechanisms different from the traditionally proposed one. In the calculated mechanism of the proton abstraction, the formation of a novel intermediate comprised of both the nucleotide and the folate was predicted, while for the hydride transfer a concerted process was favored^{1,2}. The calculations proposed that a highly conserved arginine (R166) stabilizes the transition state of both H-transfers. These computationally proposed mechanisms were tested using primary and secondary kinetic isotope effects (KIEs) and mutagenesis studies^{3,4}. The findings supported the QM/MM-predicted proton abstraction and hydride transfer mechanisms and thus provide a experimental support for the nucleotide-folate intermediate that holds promise for a new class of mechanism-based inhibitors as leads for rational drug design.

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SYSTEM-SPECIFIC RESPONSE OF POLYSTYRENE PARTICLES IN THE BODY

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Upon delivery to the body and interaction with bodily fluids, drug carriers are instantaneously coated with proteins and other biomolecules, forming a protein corona on the particle surface. It is this protein-coated surface that the body "sees" and that determines the subsequent fate of the particles within the body. Protein corona formation in lung fluid and blood are pathways which may allow a means of controlling particle behavior. This study focuses on how exposure to blood or lung fluid results in different particle behavior, indicating that particle behavior in one biological system or organ is not necessarily translatable to other systems. Lung fluid and serum, two biological fluids with many proteomic similarities, interact with particles in markedly different ways, indicating that depending on route of exposure a particle system may have unique cellular and tissue responses. Polystyrene was exposed to either bronchoalveolar lavage fluid or serum and characterized prior to exposure to lung cells. The effect of exposure to each of these fluids was studied to investigate the significance of fluid environment on model drug carrier behavior.

NOVEL N-1 SUBSTITUTED FLUOROQUINOLONES AND QUINAZOLINE-2,4-DIONES DESIGNED TO TARGET THE ACTIVE SITE TYROSINE OF BACTERIAL TYPE-II TOPOISOMERASES

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Fluoroguinolones are broad spectrum antibiotics that are widely used to treat bacterial infections. These antibiotics target bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) and inhibit these enzymes by forming a 'cleaved complex' (also called ternary complex) consisting of the drug, nicked DNA, and the enzyme. In cleaved complex, the C-3, C-4 dicarbonyl moiety of fluoroguinolones is complexed with a magnesium ion and a critical binding contact is formed with serine and aspartate/glutamate residues through a magnesium-water bridge. Mutation of either of these residues prevents bridge formation, thereby decreasing the binding of fluoroguinolones in the cleaved complex and leads to resistance. In recent years there has been a rise in the number of bacterial strains that are resistant to fluoroguinolones, thereby emphasizing the need to develop new molecules active against these resistant mutants. One way to do so is by forming new drug-topoisomerase binding contacts in ternary complex. Evaluation of crystal structures of ternary complexes for clinically established fluoroquinolones, like moxifloxacin, showed that modifications to the N-1 position on the fluoroguinolone scaffold might provide analogues capable of forming non-conventional hydrophobic, ionic or pi-stacking interactions in the cleaved complex. In particular, it was envisioned that select analogues could be designed to target a non-mutable, active-site tyrosine of the enzyme making it difficult to develop resistance against these analogues. Quinazoline-2,4-diones are structurally analogous to the fluoroguinolones. The N-3 amine and C-2 carbonyl groups of the quinazolinediones form a binding contact with a conserved arginine, orienting the diones in a position similar to that of fluoroguinolones. However, unlike fluoroguinolones, the quinazoline-2,4-diones do not form a magnesium water bridge, and therefore these molecules are active with fluoroquinolone resistant DNA gyrase and/or topoisomerase IV. The quinazoline-2,4-diones, however, have limited potency against wild type enzyme and thus a novel binding contact to another, non-mutable amino acid would greatly benefit this class of compounds as well. The goal of identifying novel binding contacts between fluoroquinolones and type-II topoisomerases is to eliminate the dependence on the magnesium-water bridge, thereby enhancing activity against current fluoroquinolone resistant mutants. This talk outlines the design and synthesis of N-1 modified fluoroguinolones and quinazoline-2,4-diones and their in vitro evaluation against purified enzymes.

ESTABLISHING THE ROLE OF GLOBAL NETWORKS IN HUMAN DIHYDROFOLATE REDUCTASE THROUGH EXAMINATION OF INTRINSIC ISOTOPE EFFECTS

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Enzymologists have been working for decades trying to understand the roles of protein dynamics in an enzyme catalyzed C-H (hydride, proton or atom) transfer reactions. Although some enzyme functions like substrate binding and product release are well understood, the chemical transformation at the active site is not as well defined. The isolation of chemical step is challenging since chemistry is rarely fully rate-limiting to enzymatic turnover. Examination of intrinsic kinetic isotope effects (KIEs) partly circumvents this challenge and serves as an important tool for understanding the transitional state of enzyme reactions¹. Dihydrofolate reductase (DHFR) from Escherichia coli has long been a benchmark model system for structural, kinetic and evolutionary studies². One can examine the role of protein motions in DHFR by measuring the temperature dependence of the intrinsic KIEs of the DHFR catalyzed reaction and analyzing the data within the context of activated tunneling models of hydrogen transfer³. For Escherichia coli DHFR, a global dynamic network has been mapped out using this methodology⁴. To test if the global dynamic network is a general feature of DHFR catalysis site directed, mutagenesis will be used to alter the equivalent residues in human DHFR and the temperature dependence of the intrinsic KIEs of the mutants and their double mutants will be determined and compared with that of the wild-type enzyme.

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ABUNDANCE AND ACTIVITY OF AEROBIC VINYL CHLORIDE DEGRADERS IN CONTAMINATED GROUNDWATER

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Vinyl chloride (VC) is a common and persistent groundwater contaminant formed primarily from incomplete anaerobic dechlorination of chloroethenes. Microorganisms such as etheneotrophs (ethene oxidizing bacteria) and methanotrophs (methane oxidizing bacteria) could contribute to the clean-up of vinyl chloride in the groundwater via aerobic vinyl chloride degradation pathways. Therefore it is important to understand the *in situ* aerobic VC degradation as part of the natural attenuation process.

