The University of Iowa

26th

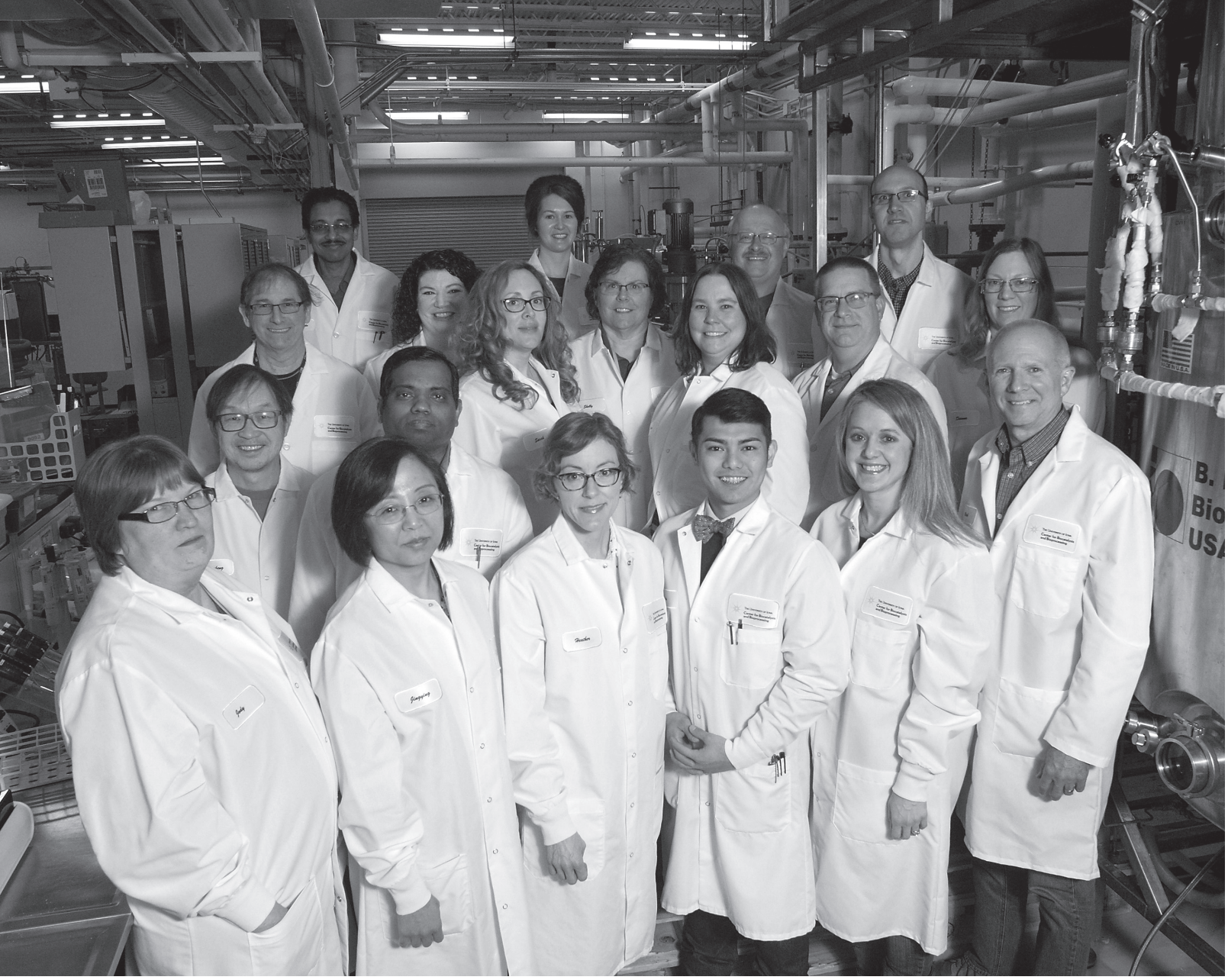
Center for Biocatalysis and Bioprocessing Conference

Advances in Biocatalytic Sciences: Attacking Societal Problems

http://cbb.research.uiowa.edu

The University of Iowa

October 17, 2017
Iowa Memorial Union
Iowa City, Iowa



Welcome from the staff of the Center's Microbal Fermentation and Processing Facility

THE UNIVERSITY OF IOWA

26th Annual Biocatalysis and Bioprocessing Conference

"Advances in Biocatalytic Sciences: Attacking Societal Problems"

Sponsored by:

October 17, 2017

http://cbb.research.uiowa.edu

26th Annual
Biocatalysis and Bioprocessing Conference

"Advances in Biocatalytic Sciences: Attacking Societal Problems"

Sponsored by:

The University of Iowa
Center for Biocatalysis and Bioprocessing

October 17, 2017

Conference Organizing Committee:

Mark Arnold, Ph.D.
Sridhar Gopishetty, Ph.D.
Shuvendu Das, Ph.D.
Daniel Quinn, Ph.D.
Mitchell Rotman, MA, MS, MHA

Director
Mark Arnold, Ph.D.

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Message from the Director

Advances in Biocatalytic Sciences: Attacking Societal Problems is the title for the 26th annual conference of the Center for Biocatalysis and Bioprocessing (CBB). A group of world-renowned speakers will present a series of seminars on topics that highlight the unique features of enzymes in the solution to long-standing problems in medicine, agriculture, and the environment. The following mix of external and internal scientists will be featured in this year's program:

Professor Ali Salem, Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT) at The University of Iowa College of Pharmacy, will talk about his research to develop gene-activated matrices to promote regeneration of musculoskeletal tissues, such as the bone and cartilage.

Dr. Susan Brockmeirer is a Research Veterinary Medical Officer in the National Animal Disease Center of the Agricultural Research Service, research branch of the USDA. She will discuss the potential of emerging rational design strategies to develop the next generation of vaccines for animal health.

Dr. Ryan Kramer is a Senior Scientist at the Infectious Disease Research Institute (IDRI) where he serves as the manager of their Characterization and Product Development group. His research focuses on developing effective vaccines to treat and prevent diseases that impact human health in developing countries.

Professor Ulrich Schwaneberg is the Head of the Institute of Biotechnology at the RWTH Aachen University, Aachen, Germany. His presentation will highlight state-of-the-art methods for engineering enzymes for targeted industrial applications and will include examples of lessons learned when extending the potential of enzymes to non-aqueous environments.

Professor Greg LeFevre is establishing a research program in the Department of Civil & Environmental Engineering at the University of Iowa. His expertise centers on exploring the role and impact of biocatalytic-mediated transformation pathways on contaminants in water supplies.

In addition, three fellows of our Predoctoral Training Program, jointly sponsored by NIH and CBB, will present their research findings.

A key feature of the Conference is a poster session, wherein students are encouraged to present their latest research findings. Given the interdisciplinary nature of the CBB and its member faculties and students, the poster session provides a stimulating atmosphere to explore the scope of biocatalytic sciences. All students on campus working in the area of Biocatalytic Sciences are encouraged to present a poster.
For this poster session, Biocatalytic Sciences are broadly defined as those areas of basic and applied research that center on enzymes and their function within an array of disciplines, including: 1) discovery of new enzymes and understanding their mechanism of action at the molecular level, 2) exploration and applications of biochemical and cellular pathways, 3) design and manufacturing of biotherapeutics, such as vaccines for both human and animal health, 4) understanding and developing enzyme-mediated chemical transformations, 5) metabolic engineering, 6) directed evolution, 7) genomics, 8) agricultural feedstock utilization, and 9) expression, production and purification of protein products.
It is my pleasure to encourage all scientists and engineers interested in biocatalytic sciences to attend our 2017 CBB Conference. The conference is scheduled for October 17th and will be located in the Iowa Memorial Union on the main campus of the University of Iowa.

Thank you for your interest,

Mark Arnold, Ph.D.
Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research & Economic Development, University of Iowa Research Park, Coralville, IA

26th Annual Center for Biocatalysis and Bioprocessing Conference
"Advances in Biocatalytic Sciences: Attacking Societal Problems"
The University of Iowa, Iowa Memorial Union, Iowa City, IA

TUESDAY, OCTOBER 17, 2017

IOWA THEATER (1ST FLOOR)

7:30 – 8:15 AM	Registration – Hubbard Commons (outside Iowa Theater 1st floor, IMU)
7:30 – 8:15	Continental Breakfast – across from Iowa Theater 1st floor, IMU
8:15– 8:30	**Program** – Iowa Theater 1st floor, IMU **Introduction and Welcome** **Mark Arnold, Ph.D.**, Director, Center for Biocatalysis and Bioprocessing, Office of the Vice President for Research 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, University of Iowa, Iowa, City , IA
8:30– 9:15	**Aliasger K. Salem, Ph.D.,** Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT), College of Pharmacy, The University of Iowa, Iowa City, IA *"Gene Activated Matrices for Bone Regeneration"*
9:15– 10:00	**Ryan Kramer**, **Ph.D.** Senior Scientist, Process and Product Development Manager, Infectious Disease Research Institute (IDRI), Seattle, WA *"Development of a Thermostable Nanoemulsion Adjuvanted Vaccine Against Tuberculosis*"
10:00– 10:15	Break – Hubbard Commons (outside Iowa Theater 1st floor, IMU)
10:15– 11:00	**Susan Brockmeier, D.V.M., Ph.D.,** Research Veterinary Medical Officer: USDA, ARS, National Animal Disease Center, Ames, IA ***"Rational Development of Veterinary Vaccines Against Swine Pathogens"***
11:00– 11:45	**Dr. Ulrich Schwaneberg,** Chair of Biotechnology, RWTH Aachen University and Member of Scientific Board of Directors, DWI-Leibniz Institute for Interactive Materials, Aachen, Germany *"Eighteen Years of Directed P450 BM3 Evolution: Lessons and Success Stories"*
11:45– 12:00	Relocation to 2nd Floor Ballroom, IMU
12:00– 12:45 PM	Lunch – Poster Session 1-Ballroom 2nd Floor, IMU S3 Product Show
12:45– 1:30	Lunch – Poster Session 2-Ballroom 2nd Floor, IMU S3 Product Show
1:30– 1:45	Relocation to Iowa Theater 1st Floor, IMU

Afternoon Session – Iowa Theater 1st Floor, IMU

1:45– 2:30 PM	**Gregory LeFevre, Ph.D.,** Assistant Professor, Department of Civil and Environmental and Engineering, College of Engineering, The University of Iowa, Iowa City, IA *"Biocatalysts in Contaminant Transformation: Because Clean Water is Pressing Societal Problem"*
2:30–3:30	**NIH/CBB Fellow Presentations**
	Jacob Poliskey, Ph.D. Candidate, Kevin G. Rice Research Group, Department of Pharmaceutics and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA "*Double Stranded mRNA Polyplexes for Liver Transfection"*
	Anh-Vu Do, Ph.D. Candidate, Aliasger K. Salem Research Group, Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA *"Combining Ultrasound and Intratumoral Administration of Doxorubicin-loaded Microparticles to Enhance Tumor Cell Killing"*
	Nitwari Tiwari, Ph.D. Candidate, Ernesto J. Fuentes Research Group, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA *"Regulation of the S. aureus SrrB Histidine Kinase by a PAS Domain"*
3:30	Closing Remarks
3:45	**Adjourn**
Approx. 3:45	Tour of CBB Fermentation Facility (Immediately following end of conference)

List of Oral Presentations

ORAL PRESENTATIONS

1. **GENE ACTIVATED MATRICES FOR BONE REGENERATION**
   Aliasger K. Salem, Ph.D.
   Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT), College of Pharmacy, The University of Iowa, Iowa City, IA

2. **DEVELOPMENT OF A THERMOSTABLE NANOEMULSION ADJUVANTED VACCINE AGAINST TUBERCULOSIS**
   Ryan Kramer, Ph.D.
   Senior Scientist, Process and Product Development Manager, Infectious Disease Research Institute (IDRI), Seattle WA

3. **RATIONAL DEVELOPMENT OF VETERINARY VACCINES AGAINST SWINE PATHOGENS**
   Susan Brockmeier, D.V.M., Ph.D.
   Research Veterinary Medical Officer: USDA, ARS, National Animal Disease Center, Ames, IA

4. **EIGHTEEN YEARS OF DIRECTED P450 BM3 EVOLUTION: LESSONS AND SUCCESS STORIES**
   Dr. Ulrich Schwaneberg
   Chair of Biotechnology, RWTH Aachen University and Member of Scientific Board of Directors, DWI-Leibniz Institute for Interactive Materials, Aachen, Germany

5. **BIOCATALYSTS IN CONTAMINANT TRANSFORMATION: BECAUSE CLEAN WATER IS A PRESSING SOCIETAL PROBLEM**
   Gregory LeFevre, Ph.D.
   Assistant Professor, Department of Civil and Environmental and Engineering, College of Engineering, The University of Iowa, Iowa City, IA

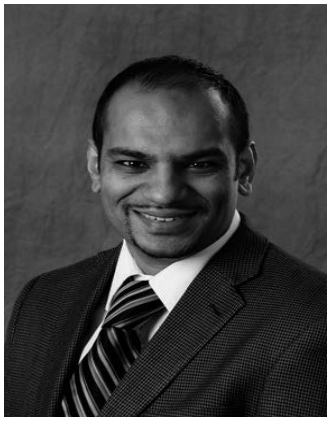
6. **DOUBLE STRANDED *m*RNA POLYPLEXES FOR LIVER TRANSFECTION**
   Jacob Poliskey
   Ph.D. Candidate
   Kevin G. Rice Research Group, Department of Pharmaceutical Sciences and Experimental Therapeutics, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA

