19th

Biocatalysis and Bioprocessing Conference

Industrial Biotechnology and Biopharmaceuticals

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THE UNIVERSITY OF IOWA

October 18-19, 2010
Iowa Memorial Union
Iowa City, Iowa


DP Recipe #2

THE UNIVERSITY OF IOWA

19th Annual
Biocatalysis and Bioprocessing
Conference

"*Industrial Biotechnology and Biopharmaceuticals*"

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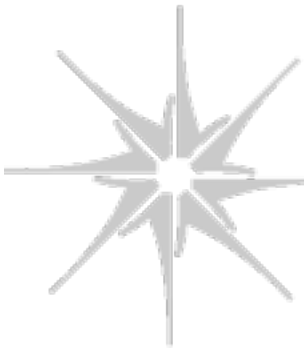

THE UNIVERSITY OF IOWA
Center for Biocatalysis and Bioprocessing

October 18-19, 2010

19th Annual
Biocatalysis and Bioprocessing Conference

"*Industrial Biotechnology and Biopharmaceuticals*"

Sponsored by

The University of Iowa
Center for Biocatalysis and Bioprocessing

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Conference Organizing Committee:

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Director
Dr. Mani Subramanian

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19th Annual Center for Biocatalysis and Bioprocessing Conference
"Industrial Biotechnology and Biopharmaceuticals"
Iowa Memorial Union, Iowa City, IA

MONDAY, OCTOBER 18, 2010

4:00 pm	Registration –Ballroom Lobby, 2nd floor IMU
4:00 - 6:00	Poster set up – Ballroom, 2nd floor IMU
4:30 pm	Tour of CBB – please registration for tour online, departure-Ballroom Lobby
6:00–7:00	Welcome Dinner Ballroom, 2nd floor IMU
7:00–8:00	**Sa V. Ho, Ph.D.,** Senior Research Fellow, Pfizer Global Biologics, Chesterfield, MO 63017 ***"Future of Biotherapeutics-Challenges and Opportunities"***

TUESDAY, OCTOBER 19, 2010

7:00 - 8:00	Registration – Bijou Commons, 1st floor IMU
7:30	Continental Breakfast – Bijou Commons, 1st floor IMU
	Program – Bijou – 1st floor IMU
8:30	**Introduction and Welcome** **Mani Subramanian, Ph.D.**, Director, Center for Biocatalysis and Bioprocessing, The University of Iowa Research Park, Coralville, IA 52241 Professor, Chemical and Biochemical Engineering, The University of Iowa, Iowa City, IA 52242
9:00	**Anthony J. Sinskey, Sc.D.**, Professor, Department of Biology, Health Science & Technology, and Engineering Systems, Massachusetts Institute of Technology, Cambridge, MA 02139 ***"Challenges in Microbial Production of Biodiesel and Biojet Fuel"***
9:45	**Christopher K. Miller,** Research BioTecnologist, Cargill BioTDC Freshwater, Navarre, MN 55331 ***"Developing Microbial Biocatalysts for Low Cost Lactic Acid Production"***
10:30	**Break** – Bijou Commons, 1st floor IMU
10:45	**Vitali Svetlitchnyi, Ph.D**., Senior Scientist, DIREVO Industrial Biotechnology GmbH, D-50829, Cologne, Germany ***"Tailor-made Enzyme and Microbial Systems"***
11:30	**Lunch** –Ballroom 2nd floor IMU CBB Advisory Board Meeting - South Room 1st floor IMU

Afternoon Session – Bijou-1st floor IMU

1:15	**Neil L. Kelleher, Ph.D.**, Professor, Department of Chemistry and Chemical Life Processes Institute, Northwestern University, Evanston, IL 60208 ***"Using Proteomics for Discovery and Targeted Analysis of Thiotemplate Biosynthetic Systems"***
2:00-3:00	**CBB/NIH/NSF Fellow Presentations** Four 10 minute talks plus 5 minutes for questions, by selected graduate students.
3:00 – 5:00	**Poster Session** – Wine/hors d'oeuvres –Ballroom, 2nd Floor IMU
5:00	Announcement of Usha Prize winner for best poster - Adjourn

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   Sa V, Ho. Ph.D.
   Senior Research Fellow, Pfizer Global Biologics, Chesterfield, MO 63017

2. **CHALLENGES IN MICRIOBOLISM PRODUCTION OF BIODIESEL AND BIOJET FUEL**
   Anthony J. Sinskey, Sc.D.
   Professor, Department of Biology, Health Science & Technology, and Engineering Systems, Massachusetts Institute of Technology, Cambridge, MA 02139

3. **DEVELOPING MICROBIAL BIOCATALYSTS FOR LOW COST LACTIC ACID PRODUCTION**
   Christopher K. Miller
   Research BioTechnologist, Cargill BioTDC Freshwater, Navarre, MN 55331

4. **TAILOR-MADE ENZYME AND MICROBIAL SYSTEMS**
   Vitali Svetlitchnyi, Ph.D.
   Senior Scientist, DIREVO Industrial Biotechnology GmbH, D-50829, Cologne, Germany

5. **USING PROTEOMICS FOR DISCOVERY AND TARGETED ANAYLYSIS OF THIOTEMPLATE BIOSYNTHETIC SYSTEMS**
   Neil L. Kelleher, Ph.D.
   Professor, Department of Chemistry and Chemical Life Processes Institute, Northwestern University, Evanston, IL 60208

6. **STRUCTURE AND REACTIVITY OF OXIDIZED 3,4-DIHYDROXYPHENYLACETALDEHYDE**
   David G Anderson, Virginia Florang, and Jonathan A Doorn*
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

7. **MODULATION OF THE ACTIVITY OF FLUOROQUINOLONES AND QUINAZOLINE DIONES BY C-2 THIO FUNCTIONALIZATION**
   Kevin R. Marks, Robert J. Kerns*
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

8. THYMIDYLATE SYNTHASE CATALYZED H-TRANSFERS: TWO CHAPTERS IN ONE TALE

Zhen Wang and Amnon Kohen*
Department of Chemistry, The University of Iowa, Iowa City Iowa 52242

9. CHARACTERIZED WET CHEMICAL ETCHING OF InAsGaSb WITH H_3Cit: H_2O_2: HCl ETCHANT FOR THE PURPOSE OF IMPROVED PERFORMANCE OF LIGHT EMITTING DIODES

Deandrea L. Watkins, Jonathon T. Olesberg, Thomas F. Boggess, and Mark A. Arnold*
Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

Abstracts of Oral Presentations

Presenter 1

FUTURE OF BIOTHERAPEUTICS – CHALLENGES AND OPPORTUNITIES

Sa V. Ho, Ph.D.
Biotherapeutics, Pharmaceutical Sciences Pfizer, Inc., Chesterfield, MO 63017

With the advent of molecular biology and supported by enhanced large-scale bioprocessing capabilities, biotherapeutics have emerged in the last two decades as an important class of drugs and are now an integral part of product portfolios in most if not all major pharmaceutical firms. Biotherapeutics complement small molecule drugs by expanding accessible targets and, for many indications, provide uniquely effective therapies. Clinically, therapeutic proteins have contributed essential therapies to critical diseases, many life-threatening, including diabetes, growth anomaly, clotting disorders, rheumatoid arthritis, multiple sclerosis, and various types of cancer. Therapeutic vaccines represent an emerging area in which biologics are used to treat infectious diseases, autoimmune diseases and cancer. Biotherapeutics span a very broad range of compounds including peptides, proteins, antibodies, nucleotides, and many forms of vaccines, with highly diverse properties and correspondingly varied manufacturing processes. Additionally, increasingly complex biotherapeutics are being developed in which multiple modalities and functions are combined into individual entities, such as many forms of protein-drug conjugates. While this can greatly expand the therapeutic range and efficacy of biotherapeutics, it also exerts much pressure on manufacturing in terms of cost and process complexity. The field is further complicated with the arrival of biogenerics on the heel of patent expiration for many biotherapeutic blockbusters, and, down the road, with stem cells as potential therapeutic agents and transgenic plants and animals as alternative production factories. Both the molecular complexity of biotherapeutics and the competitive pressure to bring them to the market more rapidly and cost-effectively will require diverse and dedicated talents from scientists and engineers for many years to come. A general survey of the field will be presented, followed with a few examples illustrating the complex issues involved in producing biotherapeutics and some innovative solutions used to overcome them.

CHALLENGES IN MICROBIAL PRODUCTION OF BIODIESEL AND BIOJET FUEL

Anthony J. Sinskey, Sc.D.
Department of Biology, Health Sciences & Technology, and Engineering Systems
Massachusetts Institute of Technology, Cambridge, MA 02139

Biodiesel derived from triacylglycerols (TAGs) can be produced by all oleaginous microorganisms such as yeast, fungi, bacteria, and algae. The Sinskey Laboratory has been utilizing both a genetic and genomic approach to study TAG metabolism in bacteria using *Rhodococcus opacus* PD630 as our primary investigative organism. We will describe how we have been able to genetically modify *R. opacus* PD630 to utilize mixed sugars as well as glycerol. Studies on cellulosic feedstocks will also be presented. In addition, we have isolated several genes that control lipid body assembly, and a key metabolic gene whose function appears to regulate redox pools thus regulating fatty acid biosynthesis, and hence TAG metabolism, to lipid bodies.

Presenter 3

DEVELOPING MICROBIAL BIOCATALYSTS FOR LOW COST LACTIC ACID PRODUCTION

Christopher K. Miller
Cargill Biotechnology, Development Center (BioTDC), Navarre, MN 55331

With an annual worldwide production in the range of 370,000MT, lactic acid ranks among the highest volume chemicals produced via microorganisms. In the last decade alone, worldwide demand for lactic acid has increased ten-fold, due in large part to increased demand for green products such as ethyl lactate and poly-lactic acid (PLA). To improve the cost competitiveness of these products, research efforts in academia and industry have focused on yeast as an alternate host for the production of lactic acid. Yeast offer several advantages over bacteria traditionally used in lactic acid fermentations. Many types of yeast are able to grow rapidly on inexpensive defined salts media and yeast lack susceptibility to phage infections, features that contribute to a cost competitive process. In addition, some yeast are naturally acid tolerant, which avoids high costs of neutralization and downstream processing and purification. Metabolic engineering yeast to divert carbon from ethanol to lactic acid has been successfully achieved by several groups. Until recently, metabolically engineered yeast lacked sufficient performance to replace the traditional bacterial process. By combining both directed and random approaches, Cargill successfully implemented a yeast biocatalyst process at commercial scale for the production of lactic acid at low pH.

Presenter 4

TAILOR-MADE ENZYMES AND MICROBIAL SYSTEMS

Vitali Svetlitchnyi, Ph.D.
DIREVO Industrial Biotechnology GmbH, D-50829 Cologne, Germany

In the recent years, the development of biobased industrial platforms has become a key ingredient for many industries to improve their carbon footprint while maintaining or enhancing product performance and profitability. On this path to a biobased economy it is of highest importance to get instant and robust access to tailor-made enzymes and microbial strains.