In this study, we used quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) to estimate the *in situ* abundance and activity of aerobic VC degraders in the groundwater at two sites with different levels of VC contamination. NAS Oceana, VA SWMU 2C has a relatively dilute VC plume (concentration range 2-50 μ g/L), while MCRD Parris Island, GA Site 45 has higher level of VC contamination (10^2 - 10^4 μ g/L).

At NAS Oceana, we observed relatively high abundance and activity of methanotrophs and less abundant and active etheneotrophs from 2008 to 2014. Illumina high-throughput sequencing revealed that methanotrophs comprised 2.5-39.3% of the total bacterial community and were 3.0-62.6% of the active bacterial community. At MCRD Parris Island, GA Site 45, high abundance of etheneotrophs and relatively low abundance of methanotrophs were observed in 2013 and 2014. A positive relationship between etheneotroph functional gene abundance and VC concentration was observed, indicating that VC at Parris Island Site 45 possibly supports the growth of etheneotrophs. Our results help clarify the contributions of etheneotrophs and methanotrophs in aerobic VC degradation and provide rapid and useful information to evaluate and monitor VC natural attenuation processes.

PROTEIN SEQUENCE OPTIMIZATION WITH A POLARIZABLE FORCE FIELD

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Computational protein design success is currently limited by a number of approximations, which often results in lists of candidate sequences with only a handful of genuine hits. Using a recent extension of the dead-end elimination (DEE) algorithm to many-body potentials, we are able to replace pairwise energy functions with the polarizable AMOEBA force field in a self-consistent reaction field generalized Kirkwood continuum solvent. Stabilizing and destabilizing combinations of residues were found by brute-force enumeration of all three-residue combinations (trimers) for canonical structures such as an α -helix; stabilizing trimers are recapitulated by DEE sequence optimization. We have additionally optimized a peptide ligand for the Tiam1 PDZ domain. Many of the discrepancies between many-body results and results with a strictly pairwise force field can be explained by the presence of stabilizing or destabilizing trimers (i.e. electrostatic and/or hydrophobic cooperativity). To the best of our knowledge, this is the first rigorous sequence optimization based on a many-body energy function and helps to establish a protein design paradigm that is transferable across dielectric environments

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MICROBIAL ADAPTATION TO VINYL CHLORIDE IN GROUNDWATER MICROCOSMS AS REVEALED BY METAGENOMICS

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Vinyl Chloride (VC), as a known human carcinogen, often appears in groundwater as a result of incomplete reductive dechlorination of the higher chlorinated ethenes and ethanes at contaminated sites. Certain indigenous bacteria can adapt to an aerobic VC-degrading lifestyle with low oxygen demand at these sites, however, the mechanisms by which the community adapts to VC remain elusive. Metagenomics, which can provide both taxonomic and functional information of a microbial community, is applied here as a primary means of tracking microbial community changes in groundwater microcosms as they adapt to VC as the sole carbon and energy source. Groundwater from a trichloroethene (TCE)- and *cis*-1,2-dichloroethene (cDCE)-contaminated site (Wendell Ave., Fairbanks, AK) was used to construct aerobic VC- and ethenefed microcosms. Cultures were maintained for about 256 days and four critical time points were selected for 16S rRNA gene amplicon and complete metagenomic sequencing. Based on the sequencing results, we are in the process of isolating VC-assimilating bacteria in the culture for additional characterization.

Both 16S rRNA gene and metagenomic sequencing showed that the community diversity significantly decreased during adaptation to VC, with species of *Nocardioides*, *Pseudomonas*, Pedobacter, and Sediminibacterium becoming more dominant in the microcosms. The genomes of several of the more abundant bacteria in VC-fed microcosms were successfully binned from metagenomics data including genes from species of *Nocardioides*, *Sediminibacterium*, Candidatus Chloracidobacterium thermophilum, Mesorhizobium, Polaromonas, Pseudomonas nitroreducens and Candidate division TM7. Metagenomic analysis showed that, when compared to the groundwater metagenome, ethene- and VC-fed microcosms were significantly enriched in genes encoding i) known ethene and VC aerobic assimilation pathway enzymes, ii) potentially novel ethene and VC aerobic assimilation pathway enzymes iii) regulatory proteins and iv) phage proteins. This includes genes encoding AkMO (>100-fold), EaCoMT (>100-fold), methane monoxygenase (18- to 71-fold), regulatory protein LuxR (13- to 49-fold), TetR (41- to 144-fold) and phase integrase (5.1- to 12-fold). Portions of the metagenome that binned with *Nocardioides* were similar to the genome of a previously isolated VC-assimilating bacteria, *Nocardioides* sp. JS614 (~70-99%), however, the morphology of this bacterium is distinct from JS614. We observed higher alpha diversity in all VC-fed microcosms when compared to ethenefed microcosms cultured in parallel. Overall, our experiment has revealed several bacteria and functional

MODELING THE FORMATE DEHYDROGENASE TERNARY COMPLEX: A MOLECULAR DYNAMICS APPROACH TO 2DIR SPECTROSCOPY

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It is expected that the motions of a protein have a relationship with the rate of catalysis, but current models do not reasonably predict how perturbations to dynamics on the timescale of bond vibrations – femtoseconds to picoseconds – correlate with effects on the kinetics of the chemical step. Because formate dehydrogenase (FDH) catalyzes a hydride transfer, its kinetics are readily probed through kinetic isotope effects (KIEs). In order to probe the dynamics of the active site and connect them to the kinetics, however, two-dimensional infrared (2DIR) spectra of the azide-NAD⁺-FDH ternary complex were taken. Azide is both a strong infrared chromophore and an analogue of the H-transfer transition state. 2DIR experiments, then, are arguably probing the structure of the reaction transition state. The resulting spectra show oscillations in the frequency-frequency correlation function that have thus far not been conclusively attributed to any particular motion. It is therefore necessary to calculate the 2DIR spectrum from molecular dynamics simulations in order to further resolve the picture presented by experimental data. In general, one can calculate the infrared spectra of a chromophore by calculating its instantaneous vibrational frequencies for each time step in a molecular dynamics simulation, and accounting for its coupling to nearby atoms in addition to through-bond vibrational shifts. This method has been demonstrated on azide in water, as well as on human amylin fibrils^{1,2}. Current work is focused on parameterization of the azide ligand within the AMBER14 force field and subsequent calculation of electrostatic potentials in order to calculate the desired frequencies.