7. **COMBINING ULTRASOUND AND INTRATUMORAL ADMINISTRATION OF DOXORUBICIN-LOADED MICROPARTICLES TO ENHANCE TUMOR CELL KILLING**
   Anh-Vu Do
   Ph.D. Candidate
   Aliasger K. Salem Research Group, Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, IA

8. **REGULATION OF THE *S. AUREUS* SrrB HISTIDINE KINASE BY A PAS DOMAIN**
   Nitija Tiwari
   Ph.D. Candidate
   Ernesto J. Fuentes Research Group, Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA

Speakers' Profiles

Aliasger K. Salem, Ph.D.
Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT)
College of Pharmacy
The University of Iowa
Iowa City, IA



Aliasger Salem, Ph.D is the Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT) at The University of Iowa College of Pharmacy. From 2009 to 2013, Aliasger Salem was an American Cancer Society Research Scholar. Since 2012, Aliasger Salem has also been Leader of the Experimental Therapeutics Program at the Holden Comprehensive Cancer Center (HCCC), University of Iowa Hospitals and Clinics. Since 2016, Aliasger Salem has been the Co-Director of the Nanotoxicology Core at the Environmental Health Sciences Research Center, University of Iowa. Aliasger Salem was educated in Applied Chemistry at Aston University of Science and Technology, Birmingham, UK (BSc 1998; 1st Class Honors). He received his Ph.D. in Pharmacy at the University of Nottingham, UK in 2002. He then received postdoctoral training at the Johns Hopkins School of Medicine in Baltimore until 2004. Aliasger Salem's research is currently focused on exploring the synergistic application of new polymers and nanotechnology for regenerative medicine and cancer vaccines. He is the author of over 250 scholarly publications, patents and presentations and has published in journals that include Nature Nanotechnology, Nature Materials, Nature Reviews Urology, the Proceedings of the National Academy of Sciences (PNAS), Advanced Materials, Bioconjugate Chemistry, Biomaterials, Journal of Pharmaceutical Sciences, Pharmaceutical Research, Journal of Controlled Release and the International Journal of Pharmaceutics. His research is supported by extensive past and current funding from organizations such as but not limited to the National Cancer Institute at the National Institutes for Health, the National Institute of Dental and Craniofacial Research at the National Institutes for Health, the Department of Defense Congressionally Directed Medical Research Programs, the American Cancer Society, the US National Science Foundation and the Pharmaceutical Research & Manufacturers of America Foundation. Aliasger Salem is an associate editor and editorial board member for a number of well-regarded pharmaceutical sciences journals including Associate Editor for the AAPS Journal (flagship journal for the American Association for Pharmaceutical Scientists) and editorial board member for the International Journal of Pharmaceutics. Aliasger Salem is an elected Fellow of the AAPS. Aliasger Salem has participated on a regular basis on a number of grant review study sections including panels for the American Cancer Society, the National Institutes of Health, the National Science Foundation, and the Department of Defense (DoD): Congressionally Directed Medical Research Programs (CDMRP) Prostate Cancer Research Program. Prof. Salem enjoys mentoring graduate students, research scientists and postdocs and teaching in the PharmD and graduate programs. To date, Aliasger Salem has mentored 27 graduate students, 3 visiting professors, 4 postdoctoral fellows, 3 masters' students and more than 100 undergraduate, PharmD and high school students in research. Prof. Salem has been an active mentor on the PharmD mentoring program since the program's inception. He is frequently nominated for collegiate teacher of the year and has previously received the 2006 College of Pharmacy Teacher of the Year award, the 2008 University Of Iowa Council Of Teaching Instructional Improvement Award and the 2012 College of Pharmacy Collegiate Teacher of the Year award.

Ryan Kramer, Ph.D.
Senior Scientist, Process and Product Development Manager
Infectious Disease Research Institute (IDRI)
Seattle, WA

Ryan Kramer is a Senior Scientist at IDRI and Manages the Process and Product Development Department. Ryan's department is responsible for moving vaccine candidates into the clinic, including bioprocess development, characterization of vaccine antigen and adjuvant components, and designing drug product formulations that are thermostable and do not depend on the cold-chain for distribution to developing countries. Ryan has worked in a wide range of disease fields including cancer, ETEC, HIV, tuberculosis, leishmaniasis, anthrax, West Nile virus, leprosy, hookworm disease, schistosomiasis, chikungunya virus, and shigellosis.

Before joining IDRI, Ryan was a postdoctoral fellow at the Macromolecule and Vaccine Stabilization at the University of Kansas, under the direction of Russ Middaugh and David Volkin. He worked on numerous contract and research formulation development projects characterizing macromolecular systems and biotherapeutics, using state of the art analytical biophysical instrumentation.

Ryan obtained his Ph.D. in biochemistry from Texas A&M University where he developed a mutational approach for increasing protein solubility. As a graduate student, Ryan received a Molecular Biophysics NIH predoctoral training grant and Texas A&M Regents' Graduate Fellowship.

Ryan graduated summa cum laude from Washington State University and received his B.S. in biochemistry. Ryan is an Eagle Scout and member of Order of the Arrow.

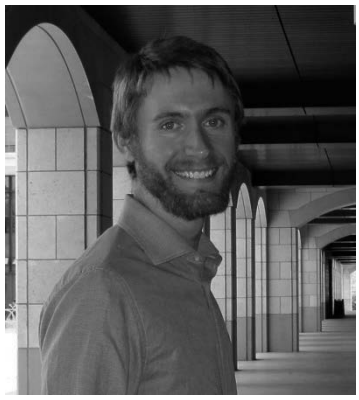
Susan Brockmeier, D.V.M., Ph.D.
Research Veterinary Medical Officer Lead Scientist - Non-antibiotic Strategies to Control Priority Bacterial Infections in Swine Virus and Prion Research Unit
USDA, Agricultural Research Service, National Animal Disease Center
Ames, IA

Susan Brockmeier obtained her D.V.M. from the University of Missouri-Columbia. After spending several years in private practice, she acquired a Ph.D. in Veterinary Microbiology from Iowa State University. She then joined the research team at the National Animal Disease Center in Ames, Iowa, part of the Agricultural Research Service, which is the research branch of the USDA. Her research focuses on infectious diseases of swine including both viral and bacterial pathogens. With the disease models she has developed, she studies virulence mechanisms and pathogenesis, innate and adaptive immune responses, biotherapeutic interventions, and vaccine development and efficacy of common swine pathogens.

Dr. Ulrich Schwaneberg
Chair of Biotechnology RWTH Aachen University
Member of Scientific Board of Directors DWI-Leibniz Institute for Interactive Materials
Aachen, Germany

Ulrich Schwaneberg graduated at the University of Stuttgart in the Institute of Technical Biochemistry in 1999. After a post-doc at Caltech (USA) he was in January 2002, he was appointed as Assistant Professor at the Jacobs University Bremen and promoted in 2006. In January 2009, he moved to the RWTH Aachen University as Chair of the Institute of Biotechnology and since 2010 he is co-appointed in the Scientific Board of Director at the DWI Leibniz Institute for Interactive Materials. Furthermore he has been appointed in the board of directors in the Bioeconomy Science Center to advance and to focus NRW's (local government) research efforts in the area of bioeconomy and servers as speaker of the industry lab HICAST (Henkel Innovation Campus for Advanced and Sustainable Technologies) at RWTH Aachen University. In 2008 he founded with coworkers the startup company SeSaM-Biotech GmbH, which commercialized developed mutagenesis technologies and four reengineered enzymes are in industrial use. He has a special interest in method development and tailoring of proteins for industrial applications, hybrid catalysis, and interactive biomaterials. In 2016 he received from the BMBF the prestigious award for the next generation of biotechnological processes (1.5 Mio€), published >180 manuscripts and is an inventor/coinventor in 18 patent families.

Gregory LeFevre, Ph.D.
Assistant Professor
Department of Civil and Environmental Engineering
College of Engineering
The University of Iowa
Iowa City, IA



Greg LeFevre is an Assistant Professor in Civil & Environmental Engineering at the University of Iowa where he started in 2016. He received his BS from Michigan Tech, MS and PhD from the University of Minnesota, and Postdoc from Stanford University, all in environmental engineering. Broadly, his research focuses on understanding mechanisms related to the biotransformation of emerging organic contaminants in the environment and engineered water systems. LeFevre has worked with bacterial, fungal, and phyto- transformation and applies a combination of analytical chemical and biological techniques to elucidate novel contaminant transformation products and pathways (for example, high resolution mass spectrometry). The LeFevre Lab's research integrates well with the CBB by illuminating and exploiting natural enzymatic processes to remediate pollution and develop pioneering, scalable coupling approaches to biological and abiotic catalyzed redox-based water treatment processes.

Jacob Poliskey
Ph.D. Candidate
Kevin G. Rice Research Group
Department of Pharmaceutical Sciences and Experimental Therapeutics
Division of Medicinal and Natural products Chemistry
College of Pharmacy
The University of Iowa
Iowa City, IA

Originally from Michigan, Jacob attended Alma College and earned a Bachelor of Science in Biochemistry in 2014. After graduation, he came to the University of Iowa to obtain his Ph.D. in Medicinal and Natural Products Chemistry from the College of Pharmacy. Jacob joined the laboratory of Kevin Rice and has explored different aspects of non-viral delivery of nucleic acids. He has focused on utilizing mRNA for gene delivery by modifying its structure for improved stability and translatability. He plans to pursue his M.D. from the University of Michigan after graduating from Iowa.

Anh-Vu Do
Ph.D. Candidate
Aliasger K. Salem Research Group
Department of Chemical and Biochemical Engineering
College of Engineering
The University of Iowa
Iowa City, IA

Anh-Vu came to the United States at the age of six and grew up in Atlanta, Georgia. He has received his B.S. Degrees in Psychology and Biology as well as a B.S.Bch.E.in Biochemical Engineering from the University of Georgia. He is currently pursuing a PhD in Chemical and Biochemical Engineering under the supervision of Prof. Aliasger Salem at the University of Iowa. His research interests include 3D printing and controlled drug delivery, specifically in conjunction with designing functional and novel 3D printed devices for tissue engineering and cancer vaccinations. Post-graduation, Anh-Vu intends to pursue a career in device development with the focus on drug delivery.

Nitija Tiwari
Ph.D. Candidate
Ernesto J. Fuentes Research Group
Department of Biochemistry
Roy J. and Lucille A. Carver College of Medicine
The University of Iowa
Iowa City, IA

I am originally from Kathmandu, Nepal and came to the United States to pursue my undergraduate degree in 2008. I completed my Bachelors of Science in Biochemistry with honors from Loras College in Dubuque, Iowa in May 2012. Then I worked as a research assistant in Dr. Ernesto Fuentes's lab for two years. During this time, I worked on a collaboration project with Dr. John Kirby's lab in microbiology studying protein-protein interactions in bacterial two-component signal transduction systems. In 2014, I joined the department of biochemistry as a graduate student and eventually joined Dr. Fuentes's lab for my graduate work. My thesis work involves studying regulatory mechanisms of *S. aureus* two-component system SrrAB using biochemical and structural approaches.

Oral Presentation Abstracts

Presenter 1

GENE ACTIVATED MATRICES FOR BONE REGENERATION

Aliasger K. Salem, Ph.D.
Bighley Chair of Pharmaceutical Sciences and Head of the Division of Pharmaceutics and Translational Therapeutics (PTT), College of Pharmacy, The University of Iowa, Iowa City, IA

There is a major need to promote regeneration of musculoskeletal tissues such as the bone and cartilage. Approaches to regenerating musculoskeletal tissues include the use of programmed stem cells, growth factors, gene therapies, and scaffold matrix materials. These approaches are being developed as standalone therapeutic strategies and in combination.

With respect to gene therapy applications, it is critical that clinically beneficial amounts of proteins are synthesized endogenously within and around the lesion in a sustained manner. By implanting gene-activated matrices (GAMs) in animal models, we have shown that sustained gene expression and continuous osteogenic protein production in situ can be achieved in a way that stimulates osteogenesis and bone repair within osseous defects. We have also shown that critical parameters substantially affecting the therapeutic efficacy of gene therapy include the choice of osteogenic transgene(s), selection of vectors, the scaffold material, the wound environment, and the selection of delivery strategies. For example, we have developed a non-viral gene delivery system for bone regeneration utilizing a collagen scaffold to locally deliver complexes encoding for platelet-derived growth factors (PDGF-B). The in vivo regenerative capacity of the system was assessed in 5-mm diameter critical-sized calvarial defects in rats. In vivo studies showed significantly higher new bone volume/total volume in calvarial defects treated with GAMs following 4 weeks of implantation (14- and 44-fold higher) when compared to empty defects or empty scaffolds, respectively. Together, these findings suggest that non-viral gene-activated scaffolds are effective for bone regeneration and are an attractive therapeutic strategy with significant potential for clinical translation. Furthermore, simultaneous delivery of multiple genes is possible and customization is relatively straightforward. For example, we have shown that co-delivering genes encoding fibroblast growth factor (FGF-2) and bone morphogenetic protein-2 (BMP-2) significantly enhances osteogenesis in human adipose-derived mesenchymal stem cells (hADMSC) over either gene alone. We also show that this combination of genes is effective at regenerating bone in a diaphyseal long bone radial defect diabetic rabbit model. More recently, we have been investigating the bone regenerative capacity of chemically modified ribonucleic acid (cmRNA). The osteogenic potential of BMSCs treated with cmRNA encoding for BMP-2 was validated by the enhanced expression of the bone-specific genes, osteocalcin and alkaline phosphatase as well as through the promotion of bone matrix deposition in vitro. In addition, using a calvarial bone defect model in rats, we have shown that cmRNA (encoding BMP-2)-activated matrices promoted significantly enhanced bone regeneration compared to plasmid DNA (BMP-2)-activated matrices.