In the evolving markets for biofuels and biochemicals novel processes are under development targeting environmental sustainability and a decreased dependence from fossil resources while more traditional applications of industrial biotechnology in the food & feed markets emphasize process optimization and the development of natural ingredients.

Since inception, DIREVO Industrial Biotechnology GmbH (DIREVO) has taken several measures to build, partner or acquire capabilities to become a powerhouse for biocatalyst discovery and optimization. On the input side, DIREVO built strong bonds with world-class academic institutions to source biodiversity from extreme environments around the globe. DIREVO also expanded most recently a high-throughput enzyme optimization platform by establishing a direct and rapid approach to mutagenize and screen whole microorganisms for improved growth and biotransformation yields.

Effectiveness and competiveness of the DIREVO Technology Platform has been proven numerous times. As an example, DIREVO has developed the best-in-class Phytase enzyme for Genencor. The product outperforms all known Phytase products for animal nutrition with respect to heat-stability, resistance to proteases in acid environment and phosphate release from phytate. In another example DIREVO has demonstrated its capabilities in improving cellulase enzymes which are used in the degradation of lignocelluloses material for biofuels production.

We have generated a strong pipeline of own and partnered product candidates that will generate a continuous flow of novel product for the next years.

Presenter 5

USING PROTEOMICS FOR DISCOVERY AND TARGETED ANALYSIS OF THIOTEMPLATE BIOSYNTHETIC SYSTEMS

Bradley S. Evans, Ioanna Ntai, Jessica Albright, Sarah Robinson, Yunqiu Chen, Stefanie B. Bumpus and Neil L. Kelleher*
Department of Chemistry and Chemical Life Processes Institute, Northwestern University, Evanston, IL 60208

Non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) are large, multi-modular enzyme complexes (often >200 kDa) that synthesize complex natural products in an assembly line fashion, with incorporation of (amino) acyl monomer units one at a time. Specific domains are responsible for activation, condensation and tailoring of monomer units, while the growing natural product is covalently tethered to carrier proteins (thiolation (T) domains) at a phosphopantetheinyl (Ppant) arm post-translationally donated from coenzyme A (CoA) to an active site serine in the T domain through a phosphoester linkage. High-resolution Fourier-transform mass spectrometry (FTMS) has proven an invaluable tool for the analysis of NRPS- and PKS-directed biosynthesis, as each addition of an acyl unit and almost all tailoring activities can be measured as mass shifts to the *apo* T domain. Additionally, specific mass spectrometric assays have been developed for analysis of T domains and their covalent intermediates, including the Ppant ejection assay which is targeted at the characterization of peptides harboring the Ppant arm. The Ppant ejection assay has been extended for application into liquid chromatography (LC)-MS applications, and because the Ppant ejection assay provides such selective and specific identification of Ppant-containing proteins and peptides, its extension into bacterial proteomic applications was examined.

We have developed a hybrid method, ***PrISM*** (*Pr*oteomic *I*nvestigation of *S*econdary *M*etabolism), for the detection and characterization of NRPS and PKS biosynthetic pathways in bacterial species, including those with unsequenced genomes. Building upon established techniques in bacterial Bottom Up proteomics, including a variety of peptide separation techniques, adapting the Ppant ejection assay for detection of peptides of interest in complex mixtures, and employing standard techniques in genetics and microbiology, this method provides exquisitely specific identification of proteins involved in NRPS-PKS biosynthetic pathways that are actually expressed by the organism. The *de novo* sequenced peptides identified allow for design of primers for use in probing the organisms genomic DNA, and sequencing of the identified gene cluster guides the search for the secondary metabolite produced.

Proof-of-concept experiments were successful in identification in various *Bacilli*, with five distinct systems uncovered and sequenced to date. Extension of the ***PrISM*** approach to *Streptomycetes* and fungi is now in progress. In no case where the NRPS or PKS proteins detected that the metabolite could also not be detected as well. We look forward to presenting our latest/greatest results later this year at the Conference.

Presenter 6

STRUCTURE AND REACTIVITY OF OXIDIZED 3,4-DIHYDROXYPHENYLACETALDEHYDE

David G Anderson, Virginia Florang, and Jonathan A Doorn*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra of the brain. PD currently affects over a million Americans and results in both motor and cognitive deficits. Among the many factors believed to be involved in the pathogenesis of PD are aberrant dopamine (DA) metabolism and the inherent toxicity of DA and certain DA metabolites. DA is known to exert toxicity via oxidation to deleterious reactive species such as an ortho-quinone and a semi-quinone radical. Formation of these species results in deleterious outcomes that could be toxic for cells and neurons, thereby contributing to the pathogenesis of PD. Oxidation of DA to a quinone can result in nucleophilic protein reactivity and cross-linking, as well as depletion of cellular thiols and reducing equivalents, all known mechanisms of toxicity. Similarly, oxidation of DA to a radical species results in oxidative protein modification or inactivation, interference with proper DA storage, and oxidative DNA damage. Both the radical and the quinone are capable of redox cycling and reactive oxygen species production. 3,4-Dihydroxyphenylacetaldehyde (DOPAL) is the first order oxidative metabolite of DA and is significantly more toxic than its parent amine. Established mechanisms of toxicity for DOPAL includes protein adduct formation via a hypothesized Schiff-base mechanism. Little is known about the ability of DOPAL to undergo oxidation to reactive species such as a quinone or a radical similar to DA. However, the formation of such a species could be key for understanding DOPAL toxicity and could potentially be involved in DOPAL induced protein reactivity. Here, structural evidence is presented indicating that oxidation of DOPAL results in the formation of both a semiquinone radical and an ortho-quinone. Oxidized DOPAL is reactive with thiols such as GSH and is capable of inducing cross-linking with model proteins. Also, reactivity of DOPAL with nucleophiles is greatly attenuated by anti-oxidants or reducing agents. These results indicate that the oxidation of DOPAL may be vital for its reactivity and toxicity. Investigating these oxidized species is important for determining the biological relevance of DOPAL and its potential involvement in PD.

Presenter 7

MODULATION OF THE ACTIVITY OF FLUOROQUINOLONES AND QUINAZOLINE DIONES BY C-2 THIO FUNCTIONALIZATION

Kevin R. Marks, Robert J. Kerns*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

Fluoroquinolones are broad-spectrum antibacterial agents structurally based upon nalidixic acid. Each fluoroquinolone generation has been characterized by a structural change to the core ring system, including addition of fluorine at the C-6 position and electron donating groups at the C-8 position, or alteration to the functional group at N-1. Ulifloxacin, a fourth generation fluoroquinolone, is structurally unique in that it is modified by inclusion of a thiazetidine between the N-1 and C-2 positions. Presented here is an effort to understand the value of the thioether and its effect on antibacterial activity. An initial molecular modeling study suggested that the sulfur may form a point of contact in the binding pocket between the fluoroquinolone and DNA gyrase or Topoisomerase IV, the enzymes responsible for unwinding supercoiled DNA, depending on species. The ternary complex formed between DNA, gyrase/TopoIV, and fluoroquinolone is known to inhibit cellular growth and may lead to rapid cell death by a mechanism resulting in chromosome fragmentation. In an attempt to determine if this binding interaction is a factor in the increased activity of ulifloxacin, we synthesized and tested C-2 thioalkyl fluoroquinolones. Common knowledge being that the 3-carboxylate group is also important for binding, we prepared 3-descarboxy derivatives of clinical fluoroquinolones and of the fluoroquinolones synthesized here for comparison. Antimicrobial studies of the fluoroquinolones showed, surprisingly, that the 3-carboxylate was not required to maintain activity. Additionally, while all of the 2-thioalkyl derivatives were less potent than 2-H compounds, those lacking the 3-carboxylate were far more active than their carboxylate containing counterparts. The loss of activity was attributed to a loss in planarity caused by the steric clash between the 2-thioalkyl and 3-carboxylate groups. Planarity is maintained in ulifloxacin due to the thioether linkage being constrained by the thiazetidine ring. Currently under investigation is the effect of thiocarbonyl substitution for the carbonyl groups of 2,4-quinazoline diones. The quinazoline diones are also potent antibacterials and have been shown not to select for resistant mutant bacteria.

Presenter 8

THYMIDYLATE SYNTHASE CATALYZED H-TRANSFERS: TWO CHAPTERS IN ONE TALE

Zhen Wang and Amnon Kohen*
Department of Chemistry, The University of Iowa, Iowa City Iowa 52242

Examination of the nature of different bond-activations along the same catalytic path is of general interest in chemistry and biology. Here we report a study that compares the physical nature of two sequential H-transfers in the same enzymatic reaction.[1] Thymidylate Synthase (TSase) catalyzes the biosynthesis of 2'-deoxythymidine-5'-monophosphate (dTMP, one of the four DNA bases) in nearly all eukaryotes including humans. The mechanism of TSase comprises a series of bond cleavages and formations including two different C-H bond activations: a rate-limiting C-H-C hydride transfer and a non-rate-limiting C-H-O proton transfer. Although the large kinetic complexity imposes difficulties in studying the proton transfer, we are able to experimentally extract the intrinsic kinetic isotope effects (KIEs) on both steps. We found that the hydride transfer has temperature-independent KIEs while the proton transfer has temperature-dependent KIEs. The results are interpreted within the framework of the Marcus-like model,[2, 3] which suggests that TSase has evolved to optimize the active site better for the hydride transfer than the proton transfer. We hope the current findings will invoke theoretical calculations and high-level simulations that may reveal the molecular details of these two C-H activation steps. Since the detailed molecular mechanisms of both H-transfers are still not clear, further investigation on these steps will discern the transition-state features and may assist in rational drug design.

References:

1. Wang, Z.; Kohen, A., Thymidylate Synthase Catalyzed H-Transfers: Two Chapters in One Tale. *J Am Chem Soc* **2010** *132* (28), 9820-9825.
2. Marcus, R. A., Enzymatic catalysis and transfers in solution. I. Theory and computations, a unified view. *J Chem Phys* **2006,** *125* (19), 194504.
3. Marcus, R. A., H and other transfers in enzymes and in solution: theory and computations, a unified view. 2. Applications to experiment and computations. *J Phys Chem B* **2007,** *111* (24), 6643-6654.

Presenter 9

CHARACTERIZED WET CHEMICAL ETCHING OF InAsGaSb WITH H_3Cit: H_2O_2: HCl ETCHANT FOR THE PURPOSE OF IMPROVED PERFORMANCE OF LIGHT EMITTING DIODES

Deandrea L. Watkins, Jonathon T. Olesberg, Thomas F. Boggess, and Mark A. Arnold*
Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

Near infrared spectroscopy is under development for measuring glucose and other bio-molecules in biological fluids at wavelengths between 2.0 and 2.5 μm. High quality spectra are needed to successfully extract analytical information from near infrared spectra collected from clinical samples. A solid-state near infrared spectrometer would advance the field by providing a means for collecting high quality spectra under non-laboratory conditions. We are developing solid-state light emitting diode (LED) sources from unique InAsGaSb semiconductor materials. Understanding the chemical reactions involved in the etching process of III-V semiconductor materials is key to improving light extraction from these LEDs and thereby enhancing instrument performance. Wet chemical etching with H_3Cit: H_2O_2: HCl as the etchant is used in this work to obtain sidewall geometric patterns that favor increased light extraction. Each component of this etchant system has a function in the etching process. Certain products can be formed during etching process that can adversely affect quality of the semiconducting technology and radiant output of the LEDs. It is well known that oxides, such as M_2O_3 where M represents either the group- III or group-V atom, may form on the surface of the material being etched. These oxides can decrease the performance of an LED.[1,2] Knowing what oxides are formed, how many oxides and how the chemistry of the etching systems affects the formation of oxides or other chemical species Is under investigation as a means to optimize LED performance.