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VALIDATION OF A PROTEIN FORCE FIELD WITH OSMOTIC PRESSURE MEASUREMENTS OF ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS

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It is important that molecular dynamics (MD) simulations be able to reproduce experimental results. Other groups have reported that MD simulations of the highly soluble villin headpiece resulted in rapid protein aggregation, and that MD simulations of intrinsically disordered proteins (IDPs) resulted in protein structural collapse. In an attempt to understand the cause of these inconsistencies between experiment and simulation, we have probed the degree of solute-solute interactions in MD. To do this we have determined the osmotic coefficients of systems of amino acids over a range of concentrations using two different water models. Perhaps surprisingly, the osmotic coefficients from MD agreed quite well with those from experiment. We also observed a systematic dependence of the osmotic coefficient on the water model used. Additionally we probed solute-solute interactions in systems of mixed charges. Finally, we tested solute-solute interactions using recently derived force fields that have been shown to maintain the disorder of IDPs. Overall, the study highlights how osmotic pressure measurements can give insights into solute-solute interactions and suggests that further parameterization efforts may need to be focused on specific amino acids.

PHOTO-CROSSLINKABLE UNNATURAL AMINO ACID WITH "CLICK" FUNCTIONALITY FOR PROTEIN INTERACTION STUDIES

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Nonsense suppression methodologies hold promise for the discovery and characterization of protein complexes. The photoreactive crosslinking non-canonical amino acid (ncAA), pbenzoyl-L-phenylalanine (Bpa), is genetically encoded in a target protein via the co-expression of an evolved orthogonal synthetase. This approach has been successful in capturing and identifying transient protein interactions in reconstituted complexes purified from *E. coli* and mammalian cells. One shortcoming of this approach is the lack of robust purification strategies to identify crosslinked groups by mass spectroscopy. We therefore synthesized a bifunctional Bpa unnatural amino acid containing an additional alkyne moiety for "click" functionality (Bpa-Alk). Our data show that Bpa-Alk in conjunction with the existing Bpa synthetase exhibits robust genetic incorporation within a tandem protein (mCherry-TAG-GFP) and thus this encoded ncAA may be captured via clicked-biotin streptavidin pulldown. Future studies will employ Bpa-Alk to characterize the interactions between pore conducting and auxiliary subunits in voltage-gated sodium channels.

SYNTHESIS OF SMALL-MOLECULES AS MECHANISTIC PROBE OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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Flavin-dependent thymidylate synthase (FDTS) encoded by *thyX* provides an alternative biosynthetic path for the *de novo* biosynthesis of thymidylate (dTMP) in several human pathogens (e.g., those causing anthrax, tuberculosis, typhus, and more,). The gene *thyX* is absent in humans thus making FDTS an attractive antibiotic target. This enzyme utilizes a noncovalently bound flavin adenine dinucleotide (FAD) prosthetic group to catalyze the chemistry and unlike human TSase, FDTS produces tetrahydrofolate (H₄folate) rather than dihydrofolate (H₂folate), thus FDTS dependent organisms do not contain the *folA* gene encoding for dihydrofolate reductase.

Several mechanisms have been proposed for the FDTS catalyzed reaction. A strong evidence for any proposed enzymatic mechanism can be obtained from the synthesis and examination of proposed intermediates. One of these intermediate is a putative exocyclic methylene intermediate; an isomer of thymidine, which has been suggested by several different hypothetical mechanims. The initial aim is to synthesize this intermediate and test it as alternative substrate for FDTS. The outcome of these tests will help us to understand the chemical mechanism of FDTS and validate the proposed mechanism. Derivatives of that intermediate might serve as potent inhibitors.

Figure 1 present two mechanisms proposed in Reference 3:

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[2+2] PHOTOCYCLOADDITIONS DIRECTED BY 2-BUTYNE-1,4-DIOL

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Our group has interests to direct chemical reactions in crystals with molecules that act as templates and serve as small-molecule catalysts. The directed reactions occur in cocrystals. The templates form discrete assemblies through reliable hydrogen-bonding synthons, facilitating geometrically demanding reactions in solids. We have previously used a templated approach to achieve [2+2] photocycloadditions of olefins within discrete assemblies. Here, we describe the use of 2-butyne-1,4-diol as a template to pre-organize olefins in the solid state within 1D hydrogen-bonded polymers. The assemblies undergo photoreactions to yield cyclobutane products.

STEROID HORMONE SULFATION CATALYZED BY HUMAN SULFOTRANSFERASES IS INHIBITED BY METABOLITES OF COMMONLY OCCURRING AIRBORNE POLYCHLORINATED BIPHENYLS

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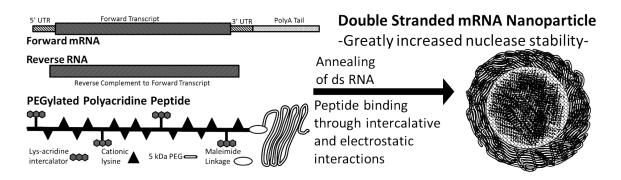
Exposure to polychlorinated chlorinated biphenyls (PCBs) has been associated with the risk of developing cancer, thyroid dysfunction, neurotoxicity, metabolic syndrome, diabetes, and other disease states. PCBs are persistent, lipophilic environmental toxins. They were widely used in the mid-twentieth century for industrial and commercial applications, but remain prevalent both from those sources and from current production as unintentional byproducts of industrial processes. Sulfation is catalyzed by cytosolic sulfotransferases (SULTs) and represents an important process both for transport and for signal termination of steroid hormones. Sulfotransferases SULT1E1 and SULT2A1 regulate the homeostasis of estrogens and androgens. respectively. We hypothesize that inhibition of the catalytic activity of these enzymes by hydroxylated and sulfated metabolites of commonly encountered airborne PCBs will inhibit the catalytic activity of these sulfotransferases with steroid hormones. This would potentially lead to disruption of the relative cellular concentrations of active and inactive hormones. We utilized radiolabeled estradiol (7 nM) and dehydroepiandrosterone (1.0 µM) to determine catalytic rates for purified SULT1E1 and SULT2A1, respectively. We found that the sulfotransferase activity of SULT1E1 was decreased at nanomolar concentrations of hydroxylated PCBs (OH-PCBs), and the activity of SULT2A1 was decreased at micromolar concentrations of OH-PCBs. The PCBsulfates were less inhibitory. Thus, the inhibition of SULT1E1 and SULT2A1 by these and other related OH-PCBs may have implications for alterations in steroid hormone signaling. We are beginning to explore such changes in a cell culture model of human adipocyte differentiation, since previous reports indicate that SULT1E1 plays an important role in adipogenesis. Our preliminary findings with cytosolic preparations from these cells indicate that OH-PCB 11 is effective in inhibiting SULT1E1 activity at nanomolar concentrations. Future studies will focus on determining the intracellular effects of this inhibition of SULT1E1 by OH-PCB 11 and other metabolites of airborne PCBs as well as understanding the potential effects of this inhibition on adipogenesis. (Supported by NIH P42 ES013661 and R25 GM058939)