Presenter 2

DEVELOPMENT OF A THERMOSTABLE NANOEMULSION ADJUVANTED VACCINE AGAINST TUBERCULOSIS

Ryan M. Kramer**,** Ph.D.
Senior Scientist, Process and Product Development Manager, Infectious Disease Research Institute (IDRI), Seattle WA

Words: Adjuvant, Lyophilization, Tuberculosis, Formulation Development

Next-generation rationally-designed vaccine adjuvants represent a significant breakthrough to enable development of vaccines against challenging diseases including tuberculosis, HIV, and malaria. New vaccine candidates often require maintenance of a cold-chain process to ensure long-term stability and separate vialing to enable bedside mixing of antigen and adjuvant. This presents a significant financial and technological barrier to worldwide implementation of such vaccines. Herein we describe the development of a single-vial lyophilized thermostable tuberculosis vaccine comprised of an antigen (ID93) and an oil-in water emulsion adjuvant (GLA-SE), using a design of experiment (DoE) approach. Stabilizing excipients were identified, and the effect of various factors were evaluated to determine optimized formulations that minimized GLA and ID93 degradation, particle size growth, and pH change, while optimizing cake quality. Formulations were identified that are stable at elevated temperatures. Further this vaccine retains the ability to elicit both antibody and TH1 responses against the vaccine antigen and protect against experimental challenge with Mycobacterium tuberculosis. These results represent a significant breakthrough in the development of vaccine candidates that can be implemented throughout the world without being hampered by the necessity of a continuous cold chain or separate adjuvant and antigen vials.

Presenter 3

RATIONAL DEVELOPMENT OF VETERINARY VACCINES AGAINST SWINE PATHOGENS

Susan Brockmeier, D.V.M., Ph.D.
Research Veterinary Medical Officer: USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA

Vaccine development against common veterinary pathogens has traditionally been via inactivation or passaged attenuation of viruses and use of whole cell bacterins. This method of vaccine development has not been successful against many common swine pathogens due to rapid evolution of some of the viruses and the existence of many serotypes and high genotypic variability of some bacterial pathogens. In addition, there is an emphasis in veterinary medicine on development of DIVA (Differentiation of Infected from Vaccinated Animals) vaccines to immunologically differentiate infected from vaccinated animals. Thus new strategies based on rational design using an understanding of the virulence, pathogenesis, and the host immune response of each pathogen is necessary to improve the next generation of vaccines. We have used various strategies such as functional genomic screens, immunoproteomics, and rational attenuation to develop and test potential vaccine candidates against common swine pathogens. For example, we have selected protein candidates for inclusion in bacterial vaccines by identifying fitness genes through a functional genomics screen, selecting predicted surface-associated proteins, and identifying proteins conserved across isolates to enhance the prospect of cross-protection. Immunoproteomic methods are being used to detect proteins that are reactive with antisera from pigs that are more broadly protected compared to antisera from pigs only protected against homologous challenge in order to identify potential targets responsible for heterologous protection. Mutants in identified virulence factors are also being tested for attenuation and possible use as live-attenuated vaccines. Through this rational approach we have identified several promising vaccine candidates against important swine diseases.

Presenter 4

EIGHTEEN YEARS OF DIRECTED P450 BM3 EVOLUTION: LESSONS AND SUCCESS STORIES

Dr. Ulrich Schwaneberg
Faculty of Mathematics, Computer Science and Natural Science; RWTH Aachen University and DWI-Leibniz Institute for Interactive Materials, Aachen, NRW, Germany

Directed evolution of biocatalysts has become a widely accepted and broadly applied method in academia and industries. On the example of monooxygenase P450 BM3, basic concepts, challenges in diversity generation and screening as well as success stories of P450 BM3 reengineering will be presented[(1)-(6)]. The latter includes novel screening systems in microtiter plate and flow cytometry format as well as P450 BM3 reengineering for improved organic cosolvent resistance, mediated electron transfer, inverted enantioselectivity, broaden substrate specificity, and implementation in cascade reactions.

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Presenter 5

BIOCATALYSTS IN CONTAMINANT TRANSFORMATION: BECAUSE CLEAN WATER IS PRESSING SOCIETAL PROBLEM

Gregory LeFevre, Ph.D.
Assistant Professor, Department of Civil and Environmental and Engineering, College of Engineering, The University of Iowa, Iowa City, IA

Society is facing a tipping point in terms of availability of clean water for drinking and irrigation, forcing increased recycling and use of less pristine sources that often contain contaminants of emerging concern. Biological processes impact the fate and transformation of contaminants, and can also generate unanticipated transformation products. Many of these transformation products retain some biological activity or may even be more toxic to human consumers. Understanding the role of biocatalysts in transforming contaminants of emerging concern and the products formed is thus critical to protecting environmental and human health. In this talk, two topics will be presented with the overarching theme of biologically mediated transformation of emerging contaminants into novel metabolites.

First, the uptake and plant transformation of the industrial anticorrosive compound benzotriazole will be presented. We discovered that benzotriazole is rapidly assimilated into the tryptophan biosynthesis pathway, acting as an indole mimic, and yields weak synthetic structural analogues of plant hormones. This reveals that the enzymes responsible for tryptophan synthesis *in planta* are more promiscuous than previously believed, and is now thought to relate to other amino acid conjugation reactions subsequently discovered. Proteomics, however, demonstrated that the synthetic tryptophan was not integrated into higher plant proteins. Following synthesis of novel compounds, we discovered previously unmeasurable benzotriazole metabolites in strawberry and lettuce crops irrigated with recycled water.

Second, the concept of exploiting coupled microbial and abiotic reactions for contaminant transformation on electrochemically active black carbon surfaces will be presented. This work is being developed to enhance the electron transfer efficiency of biofilms in water treatment processes for highly soluble pesticides. We envision scaling applications and integration of novel materials as a platform technology for biofilm-based pollutant capture and biocatalyst delivery. Motivation, early work, and context will be presented.

Presenter 6

DOUBLE STRANDED *m*RNA POLYPLEXES FOR LIVER TRANSFECTION IN VIVO

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

In vivo transfection of liver hepatocytes using *m*RNA potentially offers an advantage over delivering plasmid DNA because it eliminates the need for *m*RNA delivery to the nucleus. However, the metabolic instability of *m*RNA in the circulation limits the potency of *m*RNA nanoparticle gene delivery systems. Here we find that hybridization of *m*RNA with a complementary reverse strand *m*RNA results in a novel double stranded (ds) *m*RNA possessing dramatically increased serum stability. Hydrodynamic dosing of ds *m*RNA nanoparticles demonstrates equivalent translational efficiency to single stranded (ss) *m*RNA nanoparticles. Optimal ds *m*RNA mediated gene expression in liver was achieved by tailoring the length of the reverse *m*RNA strand. The circulatory stability of ds *m*RNA nanoparticles was also found to be significantly greater than ss *m*RNA nanoparticles as determined by gene expression in the liver. The development of this novel form of metabolically stabilized and translationally active ds *m*RNA provides a new platform to advance applications of *m*RNA nanoparticle delivery to achieve gene expression.

Presenter 7

COMBINING ULTRASOUND AND INTRATUMORAL ADMINISTRATION OF DOXORUBICIN-LOADED MICROPARTICLES TO ENHANCE TUMOR CELL KILLING

Anh-Vu Do[1], Sean M. Geary[1], Dongrim Seol[2], Phillip Tobias[1], Daniel Carlsen[1], Nattawut Leelakanok[1], James Martin[2], and Aliasger K. Salem[1]*

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Metastatic melanoma is currently an incurable disease for which alternative treatments to chemotherapy alone are sought. In this study, using a melanoma model, we investigated the antitumor potential of combining ultrasound (US) with poly(lactic-co-glycolic acid) (PLGA) microparticles loaded with doxorubicin (DOX). The aim was to achieve synergistic tumoricidal activity through direct and indirect US-mediated damage of tumor cells combined with sustained and potentially controllable release (when combined with US) of DOX from microparticles. DOX is known to not only directly kill tumor cells but to also induce an immunogenic form of cell death therefore potentially increasing the overall cytotoxicity of the treatment. An *in vitro* release assay demonstrated an ability of US to affect the release kinetics of DOX from DOX-loaded PLGA particles by inducing a 27% increase in rate of release. *In vitro* viability assays demonstrated that combining US with DOX-loaded PLGA microparticles caused greater tumor cell (B16-F10 melanoma cells) killing (76%) compared to US alone (23%), DOX-loaded microparticles alone (29%) or blank microparticles plus US (54%). Melanoma bearing mice were treated intratumorally with DOX (8 μg)-loaded microparticles and subjected to US treatment at the tumor site. This treatment proved to be significantly better at extending survival (mean survival (MS) = 22.1 days) compared to untreated mice (MS = 10.4 days) and most other treatments, such as blank microparticles plus US (MS = 11.5 days) and DOX (8 μg)-loaded microparticles alone (MS = 13 days). The findings that immune checkpoint blockade did not significantly extend survival of mice treated with DOX (8 μg)-loaded microparticles plus US, and that tumor-free ("cured") mice were not protected from subsequent tumor rechallenge suggests minimal involvement of the adaptive immune response in the observed antitumor activity. Nevertheless, the synergistic increase in survival of melanoma-challenged mice treated with the combination of US and DOX-loaded microparticles implicates such a treatment methodology as a promising additional tool for combatting otherwise currently incurable cancers.

Presenter 8

REGULATION OF THE *S. aureus* SrrB HISTIDINE KINASE BY A PAS DOMAIN

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Antibiotic resistant *Staphylococcal aureus,* commonly known as Methicillin-Resistant *Staphylococcus aureus* (MRSA) are a major cause of hospital infections. Thus, there is an urgent need for identifying novel targets/mechanisms for antibiotic development. Bacterial two-component systems (TCS) contribute to infections by regulating toxin production, antibiotic resistance and survival. TCSs are composed of a membrane bound histidine kinase (HK) and a cytoplasmic response regulator (RR). The HK senses extracellular stimuli through its extracellular sensor domain and undergoes autophosphorylation at a conserved histidine residue in the cytoplasmic kinase domain. The phosphorylated HK then transfers the phosphoryl group to a conserved aspartate residue in the RR, which in turn binds DNA to control gene expression. SrrAB TCS is a regulator of *S. aureus* virulence factors including toxic shock syndrome toxin-1 (TSST-1), which causes toxic shock syndrome. SrrB is a dual function kinase/phosphatase capable of regulating the level of phosphorylation of SrrA. SrrB is composed of an extracellular Cache domain and several cytoplasmic domains, including a HAMP, PAS and DHp-CA catalytic domains. Here, we present enzyme kinetics data showing that the PAS domain effects SrrB kinase and phosphatase function. Our data indicates that the PAS domain increases the rate of autophosphorylation and has an inhibitory effect on SrrB phosphatase activity. In addition, we used SAXS and ensemble modeling to show that the PAS and catalytic ATP binding (CA) domain of SrrB are flexible, suggesting a mechanism for SrrB regulation. Moreover, we identified heme as a ligand for the PAS domain, which could be a potential secondary mechanism of regulating the SrrAB TCS. The crystal structure of the apo PAS domain shows a putative binding pocket, which provides insights into potential residues involved in heme binding. Design of PAS mutants deficient in heme-binding for biochemical and biological analyses is ongoing. Together, our data suggest the PAS domain is critical for determining the balance of kinase and phosphatase activity and that heme binding may be involved in redox regulation of the SrrAB TCS.

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Poster Abstracts

Poster 1

NOVEL TYPE-II TOPOISOMERASE INHIBITORS FOR OVERCOMING FLUOROQUINOLONE-MEDIATED BACTERIAL RESISTANCE

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Fluoroquinolones are a class of antibiotics used clinically to treat a wide array of bacterial infections. Fluoroquinolones act by forming a ternary complex with bacterial type II topoisomerases (DNA gyrase or topoisomerase IV) and nicked DNA; religation of DNA is subsequently blocked. In ternary complex the keto-acid moiety of the fluoroquinolone is complexed with a divalent magnesium ion, forming a drug-magnesium-water bridge to a serine and/or an aspartate (or glutamate) residue on helix-4 of the topoisomerase enzyme. Mutation-mediated resistance arises through substitution of the serine or aspartate/glutamate residues, therefore preventing formation of the magnesium-water bridge and reducing stability of the cleaved complex. Quinazoline-2,4-diones (diones) are structurally similar to fluoroquinolones; diones form ternary complex similar to fluoroquinolones, however, these complexes are less stable because the quinazoline-2,4-diones do not contain the keto-acid moiety and therefore do not form a magnesium-water bridge to helix-4. While diones are therefore less potent antibiotics, their non-reliance on the magnesium water bridge generally affords equipotent activity with wild-type and fluoroquinolone-resistant strains of bacteria. We hypothesized that the quinazoline-2,4-dione structural scaffold, and crystal structure, provide preliminary structural data to generate novel inhibitors of bacterial type-II topoisomerases that act on wild-type and fluoroquinolone resistant bacteria. In this presentation, the design and synthesis of novel quinazoline-2,4-dione derivatives expected to have additional binding contacts in ternary complex will be discussed.