References

1. Chaghi, R., Cervera C., Ait-Kaci, H., Grech, P., Rodriguez, J.B., Christol, P. *Semiconductor Science and Technology* **2009**, *24*(6) 065010.
2. Kadhim, N., Laurie, S., Mukherjee, D. *Journal of Chemical Education* **1998** 75 (7), 840.

LIST OF POSTERS

1. **AN ENZYME FOR ENANTIOSELECTIVE LACTONE PRODUCTION OUT OF A POLLUTANT-DEGRADING ORGANISM**
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   Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City 52242

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   Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

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   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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   Bhakti Bapat, Sucheta Vajrala and David Murhammer*
   Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

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   Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

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   Samuel T. Crowley, Mark Ericson, and Dr. Kevin G. Rice*
   Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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   Department of Microbiology, The University of Iowa, Iowa City, Iowa 52242

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   Center for Biocatalysis and Bioprocessing and the Office of Vice President for Research, The University of Iowa, Iowa City, Iowa 52242

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Carolyn K. Dong and John R. Kirby*
Department of Microbiology, The University of Iowa, Iowa City, Iowa 52242

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Laurie L. Eckert, Virginia R. Florang, Jonathan A. Doorn*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Mark Ericson, Kevin Rice*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy,
The University of Iowa, Iowa City, Iowa 52242

13. SEPARATION AND CHARACTERIZATION OF SULFATED *N*-ARYL OLIGOSACCHARIDES BY ION PAIRING LIQUID CHROMATOGRAPHY

Amanda M. Fenner and Robert J. Kerns*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Fabian A. Grimm, Hans-Joachim Lehmler, Larry W. Robertson and Michael W. Duffel*
Interdisciplinary Graduate Program in Human Toxicology, Department of Occupational and Environmental Health, College of Public Health, and Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Victoria A. Henry, Tonya L.Peeples*, and Julie L. Jessop*
Department of Chemical & Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

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Reza Hussain*, Eric Crock and Bryce Cunningham
Bio-Research Products, Inc., North Liberty, Iowa 52317

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Yang Oh Jin and Timothy E. Mattes*
Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

18. STRUCTURAL STUDIES OF THETIAM1 PHn-CC-EX DOMAIN ALONE AND IN COMPLEX WITH THE PAR3-CC

Monika Joshi[1], Lokesh Gakhar[2] and Ernesto J. Fuentes[1*]
[1]Department of Biochemistry and [2]CCOM Crystallography Core, The University of Iowa, Iowa City, Iowa, 52242

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Ashley D'Ann Koh and Gary A. Aurand*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

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Yi Liang and Tim Mattes*
Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

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Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

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Department of Chemical and Biochemical Engineering and the Center for Biocatalysis and Bioprocessing, The University of Iowa, Iowa City, Iowa 52242

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Duncan I. Mackie and David L. Roman*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Lydia M. Mexas, Virginia R. Florang, Jonathan A. Doorn*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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John R. G. Sander,[1] Dejan-Krešimir Bučar,[1] Rodger F. Henry,[2] Geoff G. Z. Zhang[3]* and Leonard R. MacGillivray[1]*
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Heidi A. Schwanz, Jonathan D. Rosen, Robert J. Kerns*
Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Tyson R. Shepherd, Ann M. Murray, Ernesto J. Fuentes*
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Kristan L. Sorenson, C. Allan Guymon*, and Aliasger K. Salem*
Department of Chemical and Biochemical Engineering, College of Engineering, The University of Iowa, Iowa City, Iowa 52242

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Ryan M. Summers, Chi Li Yu, Michael Louie, and Mani Subramanian*
Department of Chemical and Biochemical Engineering and the Center for Biocatalysis and Bioprocessing, The University of Iowa, Iowa City, Iowa 52242

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Michael Toraason, Zhen Wang, Calvin Luzum, and Amnon Kohen*
Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242

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Tyrell R. Towle, Robert J. Kerns*
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Michael J. Toraason, Arundhuti Sen and Amnon Kohen*
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43. NOVEL ANTIMUTANT C-7 ARYL FLUOROQUINOLONES

Benjamin H. Williamson, Heidi A. Schwanz, Jonathan D. Rosen, Robert J. Kerns*
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44. PURIFICATION AND CHARACTERIZATION OF A NAD(P)H-DEPENDENT TRIMETHYLURIC ACID (TMU) OXIDOREDUCTASE FROM CAFFEINE-DEGRADING STRAIN *PSEUDOMONAS* sp. CBB1

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Abstracts of Posters

Poster 1

AN ENZYME FOR ENANTIOSELECTIVE LACTONE PRODUCTION OUT OF A POLLUTANT-DEGRADING ORGANISM

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Soil bacterium *Polaromonas* sp. strain JS666 is the only isolated organism capable of growth using the groundwater pollutant *cis*-dichloroethene (cDCE) as a sole carbon and energy source. Genome sequencing revealed that an assimilation pathway analogous to that for microbial growth on vinyl chloride (1-chloroethene) does not exist in strain JS666, so several novel degradation mechanisms have been proposed. Proteomics, kinetic studies, and gene expression analysis pointed to the involvement of a monooxygenase, and a plasmid-encoded cyclohexanone monooxygenase (CHMO) was found to be upregulated in response to cDCE exposure.

The CHMO gene was cloned and successfully expressed at high levels in an *E. coli* host, following optimization of expression conditions. Activity of the CHMO was confirmed by observing the conversion of cyclohexanone to ε-caprolactone in transformed *E. coli* cells. Under these reaction conditions, no activity on cDCE was observed, so the enzyme may be coincidentally upregulated or may play some other role in strain JS666's response to cDCE exposure.

The successful heterologous expression system was also used to study the enantioselective behavior of strain JS666's CHMO. CHMOs are known to exhibit varying degrees of enantioselectivity when presented with racemic cyclic ketones, yielding valuable chiral lactone products used as building blocks in the pharmaceutical industry. *Polaromonas* sp. strain JS666's CHMO was found to have extremely high enantioselectivity ($E > 200$) for several 2-substituted cyclohexanones chosen as model compounds. This enzyme is therefore a valuable target for future research into environmentally benign and highly selective biological production of chiral lactones.

Poster 2

MICROSPECTROSCOPIC CHARACTERIZATION OF HUMAN SKIN TISSUE HETEROGENEITY AND ITS EFFECT ON NONINVASIVE GLUCOSE DETECTION

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Ability to measure glucose through a fold of skin noninvasively is an exciting prospect. The measurement is performed in the combination range of the near-infrared spectrum (4000-5000 cm^{-1}; 2.0 -2.5 μm) and uses two optical fibers in a transmission geometry. However, precise glucose quantification is hampered by effects of fiber repositioning. Movement of the interface has been shown to increase prediction errors more than 2.5-fold when the experiment was done on a stationary animal model. Microspectroscopy of excised rat skin samples elucidated spatial heterogeneity in the major components of the tissue matrix and this heterogeneity appears to interfere with noninvasive glucose measurements. On the basis of our measured skin heterogeneities, sets of skin spectra were simulated to investigate the potential impact of fiber repositioning. The resulting model degradation was in most cases larger than 2.5-fold.

In the present work, we extend the study to human skin including skin samples from two male and two female subjects. Four microspectroscopic maps were collected for each subject to compute amounts of water, collagen protein I, fat, and keratin protein within each sample. Constant offset and slope terms serve to model scattering and temperature changes. These values are then used to simulate near-infrared spectra at various locations across skin tissue. These simulated spectra included Gaussian distributed noise and spectra associated with glucose concentrations in the range from 5 to 35 mmol/L.

These preliminary sets of data obtained from human skin demonstrate the presence of domains of major skin components comparable to the diameter of the optical fibers. These domains are slightly larger than those found in rat tissue. The possible reason is the difference in the site of body investigated as well as differences in skin anatomy between species. Nevertheless, when fiber movement was simulated across these human skin samples, the standard errors of prediction (SEP) of multivariate models also increased more than 2.5-fold for some locations compared to a constant location of the interface. This study implicates that chemical heterogeneity of human skin tissue should be accounted for in multivariate models used for noninvasive glucose measurements.

Poster 3

STRUCTURE-SPECIFIC EFFECTS OF DIELDRIN, AN ORGANOCHLORINE INSECTICIDE IN A PARKINSON'S DISEASE MODEL

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Parkinson's disease (PD) is a progressive disorder that leads to the degeneration of dopaminergic neurons in the substantia nigra. This neurodegeneration has been shown to significantly correlate with a number of environmental factors, including exposure to pesticides such as the organochlorine insecticide, dieldrin. This pesticide is ranked one of the twelve most persistent, bioaccumulative and toxic chemicals by the US EPA. Previous studies found an increased concentration of dieldrin in the striatal region of brains of PD patients, and that dieldrin adversely affects a number of cellular processes hypothesized to increase the likelihood to develop PD. However, the mechanism responsible for dieldrin-mediated cellular dysfunction and the structural components contributing to the toxicity of the organochlorine have not been defined. In order to identify the toxicophore of dieldrin, a structure-activity approach was used, with the toxicity profiles of a number of structural analogs of dieldrin (including aldrin, cis aldrin diol, endrin, and isodrin) assessed in differentiated, dopaminergic PC6-3 cells. Cellular assays monitoring mitochondrial activity, cytotoxicity, production of reactive oxygen species, and extracellular dopamine metabolites were used. It was determined that aldrin, dieldrin, and cis aldrin diol substantially inhibited mitochondrial activity, as assessed with an MTT assay. An LDH assay was conducted to evaluate compound cytotoxicity, and aldrin and cis aldrin diol were found to be significantly cytotoxic. In addition, all of the compounds tested were found to disrupt dopamine catabolism, indicated by significant changes in the production of downstream metabolites of dopamine. Comparisons of the toxicity profiles for each dieldrin analog indicate a structure-specific effect that will be important for elucidating the mechanisms of dieldrin toxicity as they relate to PD.