DEVELOPMENT OF STABILIZED mRNA NANOPARTICLES FOR IN VIVO GENE DELIVERY

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Gene therapy may one day be the first option for the treatment for many acquired and genetic diseases. By delivering genes directly, dysfunctional proteins can be replaced. We have previously developed a PEGylated polyacridine peptide (PEG-peptide) that stabilizes DNA in circulation for up to 12 hours¹. However, successful delivery of stabilized DNA to the nucleus of cells in animals remains highly challenging. The primary barrier to DNA delivery is the nuclear membrane. Therefore, mRNA may offer an advantage over DNA because delivering mRNA bypasses the need for nuclear translocation. However, mRNA is much more sensitive to metabolism compared to DNA². The goal of this project is to develop a stabilized form of mRNA that can be dosed i.v. to mice and targeted to hepatocytes in the liver to achieve gene expression. To this end, we have found that double stranded mRNA (dsRNA) is much more stable to serum challenge as compared to single stranded mRNA (ssRNA). dsRNA is able to produce similar level and persistence of luciferase expression when hydrodynamically dosed in mice. dsRNA condenses with PEG-peptide, resulting in increased stability in the circulation. Progress toward developing a stable mRNA formulation for non-viral gene delivery will be presented.



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PROBING THE DYNAMICS OF INTRINSICALLY DISORDERED PEPTIDE STRETCHES

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In recent years there has been a growing interest in the concept of Intrinsically Disordered Peptides (IDPs). Typical structured proteins have free energy landscapes that can be described as having a single or few well defined and deep funnels corresponding to a single or few conformations. Conversely, IDPs are characterized by a shallow free energy surface in which many local minima exist which are separated by low energetic barriers allowing rapid transition between states. Traditional methods of structure determination have provided high resolution information on regions which are well folded with a single or few local energy minimum. However, they fail to provide any information in regions which no intrinsic structure exists. Translesion synthesis (TLS), which is the pathway which replicates beyond damaged DNA sites permitting progression of replication when encountering damaged DNA bases, has several proteins which have large stretches of IDPs. All polymerases of this pathway have known structured domains which are flanked either on one or both ends by long stretches of IDPs which, if truncated, lead to a phenotype like full knockout of the protein. Polymerase eta, a TLS member, was chosen as an initial candidate to predict behavior of its disordered C-terminal tail. We are developing a dual experimental approach which uses 1) Brownian dynamic (BD) simulations which can been utilized to provide predictions on the flexibility and dynamics and 2) a Förster Resonance Energy Transfer (FRET) system which will provide not only intensity information of the FRET transfer (which can be correlated to distance between FRET probes as energy transfer efficiency) as well as conformation populations from variance of signals intensity and frequency of occurrence. These data can be used to validate, or modify, simulation predictions providing an unprecedented representation of dynamics of these unique peptide stretches.

MASS MODULATED EFFECTS ON FUNCTIONAL DYNAMICS OF FORMATE DEHYDROGENASE

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Understanding the correlation between fast (femtosecond (fs) to picosecond (ps) timescale) dynamics and enzyme catalysis is an area of great interest in contemporary enzymology. Recent studies of heavy enzymes have addressed this issue, but have only focused on kinetics and did not examine dynamics. In such experiments all C, N and non-exchangeable H atoms in the native enzyme are substituted by C-13, N-15 and H-2 to obtain a vibrationally perturbed heavy enzyme. Formate dehydrogenase from *Candida boidinii* (CbFDH) is a unique model system since its inhibitor, azide, is a transition state (TS) analog, and is also a good infrared (IR) chromophore. As a result, the active site fast dynamics could be directly captured via two-dimensional infrared spectroscopy (2D-IR). Steady state and pre-steady state parameters were compared for light and heavy FDHs, and higher Michaelis constant (K_M) of formate in heavy-FDH was observed, indicating that heavy enzyme affects events on slower time scale motions other than the expected fs-ps. Intrinsic kinetic isotope effects (KIEs) were measured and 2D-IR experiments are under way. The effects of heavy FDH on these kinetic parameters will be discussed further.