Poster 2

HOMOLOGOUS RECOMBINATION INITIATION AND CONTROL VIA RAD51-DNA SPECIFICITY

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Homologous recombination is a vital process for maintaining genome stability. This process is essential when the cell encounters double-strand breaks during replication. During homologous recombination RAD51 recombinase binds single-strand DNA, forming a nucleoprotein filament. Strand invasion is initiated, displacing the nascent complimentary strand of nearby double-strand DNA and the RAD51 coated ssDNA is brought into proximity of the newly single-stranded DNA. A homology search, for complimentary DNA, is carried out and recombination proceeds when the D-loop is formed. Without RAD51, persistent double-strand breaks are formed, and the cell is marked for death, apoptosis. We use single molecule total internal reflection microscopy techniques to examine the ability of RAD51 to form nucleoprotein filaments on the many DNA structures that are found in the cell, such as collapsed replication forks, resected double-strand DNA, and D-loops, with the goal of determining if RAD51 has preference for certain DNA structures, that point to a mechanism of controlling homologous recombination.

Poster 3

EFFICIENT AND ACCURATE SAMPLING OF THE THERMODYNAMIC PATH BETWEEN MOLECULAR STATES USING A GPU ACCELERATED HYBRID MOLECULAR DYNAMICS ALGORITHM

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Efficient and accurate calculation of free energy differences between two atomic resolution molecular states by sampling the thermodynamic path between them is of central importance to predicting the effect of missense mutations and to designing new therapeutics. In the former case, the change in folding stability of a protein due to an amino acid change can explain disease phenotypes, while in the latter case the relative binding affinity of ligands to a protein can inform a lead optimization campaign. A prominent hurdle in the exploration of the thermodynamic path between states of interest has been the large computational expense to converge ensemble averages (especially for large systems). Here we introduce a novel algorithm that takes advantage of the processing power of GPU coprocessors to dramatically accelerate the energy and force evaluations associated with molecular dynamics simulations, while the synchronization between processes and thermodynamic integration are executed on CPU cores. This hybrid approach combines the molecular dynamics packages Force Field X (FFX) and OpenMM to offer a sharp increase in computational speed and faster convergence of the free energy landscape (i.e. the ensemble average thermodynamic force along the path between states). For instance, for a 5000 atom system, the amount of time needed to simulate a 100 step molecular dynamics trajectory (at 1 femtosecond timesteps) on the normal FFX software is roughly 15 seconds. The same molecular dynamics trajectory finishes running in .44 seconds using the new hybrid method resulting in a 35 times speed up of simulation power. Ultimately this new hybrid algorithm will cause a decrease in turn-around time for the investigation of computational biophysical hypotheses such as the degree of pathogenicity missense mutations have on the folding stability of Connexon 26, a protein that plays a central role in the transduction of sound. Changes in free energy, between wild type and mutated protein states, on the order of 1 kcal/mol indicates destabilization of the native folding of the protein that can impair protein function that ultimately leads to hearing loss. The use of computational biophysics techniques to model protein systems offers a quantitative method towards predicting the pathogenic affects a variant can have on protein function. The quantitative computational approach adds a complementary layer to the qualitative prediction methods employed by bioinformatics studies with the aim of creating a robust protocol to predict the role protein variants have in diseases. (Supported in part by NIDCD R01 DC012049)

Poster 4

EFFECT OF THERMAL SHOCK ON *PSEUDOMONAS AERUGINOSA* BIOFILM

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When bacteria colonize a medical implant surface, they form a biofilm which cannot be eradicated chemically. The current standard of care is surgical explantation of the device and surrounding tissue, with eventual reimplantation of a replacement device with twice the probability of infection. These infections are a $5 billion problem in the U.S. alone, impacting over 100,000 patients annually. By applying a localized thermal shock, we have shown that these biofilms can be eliminated, though the mechanism of elimination has been unclear. In the presence of antibiotics which have little effect on the biofilm by themselves, biofilms are eliminated by heat shocks which are too mild to have any noticeable effect in the absence of antibiotics. One hypothesis for this synergistic efficacy is that the heat shock triggers a change in bacterial phenotype, prompting the bacteria to disperse from the biofilm in the planktonic state, and thereby making them susceptible to antibiotic elimination.

Reincubation studies, however, indicate that biofilm elimination by thermal shock is not instantaneous. Biofilms were cultured to a steady-state population density of 10^7 colony forming units (CFU) per cm^2 and subjected to heat shocks of varying intensity (60, 70, or 80 °C for 1, 5, 7.5, or 30 min). Their population density was quantified immediately following thermal shock, demonstrating populations reductions ranging from none (no statistically significant reduction) after mild heat shock to complete (no viable bacteria detected) after aggressive heat shocks. Reincubation of thermally shocked biofilms for 2, 4, 12, 24 and 96 hours followed by resuspension and plating showed a bimodal response. Following heat shock, biofilms slowly regrew, eventually reaching their original 10^7 CFU/cm^2 population density—if the population density immediately following these heat shocks was typically 10^3 CFU/cm^2 or above. However, if the population density immediately following these heat shocks was typically below 10^3 CFU/cm^2 (yet clearly detectable and quantifiable), the biofilms would continue dying off, with no viable CFU after a few hours, despite being able to form colonies when immediately resuspended and plated. This supports an opposing hypothesis that the bacteria do not flee the biofilm, but rather die in place, leaving a matrix full of toxic enzymes that proceed to kill off the surviving bacteria. These hypotheses are further examined by applying thermal shock in a flow cell system in which any fleeing bacteria are collected in the flowing fluid and quantified as a function of time.

Poster 5

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL ANTIMALARIALS TARGETING APICOPLAST DNA POLYMERASE (apPOL) FROM *P. FALCIPARUM*

Pratik Rajesh Chheda and Robert J. Kerns*
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Plasmodium spp. are the causative agents of malaria, killing nearly 600,000 people each year. Resistance of *Plasmodium* to chloroquine, artemisinin-based therapies as well as other antimalarial agents accentuates the need for new drugs that target novel aspects of the parasite's biology. Nearly all parasites in the phylum *Apicocomplexa* have an unusual organelle called the apicoplast, which is a relic chloroplast, acquired through a secondary endosymbiotic event with algae. It participates in the biosynthesis of fatty acids, heme, iron-sulfur clusters, and isoprenoids and any defect in apicoplast metabolism or failure of the apicoplast to replicate and divide leads to the death of the organism. Additionally, lack of a human counterpart to the apicoplast makes apicoplast functions and enzymes promising drug targets. The 35-kb genome of apicoplast is replicated by select DNA replication enzymes of which the apicoplast DNA polymerase (apPOL) is unique to the parasite. The apPOLs from *P. falciparum* and *P. vivax* have 84% homology, while the most similar human DNA polymerases are the lesion bypass polymerases theta and nu (23 and 22% identity, respectively). This suggests that drugs targeted against the *Pf*-apPOL would also be effective in treating *P. vivax* infections with low human toxicity. Towards identifying inhibitors of apPOL, a high throughput screen of 400 compounds from the Open Malaria Box provided by Medicines for Malaria Ventures identified an inhibitor of apPOL with an IC_{50} of 0.8 ± 0.3 μM (MMV666123, Fig. 1). Preliminary studies indicate that MMV666123 is specific for apPOL, with no inhibition of human DNA Pol or *E. coli* DNA Pol I. Also, MMV666123 inhibits the polymerase activity of apPOL but not its exonuclease activity, suggesting binding to the C-terminal polymerase domain of apPOL. This is supported by initial docking studies of MMV666123 onto apPOL X-ray crystal structure (PDB: **5DKT and 5DKU**). Additionally, being from the malaria box substantiates anti-malarial activity of MMV666123. Presented here are our current design, synthesis and *in vitro* evaluation efforts toward understanding the structural requirements of MMV6661213 for inhibition of apPOL and identifying more potent and drug-like apPOL inhibitor derivatives.

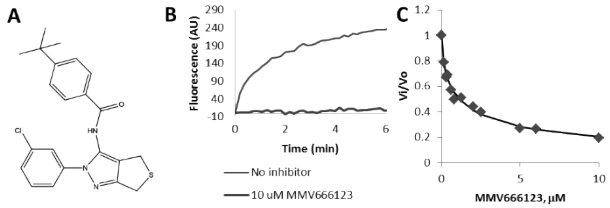

Figure 1. *A)* Structure of MMV666123. *B)* Effect of MMV666123 on apPOL activity as measured with the HT substrate. *C)* Dose/Response curve showing IC_{50} of 0.8 μM.

Poster 6

IDENTIFICATION OF MODIFIED-HEPARIN DERIVATIVES AS NOVEL DIRECT ALLOSTERIC INHIBITORS OF THROMBIN

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Regulation of thrombin commands a prominent interest in the medical community because it is a key enzyme involved in coagulation, being an important target for preventing thrombotic disorders such as myocardial infarction, venous throm-boembolism and stroke. Thrombin is a plasma serine protease that catalyzes the conversion of fibrinogen to fibrin, which together with platelets forms hemostatic clots. Heparin, a widely used anticoagulant, catalyzes inhibition of thrombin indirectly by forming a ternary complex with thrombin and antithrombin (AT), thus activating AT and facilitating thrombin inhibition. However, clinical utility of heparin is plagued by significant adverse reactions, most of which are associated with the chemical nature of heparin itself. Heparin is a heterogeneous polysulfated polysaccharide that nonselectively bind many glycosaminoglycan-binding proteins, resulting in considerable off-target effects. Based on the molecular interactions observed in heparin-protein binding, it is hypothesized that charge-reduced modified heparin derivatives substituted with aromatic moieties in place of the N-sulfo or carboxylate groups will exhibit improved protein binding specificity, particularly direct binding and direct inhibition of heparin-binding proteases, offering a novel type of anticoagulant agent. Considering this initial hypothesis, a series of charge-reduced heparin derivatives was synthesized and each derivative was evaluated for activity towards direct inhibition of thrombin. Modified-heparin derivatives possessing 3-(4-hydroxyphenyl)propionate moieties in place of N-sulfo groups were found to be potent, dose-dependent, direct inhibitors of thrombin. Tridimensional distribution of negative charges or degree of sulfation played a secondary role for inhibition. Initial velocity studies revealed these heparin derivatives are allosteric thrombin inhibitors. Cell viability studies demonstrated that at concentrations as high as 100-1000 fold the IC_{50} values, the modified-heparin derivatives evaluated displayed a remarkable percentage of cell survival. Overall, the results of this study indicate that non-selective ionic interactions between anionic groups on heparin and cationic amino acid clusters within heparin-binding proteins are being effectively replaced by more selective interactions with select N-arylacyl groups that have been introduced into modified-heparin derivatives. These novel N-desulfonated/N-acylated heparin derivatives were successfully identified as lead structures for further optimization towards the development of new allosteric inhibitors of thrombin as anticoagulant agents.

Poster 7

THE EFFECT OF VARIOUS NITROGEN SOURCES ON THE EXPRESSION OF ATRAZINE-DEGRADING GENES IN *PSEUDOMONAS SP.* ADP

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Atrazine is an herbicide used throughout the midwestern United States to control the growth of broad leaved and grassy weeds. The maximum contaminant level set by the Environmental Protection Agency is 3 parts per billion of atrazine in drinking water. Elevated levels of atrazine present in drinking water poses a human health risk, as atrazine is classified as an endocrine disruptor compound by U.S. regulatory agencies. Bioremediation, the method of degrading recalcitrant compounds in the environment by microbial action, is considered an emerging, environmentally conscience, and cost-effective strategy to pollutant removal. *Pseudomonas* sp. ADP, a bacterial strain isolated from an atrazine-spill site, is capable of degrading atrazine in six enzymatic steps via a catabolic pathway. Studies on the degradation efficiency and expression of the genes encoding for the enzymatic pathway have been performed using planktonic *Pseudomonas* sp. ADP cells, however, the cells have not been extensively characterized in the biofilm mode of growth. Using reverse transcription quantitative real-time PCR, we evaluated the expression of the genes involved in the catabolic pathway of atrazine degradation in the planktonic cell and biofilm cell mode of growth. In addition, we evaluated the expression of the genes in the pathway from *Pseudomonas* sp. ADP cells grown on different nitrogen sources. This was done to determine if the expression of the atrazine-degrading genes were downregulated or upregulated in response to the availability of nitrogen from various sources in the natural environment in addition to atrazine. The results of our gene expression studies aid in the development of biofilm-based bioremediation and the optimal conditions for the bacteria to thrive in for a continuous degradation of atrazine.