Poster 4

CARBON DIOXIDE INHIBITORY EFFECT ON INSECT CELL GROWTH AND THE ROLE OF INTRACELLULAR pH

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Recombinant protein and biopesticide production with the baculovirus/insect cell system are becoming widespread. Large scale production of these products requires the use of bioreactors with high cell densities and large working volumes. These high cell densities increase the potential for CO2 accumulation in the culture medium to inhibitory levels, thereby reducing the cell growth rate and product production. The aim of the present study was to quantify the effect of elevated CO2 concentration on cellular growth and metabolism, and to determine the mechanism leading to the inhibitory effect of CO2 accumulation.
The effect of CO2 concentration on the growth and metabolism of the *Spodoptera frugiperda* Sf-9 cell line was evaluated in a well controlled agitated bioreactor. The bioreactor was maintained at 27ºC, pH 6.2, and a dissolved oxygen concentration of 20% air saturation. The population doubling time of the Sf-9 cells increased from 23.2 ± 6.7 h (95% confidence level, n=3) to 71.0 ± 24.7 h (95% confidence level, n=5) as the CO2 concentration increased from 0-37 mm Hg to 220 mm Hg. The specific glucose consumption rate did not vary significantly with increasing CO2 concentration (i.e., ~ 3 x 10-17 mol/cell-s throughout), but the lactate production rate increased from -3.0 x 10-19 to 10.2 x 10-19 mol/cell-s.
It was hypothesized that the mechanism through which elevated CO2 could either be (i) oxidative stress (through the production of carbonate and/or peroxymonocarbonate anions) or (ii) intracellular acidification. It was demonstrated that elevated CO2 concentration did not lead to a measurable increase in oxidative stress. A protocol is currently being developed using 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM) to measure the intracellular pH.

Poster 5

SOLUBILIZATION OF HYDROPHOBIC NANOPOROUS PARTICLES WITH SURFACTANTS

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Hydrophobic nanoporous particles are currently pursued as platforms for distinctive biological domains in tissue phantoms and as vehicles for drug delivery. A challenge for their applications is their insolubility in the aqueous biological matrices. We have developed methodologies to improve the solubilization of hydrophobic particles with surfactant molecules. The protocols for solubilization and studies of the surfactanthydrophobic surface interaction are presented. Selective solubilization with and without nanopore wetting generates well separated fractions in the solution preparation. Wetting of the outer surface of hydrophobic C18derivatized silica particles without pore wetting creates a stable layer on top of the aqueous solution. The wetting of both the outer surface and the nanopores generates a layer sedimented from the aqueous solution. Comparative confocal imaging experiments are conducted to probe the wetting dynamics. Images show that the top fraction incorporates particles with wetted outer surface and dry hydrophobic nanopores while the bottom fraction consists of particles with completely wetted nanopores. By preloading the particles incorporated in the top fraction with desired molecules, one can disperse the particles in aqueous matrix while completely preserving the molecular compositions inside. Successful loading of the hydrophobic silica particles with the probe fluorophore Rhodamine 6G (R6G) is confirmed by confocal microscopy. The probe molecules remain encapsulated in the particles in the top fraction at the end of the wetting procedure. The ease of dispersibility of the top-fraction particles in aqueous matrices is tested. The kinetic stability of the dispersed particles is investigated with light scattering. This study has direct implications in tissue phantom preparation in the field of biomedical imaging, and in the development of molecular containers in the field of drug delivery. We demonstrate that the established wetting protocols can efficiently generate particles with dry hydrophobic nanopores that constitute ideal biological domains with well-delimited boundaries in aqueous matrices.

Poster 6

FEASIBILITY OF USING NET ANALYTE SIGNAL CALIBRATION MODELS FOR ON LINE NEAR INFRARED MONITOING OF UREA DURING HEMODIALYSIS

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More than 527,000 people in the United States experience renal failure and must undergo repeated hemodialysis treatments. The total health care expenditures for these treatments was estimated to be $410 billion in 2007. Treatment options for these patients are limited and include kidney transplantation and hemodialysis; however, the limited number of available kidneys makes hemodialysis the more common method. The optimum hemodialysis dose is a key factor in improving patient outcomes. For that reason, frequent monitoring of urea concentration is significant because it provides a means to track hemodialysis dosage. In addition, monitoring urea can be used to identify the problems associated with the dialysis process and, potentially, take corrective measured before adversely affecting the patient. Therefore, development of a continuous and rapid urea sensing monitor will be beneficial to patients with renal failure.

A near-infrared spectrometer based on an acousto-optic tunable filter (AOTF) has been constructed specifically for monitoring the concentration of urea in spent dialysate collected during the hemodialysis process. Near-infrared spectroscopy can be used to quantify multiple species within complex biological samples. The AOTF is a rugged, fast scanning solid state device that is well suitable for clinical application. In this technique, the selective analytical signature of the targeted analyte, such as urea, can be used to measure its concentration. The net analyte signal (NAS) presents this selective signature and can be determined from a multivariate analysis of the near infrared spectra.

To test the feasibility of online urea monitoring pure components spectra of urea and other major active components in spent dialysate, such as glucose, lactate, creatinine, alanine, and glutamine were collected. A NAS calibration model was built for urea based on absorbance spectra of these pure components. To validate the calibration model, spectra of mixtures of these pure components were collected and the concentration of urea was predicted from these mixture spectra. Urea was predicted with a standard error of 0.37 mM, indicating adequate analytical performance. Details of the NAS calibration models will be presented, including selectivity, sensitivity, and stability over time.

A STRATEGY TO NONCOVALENTLY ATTACH AN ENZYME TO DNA

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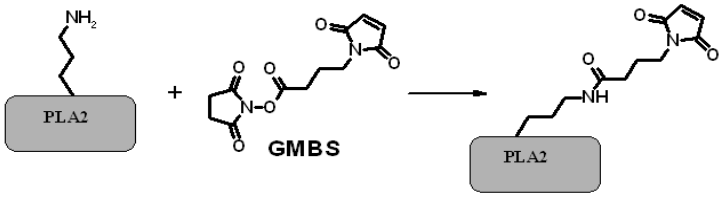
DNA plays a vital role in all cellular functions, storing the information that codes for proteins, RNAs, and their regulation. As such, DNA is a valuable target in therapies against cancer, hereditary disease, and other conditions. Many small molecules are available for binding DNA and the enzymes that process it, but methods for binding whole proteins to DNA would also be useful. Therefore, we have developed a method to noncovalently bind enzymes to DNA.

PLA2 was modified with the heterobifunctional linker γ-maleimide-butyl-succinimide, GMBS, (Step1), which was then used to attach the DNA binding peptide $(Acridine\text{-}Lysine)_6$-cys[1], $(AK)_6$, (Step 2). Reaction progress was monitored with reverse-phase HPLC and SDS-PAGE. PLA2 activity was monitored with a bromothymol blue assay, using phosphatidylcholine as the enzyme's substrate.

Step 1:



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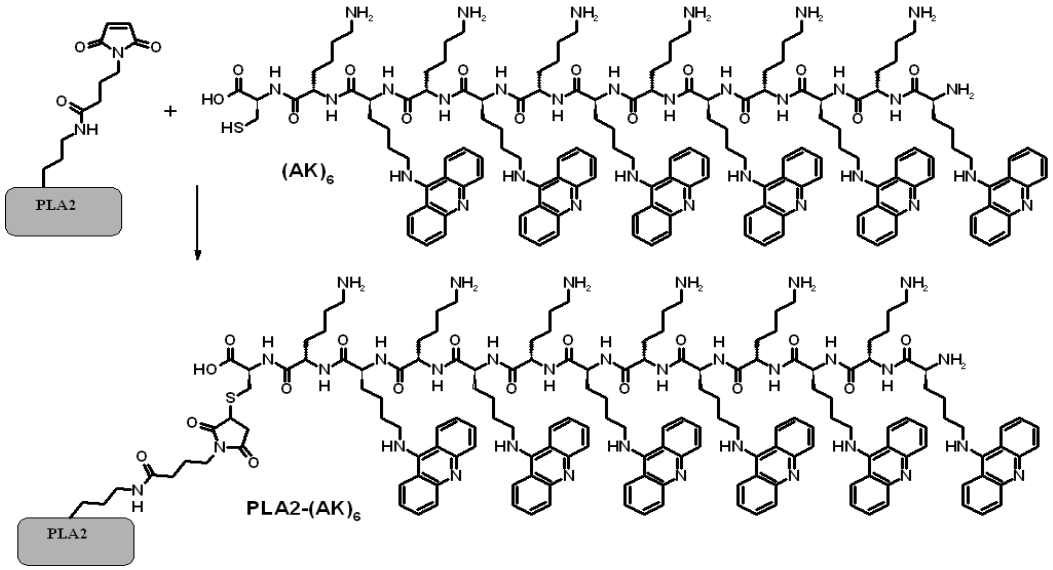

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Poster 8

THE MYXOCOCCUS XANTHUS Che7 SYSTEM REGULATES CAROTENOIDS

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The soil bacterium *Myxococcus xanthus* produces carotenoids in response to stressors including light, copper, and high levels of oxygen. To keep carotenoid levels balanced in the cell, a complex regulatory network, named Car, keeps tight control over transcription of carotenoid biosynthetic genes. In addition to the many described regulators, we have discovered new genes that appear to be involved in maintaining proper carotenoid levels. The Che7 system would represent an emerging role for chemosensory systems in regulation of secondary metabolites. Chemosensory systems function as modified two-component phosphorelay with a derived adaptation feature. Insertional mutations in *che7* genes leads to disregulation of carotenoids. Mutations in *cheB7*, *mcp7*, and *cpc7* lead to overproduction of carotenoids while a mutation in *cheR7* results in lowered production compared to the parent strain. By combining these mutations with known regulators of the Car pathway, we were able to establish epistatic relationships. A strain deleted for the *carR* gene constituatively produces carotenoids, even in the absence of inducing signal. A *carF* mutation results in a strain that cannot produce carotenoids in response to light, but can upon addition of copper. Further studies are being done with additional regulators in the pathway to determine a converging point. By dissecting the role of Che7 in the production of secondary metabolites such as carotenoids, we can more fully understand how to manipulate these systems to maximize the output.