EVALUATING MESOPOROUS SILICA NANOPARTICLES FOR DRUG DELIVERY THERMAL MITIGATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS

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Bacterial biofilms are a longstanding problem in both medical care and food processing. Heating is a well-established approach to biofilm sterilization, though many applications cannot tolerate the high temperature and pressure seen in autoclaves. An *in situ* heating mechanism using iron oxide nanoparticles suspended in a polymer coating have been shown to heat surfaces remotely via an alternating magnetic field allowing for hard to reach surfaces to be heated to a specific temperature. To investigate the degree of biofilm mitigation achievable by treatment at lower temperatures, *Pseudomonas aeruginosa* PAO1 biofilms were cultured in tryptic soy broth (TSB) at 37 °C for 24 hours in a drip-flow reactor (DFR) to a population density of 3.6 x 10⁸ CFU/cm² and subjected to thermal shocks at 50, 60, 70, and 80 °C and controls at 37 °C for 1, 2, 5, 10, 15, 20, or 30 min, then quantified by serial dilution. Population reductions of up to six orders of magnitude were quantified, with the resulting biofilm populations showing an Arrhenius-style dependence on mitigation temperature and a Weibull-style dependence on exposure time fitting the following equation.

$$log(CFU/cm^2) = log(CFU/cm^2)_0 - [0.079 + 0.044 log(t)] * (T - 37 °C)$$

where $log(CFU/cm^2)_0$ is the initial biofilm population density, T is the treatment temperature and t is the exposure time. To investigate the transferability of these results to less densely populated biofilms, P. aeruginosa biofilms were cultured in TSB at 37 °C for 96 hours on a shaker table to a population density of just 4.4×10^6 CFU/cm² and subjected to thermal shocks at 50 and 80 °C and controls at 37 °C for 1, 5, and 30 min. At 80 °C these biofilms often dropped below the quantification threshold though at 50 °C population reduction was not statistically significant. Comparable trials with biofilms cultured in Mueller Hinton Broth, in-house glucose-enhanced media, and a mixture of 90% Minimum Essential Media with 10% fetal bovine serum gave similar results, indicating that thermal susceptibility is independent of growth media, though maturity and nutrient availability may impact mitigation at lower temperatures. These results are particularly relevant for treatment of temperature-sensitive materials such as low-Tg plastics and a new approach for in situ mitigation of unwanted biofilm growth.

RHIZOBIUM SP. PRODUCES NOVEL METABOLITES OF 2,4-DINITROANISOLE IDENTIFIED USING STABLE ISOTOPES

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The environmental fate of 2,4-dinitroanisole (DNAN), a component of new insensitive munitions explosives (IMX) formulations, is an emerging global issue. The new IMX formulations – which all contain DNAN – are replacing old formulations at a rapid pace. However, DNAN has a relatively unknown fate and ecosystem toxicity. DNAN is a nitro-substituted aromatic compound that is similar to-but more soluble than-2,4,6-trinitrotoluene (TNT). TNT and other nitroaromatics have been shown to cause harm to human health and ecosystems, leading to concern over the impacts of widespread DNAN use. The research presented will focus on metabolism of DNAN by a *Rhizobium sp.* isolated from willow tree tissues. The findings demonstrate the utility of stable isotope labeling in elucidating novel metabolites. We demonstrate previously unknown sulfonated and acetylated DNAN derivatives confirmed by LC-MS/QT and LC-MS/MS. The results also confirm *para*-nitro-reduction of dinitroanisole to 2-amino- (and 2-hydroxylamino-) 4-nitroanisole, a formerly unseen phenomenon.

ARGENTOPHILIC AND PERFLUOROPHENYL-PERFLUOROPHENYL INTERACTIONS SUPPORT A HEAD-TO-HEAD [2+2] PHOTODIMERIZATION IN THE SOLID STATE

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The use of principles of supramolecular chemistry to direct reactions in the crystalline state has emerged as a reliable means to facilitate highly selective reactions in a solvent-free environment. In this context, we exploit metal-organic complexes to fix spatial arrangements of molecules in crystal lattices to promote intermolecular [2+2] photodimerizations. Perfluorophenyl-perfluorophenyl ($C_6F_5\cdots C_6F_5$) interactions have recently emerged as a means to control supramolecular architectures and frameworks. However, it was not clear whether perfluorophenyl-perfluorophenyl interactions can be integrated in [2+2] photodimerizations in solids.

In this presentation, we report face-to-face $C_6F_5\cdots C_6F_5$ interactions achieved in a disilver metalorganic complex. The $C_6F_5\cdots C_6F_5$ interactions, along with argentophilic forces, support *trans*-pentafluorostilbazole to undergo a head-to-head [2 + 2] photodimerization to form a cyclobutane that sustains a fluorinated two-dimensional metal-organic framework.² In addition, recent results regarding the [2+2] photodimerizations of fluorinated olefins in organic cocrystals will be discussed.

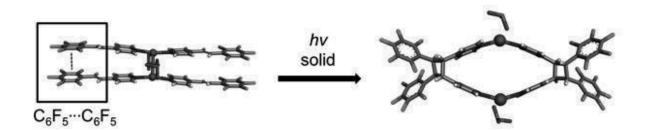


Figure 1: Face-to-face $C_6F_5\cdots C_6F_5$ interactions involving a disilver metal-organic complex support a head-to-head solid-state photodimerization.

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PHOTOCATALYTIC ADDITION OF A-BROMOPHOSPHONOACETATE ESTERS INTO INDOLE RING SYSTEMS TO ACCESS POTENTIAL THERAPEUTIC TARGETS AS WELL AS SYNTHONS FOR HORNER-WADSWORTH-EMMONS OLEFINATION

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The phosphonoacetate ester functional group is used ubiquitously in chemical synthesis for the Horner-Wadsworth-Emmons (HWE) olefination reaction. Its hydrolyzed form (phosphonocarboxylic acid) is found in an array of potentially useful therapeutic compounds, including iPEHPC (Figure 1-a) which is a geranylgeranyltransferase II (GGTase II) inhibitor. GGTase II is an enzyme involved in post-translational prenylation of Rab proteins. Rab proteins play a critical role in all facets of protein trafficking. As a result GGTase II inhibitors are potentially useful as therapeutic agents that target diseases like multiple myeloma that are characterized by an excess of secreted proteins.

The utility of phosphonoacetate esters in synthesis as well as in medicinal chemistry has led us to incorporate this functionality into indole ring systems in a novel manner. Utilizing the reductive coupling of the photocatalyst $Ru(bpy)_3Cl$ in conjunction with a tertiary amine and an α -bromophosphonoacetate ester allows for the direct incorporation of the phosphonoacetate ester at the 2' position of the indole ring (Figure 1-b). To the best of our knowledge, the indole phosphonate esters produced by this methodology are novel, and their synthetic and therapeutic value will be evaluated in the near future.