Poster 8

NOVEL BIOLOGICAL ACTIVITY OF FLUOROQUINOLONES

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Fluoroquinolones are traditionally broad spectrum antibiotics that are used clinically to treat a variety of bacterial infections. Fluoroquinolone antibiotics selectively inhibit bacterial type II topoisomerases, DNA gyrase and/or topoisomerase IV. Type II topoisomerases regulate the topology of DNA and are necessary for replication and transcription. Clinically used fluoroquinolones fall into a class of compounds termed topoisomerase poisons. Fluoroquinolones poison topoisomerase activity by forming a stable drug-topoisomerase-DNA cleavage complex, preventing re-ligation of DNA. This leads to an increase in double strand breaks and eventually cell death. In an effort to overcome bacterial resistance to fluoroquinolones novel fluoroquinolone derivatives were designed to form new binding contacts with the bacterial type II topoisomerases. By crystallographic investigation, the N-1 and C-7 positions of fluoroquinolones were identified as targets for structural modifications. These positions were chosen because of their potential to acquire additional binding contacts not utilized in current fluoroquinolone binding. During this study, it was found that incorporation of certain aryl groups at the N-1 position of the fluoroquinolone core resulted in loss of activity toward the bacterial enzyme. However, these novel N-1 aryl fluoroquinolones were found cross over and inhibit, but not poison, human topoisomerase I and II and therefore inhibited enzyme topoisomerase activity without creating DNA breaks. Human topoisomerase I and II are well established targets for several clinically used chemotherapeutics including epipodophyllotoxins, camptohecins, and anthracyclines. A common therapeutic issue with many topoisomerase targeting anticancer drugs is secondary malignancies. One potential cause of secondary malignancy stems from the genomic damage that occurs due to the poisoning mechanism of these compounds. Interrogation of structural requirements for activity, improvement of biophysical properties, and efficacy of these novel N-1 aryl fluoroquinolones as anticancer agents is presented here.

Poster 9

DESIGN, SYNTHESIS, AND CHARACTERIZATION OF A MODULAR LIVERTARGETING STRATEGY FOR NON-VIRAL GENE DELIVERY

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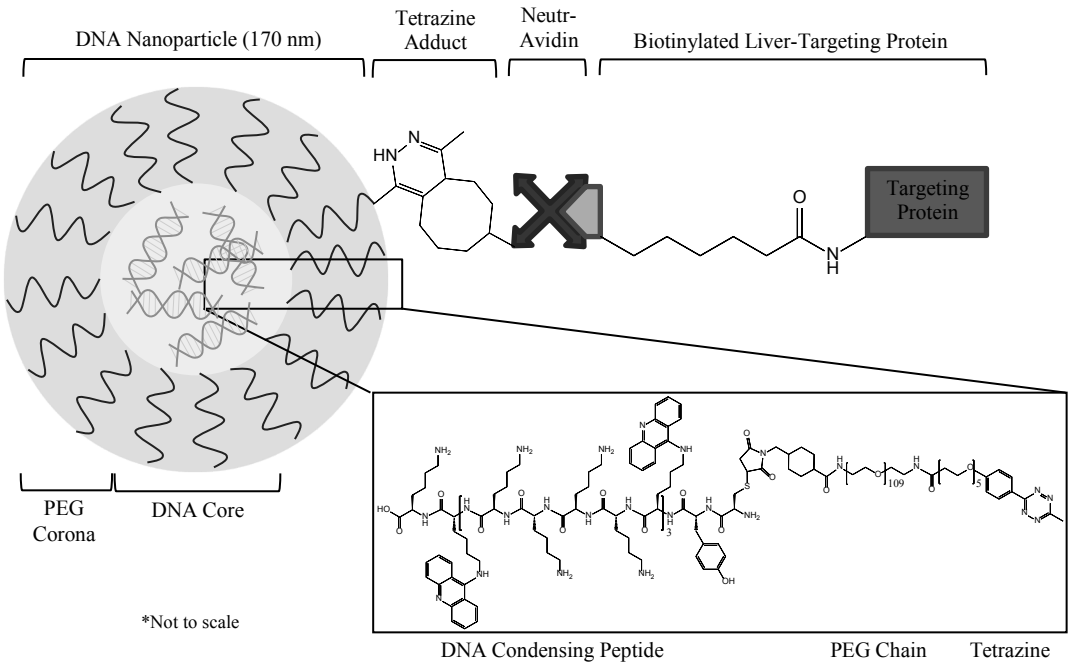
Non-viral gene delivery to the liver faces many challenges including maintaining DNA stability in circulation, avoiding macrophage uptake, and selectively inducing hepatocyte gene expression. We have previously developed a plasmid DNA carrier system utilizing a PEGylated polylysine-acridine peptide capable of condensing DNA into nanoparticles. These nanoparticles exhibit circulatory stability and hepatocyte uptake and gene expression in mice under hydrodynamic dosing, but lack intrinsic hepatocyte targeting. The present study reports on the use of tetrazine ligation "click" chemistry to append NeutrAvidin to the surface of nanoparticles as a modular system for attachment of biotinylated targeting proteins. To furnish a tetrazine ligation handle, a heterobifunctional 5-kDa PEG was synthesized containing the tetrazine moiety on one end and a maleimide on the other for attachment to the DNA condensing peptide. The counterpart, *trans*-cyclooctene (TCO) labeled NeutrAvidin, was constructed using standard N-hydroxysuccinimide (NHS) ester coupling. SDS-PAGE verified the construction of NeutrAvidin-labeled nanoparticles, and DNA condensation assays demonstrated efficient compaction of DNA into nanoparticles in the presence of bound NeutrAvidin. The sizes of NeutrAvidin-labeled nanoparticles as measured by dynamic light scattering (DLS) were found to be dependent on the mole percentage of tetrazine in the nanoparticles as well as the extent of TCO modification of NeutrAvidin. Additionally, NeutrAvidin-nanoparticle sizes were altered upon addition of the biotinylated targeting proteins apoliprotein E, *Sambucus nigra* lectin (sialic acid specific), and *Erythrina cristagalli* lectin (galactose specific), indicating successful formation of the targeted nanoparticles. Further work aims to determine the targeting efficiency of the constructs using HepG2-based binding assays and *in vivo* gene expression and pharmacokinetic experiments in mice. This novel strategy provides a modular platform for rapidly testing targeting ligands and optimizing targeted non-viral gene delivery systems.



Poster 10

COMBINING ULTRASOUND AND INTRATUMORAL ADMINISTRATION OF DOXORUBICIN-LOADED MICROPARTICLES TO ENHANCE TUMOR CELL KILLING

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Metastatic melanoma is currently an incurable disease for which alternative treatments to chemotherapy alone are sought. In this study, using a melanoma model, we investigated the antitumor potential of combining ultrasound (US) with poly(lactic-co-glycolic acid) (PLGA) microparticles loaded with doxorubicin (DOX). The aim was to achieve synergistic tumoricidal activity through d132irect and indirect US-mediated damage of tumor cells combined with sustained and potentially controllable release (when combined with US) of DOX from microparticles. DOX is known to not only directly kill tumor cells but to also induce an immunogenic form of cell death therefore potentially increasing the overall cytotoxicity of the treatment. An *in vitro* release assay demonstrated an ability of US to affect the release kinetics of DOX from DOX-loaded PLGA particles by inducing a 27% increase in rate of release. *In vitro* viability assays demonstrated that combining US with DOX-loaded PLGA microparticles caused greater tumor cell (B16-F10 melanoma cells) killing (76%) compared to US alone (23%), DOX-loaded microparticles alone (29%) or blank microparticles plus US (54%). Melanoma bearing mice were treated intratumorally with DOX (8 μg)-loaded microparticles and subjected to US treatment at the tumor site. This treatment proved to be significantly better at extending survival (mean survival (MS) = 22.1 days) compared to untreated mice (MS = 10.4 days) and most other treatments, such as blank microparticles plus US (MS = 11.5 days) and DOX (8 μg)-loaded microparticles alone (MS = 13 days). The findings that immune checkpoint blockade did not significantly extend survival of mice treated with DOX (8 μg)-loaded microparticles plus US, and that tumor-free ("cured") mice were not protected from subsequent tumor rechallenge suggests minimal involvement of the adaptive immune response in the observed antitumor activity. Nevertheless, the synergistic increase in survival of melanoma-challenged mice treated with the combination of US and DOX-loaded microparticles implicates such a treatment methodology as a promising additional tool for combatting otherwise currently incurable cancers.

Poster 11

TERAHERTZ ABSORPTION SPECTRA OF PROTEIN CRYSTALS: A CRITICAL EXAMINATION

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Terahertz time domain spectroscopy (THz-TDS) is a relatively new area of analytical chemistry that is capable of probing low frequency molecular transitions over a spectral range of 0.3 to 3.0 THz or 5 to 100 cm^{-1}. Rotational transitions are mostly probed for gases, while inter-molecular interactions and torsional modes are probed within molecular crystals. Spectra are collected in the time-domain by recording a gated-detection signal for a coherent pulse of electromagnetic radiation at THz frequencies. This signal is recorded after the radiation passes through the sample of interest. The resulting time-domain spectrum is converted to frequency space through a Fourier processing step to reveal a single-beam spectrum. The negative base-10 logarithm of the ratio of sample and air single-beam spectra results in the corresponding absorption spectrum.

Absorption spectra are reported over THz frequencies for crystals of many different class of compounds, including those of biological origin. A few research groups have reported THz absorption spectra for protein crystals of different types, including lysozyme. These reported spectra are relatively featureless over these frequencies, which raises the question if protein-protein interactions can be probed within crystals using this form of spectroscopy.

The overall objective of this research project is to understand THz spectra of protein crystals as a way to examine protein-protein interactions. The presentation will provide a critical examination of the THz spectra reported in the literature for crystals of lysozyme and other proteins. In addition, absorption spectra of amino acids will be presented as our starting point to establishing a firm understanding of THz absorption spectra of peptides and proteins.

Poster 12

PCB DECHLORINATION HOT SPOTS AND REDUCTIVE DEHALOGENASE GENES IN SEDIMENTS FROM A CONTAMINATED WASTEWATER LAGOON

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Aquatic sediments constitute a large sink for spilled and improperly disposed PCB mixtures. Biodegradation of PCBs offers a pathway for the transformation of these persistent organic pollutants to less toxic compounds. To enhance understanding of the microbial community structure and the enzymes which catalyze the PCB biodegradation process, sediment samples were collected from a PCB-contaminated lagoon in Altavista, Virginia. Total PCB congener analysis from 27 sample locations revealed profiles very similar to that of Aroclor 1248 with a range of concentrations from 6.3 mg/kg to 12,700 mg/kg. Among the 27 samples several perceived hotspots of dechlorination became evident. Illumina sequencing extracted DNA showed variation in microbial community structure, but were consistently dominated by Proteobacteria and Firmicutes. Chloroflexi, the phylum containing known anaerobic PCB dechlorinators, ranged from 1.43-10.70% in the samples. A PCR survey conducted to isolate and identify reductive dehalogenase genes resulted in 11 sequences most closely related to this found in *Dehalococcoides mccartyi* strain CG5 and PCB-dechlorinating D. mccartyi strain CBDB1. An enrichment culture study to better understand the PCB dechlorinators present at these hotspots is being undertaken. PCBs are highly hydrophobic and sorb strongly to aquatic sediments, which may limit access to dechlorinators. To better understand the bioavailable PCB congener profile SPME PDMS-fiber passive samplers were deployed in the enrichment cultures to examine congeners present in the pore water.

Poster 13

COMPARATIVE BEHAVIOR OF A THYMIDYLATE SYNTHASE MUTANT TOWARD DEOXYURIDYLATE AND DEOXYCYTIDYLATE

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All organisms depend on sufficient intracellular concentrations of thymidylate (dTMP) for their DNA synthesis. In almost all organisms, dTMP comes from uridylate (dUMP) thanks to the transfer of methylene and hydride units derived from methylene tetrahydrofolate (MTHF).[1] Chemotherapeutic drugs to combat several cancers are based on knowledge obtained from studies of *E. coli* TSase.[2]

The current work leverages existing literature[3-5] on an active-site mutant of TSase, N177D, to add detail to the current understanding of TSase catalysis. The hydride transfer step contributes to rate limitation of the overall cycle[6] and thus has been the subject of detailed study. X-ray crystal structures suggest that N177 engages in a key cyclic hydrogen bonding interaction[7] with uridylate. D177 is likely to have an altered hydrogen-bonding pattern. We investigated the implications of this for several of the enzyme's properties, including the preparation of the active site for the hydride transfer step. Additionally, based on prior publications,[3, 8] we further explored the behavior of N177D with deoxycytidylate (dCMP) – which this mutant accepts as a substrate, while WT *ec*TSase does not. Deoxycytidylate differs from deoxyuridylate solely by the replacement of the C4=O4 carbonyl group with a C4 amino group. Therefore, study of N177D with dCMP would add to our understanding of this hydrogen bonding pattern and offer an example of enzyme adaptation upon mutation. Kinetic isotope effects were employed as a key technique to weigh in on substrate binding order and on the architecture of the active site in the lead-up to the hydride transfer.

Our outcomes serve as an example of enzyme plasticity. Often, partial adaptation and compensation for a deleterious mutation can occur. Additionally, the results obtained with dCMP corroborate the mechanistic evidence for WT ecTSase arrived at through study of N177D with the natural substrate.

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Poster 14

SOLVENT ISOTOPE EFFECTS TO PROBE THE MECHANISM OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE (FDTS)

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Thymidylate (dTMP, one of the four building blocks of DNA) is synthesized *de novo* by methylation of Uridylate (2'-deoxyuridine 5'-monophosphate) at the C5 position. In most eukaryotes, including humans, the enzyme thymidylate synthase (TSase) encoded by *thyA* gene catalyzes the reductive methylation of dUMP to form dTMP (1,2). However, a novel gene *thyX,* coding for a new class of TSase was discovered (3). This new class of TSases named as Flavin-Dependent Thymidylate synthases (FDTSs) makes use of a non-covalently bound Flavin Adenine Dinucleotide (FAD) prosthetic group as a reducing agent as well as a methylene carrier (3). Several disease-causing bacteria rely on FDTS (3). Classical TSase and FDTS are substantially different in structure and chemical mechanism (4,5,6,7). Hence, understanding the FDTS mechanism may allow the development of mechanism-based antibiotics with minimal toxicity to humans. In this work we have utilized solvent isotope effects to probe the mechanism of this unique enzyme. Rate of product formation and substrate consumption followed by quench flow experiments done in isotopic solvents (H_2O and D_2O) were compared to calculate the solvent isotope effect. Information obtained by these experiments provide insight into the complex mechanism of FDTS catalyzed thymidylate synthesis.