Poster 9

CHEMO-ENZYMATIC ONE-POT DYNAMIC RESOLUTION OF 2-HYDROXY ACIDS BY GLYCOLATE OXIDASE (GO) AND CATALASE COEXPRESSED IN *PICHIA PASTORIS*

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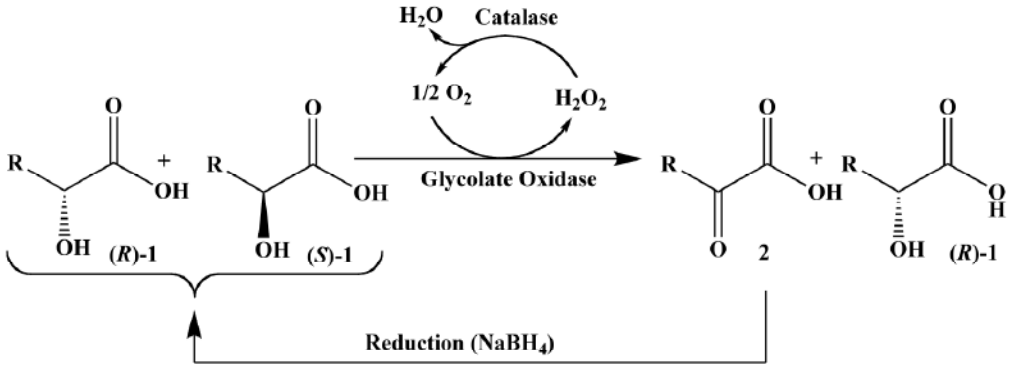
Optically active 2-hydroxy acids are important building blocks for the asymmetric synthesis of numerous bioactive molecules (e.g., A2 antagonists, Ca-channel blockers, ACE inhibitors, beta-blockers) including glycols, halo esters and epoxides. These chiral acids are used in pharmaceutical, biotechnological and chemical industries. Glycolate oxidase ((*S*)-2-Hydroxyacid oxidase, EC 1.1.3.15) is a flavin mononucleotide-dependent enzyme, which catalyzes oxidation of 2-hydroxy carboxylic acids (1) such as lactate or glycolate, to the corresponding 2-keto acids (2). Catalase has been used as cocatalyst to decompose hydrogen peroxide produced in the reaction, thus limiting peroxide-based side reactions and GO deactivation. GO from spinach and catalase T from *Saccharomyces cerevisiae* were previously coexpressed in methylotrophic yeast *Pichia pastoris* strain NRRL Y-21001. Permeabilized whole cells of *P. pastoris* were used for the oxidation of 3-phenyllactic acid, 3-indolelactic acid, 3-chlorolactic acid, 2-hydroxybutanoic acid, and 2-hydroxydecanoic acid to demonstrate high degree of selectivity to the (*S*)-enantiomers leaving (*R*)-isomers intact. Non-selective reduction (e.g., sodium borohydride reduction) of the 2-keto acids (**2**) produced by GO, created a commercially feasible dynamic process for resolution of 2–hydroxy acids (**1**) with high yield of the (*R*)-enantiomers (> 95%) in one pot. GO could be recycled 3 times with > 80% yield of the (*R*)-acids. The entire "Single pot – two step" process was carried out in water and at room temperature in order to make the process economical.



Poster 10

NOVEL REGULATION OF RIBOFLAVIN BIOSYNTHESIS BY A *C*HEMOSENSORY PATHWAY IN *MYXOCOCCUS XANTHUS*

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The predatory gram-negative soil bacterium, *Myxococcus xanthus*, is a model organism for the study of social motility, cell-cell communication, and biofilm formation. Like many bacterial species, *M. xanthus* secretes riboflavin (vitamin B_2), a water-soluble vitamin produced by plants and some bacteria, but not mammalian species. Riboflavin is a precursor for cofactors, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), and therefore essential for a number of enzymatic functions in the cell. Interestingly, the *M. xanthus* riboflavin biosynthesis genes are co-transcribed with a set of chemosensory genes (*che8*). Chemosensory pathways are known to regulate motility, but have not previously been linked to riboflavin biosynthesis. However, preliminary work in our laboratory has suggested that mutations within this operon lead to deregulation of riboflavin production by cells grown in liquid culture as well as defects in biofilm formation on surfaces. We propose that *M. xanthus* regulates riboflavin biosynthesis via the *che8* system. Such a link may have novel implications for microbial production of riboflavin in industry.

Poster 11

INTERACTION OF MICROGLIA AND AN ENDGENOUS NEUROTOXIN, 3,4-DIHYDROXYPHENYLACETALDEHYDE: METABOLISM, ACTIVATION, AND TOXICITY

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The cause of the hallmark dopaminergic cell death of Parkinson's Disease (PD) is unknown, but recent research indicates oxidative stress and the endogenous neurotoxin, 3,4-dihydroxyphenylacetaldehyde (DOPAL), to play roles in the disease pathogenesis. DOPAL is generated from dopamine (DA) by monoamine oxidase (MAO) and oxidized to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase. The dopamine metabolite is highly toxic to dopaminergic cells and needs to be rapidly metabolized to prevent toxicity. Non-neuronal cells express high levels of MAO-B, but formation and metabolism of DOPAL within these cells has not previously been measured. Microglial cells have been shown to be activated in neuronal regions containing high MAO-B activity via DA-protein adducts, specifically in the striatal region containing the substantia nigra, however the mechanism of this activation has not been demonstrated, and could be due to DOPAL or DOPAL-protein adducts. Activated microglia cause injury to dopaminergic neurons via a host of mechanisms, including reactive oxygen species production, release of cytokines, and phagocytic activity. The ability of DA, DOPAL, and other DA metabolites to activate BV-2 microglial cells was previously unknown, but DOPAL-mediated activation has been demonstrated in this work, as measured by TNF-α secretion. Metabolism of DA and DOPAL and toxicity of DOPAL, as analyzed by the MTT and LDH assays, were determined for microglia. It was found that BV-2 cells metabolize DA to DOPAL, and DOPAL further to DOPAC, and in addition, greater cytotoxicity was observed for those cells treated with DOPAL compared to DA. DOPAL-mediated activation of microglia demonstrated in this study could represent a mechanism for inflammation and dopaminergic cell death detected in patients with PD.

Poster 12

A NOVEL SOLID PHASE SYNTHESIS OF REDUCIBLE PROTEINS

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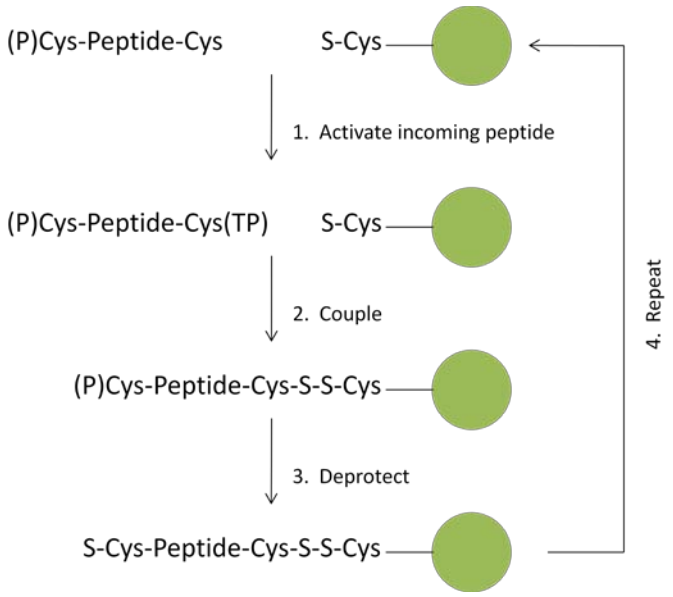
Since the first report by Merrifield on solid phase peptide synthesis (SPPS) in 1963, innumerable short length peptides and proteins have been synthesized on a solid support. With the relative simplicity of reactions, automation of the process, and ability to synthesize libraries of peptides in a parallel fashion, SPPS has become critical in the investigation of small biologically active peptides. Here we present our work in developing a new methodology of SPPS. Unlike traditional SPPS, where individual amino acids are sequentially added, we propose to add full length cysteine flanked peptides in each coupling cycle. A protein synthesized in this manner would be composed of many peptides subunits individually constructed of amide bonds, and subunits linked together through disulfide bonds. Such a monodisperse reducible protein would have many applications in gene and drug delivery.

To this end, a variety of model peptides with orthogonally protected cysteines have been synthesized and purified; the C-terminal cysteines have been selectively deprotected and activated as thiopyridines. These peptides are being reacted in solution to determine the optimal reaction conditions, which will then be applied to the solid phase synthesis. Concurrently, different resins derivatized with an array of protected cysteines are being examined to discover a loaded resin that can be deprotected yielding a free cysteine thiol. Once suitable conditions are found, we will begin synthesis of full length peptides that will be coupled to produce the target reducible proteins.



Poster 13

SEPARATION AND CHARACTERIZATION OF SULFATED *N*-ARYL OLIGOSACCHARIDES BY ION PAIRING LIQUID CHROMATOGRAPHY

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Hundreds of eukaryotic and prokaryotic proteins bind to cell surface glycosaminoglycans (GAGs) to promote biological activities. Development of compounds to block GAG-protein interactions has primarily focused on optimizing the degree and orientation of anionic substituents on a scaffold, to mimic GAG structure, but their utility is diminished by non-specific interactions with many proteins. To overcome these limitations, our lab demonstrated that replacing *N*-sulfo groups on heparin with non-anionic *N*-arylacyl groups increased affinity and selectivity for binding different heparin-binding proteins. Here we report the preparation and characterization of *N*-aryl substituted tri- and tetrasaccharides followed by sulfonation of the hydroxyl groups. An LC-MS method to analyze components of the resulting mixtures was developed using ion-pair-RP-HPLC to separate oligosaccharides based on degree and position of sulfates, and ESI-MS to characterize individual molecules. Screening for biological activity indicates the aromatic substituent, core saccharide, and degree of sulfation are all important for differentiation of function.

SULFATED METABOLITES OF POLYCHLORINATED BIPHENYLS BIND WITH HIGH AFFINITY TO THE THYROID HORMONE TRANSPORTER TRANSTHYRETIN

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Polychlorinated biphenyls (PCBs) represent a class of hazardous chemicals that were used extensively in a variety of technical applications from the 1930s until their production was banned in the United States in the late 1970s. The ban was a reaction to increasing numbers of reports relating PCB exposure to adverse human health effects. Due to their bioaccumulation and resistance to biodegradation, PCBs remain among the most widespread environmental contaminants. Among the observed hazardous effects, populations exposed to high concentrations of PCBs were shown to develop pathological abnormalities of the thyroid gland, and PCB exposure is associated with decreased serum concentrations of thyroid hormones. Previous research has revealed that certain PCBs as well as their hydroxylated metabolites (OHPCBs) are capable of displacing L-thyroxin from one of its transporter-proteins, transthyretin (TTR), by competitive binding. However, there is little information available about the potential effects of conjugated PCB metabolites. Although some OHPCBs are excellent substrates for the sulfotransferases (SULTs) that catalyze the formation of sulfate conjugates, little is known about the fate or toxicity of these sulfate esters. In the current study, three 4'hydroxylated PCB congeners, 4'OHPCB 3, 4'OHPCB 9 and 4'OHPCB 12, as well as their corresponding sulfates, were examined for their ability to bind to human TTR via the quantification of 8-anilinonaphthalene-1-sulfonate (ANS) displacement from hTTR. A comparison of the binding properties of the respective OHPCBs, PCB-sulfates, and L-thyroxin clearly indicated similar binding affinities of all tested compounds with K_d values between 2.7 and 6.7 nM for the high-affinity binding site in TTR. Initial experiments to determine appropriate concentrations for examination of the three OHPCBs as substrates for family 1 hepatic sulfotransferases included their ability to inhibit the sulfation of 0.8 µM 4-methylumbelliferone, a fluorescent substrate for family 1 SULTs. A comparison of cytosolic extract of *E.coli* BL21(DE3) expressing either human or rat SULT1A1 and purified rat SULT1A1 revealed IC_{50} concentrations between 0.3 and 0.6 µM. These results indicate strong interactions with the rat and human SULT1A1, and they will guide our current examination of the ability of these SULTs to catalyze sulfation of these OHPCBs. Thus, our current results on the binding of PCB-sulfates to TTR suggest a potential relevance in PCB-mediated thyroid hormone disruption. [Supported by NIH P42 ES013661]

Poster 15

MONITORING ATRAZINE DEGRADATION IN *PSEUDOMONAS ADP* BIOFILM WITH RAMAN SPECTROSCOPY

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Removal of xenobiotic compounds from the environment is of great concern due their adverse effects; however, current approaches used to resolve this problem are both expensive and inadequate. A more cost effective approach may be relying on microorganisms degradation of xenobiotic compounds in situ. Therefore the purpose of the research project is to examine the integration of biofilm flow devices with Raman Scattering for bioanalysis and separation. Proposed hypotheses are 1) Raman scattering can be used to identify and quantify members of biofilm communities and 2) Raman scattering can be used to evaluate the persistence of the model contaminant atrazine and metabolites in the flow systems. Using biofilm flow devices with atrazine as a model xenobiotic compound, the team will evaluate catabolic pathways that facilitate biodegradation in samples. The success of the research may lead to improved bioremediation technologies which can assist in the reduction of environmental contamination and associated risk to human health.