Figure 1 – (a) iPEHPC. (b) Representation of photocatalytic reaction

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BIOPHYSICAL STUDIES OF THE S. AUREUS SRRB-SRRA TWO-COMPONENT SYSTEM INVOLVED IN TOXIC SHOCK SYNDROME

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Methicillin-resistant Staphylococcus aureus (MRSA) is the major cause of hospital induced infections in United States. Due to the antibiotic resistant properties of some Staphylococcal aureus strains, there is an urgent need for identifying novel therapeutics. SrrB-SrrA twocomponent system (TCS) is a global regulator of S. aureus virulence factors, including Toxic Shock Syndrome Toxin-1, which is a causative agent of Toxic Shock Syndrome. Bacterial TCS, composed of histidine kinases (HK) and a response regulator (RR), connect environmental stimuli to the appropriate cellular response. SrrB is a kinase/phosphatase capable of regulating the level of phosphorylation of SrrA response regulator. SrrB-SrrA has been found to negatively regulate the expression of tst gene and TSST-1 production in the absence of oxygen. In contrast, under micro aerobic conditions (>2% oxygen), it is turned on and the toxin production increases. We have expressed and purified full-length and truncated SrrB and SrrA proteins and showed that they are biochemically active. In addition, we found that the PAS domain binds heme, suggesting that this domain may act as an O₂ sensor that plays an important role in regulation of the SrrB-SrrA system. We have solved the crystal structure of apo form of the PAS domain and found potential ligand binding sites which will be further studied. Using small angle X-ray scattering data and homology modeling, we have developed an initial molecular model of cytoplasmic region of SrrB and studied changes upon nucleotide binding. Our results are beginning to elucidate the signaling cascade that leads to the toxin TTST-1 production which might ultimately be utilized to inhibit the production of the toxin by targeting the SrrB-SrrA TCS.

NEURONAL TOXICITY DUE TO REACTIVE DOPAMINE METABOLITES AND ALDEHYDE DEHYDROGENASE INHIBITION

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Parkinsons disease (PD) 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 this selective dopaminergic neuronal death is unknown. Dopamine is metabolized by monoamine oxidase to form the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehye (DOPAL). The DOPAL is metabolized by aldehyde dehydrogenase (ALDH) to form a non-reactive metabolite and is excreted from the cell. Inhibition of ALDH is of great interest to our group as the reduction in levels of DOPAL is biologically critical, given this aldehyde is highly toxic to dopaminergic cells. However, ALDH inhibition has only recently been linked to PD occurence.

This work has confirmed linking DOPAL levels to a fungicide associated with PD risk. This benzimidazole fungicide, benomyl inhibits ALDH2 in dopaminergic neurons. The cytotoxicity of benomyl, DA, DOPAL, and the combination of DA or DOPAL with benomyl was assessed by MTT assay. This toxicity appears to be synergistic as none of the single treatments are significantly toxic to the cells. A second group of pesticides organochlorines, named *chlorpyrifos* also inhibit ALDH and are toxic to dopamingergic cells. Exposure to environmental toxins such as pesticides and fungicides have long been linked to PD risk, and only recently to DOPAL levels. This work provides a novel mechanism by which fungicide and pesticide exposure may stimulate PD pathogenesis.

HEPATOCYTE TARGETED ENDOSOMAL ESCAPE AGENTS

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Endosomal escape is a current bottleneck in the development of non-viral gene delivery vectors. We propose the use of novel endosomal escape agents to enhance transfection efficiency of our peptide-based vector, potentially leading to unstimulated gene expression. Three major strategies have emerged, one that utilizes membrane lytic peptides, a second that uses proton sponge polymers, and a third that uses an enzyme to catalyze the cleavage of phospholipids. Each novel endosomal escape agent is designed to target liver hepatocytes through the asialoglycoprotein receptor. The present study compares these approaches by conducting 384-well in vitro transfections on primary hepatocytes, as previously described.¹

Melittin (Mel), a 26-mer membrane lytic peptide, was modified to contain an N-terminal cysteine. Triantennary N-glycan (Tri), a potent high affinity asialoglycoprotein receptor ligand, was purified from bovine fetuin and chemically modified to contain a free thiol. Following bioconjugation with melittin, the membrane lytic activity of the resulting disulfide linked Tri-S-S-Mel (Figure 1) is abrogated. Therefore, the molecule can be safely i.v. dosed in mice and only upon bioactivation does the peptide regain activity, as determined in RBC hemolysis assays.

A second strategy involves the synthesis of a proton sponge polymer based on the 25 kDa branched polyethylenimine (PEI) backbone. The many 1° and 2° amines of PEI were modified with D-Lactose by reductive amination and with 4-imidazoleacetic acid using EDC/NHS coupling. These modifications serve many functions including: reduction of electrostatic potential at pH 7.5, targeting to the asialoglycoprotein receptor, reduction of toxicity, and increased buffering capacity. Early generation polymers demonstrated improved lethality profiles when i.v. dosed via mouse tail vein in comparison to PEI.

Phospholipase A₂ (PLA₂) purified from honeybee venom is a 15.2 kDa enzyme that cleaves at the sn-2 acyl bond of phospholipids releasing arachidonic acid and lysophopholipid. This action creates holes in the plasma membrane, potentially leading to the release of endosomal-entrapped cargo. A novel technique for PLA₂ modification was developed wherein it's intramolecular disulphide bonds react with a thiol pyridine modified Tri (Tri-TP) generating Tri-S-S-PLA₂ without the use of common reducing agents and without a significant loss in enzyme activity. These novel endosomal escape agents are being compared for their ability to mediate in vitro gene transfer of glycan targeted DNA and mRNA in miniaturized primary hepatocyte transfection assays.