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Poster 15

EXPLOITING THE PAF RECEPTOR TO TARGET INFECTIOUS DISEASES IN THE LUNGS

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Many bacterial pathogens that cross cell membranes in the lungs express lipooligosaccharides (LOS) laden with phosphorylcholine (CHoP) moieties. Our lab has demonstrated that these CHoP-containing LOS interact with the PAF receptor on lung cells. Previous students have explored using CHoP-containing LOS as targeting ligands for particles, but there is an inherent toxicity in these difficult-to-purify bacterial extracts. Currently, we are approaching this challenge by using CHoP-containing synthetic polymer coatings as a means of imbuing our particles with targeting to the PAF receptor for targeted uptake. We are investigating copolymer systems, so we need an easily-modified platform for study. Thus, we have designed our polymers with thiol end-groups to form self-assembled monolayers onto gold nanoparticles. By modifying chain length and ion content in these polymers we modify chain behavior and charge effects of these particle coatings. These are tools that we use to modify the way these particles interact with proteins and cells to enhance their ability to effectively carry drug and gene payloads to targeted areas. The development of systems that can target specific cell or tissues is a major goal in drug delivery, and biomaterials that minimally interact with proteins in the body are of additional value in a variety of medical devices.

Poster 16

SUPERANTIGEN BINDING SITES IMPORTANT FOR ENDOTHELIUM INTERACTION AND DYSFUNCTION

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Staphylococcus aureus is the leading cause of infective endocarditis (IE). IE is a life-threatening condition of the heart characterized by the formation of vegetative lesions on the inner leaflet of the valves. Recent studies have shown the importance of superantigens (SAgs) in the establishment of staphylococcal IE. All SAgs share multiple binding sites such as the TCR binding site, MHCII binding site, and the dodecapeptide region. Classically, SAgs crosslink the Vβ domain of the TCR to the MHCII on the APC. This crosslinking event leads to a non-antigen specific, Vβ-specific amplification of T cells that is orders of magnitude larger than normal antigen responses. The less characterized dodecapeptide region was shown to interact with and induce activation of epithelial cells. Currently, the understanding of the SAg functions that promote the pathogenesis of IE is not substantial. Preliminary data using a rabbit model of IE has Juncovered a significant pathogenic contribution of a superantigenicity deficient mutant SECN23A. This suggests that an alternative function of SAgs interacting with cells at the infection site may play an important role in the pathogenesis of IE. We show that two divergent superantigens, TSST-1 and SEC, modulate immune functions of human aortic endothelial cells, which may play a role in the development of IE. To address the regions of the SAg important for endothelial interactions, a series of binding site mutants with TSST-1 and SEC were used to treat human aortic endothelial cells and immune responses measured to help further elucidate the mechanism by which SAgs contribute to IE.

Poster 17

COUPLED MOTIONS OF DISTAL RESIDUES FOR HYDRIDE TRANSFER REACTION IN HUMAN DIHYDOFOLATE REDUCTASE

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Enzymologists have been trying to understand the roles of protein dynamics in an enzyme catalyzed reactions, which usually involves multiple steps including substrate binding, conformational changes and product release. 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 a meaningful probe for understanding the transitional state of enzyme reactions. Dihydrofolate reductase (DHFR) from *Escherichia coli* has been a benchmark system for structural, kinetic and evolutionary studies. A global dynamic network of coupled motions involving both active site and distal residues for *ec*DHFR has been mapped out through examination of temperature dependence of kinetic isotope effects (KIEs) and molecular dynamic simulations. Similarly, temperature dependence of the intrinsic KIEs measurement for two of the equivalent conserved residues in human DHFR (*hs*DHFR) have been conducted and synergistic effects have been observed. This study shows that this kind of global network of coupled motions is conserved along the evolution from relatively flexible bacterial enzyme (*ec*DHFR) to the rigid human DHFR (*hs*DHFR). A more comprehensive understanding about motions and its correlation with reactivity of this enzyme could be beneficial to rational drug design and protein engineering in biomedical studies or industrial uses.

Poster 18

DUAL HAMILTONIAN SWITCHING: ACCELERATING ACCURATE PROTEIN THERMODYNAMIC CALCULATIONS

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To obtain an accurate, precise estimate of the free energy associated with a molecular process, alchemical free energy (AFE) simulations must use an accurate, realistic potential energy surface, and thoroughly sample from it. Indirect methods first use an efficient energy surface, usually from a fixed partial charge force field, and then calculate a $\Delta G_{correction}$ to a more accurate but more expensive energy surface to avoid having to sample directly from that expensive energy surface. Dual resolution switching (DRS) grows from the dual force field (DFF) method, incorporating a tunable approximation that allows DRS to be applied to much larger systems than have been previously studied with indirect methods. DRS corrections can converge much quicker than the underlying mutational free energy of interest, with results shown for a protein-peptide binding system.

Poster 19

PROBING THE INVOLVEMENT OF SCHIFF BASES IN THE MECHANISM OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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The *de novo* synthesis of thymidylate (2'-deoxythymidine-5'-monophosphate; dTMP) is essential for the proliferation of many organisms. One biosynthetic pathway involves catalysis with classical thymidylate synthase (TSase) wherein dUMP (2'-deoxyuridine-5'-monophosphate) is reductively methylated to form dTMP. The mechanism is well-understood and currently serves as a chemotherapeutic target. A study in 2002 found many pathogenic organisms synthesize dTMP with a different enzyme, flavin-dependent thymidylate synthase (FDTS). Previous studies have shown the FDTS mechanism is dramatically different than TSase, and since FDTS is not translated from the human genome, it has potential to serve as a drug target.

The most viable FDTS mechanism of reductive methylation involves two substrates, methylene tetrahydrofolate (MTHF) and dUMP, as well as a cofactor FAD (flavin adenine dinucleotide) and two characterized intermediates. The mechanism also includes numerous Schiff base ($R_2C=NR$) intermediates which have not been validated, but a previous study has shown this moiety is susceptible to nucleophilic attack by a hydride from sodium cyanoborohydride ($NaCNBH_3$). This is the grounds for the study currently underway: quench-flow (2 ms to 400 s) with $NaCNBH_3$ as a quenching agent to reduce Schiff base-containing intermediates. Quenched reactions will be analyzed with mass spectrometry to identify chemically modified intermediates, view the lifetime of the intermediates with respect to reaction progress, and confirm, refute, or propose a new FDTS catalytic mechanism.

Poster 20

ATTEMPTS TO CORRECT THE INTERACTION THERMODYNAMICS OF CHARGED AMINO ACIDS IN MOLECULAR DYNAMICS SIMULATIONS

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A number of recent simulation studies have determined osmotic coefficients for small molecules in aqueous solutions in order to assess the strength of solute-solute interactions in molecular dynamics simulations.[1-10] In cases where these osmotic coefficients derived from simulation do not match experimental values, one effective way to correct them has been to adjust the simulation parameters that describe van der Waals forces between pairs of atoms.[1-2, 6, 8-10] In the present study, we used the same approach for simulations of charged amino acids, which we previously showed were unable to reproduce experimental osmotic coefficients.[7] After investigating these amino acids, as well as small molecule analogs that approximate their functional groups, we achieved only limited success in matching experimental data. Our results suggest that adjustments to van der Waals terms might need to be supplemented with other modifications in order to fully reproduce the experimental interaction behavior of charged amino acids.

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Poster 21

VOLTAGE-GATED SODIUM CHANNEL CROSSLINKER STUDIES WITH BETA AUXILIARY SUBUNITS

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The cardiac voltage-gated sodium channel provides the impetus for the spread of electrical potential and contraction throughout the heart. Voltage-gated sodium channels associate with and are modulated by auxiliary β-subunits that alter the channel expression, conduction and pharmacology. We have been working towards identifying regions of association and stoichiometry between the α channel subunit and the β auxiliary subunits to better understand the β-subunit interaction and significance. To go about this, we are employing multiple different crosslinkers that will be followed up with mass spectrometric analysis to identify regions of interaction between the α cardiac channel and the β-subunit. We're utilizing the amino crosslinkers disuccinimidyl suberate and bissulfosuccinimidyl suberate that can crosslink lysine residues. For bridging charged bonds, we are utilizing the zero-length crosslinkers dicyclohexylcarbodiimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide that can form a covalent bond between lysine and the carboxylic acid containing residues aspartic acid and glutamic acid. In addition, we are utilizing site specific incorporation of unnatural amino acid photocrosslinkers that upon UV stimulation can form a covalent bond.

Poster 22

REACTION INTERMEDIATES- A TOOL TO UNDERSTAND THE MECHANISM OF CATALYSIS IN FLAVIN DEPENDENT THYMIDYLATE SYNTHASE

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In humans and certain bacteria *de novo* biosynthesis of thymidylate (dTMP) is catalyzed by thymidylate synthase(TSase) encoded by the gene *thyA*. Recently is was discovered that in several human pathogens (e.g., those causing anthrax, tuberculosis, typhus, and more,) an alternative path exists for the *de novo* biosynthesis of thymidylate (dTMP). It was found the enzyme Flavin-dependent thymidylate synthase (FDTS) encoded by *thyX* does the catalysis of dUMP to dTMP. Furthermore, studies showed this gene is absent in humans thus making FDTS an attractive antibiotic target. Unlike human TSase, FDTS utilizes a noncovalently bound flavin adenine dinucleotide (FAD) prosthetic group to catalyze the chemistry and produces tetrahydrofolate (H_4folate) rather than dihydrofolate (H_2folate). Although much studies have been done still proper understanding of the mechanism is yet to be achieved.

Several mechanisms have been proposed to explain the catalysis FDTS and they all propose the existence of a non-covalently bound exocyclic methylene intermediate I_3 (Fig 1); an isomer of thymidine. Our lab aims to synthesize the intermediate and test it as alternative substrate for FDTS. These test data will increase the understanding of the chemical mechanism of FDTS and will validate the proposed mechanism. Further derivatization of this exocyclic methylene intermediate might serve as potent mechanism based inhibitors.

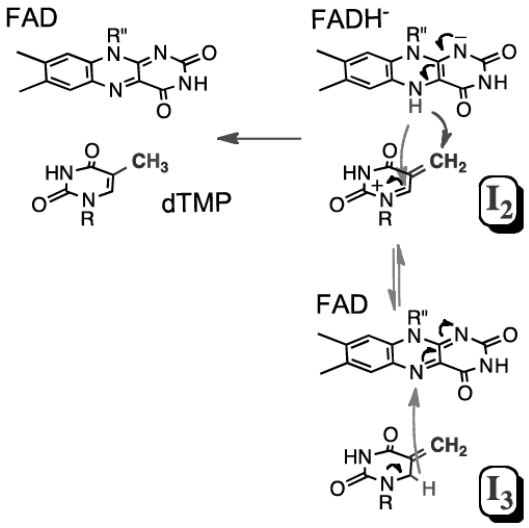

Figure 1: mechanism proposed in SI of Reference 1

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Poster 23

BIOCHEMICAL AND STRUCTURAL INVESTIGATION OF THE DYNAMIC REGULATION MECHANISM OF PYRUVATE KINASE MUSCLE ISOFORM 2 USING HYDROPHOBIC AND HYDROPHILIC AMINO ACIDS

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Pyruvate kinase muscle isoform 2 (PKM2) catalyzes the terminal step in glycolysis and is a key enzyme in cancer metabolism. In glycolysis, PKM2 converts the substrates, phosphoenol pyruvate (PEP) and adenosine diphosphate to pyruvate and adenosine triphosphate (ATP). Cancer cells require ATP and glycolytic intermediates for cell function and proliferation, respectively. PKM2 activation results in an elevated ATP level, which enhances cellular processes in cancer cells. On the other hand, PKM2 inhibition increases the concentration of upstream glycolytic intermediates that aid in cancer cell proliferation. Previous studies identified various amino acids regulate the activity of PKM2 by binding to a hydrophilic binding pocket in the enzyme. However, the mechanism of regulation by amino acids is not understood. Our activity and ligand binding studies indicate that hydrophilic amino acids aspartic acid and asparagine activate PKM2 by enhancing its PEP binding affinity. In contrast, a hydrophobic amino acid valine inhibits PKM2 by lowering its affinity for the substrate. Crystal structure of wtPKM2 bound to valine shows reduced number of H-bonds between valine and the binding pocket compared to the reported PKM2-serine complex. Using site-directed mutagenesis of residues in the amino acid binding pocket, followed by activity and binding affinity studies, we have determined that changing the polarity or charge of the environment decreases the activity of PKM2 by reducing its PEP binding affinity. Based on these structural and biochemical studies, we propose that polarity of amino acids together with the charge or polarity of the amino acid binding pocket plays an important role in the regulation of PKM2 activity

Poster 24

ASSESSING THE CYSTEINE DEPENDENCE OF SMALL MOLECULE INHIBITION OF RGS4 AND RGS14

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Regulators of G Protein signaling (RGS) proteins act as GTPase activating proteins (GAPs) effecting an increased rate of hydrolysis of GTP to GDP by active Gα subunits, thus terminating their signaling. As there are over 20 different RGS family members, understanding both highly-conserved and unique residues among RGS proteins is of key importance to their therapeutic potential, in order to understand specificity of inhibition. Previous work revealed that cysteine residues may not be crucial to the ability of RGS proteins to act as GAPs, however these sites could be exploited for small molecule targeting and inhibition. Therefore, creating cysteine null and single cysteine mutants will allow for the specific study of interfamily RGS protein cysteine dependence of inhibition. RGS4 and RGS14 were chosen based on previous work detailing that these RGS proteins are highly sensitive to small molecule inhibition. Through monitoring the release of free phosphate, the GAP activity of RGS4 and RGS14 wild type were compared to their respective single cysteine and cysteine null mutants. The results indicate that while there is no cysteine dependence for RGS4 or RGS14 for GAP activity. The characterization of small molecule inhibitors for cysteine dependent mechanisms of action is being evaluated.