IMMOBILIZED ENZYMES FOR ORGANIC SYNTHESIS

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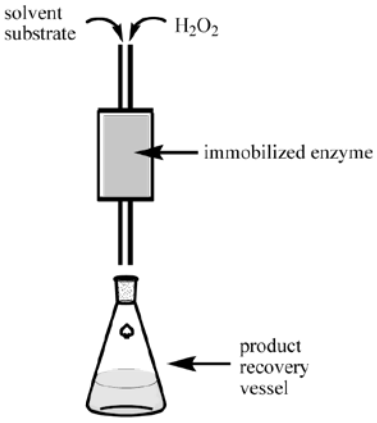
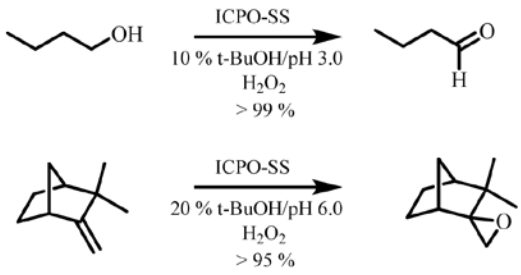
Chloroperoxidase (CPO) is a versatile enzyme for use in organic synthesis. The immobilized form of CPO (ICPO-SS) has shown increased stability, recyclability, and yield. Free CPO loses activity at increased peroxide concentrations, but the immobilized form shows improved stability and recyclability. Transformations of functional groups by CPO are as follows: allylic and benzylic carbons to alcohols, double and triple bonds to epoxides, primary alcohols to aldehydes, halogenation of activated carbons, and sulfoxidations; many of the reactions are highly enantioselective. The ICPO-SS is essentially a sponge that can be molded to fit a specific reactor and allows flow through conditions. We have used ICPO-SS for oxidations in a continuous flow system that is very effective for recyclability and product recovery.



Continuous Flow System

ICPO-SS Reactions



Poster 17

A QUANTITATIVE PCR ASSAY FOR AEROBIC, VINYL CHLORIDE- AND ETHENE-ASSIMILATING MICROORGANISMS IN GROUNDWATER

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Quantitative real-time PCR (qPCR) methods are an increasingly popular means of providing evidence for the presence and abundance of microbes involved in bioremediation of pollutants in the environment. A widespread groundwater contaminant of concern is vinyl chloride (VC), a known human carcinogen that is primarily formed via anaerobic dechlorination of chloroethenes. Recent research suggests that aerobic, VC-oxidizing bacteria in groundwater could be important in the natural attenuation of VC plumes formed under anaerobic conditions. A qPCR method for quantifying the abundance of aerobic, VC-oxidizing bacteria in groundwater would be used to support VC bioremediation strategies and assist in site closure; however, qPCR methods for VC-oxidizing bacteria are not available. In response to this need, we developed and tested degenerate real-time PCR primers that amplify two different functional genes (*etnC* and *etnE)* involved in the aerobic VC and ethene biodegradation pathways. Application of these qPCR primers to groundwater samples from three different contaminated sites revealed that *etnC* gene abundance ranged from 1.6×10^3 - 1.0×10^5 copies per L groundwater while *etnE* gene abundance ranged from 4.3×10^3 - 6.3×10^5 copies per L groundwater. This environmental measurement method indicates the potential for aerobic VC oxidation at these sites and strengthens the hypothesis that these microbes are widespread in the environment.

Poster 18

STRUCTURAL STUDIES OF THETIAM1 PHn-CC-EX DOMAIN ALONE AND IN COMPLEX WITH THE PAR3-CC

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The T-lymphoma and metastasis gene 1 (Tiam1) encodes a guanine exchange factor protein that is specific for the Rac1 GTPase. Tiam1 is a large, multi-domain protein that contains several protein-protein binding domains that are important for regulating function. The PHn-CC-Ex domain is critical for plasma membrane association and the interaction with multiple protein scaffold proteins (e.g. Par3, Spinophilin, IRSp53, and JIP2) to direct Tiam1-Rac1 signaling specificity. We have initiated studies on Tiam1 PHn-CC-Ex and Par3 coiled-coil (Par3-CC) domains in order to understand the thermodynamic and structural basis for PHn-CC-Ex/Par3 interactions and signaling specificity. We have identified the region in Par3-CC that binds the PHn-CC-Ex domain and fluorescence anisotropy data indicates the affinity (K_d) of the complex is ~30 micromolar concentration. We have obtained crystals of the Tiam1 PHn-CC-Ex domain alone and collected diffraction data to 2.5 Å resolution. The preliminary structure indicates that the PHn and the CC-Ex region form independent domains that together provide a platform for binding the Par3-CC region. Small angle X-ray scattering (SAXS) data indicates that no large structural change occurs upon formation of the Tiam1 PHn-CC-Ex/Par3-CC complex. Together, these data begin to elucidate the structural mechanism for PHn-CC-Ex/scaffold interactions and Tiam1-Rac1 signaling.

Poster 19

TWO-STEP BIODIESEL PRODUCTION USING SUPERCRITICAL ETHANOL

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Current industrial biodiesel production utilizes an alkali catalyst that can participate in saponification side reactions. The side reactions are reduced by using highly refined vegetable oil feedstocks. Also, the catalyst must be extracted from the final product in a washing step. A catalyst-free alternative for the production of biodiesel is being developed. It will involve two reaction steps: 1) triglyceride hydrolysis (fat splitting) at subcritical conditions to separate glycerol from fatty acids, and 2) fatty acid esterification in supercritical ethanol to form fatty acid ethyl esters. The catalyst-free process potentially can be used with a variety of low-cost vegetable and animal fats without undesired side reactions. Esterification experiments were carried out in a batch reaction system to obtain kinetics data. A Raman spectroscopic method was developed for the analysis of the esterification reaction products.

Poster 20

POTENTIAL OF POLYCHLORINATED BIPHENYLS (PCBs) MICROBIAL BIODEGRADATION IN SEDIMENTS FROM INDIANA HARBOR, IN

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Microbial PCB biodegradation can proceed under anaerobic and aerobic conditions; however, there is little direct evidence to indicate that PCB biodegradation occurs in the environment. The objective of this research is to use molecular microbiology techniques to assess the possibility that microbial communities in PCB-contaminated sediment samples are involved in PCB biodegradation. Genomic and proteomic analysis were performed on the sediment samples from Indiana Harbor and suggested a high potential for aerobic microbial PCB degradation. The biphenyl dioxygenase alpha subunit gene (*bphA*), which encodes part of an enzyme known to catalyze aerobic PCB biodegradation, was retrieved from three surficial samples and one core sediment sample. BphA gene sequences were found to be closely related to biphenyl dioxygenase from *Pseudomonas putida, Pseudomonas alcaligenes, Comamonas testosteroni, Pseudomonas alcaligenes, Burkholderia* sp. WBF3, and *Pseudomonas* sp. KKS102, all of which are known PCB-degraders. Furthermore, biphenyl-2,3-diol 1,2-dioxygenase from *Azorhizobium caulinodans* was detected in the core sediment sample when qTOF MS/MS spectra were searched against a protein database established in Uniprot containing all biphenyl dioxygenase sequences. However, effort is still needed to improve protein extraction from complex environmental matrix and to develop the most appropriate database for making protein identifications from tandem mass spectra.

Poster 21

KINETIC EVALUATION OF DUAL BINDING HUMAN ACETYLCHOLINESTERASE INHIBITORS

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Synthesis and kinetic evaluation of dual binding acetylcholinesterase (AChE) inhibitors targets both the cholinergic and β-amyloid plaque pathways of Alzheimer's disease (AD) treatment. Aryl-trifluoroketones, ladderane natural product derivatives, and paracylcophane moities have been evaluated as potential human AChE inhibitors. Dose response assays, using the Ellman[1] method, of quinoline and N-methylquinolinium aryl-trifluoroketones showed IC_{50} values in the 10^{-9} M range. Additionally, both aryl-trifluoroketone moities were observed to be tight binding while only the N-methylquinolinium showed time dependent inhibition. Dose response assays of chiral and achiral tetrapyridyl-5-ladderane (TPL5) showed IC_{50} values in the 10^{-6} M range. Similarly, the tetrapyridyl-paracylcophane (TPPCyc) and N-methyl tetrapyridyl-paracylcophane (Me-TPPCyc) showed IC_{50} values in the 10^{-5} M and 10^{-6} M range respectively. Lineweaver-Burk analysis of Me-TPPCyc showed its mode of inhibition to be noncompetitive.

Reference:
1. Ellman, G.L., Courtney, K.D., Andres, V., Jr. & Featherstone, R.M. *Biochem. Pharmacol.* (1961), 7, 88-95

Poster 22

CLONING AND FUNCTIONAL EXPRESSION OF NdmA AND NdmB, TWO POSITIONAL-SPECIFIC METHYLXANTHINE *N*-DEMETHYLASES FROM *PSEUDOMONAS PUTIDA* CBB5

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Pseudomonas putida CBB5 is capable of utilizing caffeine (1,3,7-trimethylxanthine) as the sole source of carbon and nitrogen. A unique *N*-demethylase that demethylates caffeine to xanthine has been purified from CBB5. This soluble *N*-demethylase is composed of two components, a reductase component and a 2-subunit *N*-demethylase component (Ndm). The two subunits of Ndm were designated as NdmA and NdmB. Ndm was deduced as a Rieske [2Fe-2S] domain-containing non-heme iron oxygenase. Therefore, we used degenerate PCR primers designed from the N-terminal protein sequences of NdmA, NdmB, and conserved domains in other Rieske, non-heme iron oxygenases and successfully identified two complete ORFs from a CBB5 genomic DNA library. The deduced protein sequences encoded by the 5′ end of the first and second ORFs were 100% identical to those of NdmA and NdmB, respectively. Both ORFs encoded 40-kDa products, in good agreement of the MWs of NdmA and NdmB. Therefore, these two ORFs were designated as *ndmA* and *ndmB*. *ndmA* and *ndmB* were cloned individually into the pET32a expression vector as C-terminal His-tagged fusion genes. NdmA-His and NdmB-His proteins were purified using a Ni-NTA column. Surprisingly, purified NdmA-His alone was capable of *N*-demethylating caffeine to theobromine (3,7-methylxanthine) stiochiometrically; theobromine was not further *N*-demethylated. Replacing caffeine with theophylline (1,3-methylxanthine) and paraxanthine (1,7-methylxanthine) resulted in the production of 3-methylxanthine and 7-methylxanthine, respectively. 3-Methylxanthine and 7-methylxanthine were not substrates for NdmA-His. These results indicated NdmA is an *N*-demethylase that specifically removes the N-1 methyl group from methylxanthines. On the other hand, NdmB-His alone was active on theobromine and 3-methylxanthine, producing 7-methylxanthine and xanthine, respectively. It was also active on caffeine and theophylline, producing paraxanthine and 1-methylxanthine, respectively. However, NdmB-His activities on these 2 substrates were less than 10% of those on theobromine and 3-methylxanthine. Paraxanthine and 7-methylxanthine were not substrates of NdmB-His. These results suggest: (i) NdmB specifically removes the N-3 methyl group from methylxanthines and (ii) the 1-methyl and/or 7-methyl groups on caffeine and theophylline could interfere to some extent with NdmB activity. To our knowledge, this is the first report of Rieske, non-heme iron oxygenases with positionally specific *N*-demethylase activities.