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SULFONIUM SPECIES AS REVERSIBLE INHIBITORS OF HUMAN ACETYLCHOLINESTERASE

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Acetylcholinesterase (AChE; EC 3.1.1.7) is one of the most catalytically powerful enzymes and, as such, it has become one of the most intensively studies enzymes. AChE functions in the central and peripheral nervous systems to terminate action potentials across nerve-nerve and neuromuscular junctions. This essential role of AChE makes it a target for drug development for Alzheimer's disease and the main target for the development of extremely toxic organophosphorus compounds (OP) such as chemical warfare agents (CWAs) and pesticides. Thus, AChE inhibitors are used in a wide range of fields. Among the AChE inhibitors, reversible AChE inhibitors are important especially in medicinal chemistry. For examples, 2-pyridine aldoxime methyl chloride, 2-PAM, a reversible AChE inhibitor, serves as an antidote by nucleophilically displacing the phosphyl moiety from the active site while the reversible inhibitor Donepezil is used in the palliative treatment of Alzheimer's disease. Therefore, we synthesized and evaluated a series of multifunctional compounds that incorporate the dimethyl sulfonium function as inhibitors of human acetylcholinesterase (AChE). The compounds contained three structural elements, arranged as Ar-spacer-S⁺(CH₃)₂, where Ar is a peripheral site targeting aromatic group that is separated from the active site targeting sulfonium group via alkyl or aromatic spacers. All compounds in this series were potent reversible inhibitors of the enzyme, with IC₅₀ values spanning a thousand-fold range from 35 µM to 28 nM. Computer modeling of the interaction of the most potent inhibitor in the series illustrates that, like the ammonium function of typical AChE substrates and ligands, the dimethyl sulfonium function is molecularly recognized by the quaternary ammonium binding locus of the active site.

RAPID IDENTIFICATION AND PRODUCTION OF METABOLITES USING NOVEL STABILIZED DRIED POWDER (SDP) OF HUMAN CYTOCHROME P450S (CYPS) ENGINEERED IN YEAST

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

Our method is extremely simple; add 20-100 mg of SDP/ mL of drug-candidate (20-1000 μ M) of interest in 50 mM phosphate or Tris-HCl buffer at 30°C. Incubate for 1 to 4 h and analyze metabolites by HPLC or LCMS. SDP is easily separated from reaction mixture just by centrifugation. Prep-scale procedure is also similarly easy, just mixing of cells with choice of drugs in the buffer solution at 30°C.

We have engineered CYP2D6, CYP3A4, CYP2C9, CYP2C19, and CYP1A2 in yeast, optimized high-cell density fermentation and made SDP of these CYPs successfully. Typical metabolite yields from FDA approved substrates in Microtiter Plate reaction with different SDP are summarized in Table 1. K_m and V_{max} of all hCYP SDP catalyzed reactions have been determined and compared. We have scaled up reaction to several 100s of ml to produce several 10s of mg metabolites. As an example, 400 ml of CYP 2C9 catalyzed reaction of diclofenac (DN) produced 53.6 mg of 4-hydroxydiclofenac (HDN) from 59.2 mg of DN in only 1.5 hours. This SDP can be used for 2-cycles of catalysis, to further increase the yield of metabolites. We are currently working with other hCYPs (CYP2E1, CYP2B6, and CYP1A1) to demonstrate facile synthesis of their respective metabolites.

Table 1: Typical metabolite yields from FDA approved substrates with different SDP

No.	Substrate, 200 μΜ	CypExpress	Metabolite	Micro Plate	μM of Metabolite ± SD (n = 3)
1	Dextromethorphan	2D6	Dextrorphan	24-Well	92.15 ± 0.95
2	Dextromethorphan	2D6	Dextrorphan	96-Well	58.17 ± 3.84
3	Testosterone	3A4	6-β-OH-Testosterone	24-Well	52.37 ± 0.76
4	Testosterone	3A4	6-β-OH-Testosterone	96-Well	46.92 ± 7.70
5	Diclofenac	2C9	4'-OH-Diclofenac	24-Well	80.55 ± 8.32
6	Diclofenac	2C9	4'-OH-Diclofenac	96-Well	184.89 ± 5.03
7	S-Mephenytoin	2C19	S-4'-OH-Mephenytoin	24-Well	28.73 ± 4.25
8	S-Mephenytoin	2C19	S-4'-OH-Mephenytoin	96-Well	18.07 ± 1.51

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EXTENTION CAFFEINE DRHYDROGENASE-BASED RAPID CAFFEINE DETECTION TECHNOLOGY TO HEALTH-CARE APPLICATIONS.

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Caffeine, the ever-popular widely-consumed psychoactive natural product, has got its own set of ill effects on human health if not consumed within FDA recommended limits. While excessive and long-term caffeine consumption (>400 mg/day/adult) can lead to adverse health-effects in normal healthy people, it is highly restricted to infants, nursing mothers, pregnant women, elderly people and people allergic to caffeine. In addition to that, recent reports on caffeine's role in drug contraindications, performance enhancing drugs (PEDs) and human death due to caffeine intoxication is of concern to FDA, health-care providers and public at-large. Currently, no commercial test kit is available in the market, which can detect caffeine in food/beverages or in human body fluids such as human urine, blood serum and nursing mother's milk, that will aid vigilant consumers and health-care providers to identify caffeine-loaded samples easily and quickly. In 2014, Professor Subramanian's laboratory at The University of Iowa developed a "Rapid Caffeine Detection Test" using caffeine dehydrogenase (Cdh) (isolated from soilbacterium *Pseudomonas* sp. strain CBB1 in 2008¹) in a 96-well microtiter plate format with each well containing all required assay components such as enzyme, dye, and buffer². In that study, Dr. Sujit Kumar Mohanty i) validated this Cdh-based test with various commercially available caffeine-containing beverages, pharmaceuticals, and caffeine-spiked milk samples (including breast milk), and ii) optimized the test to make it rapid (1-2 min), sensitive (1 ppm), specific (to caffeine) and robust (work with complex test samples/body fluids)². The major aim of that work was aimed at caffeinated foods and beverages for an "at-home" test by consumers². Current work aims at extending that Cdh-based caffeine-detection technology to human-health care applications by rapid caffeine detection in human urine and blood serum in microtiter plate format that can be used by health-care providers. The Cdh-based test is the first with the desired attributes for a rapid and robust caffeine diagnostic kit development². Thus, our current plan is to extend this caffeine detection technology to health-care applications where caffeine interference has been observed with cardiac-stress tests. Other health-care applications contemplated include PED-testing and caffeine allergenicity.