Poster 25

DOUBLE STRANDED *m*RNA POLYPLEXES FOR LIVER TRANSFECTION IN VIVO

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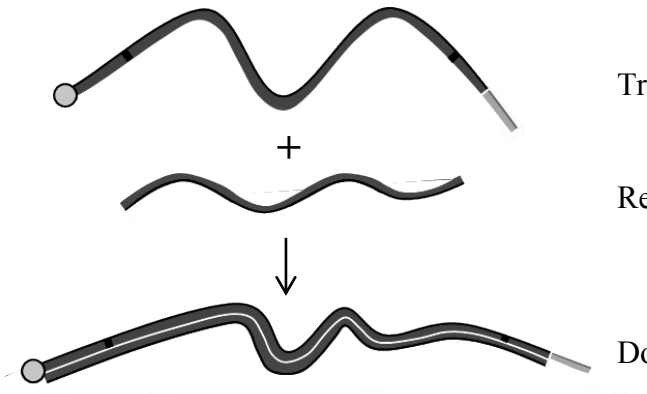
In vivo transfection of liver hepatocytes using *m*RNA potentially offers an advantage over delivering plasmid DNA because it eliminates the need for *m*RNA delivery to the nucleus. However, the metabolic instability of *m*RNA in the circulation limits the potency of *m*RNA nanoparticle gene delivery systems. Here we find that hybridization of *m*RNA with a complementary reverse strand *m*RNA results in a novel double stranded (ds) *m*RNA possessing dramatically increased serum stability. Hydrodynamic dosing of ds *m*RNA nanoparticles demonstrates equivalent translational efficiency to single stranded (ss) *m*RNA nanoparticles. Optimal ds *m*RNA mediated gene expression in liver was achieved by tailoring the length of the reverse *m*RNA strand. The circulatory stability of ds *m*RNA nanoparticles was also found to be significantly greater than ss *m*RNA nanoparticles as determined by gene expression in the liver. The development of this novel form of metabolically stabilized and translationally active ds *m*RNA provides a new platform to advance applications of *m*RNA nanoparticle delivery to achieve gene expression.



Poster 26

FORMULATING AN ASPIRIN-ELUTING DEGRADABLE POLYMER COATING FOR INTRACRANIAL FLOW DIVERTERS

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The international subarachnoid hemorrhage aneurysm trial (ISAT) transformed the treatment of intracranial aneurysms (IAs). It showed a superior outcome treating IAs using endovascular means versus microsurgical clipping. One of the advances in the field is the use of stent assisted coiling or flow diverters. These two techniques require the use of dual anti-platelet therapy (DAPT). The role of DAPT is to minimize the risk of thromboembolic events in patients, however, DAPT use is associated with increased risk of brain hemorrhage. Here we propose a novel strategy using a drug eluting biodegradable coating that can be deposited directly onto the intracranial flow diverter surface. We expect that the negative effects associated with DAPT can be decreased when delivering the drugs locally as opposed to systemically. Intracranial flow diverters [Pipeline™, Medtronic] were coated using a solution of 1% w/v poly(lactic-co-glycolic) acid (PLGA) in acetone incorporated with 1% w/v acetylsalicylic acid (aspirin) [Sigma]. The dip coating process involved leaving the flow diverter in the coating solution on a shaker for 30 minutes, removing it from the solution and allowing it to dry completely, then returning the flow diverter to the solution for the next coat. This process was repeated several times and the success of the coating procedure determined using scanning electron microscopy (SEM) imaging [Hitachi S-4800]. Before a release study could be performed on the coated flow diverters, it was necessary to develop a method to accurately determine the concentration of aspirin. UV-Vis spectroscopy was used to verify appropriate wavelengths to use as well as the linear range for each wavelength using known standard solutions. High performance liquid chromatography (HPLC) was used to determine the amount of aspirin loaded onto the stent and the concentration of aspirin in each sample of the release study.

The amount of aspirin loaded onto the stent was determined by dissolving the aspirin/PLGA coating in acetone, using a rotary evaporator to remove the acetone, then reconstituting the solid in the HPLC mobile phase. The release study design involved having the coated flow diverters placed in a sealed 6 well plate filled with sufficient 1X PBS solution to cover it completely. The plate was left in a 37°C incubator for 7 days and each day, 200µL samples were taken from the well and replaced with fresh release media. The samples were stored in a 4°C fridge until the last day of the experiment, at which point the concentrations for each sample was determined and a release profile compiled. All HPLC samples were measured using a UV-Vis detector measuring dual wavelengths of 230 and 270nm.

The intracranial flow diverters were successfully coated with PLGA/aspirin and the coating was confirmed using SEM imaging. Aspirin loading and release profile from the coated flow diverters results will be shown. In the field of neurosurgery, there is a medical need to find an alternative to systemic use of DAPT. Here we demonstrate the ability to coat intracranial flow diverters with PLGA incorporated with aspirin which we believe will be able to deliver a controlled and sustained release of the anti-platelet drug at the implantation site and reduce the risks associated with administration of a systemic antiplatelet therapy.

Poster 27

C-H BOND ACTIVATION CATALYZED BY FORMATE DEHYDROGENASE AS A MODEL TO INVESTIGATE THE ROLE OF PROMOTING VIBRATIONS IN ENZYME CATALYSIS

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The importance of fast vibrations coupling between enzyme catalyzed chemistry at pertinent timescales has been a subject of open debate for many decades. In this regard, enzyme catalyzed C-H bond activations have been of great interest as such studies provide more direct and accessible means to investigate the role of fast dynamics on the chemical step (C-H→C transfer). Formate dehydrogenase (FDH) is a unique model system that can be tailored to serve the above purpose by mutagenesis studies and two-dimensional infrared spectroscopy (2D-IR). FDH catalyzes the hydride transfer from formate to NAD^+ giving rise to CO_2 and reduced cofactor. Previous work on FDH mutagenesis from our lab involved active site mutations - V123A and I175A which showed that the femtosecond-picosecond vibrational contributions from amino acid residues that are located behind the nicotinamide ring of the NAD^+ cofactor are assisting the modulation of the donor-acceptor-distance (DAD) during the hydride transfer. Current work on F69A mutation would shed light on the vibrational contribution from a residue located within van der Waals distances from the substrate, formate, in achieving the optimized DAD during C-H bond activation. Temperature dependence of kinetic isotope effects is used to assess the nature of hydride transfer at the tunneling ready state (quantum mechanically delocalized transition state) of the catalyzed reaction, while 2D-IR is used to directly probe the relevant fast timescale motions by using the transition state analog ternary complex, FDH-NAD^+-azide. Together, the current and previous studies will reveal the role of fast dynamics in FDH catalyzed chemistry.

Poster 28

DEVELOPMENT OF NORMALIZING COVALENT PROBES FOR GLYCOSAMINOGLYCAN ACTING ENZYMES

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Glycosaminoglycans (GAGs) are highly sulfated linear polysaccharide residing in the extracellular matrix. GAGs serve a variety of functions, ranging from growth factor regulation to nutritional sources for the intestinal microbiota.[1] Such a diverse range of function has resulted in vast array of enzymes acting on the glycosaminoglycan family, many of great interest to researchers.[1,2] Current probes for the study of these enzymes are highly specialized, capable of probing only one class of enzyme.[2] The vast array of bacteria composing the human microbiota calls for a more universal tool in studying the structure and functions of such enzymes.

The synthesis of a more generalized covalent probe is currently underway in the Nguyen Lab. These probes feature the uniquely stable sulfonyl fluoride group as an electrophilic trap. Sulfonyl fluorides are hydrogen bonding activated electrophiles, making them particularly adept at probing the electrostatic surface of an enzyme. The sulfonyl fluoride moiety reacts with an appropriate amino acid when the substrate is hydrogen bonded for an extended period.[3,4] Computational studies have shown the sulfonyl fluoride probe residing within an oxyanion hole, adjacent to a tyrosine residue in the active site of glycosidase *human heparanase*. Progress on the current synthesis stands three steps from completion. First tests will determine the stability of the sulfonyl fluoride in variety of physiological conditions.

Despite these powerful and well-known properties, sulfonyl fluorides have never been applied to carbohydrate acting enzymes. It is the hope of the authors, that this probe will aid structural biologists and medicinal chemists in the study of GAG acting enzymes. The plethora of enzymes produced by the human microbiota, including glycosidase, sulfatase, and epimerase enzymes require a more diverse probe for time effective study.

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Poster 29

SYNTHESIS OF AMIDE ISOSTERES OF SCHWEINFURTHIN-BASED STILBENES

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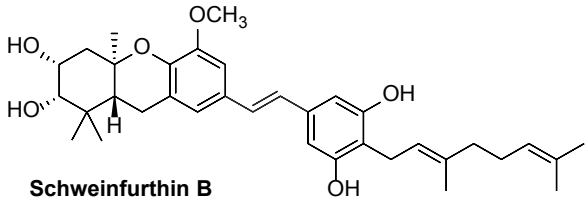
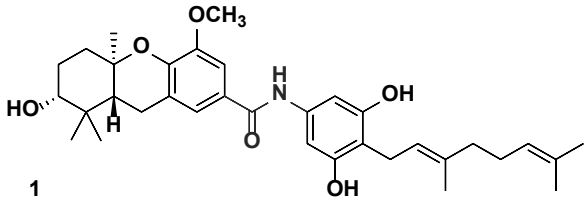
The schweinfurthins are a family of natural products that were originally isolated from extracts of Macaranga schweinfurthii. They are pharmacologically intriguing because they have shown strong differential anti-proliferative activity against a variety of human-derived tumor cell lines, and in some cases display activity at nanomolar concentrations. The mechanisms of action of the schweinfurthins are not entirely known, which has led our lab to conduct extensive structure-activity studies. In continuation of those studies, four novel compounds with the overall schweinfurthin motif have been prepared, where an amide linkage has replaced the stilbene olefin present in the natural products. Assays in the NCI 60-cell line screen have shown that these compounds have activities comparable to their parent structures, with one amide analogue (**1**) showing activity at nanomolar levels. The synthesis of these compounds will be presented, along with some information on their biological activity.



Schweinfurthin B



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Poster 30

REGULATION OF THE *S. AUREUS* SrrB HISTIDINE KINASE BY A PAS DOMAIN

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Antibiotic resistant *Staphylococcal aureus,* commonly known as Methicillin-Resistant *Staphylococcus aureus* (MRSA) are a major cause of hospital infections. Thus, there is an urgent need for identifying novel targets/mechanisms for antibiotic development. Bacterial two-component systems (TCS) contribute to infections by regulating toxin production, antibiotic resistance and survival. TCSs are composed of a membrane bound histidine kinase (HK) and a cytoplasmic response regulator (RR). The HK senses extracellular stimuli through its extracellular sensor domain and undergoes autophosphorylation at a conserved histidine residue in the cytoplasmic kinase domain. The phosphorylated HK then transfers the phosphoryl group to a conserved aspartate residue in the RR, which in turn binds DNA to control gene expression. SrrAB TCS is a regulator of *S. aureus* virulence factors including toxic shock syndrome toxin-1 (TSST-1), which causes toxic shock syndrome. SrrB is a dual function kinase/phosphatase capable of regulating the level of phosphorylation of SrrA. SrrB is composed of an extracellular Cache domain and several cytoplasmic domains, including a HAMP, PAS and DHp-CA catalytic domains. Here, we present enzyme kinetics data showing that the PAS domain effects SrrB kinase and phosphatase function. Our data indicates that the PAS domain increases the rate of autophosphorylation and has an inhibitory effect on SrrB phosphatase activity. In addition, we used SAXS and ensemble modeling to show that the PAS and catalytic ATP binding (CA) domain of SrrB are flexible, suggesting a mechanism for SrrB regulation. Moreover, we identified heme as a ligand for the PAS domain, which could be a potential secondary mechanism of regulating the SrrAB TCS. The crystal structure of the apo PAS domain shows a putative binding pocket, which provides insights into potential residues involved in heme binding. Design of PAS mutants deficient in heme-binding for biochemical and biological analyses is ongoing. Together, our data suggest the PAS domain is critical for determining the balance of kinase and phosphatase activity and that heme binding may be involved in redox regulation of the SrrAB TCS.