Poster 23

HIGH-THROUGHPUT SCEENING FOR SMALL MOLECULE INHIBITORS OF RGS17 FOR TREATMENT OF LUNG AND PROSTATE CANCERS

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G-Protein Coupled Receptors are one of the most important targets in drug development with over 50% of all drugs on the market targeting them. Recent studies have implicated a role of Regulator of G-Protein Signaling (RGS) proteins in the development and progression of pathologies, including some cancers. RGS17, the most-recently identified family member of the RZ family of RGS proteins has been implicated in the growth, proliferation, metastasis and migration of prostate tumors as well as small-cell and non-small cell lung cancers. In this study, we developed a novel high-throughput screening method for interrogating small molecule libraries for inhibitors of RGS-17. The novel screening method utilizes a measurement of the RGS-17/ G protein interaction, and is a very robust method, with small amounts of purified protein (20 ng) and excellent Z-scores exceeding 0.7. Here we present the development and validation of this novel high-throughput screening for RGS protein targets. This method will allow for further high-throughput screening of the RGS-proteins while using a faster and more robust technology. This screen has established lead pharmacophores for further optimization of structure focused on activity in enzymatic, whole cell, xenograft and whole animal models as well as potential new avenues for anticancer therapies.

3, 4-DIHYDROXYPHENYLACETALDEHYDE TOXICITY, METABOLISM, AND EFFECT ON TYROSINE HYDROXYLASE ACTIVITY

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Parkinson's Disease (PD) is a progressive neurodegenerative disorder, characterized by the loss of dopaminergic neurons. This leads to motor symptoms including resting tremors, bradykinesia, and muscle rigidity. Dopamine (DA) is an important neurotransmitter which undergoes catabolism to form 3, 4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is structurally analogous to DA, but is a reactive intermediate; therefore, it has the potential to interact with proteins containing DA-binding sites. Recent studies have shown that DOPAL, at pathological levels, modifies proteins in dopaminergic cells. Currently, the identity of these target proteins and the effect on function are unknown. Therefore, it is hypothesized that DOPAL is a toxic dopamine metabolite which modifies and inhibits enzymes that are important to dopamine biosynthesis and trafficking. Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in DA synthesis, converting tyrosine to 3, 4-dihydroxyphenylalanine (L-DOPA). To study DOPAL metabolism and toxicity, nerve-growth factor differentiated PC6-3 cells were incubated in the presence of DOPAL (5-50 μM) for 2 hours and aliquots were obtained at 30 minute intervals. HPLC analysis indicated metabolism to both the acid 3, 4-dihydroxyphenylacetic acid (DOPAC), as well as the alcohol 3, 4-dihydroxyphenyl-ethanol (DOPET). Furthermore, toxicity studies showed a decrease in cell viability with increasing concentrations of DOPAL present. Tyrosine hydroxylase activity was studied using PC6-3 cell lysate. Lysate was treated with either tyrosine or tyrosine and DOPAL (0.5-10 μM) and the formation of L-DOPA was quantified using HPLC analysis over a 150 minute time course. Results showed a smaller increase in L-DOPA formation when DOPAL was present, as compared to controls. Overall, these results indicate that DOPAL may adversely affect not only cell viability, but the function of important DA biosynthesis enzymes, such as tyrosine hydroxylase.

Poster 25

HUNTING FOR AN INTERMEDIATE: STUDIES OF CHEMICAL MECHANISM OF FLAVIN-DEPENDENT THYMIDYLATE SYNTHASE

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Thymidylate synthase (TS) enzymes catalyze the *de novo* production of thymidylate, a DNA nucleotide, from deoxyuridine monophosphate and N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate. In nearly all eukaryotes, including humans, this reaction is carried out by classical TS encoded by *thyA* gene. Chemical and kinetic mechanisms of classical TS have been thoroughly studied, and several mechanism-based TS inhibitors (e.g. 5-fluorouracil, raltitrexed, pemetrexed) are used as anti-cancer drugs. Thymidylate production in many human pathogenic bacteria (e.g. typhus-causing *R. prowazekii*, *M. tuberculosis*, and *B. anthracis*) depends on a *thyX* enzyme, analogous in function but dissimilar in structure and mechanism from classical TS. The *thyX* catalysis relies on a bound flavin cofactor, which cycles between oxidized and reduced forms in the course of the reaction. Little is known about the chemistry of the flavin-dependent thymidylate synthesis. The current work presents the effort towards trapping, isolation and characterization of any intermediate(s) in *thyX* TS-catalyzed reaction, which will illuminate this enzyme's chemical mechanism and assist in inhibitor and drug design. In particular, the results of rapid-quench experiments using cold and radiolabeled substrates are reported.

Poster 26

OXIDATIVE STRESS MODIFIES RGS4

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Regulators of G-protein signaling (RGS) proteins are responsible for regulating the signal transduction cascades initiated by G-protein coupled receptor (GPCR) activation. RGS proteins temporally regulate the signal transduction from GPCRs by acting as GTPase accelerating proteins (GAPs), increasing the rate of hydrolysis of GTP to GDP by the Gα subunit. RGS proteins GAP Gαo, Gαi, and Gαq but not Gαs. RGS4 is widely expressed member of the RGS family which has been shown to regulate M4 muscarinic autoreceptors that act to slow the autonomous pacemaking of the cholinergic interneurons in the striatum. Downregulation of M4 autoreceptor function could lead to increased acetylcholine release which has been implicated as a critical factor for many of the motor symptoms associated with Parkinson's disease. Oxidative stress in the striatum is another important marker in the pathology of Parkinson's disease. One of the effects of oxidative stress in cells is the formation of lipid peroxidation products. These products may form adducts on reactive amino acids such as cysteine. Key cysteine residues have been recognized in RGS4 at both catalytic face as well as allosteric sites. We hypothesize that oxidative stress modifies RGS4 functionality within the cell by covalent modification at cysteine residues. Using 4-hydroxynonenol, a reactive aldehyde product of lipid peroxidation, as a model for oxidative stress we observed the purified RGS4 for the formation of adducts. A western blot detecting 4HNE adducts on cysteine, lysine, and arginine detected only RGS4 and the cysteine-null mutant. Confirmation with mass spectrometry showed that the cysteine-null mutant formed no adducts. This confirms that modification by 4HNE occurs only at cysteine residues. Future work will focus on identifying which cysteine residues are readily modified by oxidative stress as well as the extent of modification in whole cells.

SECONDARY METABOLITES FROM A FUNGICOLOUS ISOLATE OF *ASPERGILLUS FLAVIPES*

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During our continuing studies of mycoparasitic and fungicolous fungi, an isolate of *Aspergillus flavipes* (NRRL 58569) was collected from basidiomata of *Earliella scabrosa* in an alien wet forest on the island of Hawaii. Several metabolites, including a new tetrapeptide were isolated from this extract and described in an earlier presentation. Further studies of this extract afforded another new compound, a modified tripeptide consisting of three amino acids, including an uncommon 3-hydroxyanthranilic acid unit, as well as a *p*-hydroxybenzoic acid unit. Although not a complex metabolite, there are no particularly close analogs to this compound in the literature. The structure was determined by analysis of ^{1}H and ^{13}C NMR, HRESITOFMS, HMBC, and HMQC data. The absolute configurations of the individual amino acid units were determined by GCMS analysis of the corresponding trifluoroacetyl *sec*-butyl ester derivatives. An additional known bioactive compound (PF1233B) was also isolated from this extract, but the corresponding structure has appeared only in the patent literature, and complete data for it have not been previously reported. PF1233B incorporates a modified tryptophan unit and a phenyllactic acid moiety, united to form a dioxomorpholine ring, and a prenyl group substituent. It was identified primarily through analysis of HRESITOFMS, HMBC, and HMQC data. Although known, a full complement of spectroscopic data was used to identify PF1233B because of unusual NMR signals that were observed and the lack of detailed spectral information available in the literature. Details regarding the isolation and identification of these two natural products will be presented.

Poster 28

REACTION KINETICS OF CELLULOSE HYDROLYSIS IN SUPERCRITICAL AND SUBCRITICAL WATER

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More efficient conversion technologies are needed to utilize cellulosic biomass for the production of fuels and chemicals. Cellulose can be hydrolyzed very rapidly in supercritical water without the need for an enzyme or other catalyst. Fermentable sugar yield and selectivity obtained from this continuous flow process are low due to monosaccharide decomposition at high temperatures. High selectivity could be achieved by ensuring that the rate of cellulose hydrolysis is high relative to the rate of monosaccharide decomposition. However, treatment at lower, subcritical temperatures is ineffective because of cellulose insolubility. Results of hydrothermal oligosaccharide reactions suggest that monosaccharide yield and selectivity may be enhanced significantly via control of the reactor temperature. More detailed understanding of hydrothermal cellulose dissolution and hydrolysis kinetics is required to develop an efficient treatment process. Computer simulations of cellulose hydrolysis have been performed based on a variety of hydrolytic scission modes. The simulation results will be compared to cellulose molecular weight distributions from hydrolysis experiments in subcritical and supercritical water. The objective is to determine the mode(s) of scission and the corresponding reaction kinetics parameters. The results will be incorporated into a comprehensive model describing the process of cellulose conversion in hydrothermal systems. The model subsequently will be used in the development of an improved process design.

Poster 29

A NEW CYCLIC PEPTIDE FROM A FUNGICOLOUS HAWAIIAN ISOLATE OF *SESQUICILLIUM MICROSPORA*

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Our studies of mycoparasitic and fungicolous fungi have yielded a variety of novel bioactive compounds. In the course of our ongoing studies of such species, an isolate of *Sesquicillium microspora* (MYC-1881) was obtained from a basidioma of *Phellinus gilvus* growing on a dead branch collected in a Hawaiian coastal forest. The ethyl acetate extract of solid-substrate fermentation cultures of this isolate showed antifungal activity against *Fusarium verticillioides*. A new cyclic decapeptide was isolated from the extract using column chromatography and HPLC. There are only a few prior reports of metabolites from members of the genus S*esquicillium*, none of which are cyclic peptides. The amino acid composition was determined by GCMS analysis of the N-trifluoroacetyl-*sec*-butyl ester derivatives of the amino acids obtained upon hydrolysis. The units present include N-methylated alanine, threonine, and phenylalanine units, as well as other, more common amino acids. The peptide sequence was determined by analysis of MS, 1D-NMR, and 2D-NMR data. Details of the isolation, structure determination, and biological activity of this compound will be presented.