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COORDINATED EFECT OF DISTAL MUTATIONS ON ACTIVE SITE IN DIHYDROFOLATE REDUCTASE

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Enzyme motions on a broad range of time scales can play an important role in various intra- and intermolecular events, including substrate binding, catalysis of the chemical conversion, and product release. The relationship between protein motions and catalytic activity is of contemporary interest and heated controversy. Enzyme catalysis has been studied extensively, but the role of enzyme dynamics in the catalyzed chemical conversion is still an enigma. The enzyme dihydrofolate reductase (DHFR) is often used as a model system to assess a network of coupled motions across the protein that may affect the catalyzed chemical transformation.^{1,2} Molecular dynamics calculations³ and bionformatic³ studies of DHFR have suggested the presence of such network, experimental studies testing this idea are scarce. Recent experimental studies demonstrated that distal residues G121, M42 and F125 in E. coli DHFR participate in that network.⁴ The missing link in our understanding of DHFR catalysis is the lack of a path by which such remote residues can affect the catalyzed chemistry at the active site. Here, we present a study of intrinsic kinetic isotope effects that indicates catalytic synergism between a remote residue in that dynamic network, G121, and the active site's residue I14. This finding links the remote residues in the network under investigation to the enzyme's active site, providing a path by which these residues can be coupled to the catalyzed chemistry. This experimental evidence validates calculations⁵ proposing that both remote and active site residues constitute a network of coupled promoting motions correlated to the bond activation step (C-H \rightarrow C hydride transfer in this case).

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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
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Montgomery, Rex Emerit		5-7897	Cox, Charles, Emeritus	3752 BSB	5-7779	
Plapp, Bryce, Emeritus	4-550 BSB	5-7909	Feiss, Michael Emeritus	3-352 BSB	5-7782	
Schnieders, Michael	4-516 BSB	5-7891	Horswill, Alexander	540F EMRB	5-7783	
Shea, Madeline	4-450 BSB	5-7885		3-632 BSB	5-7818	
Spies, M. Asley	4-430 DSD	3-5645	Kirby, John		5-7818 5-9721	
Washington, M. Todd	4-516 BSB	5-7518	McCarter, Linda	3-430 BSB		
washington, w. rodu	4-310 DSD	3-7310	Stauffer, George Emeritus		5-7791	
DIOLOCV			Weiss, David Yahr, Timothy	3-452 BSB 540B EMRB	5-7785 5-9688	
BIOLOGY Cheng, Chi-Lien	143 BB	5-2583	rum, rumoury	C TOD ENTED	2 7000	
		5-1542	MOLECULAR PHYSIOLGY &			
Fassler, Jan 324 BBE Gussin, Gary, Emeritus 318 BB		5-1342				
Gussin, Gary, Emeritus		5-2071	BIOPHYICS			
Shih, Ming-Che, Emeritu			Ahern, Christopher	4256 CBRB	5-6964	
Soll, David	302 BBE	5-1117				
			PHARMACEUTICAL SCIENCES &			
BIOMEDICAL EN	GIMEERING		EXPERIMENTAL THERAPEUTICS			
Schnieders, Michael	5013 SC	5-7891	Doorn, Jonathan	S328 PHAR	5-8834	
			Duffel, Michael	S325 PHAR	5-8840	
CHEMICAL & BIO	CHEM ENG		Fiegel, Jennifer	S215 PHAR	5-8830	
Aurand, Gary	3324SC	4-0970	Jin, Zhendong	S315 PHAR	3-5359	
	4140SC	5-5191	◆Kerns, Robert J.	S321 PHAR	5-8800	
Carmichael, Greg			Olivo, Horacio	S319 PHAR	5-8849	
Fiegel, Jennifer	4124 SC	5-8830	Rice, Kevin	S300 PHAR	5-9903	
Murhammer, David	4132SC	5-1228	Rosazza, Jack, Emeritus	C106 MTF	5-4908	
♦Nuxoll, Eric	4140SC	3-2377		5228 PHAR		
♦Peeples, Tonya	4128SC	5-2251	Salem, Aliasger		3-8810	
Rethwisch, David	4139SC	5-1413	Spies, M. Asley	S313 PHAR	3-5645	
Subramanian, Mani Emeritu	s 4139SC	5-5813				
			RADIATION ONCOLOGYN (FRRB)			
CHEMISTRY			Schultz, Michael	B180 ML	5-8017	
♦Arnold, Mark	230 IATL	5-1368				
Cheatum, Christopher	326 IATL	3-0379	CBB STAFF			
Dey, Mishtu	W285 CB	4-1319	Main	C100MTF	5-4900	
Geng, Lei	319 CB	5-3167	Arnold, Mark A	ssociate and Interi	m Director	
Gloer, Jim	E515 CB	5-1361	Gopishetty, Sridhar	Technical Director		
Grassian, Vicki	244 IATL	5-1392	Das, Shuvendu	Resea	rch Leader	
♦Kohen, Amnon	E274 CB	5-0234	Rotman, Mitchell	Administrator		
MacGillivray, Leonard	E555 CB	5-3504	Spidle, Kay	Secretary		
Margulis, Claudio	118 IATL	5-0615	Garner, Mary	Quality Associate		
Messerle, Louis E45 CB		5-1372	McCarthy, Troy	QA Assistant		
Nguyen, Hein	E457 CB	4-1887	Coeur, Melissa	•	h Assistant	
♦Quinn, Dan			Dostal, Larry	Research Assistant		
Tivanski, Alexei	E272 CB	4-3692	Ehler, Jolene			
Wiemer, Dave %531 CB		5-1365		Research Associate Research Associate		
,			Garner, Mary Kasperbauer, Sarah	Research Associate		
CIVIL&ENVIRON	MENTAL EN	I G	Lashmit, Philip	Research Associate		
Just, Craig	4111 SC	5-5051	Lettington, Deanna	Research Assistant		
♦Mattes, Tim	4111 SC 4105 SC	5-5065	Liu, Wensheng		n Assistant	
Parkin, Gene Emeritus	4106 SC	5-5655	Railsback, Michelle		Associate	
Schnoor, Jerald	4112 SC	5-5649	Xu, Jingying		h Assistant	
Valentine, Richard	4112 SC 4118 SC	5-5653	maying	Research		
v alchume, Menalu	7110 50	J-J0JJ				

[◆]Coordinating Committee Updated 09/16/15

Notes