Poster 31

ACCELERATED PROTEIN OPTIMIZATION AND PHYSICS-BASED ANALYSIS OF GENETIC MISSENSE VARIANTS ASSOCIATED WITH DEAFNESS

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Accurate classification of missense variants as pathogenic or benign is a crucial step in translational genetic research. Current classification schemes often use a combination of bioinformatics tools such as SIFT, CADD, and FoldX to analyze genetic variants of unknown significance in patients with hearing loss. These prediction tools assess the pathogenicity of a variant based primarily on sequence conservation; however, they often yield ambiguous or even contradictory results. Computational molecular biophysics provides a complementary approach by incorporating three-dimensional protein structures and first principles thermodynamic assessment into variant classification algorithms. We applied free energy perturbation (FEP) to calculate the mutational folding free energy ($\Delta\Delta G_{WT>Variant}$) of sixty USH2A variants from the Deafness Variation Database (DVD), which are associated with hearing loss. We hypothesized that a significant (>1 kcal/mol) free energy change, indicative of substantial protein folding destabilization and/or misfolding, correlates with pathogenicity. Assessment of this hypothesis was tested using thermodynamic predictions on positive and negative control variants of known status, *i.e.* those classified specifically as benign or pathogenic according to the DVD. FEP calculations on positive and negative controls showed agreement with the DVD. We recently designed a parallelization scheme utilizing Nvidia graphical processing units (GPUs) to accelerate our atomic resolution assessment algorithms. With the use of one GPU, our side-chain optimization algorithm achieved a 67 times speed-up compared to using two Intel Xeon E5-2680v4 central processing units (CPUs). These simulations remain time consuming to prepare, troubleshoot, and analyze, and thus require the attention of experienced computational scientists. To address this challenge, we designed a web-based application called "OtoProtein", the goal of which is to provide user-friendly access to biophysical algorithms while requiring little specialized knowledge of simulation methods. In its current state, OtoProtein provides intuitive access to cutting-edge biophysical optimization algorithms. Ultimately, we expect OtoProtein to provide access to missense variant classification algorithms, which will complement and augment diagnostic capabilities.

Poster 32

PROBING ALLOSTERIC REGULATION OF AN EXECUTIONER CASPASE

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Due to their essential roles in apoptosis and inflammation pathways, the caspase family of cysteine-dependent, aspartate-specific endoproteases have received widespread attention as drug targets. The caspases involved in apoptosis are further segregated into initiator and executioner caspases. Initiator caspases (-8, -9) activate the executioner caspases (-3, -6, -7) in order to carry out the final steps of apoptosis. This family of enzymes employs a catalytic cysteine-histidine dyad to hydrolyze peptide bonds with a high degree of specificity, in order to carry out their essential cellular functions. Their aberrant activation has been implicated in numerous disease states related to neurodegeneration and cardiovascular diseases.

Numerous computational and high-throughput screening (HTS) efforts have been directed at the development of caspase inhibitors. To date, the overwhelming majority of these compounds target the active site through a form of mechanism-based inhibition. These molecules lack drug-like properties, as molecules binding the active site of caspases are inherently negatively charged due to the preference of aspartate containing peptide substrates. It has been hypothesized that targeting a known allosteric site at the dimer interface of the caspases will yield molecules with superior drug properties. We also hypothesized that current HTS libraries do not contain the complementary chemical space for the allosteric site of the caspases, and that fragment screening will provide the necessary diversity to discover allosteric caspase inhibitors.

We employed fragment-based drug discovery in an effort to leverage superior screening diversity. A fragment library was screened against caspase-7 by differential scanning fluorimetry. Compounds identified from the screen were further tested by differential scanning fluorimetry, functional assays, and surface plasmon resonance. Hits were identified capable of inhibiting caspase-7 in a non-competitive fashion. Compounds in a similar chemical space (tanimoto > 0.7) were purchased and resulted in non-competitive inhibitors with improved ligand efficiency and potency. Two hits have been characterized by X-ray crystallography and were confirmed to bind at the aforementioned allosteric site. Further work is being done to understand the mechanism by which these compounds are allosterically inhibiting caspase-7.

Poster 33

SORPTION AND BIOTRANSFORMATION OF NEONICOTINOID INSECTICIDES ON GRANULAR ACTIVATED CARBON DURING DRINKING WATER TREATMENT

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Neonicotinoid pesticides (neonics) are among the most widely used pesticides in the world due to their targeted toxicity towards insects. The widespread use of neonics has led to their detection in waters across the U.S., including those used as drinking water sources. Although neonics exhibit relatively low toxicity towards mammals, formation of toxic metabolites is an exposure concern. Previous studies comparing neonic detection at the University of Iowa and Iowa City drinking water treatment plants have identified granular activated carbon (GAC) as a possible method for removing the neonics imidacloprid, clothianidin, and thiamethoxam from drinking water. The goal of the present study was to determine the efficacy of GAC to remove neonics from drinking water. Adsorption isotherms were conducted with imidacloprid, clothianidin, and thiamethoxam to assess their affinity towards GAC. Desorption tests were also conducted using five-year-old used GAC from the Iowa City drinking water plant to determine the likelihood of neonic desorption and concentrations of neonics on GAC granules. Results indicate neonics (despite high polarity / solubility) are readily adsorbed to GAC and minimally desorbed in buffered solutions at environmental pH. The greatest extent of neonic desorption occurred in solvents of medial polarity (acetonitrile, acetone, and dichloromethane). In solvents with the greatest desorption of neonics from GAC, concentrations of up to 600 ng neonic/ g GAC were determined. These sorption and desorption tests suggest the long-term use of GAC is a viable option for neonic removal from drinking water. Nevertheless, over time, GAC filters are prone to biofilm formation, which may enhance the biotransformation of adsorbed neonics into metabolites of enhanced mammalian toxicity. Studies are currently underway concerning the biotransformation of these neonics in environmental and engineered systems that contain electron transporters such as GAC.

Poster 34

NOVEL C1 PROTEIN EXPRESSION SYSTEM FOR HIGH PRODUCTION YIELDS BY USING A REAL-TIME PAT BIO-MONITOR

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[3]Dyadic International, Inc., Jupiter, FL

High yield of protein production (> 25 g/L) is a major challenge in the pharmaceutical industry, specifically to enhance the production of biologics or enzymes required for the chiral synthesis of small molecule drugs. A novel protein expression platform capable of such high production yields is under investigation. This expression system uses a proprietary strain of *Myceliopthora thermophila* fungal cells (C1) developed by Dyadic International for production of enzymes used by the biofuels industry. Recombinant protein is secreted by this C1 cellular factory, thereby producing a relatively clean supernatant product with yields of 80 g/L for a single enzyme within a matrix yield of 100 g/L of total protein. We are interested in translating this C1 protein expression platform for pharmaceutical applications, including for the manufacturing of biologic proteins such as enzyme reagents or vaccines. In addition, utility of a novel bioprocess monitor is being evaluated as a tool to follow and optimize C1 fermentations in real-time. This bioprocess monitor tracks the concentrations of glucose continuously during the fermentation process. This real-time PAT information can be used to optimize protein production yields while documenting quality of manufacturing.

This presentation highlights findings from three initial C1 fermentations designed to express the enzyme cellulase. The PAT real-time monitor was calibrated from readings collected during the first fermentation. Once calibrated, this monitor was used to follow and control glucose concentrations in the two subsequent fermentations. Glucose readings from the monitor were comparable with off-line YSI & GluCell measurements. An 8-day C1 fermentation process produced 286 g/L (wet cell weight) of biomass and a total protein level of 1.56 g/L in the supernatant. Cellulase activity was quantified as 43.51 Units/mL in this supernatant fraction. Future efforts will focus on increasing protein yields and assessing utility of the PAT monitor in enhancing yields and production repeatability

CBB/NIH Fellowships

YEAR	CBB Fellowship	*NIH Trainee Fellowship
1990-91	10	4
1991-92	12	8
1992-93	12	6
1993-94	12	6
1994-95	13	10
1995-96	13	6
1996-97	12	6
1997-98	12	6
1998-99	12	6
1999-00	13	7
2000-01	14	7
2001-02	12	7
2002-03	11	8
2003-04	14	8
2004-05	12	7
2005-06	11	7
2006-07	10	7
2007-08	10	7
2008-09	10	8
2009-10	6	8
2010-11	7	8
2011-12	6	8
2012-13	5	6
2013-14	4	5
2014-15	4	6
2015-16	5	6
2016-17	6	0
2017-18	6	3
TOTAL	274	181 *Partially Sponsored by CBB

The Center for Biocatalysis and Bioprocessing
Gratefully Acknowledges
The Following Contributors for Their
Support of the
26th Biocatalysis and Bioprocessing Conference
"Advances in Biocatalytic Sciences: Attacking Societal Problems"
October 17, 2017

and

Usha Balakrishnan

Center for Biocatalysis and Bioprocessing
2017-2018 Fellowship Award

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Nicholas Luedtke	Kohen and Cheatum	Chemistry
Julia Quarterman	Salem	Pharmaceutics
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Elcock, Adrian	4-530 BSB	5-6643
Fuentes, Ernesto	4-362 BSB	3-4244
Musselman Catherine	4-452 BSB	5-8888
Montgomery, Rex Emerit.	4-772 BSB	5-7897
Plapp, Bryce, Emeritus	4-550 BSB	5-7909
Schnieders, Michael	4-516 BSB	5-7891
Shea, Madeline	4-450 BSB	5-7885
Washington, M. Todd	4-516 BSB	5-7518

BIOLOGY

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

BIOMEDICAL ENGINEERING (ENG)

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CHEMICAL & BIOCHEM ENG

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CHEMISTRY

◊♦Arnold, Mark	230 IATL	5-1368
Cheatum, Christopher	326 IATL	3-0379
Dey, Mishtu	W285 CB	4-1319
Geng, Lei	319 CB	5-3167
Gloer, Jim	E515 CB	5-1361
Grassian, Vicki	244 IATL	5-1392
Hass, Amanda	204 IALT	4-3695
♦Kohen, Amnon	E274 CB	5-0234
MacGillivray, Leonard	E555 CB	5-3504
Margulis, Claudio	118 IATL	5-0615
Messerle, Louis	E45 CB	5-1372
Nguyen, Hein	E457 CB	4-1887
♦Quinn, Dan	W333 CB	5-1335
Tivanski, Alexei	E272 CB	4-3692
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♦Mattes, Tim	4105 SC	5-5065
Parkin, Gene	4106 SC	5-5655
Schnoor, Jerald	4112 SC	5-5649
Valentine, Richard	4118 SC	5-5653

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Cox, Charles, Emeritus	3752 BSB	5-7779
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Stauffer, George	3-315A BSB	5-7791
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Weiss, David	3-452 BSB	5-7785
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♦Kerns, Robert J.	S321 PHAR	5-8800
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Updated 09/12/17

Notes

The University of Iowa
Center for Biocatalysis and Bioprocessing

Winter Term Course Offering

CBB Offers a Course in Upstream Biotechnology Processing

The biotechnology industry is rapidly growing in the U.S. and beyond and represents a major manufacturing arm for the production of novel products, including industrial enzymes, food supplements, biotherapeutics, and renewal fuels. Bioprocessing describes the production of materials through fermentation with subsequent purification. Fermentation processes are generally referred to as upstream processing (USP) while purification is considered as downstream processing (DSP).

This new course entitled Upstream Biotechnology Processes is designed to provide students with hands-on experience in basic upstream fermentation processes applicable to the biotechnology industry. Basic concepts and general designs of upstream fermentations will be covered through a series of lectures. The bulk of the course, however, will be spent in the laboratory where students will have an opportunity to perform a fermentation on a 5-liter scale. All the fundamental steps used within the fermentation industry will be experienced, including selection of clones, growth of microorganism, monitoring purity of the culture, and bioassay to document product yield.

The course will be organized as 20% lectures and 80% laboratory experience. This format is designed to broaden student knowledge and understanding of upstream biotechnology processes while emphasizing practical laboratory experience.

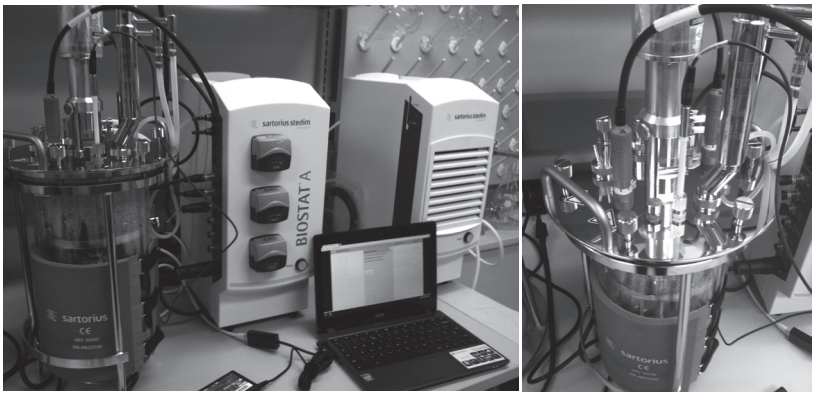

Figure: Sartorius Biostat A MO UniVessel 5-L Fermenters. These fermenters are dedicated for Upstream Biotechnology Processes course.

Course Details

Course Title: Upstream Biotechnology Processes

Course number: CHEM:4850:0001

Semester hours: 2

Instructors: Shuvendu Das, Sridhar Gopishetty, and Mark Arnold

Dates: 12/27/2017 – 1/12/2018

Meeting: TWTh, 9:00-12:10 PM

Location: E120 MTF (Lecture) and E105 MTF (Lab), Oakdale Campus

Course prerequisites: None

Target student: Students majoring in chemistry, biochemistry, chemical and biochemical engineering, biology, microbiology, and pharmaceutical science with interests in biotechnology

Topics covered: Hands-on experience with basic cloning, shake-flask growth, and microbial fermentation emphasizing reactor preparation, inoculation methods, reactor operation and control, product collection, and bioassay

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