Poster 30

TEMPERATURE-INSENSITIVE GLUCOSE MEASUREMENTS IN BOVINE BLOOD ULTRAFILTRATE WITH NEAR-INFRARED SPECTROSCOPY

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Temperature is a critical parameter for near infrared (NIR) spectroscopic analysis of aqueous based samples because of the strong temperature dependent absorption properties of water. This temperature sensitivity can reduce the effectiveness of multivariate calibration models based on near infrared spectra. Digital Fourier filters can be used to reduce this sensitivity and can improve accuracy of calibration models based on the partial least squares (PLS) algorithm.

The use of digital Fourier filtering is examined for measurements in samples of bovine blood ultra filtrate. In this experiment, eighty samples were prepared in a matrix of bovine blood ultra filtrate with random concentrations of glucose, urea and triacetin. Endogenous levels of glucose and urea in these samples were determined by using an ACE analyzer (Alfa Wassemann, Inc. West Caldwell, NJ). Triplicate NIR spectra were collected over the combination spectral region (5000–4000 cm^{-1}) at 25, 30, 35, 37, and 40°C (± 0.2°C). Two type of absorbance spectra were created for calibration models: temperature matched and temperature mismatched, where absorbance spectra were based on the ratio of the single beam spectra of sample and buffer at same or different temperatures, respectively.

As expected, large baseline variations were observed for the temperature mismatched spectra. These large baseline variations could be effectively eliminated and the desired analytical information could be retained by using the appropriate digital Fourier filter. The basic parameters of this digital filter include the position and width of a Gaussian shaped response function and these parameters could be optimized to realize the best analytical properties. The optimized digital filters coupled with PLS modeling created temperature insensitive calibration models with measurement errors of 0.40 mM. The effectiveness of the digital filtering step will be presented.

DIELS-ALDER CYCLOADDITIONS OF *N*-ENOYL THIAZOLIDINETHIONES TOWARDS THE SYNTHESIS OF ANTI-TB DITERPENES ESCOBARINES

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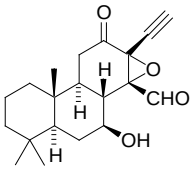
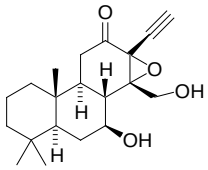
We have developed an inexpensive chiral indene-based thiazolidinethione auxiliary that has found value in acetate aldol reactions.[1] This versatile auxiliary is prepared from indene employing a lipase-mediated perhydrolysis to epoxidize indene in a green manner. This chiral auxiliary can also be removed effectively with different nucleophiles which make it superior to other known chiral auxiliaries.



escobarine A



escobarine B

Escobarines A and B, are two new diterpenes that were isolated from the roots of *Calliandra californica,* which were shown to possess promising activities against two *Mycobacterium tuberculosis* strains.[2] These diterpenes showed activities against *M. tuberculosis* H37Rv and *M. tuberculosis* CIBIN/UMF15:099 strains, the latter of which is resistant to first-line antituberculosis drugs. Escobarine-A was active against a clinical isolate resistant to all five active first-line anti-tuberculosis drugs, with the corresponding MIC being eight times lower than that of rifampin for the same strains.

We envision building the decalin ring system of escobarines employing a Diels-Alder cycloaddition reaction. We will report the synthesis of the desired diene and dienophile and our preliminary results in this reaction.

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[1] (a) A. Osorio and H. F. Olivo. *Org. Lett*. **2008**, *10*, 617-620.
(b) R. Tello-Aburto and H. F. Olivo. *Org. Lett*. **2008**, *10*, 2191-2194.
(c) A. Osorio-Lozada and H. F. Olivo. *J. Org. Chem.* **2009**, *74*, 1360-1363.
(d) R. Sabala, L. Hernandez-Garcia, A. Ortiz, M. Romero, and H. F. Olivo. *Org.Lett*. **2010**, *12*, in press.

[2] R. Encarnacion-Dimayuga, J. Agundez-Espinoza, A. Garcia, G. Delgado, G. M. Molina-Salinas, S. Said-Fernandez. *Planta Medica* **2006**, *72*, 757-761.

Rh(II)-CATALIZED CARBENOID INSERTION TO AROMATIC RINGS TOWARDS THE SYNTHESIS OF ANTI-TB DITERPENES EROGORGIAENE

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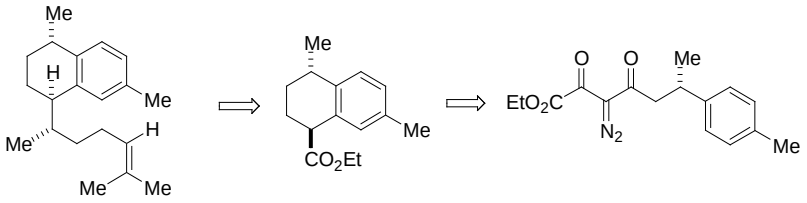
We have developed inexpensive chiral indene-based oxazolidinethione and thiazolidinethione auxiliaies that have found value in acetate aldol reactions.[1] This versatile auxiliary is prepared from indene employing a lipase-mediated perhydrolysis to epoxidize indene in a green manner. We have recently reported the conjugate addition of organocuprates to *N*-enoyl oxazolidinethiones[2]

Bioassay-guided fractionation from the West Indian gorgonian octocoral *Pseudoterogorgia elisabethae* Bayer led to the isolation of the diterpene, erogorgiaene which possess potent inhibitory activity against *Mycobacterium tuberculosis*.[3] We are interested in preparing this diterpene and a large number of analogues.



erogorgiaene

In this context, we are investigating the Rh(II)-catalyzed insertion of carbenoids to aromatic rings. Details of our work will be presented.

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[1] (a) A. Osorio and H. F. Olivo. *Org. Lett*. **2008**, *10*, 617-620.
(b) R. Tello-Aburto and H. F. Olivo. *Org. Lett*. **2008**, *10*, 2191-2194.
(c) A. Osorio-Lozada and H. F. Olivo. *J. Org. Chem.* **2009**, *74*, 1360-1363.

[2] R. Sabala, L. Hernandez-Garcia, A. Ortiz, M. Romero, and H. F. Olivo. *Org. Lett*. **2010**, *12*, in press.

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Poster 33

KINETIC ISOTOPE EFFECTS AS A PROBE OF DONOR-ACCEPTOR DYNAMICS IN ENZYME-CATALYZED H-TRANSFERS

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Quantum mechanical tunneling in enzyme-catalyzed hydrogen transfers is highly sensitive to the distance between the heavy atom donor and acceptor. The shorter donor-acceptor distance necessary for heavy isotopes to tunnel through the reaction barrier leads to secondary kinetic isotope effects (KIEs) that have been difficult to interpret by traditional models. However, by assuming a "tunneling ready state", which is the geometry from which H-tunneling occurs, the secondary KIEs yield detailed structural information about the reaction coordinate. Notably, the difference in secondary KIEs when a heavier isotope is at the primary position indicates the difference in average donor-acceptor distance for the two isotopes and thus information on dynamic heavy atom fluctuations along the reaction coordinate. (Roston and Kohen, *PNAS*, 2010) Current efforts seek to test some of the predictions of the recently published model for the tunneling ready state, and also to develop a direct computational link between the temperature dependency of primary KIEs and the nature of donor-acceptor fluctuations.

EXPANSION OF THE PHARMACEUTICAL NANO-COCRYSTALS LIBRARY BY SOLVENT SELECTION AND USE OF SURFACTANT

John R. G. Sander,[1] Dejan-Krešimir Bučar,[1] Rodger F. Henry,[2] Geoff G. Z. Zhang[3]* and Leonard R. MacGillivray[1]*
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The unique size-dependent properties of crystals of nanometer-scale dimensions make such solids of great interest to areas ranging from synthetic chemistry, materials science, and medicine. Whereas inorganic nanocrystals have experienced utility in a variety of areas (e.g. semiconductors, medical diagnostics), nanocrystals comprised of purely organic components have remained relatively unexplored despite unique physiochemical properties. In recent years cocrystals (i.e. multi-component molecular crystals) have drawn immense attention in the field of pharmaceutics. Cocrystals of pharmaceutical agents (PAs) and complementary molecules in the form of cocrystal formers (CCFs) have been shown to improve the physiochemical properties (i.e. stability, solubility/dissolution rate, and mechanical properties) of PAs. Coupling the benefits of pharmaceutical cocrystals with a significant decrease in particle size to the nanoscale can be expected to further improve the properties of PAs. To achieve pharmaceutical nano-cocrystals we turned to sonochemistry. The technique, which is harsh yet transient, has been successfully applied to afford cocrystal with components comprised of relatively simple organic molecules. To apply the technique to the preparation of the more complex pharmaceutical cocrystals we employed a combination of multiple-solvents and the use of the surfactant Span-85. The cocrystal that is the focus of our initial work is caffeine 2,4-dihydroxybenzoic acid monohydrate (caff)·(dhba)·(H_2O). The results demonstrated the ability of a two-solvent to synthesize pharmaceutical nano-cocrystals with the opportunity to achieve further size reduction *via* incorporation of surfactant in the antisolvent to obtain an average particle size of 136.4 nm ± 65.05. We look to expand our success with the (caff)·(dhba)·(H_2O) system to include other cocrystal systems composed of PAs with poor aqueous solubility. The PAs will vary with respect to molecular weight, functional groups, and flexibility to test the ability of multiple solvents and surfactant to afford cocrystals of nanometer scale dimensions.

Poster 35

ACTIVITY AGAINST MUTANT *E. COLI* STRAINS AND *IN VITRO* EFFECTS OF NOVEL C-7 ARYL FLUOROQUINOLONES ON DNA GYRASE AND DNA

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Fluoroquinolones (FQs), broad-spectrum bactericidal antibiotics, exert their effects by inhibiting DNA gyrase and/or topoisomerase IV through the formation of a ternary complex with the enzyme and DNA. Molecular modeling and crosslinking studies with ciprofloxacin-derived derivatives revealed a potential binding pocket on gyrase. Based on this model, we reasoned that C-7 aryl substituents on FQs could potentially fit into a pocket between Arg121 and Tyr122 and increase binding contacts in the quinolone-resistance determining region on gyrase. The effects of aryl groups added to the C-7 end of FQs on activity with FQ-resistant gyrase mutants were examined against mutant strains of *E. coli*. Computed properties of the C-7-aryl groups were determined to look for correlations with activity against different gyrase mutants. Lead compounds were identified. *In vitro* assays with DNA revealed that C-7 aryl FQs do not intercalate and unwind DNA like other FQs do. Binding studies showed that C-7 aryl FQs have a stronger binding affinity for nicked DNA.

STRUCTURAL AND THERMODYNAMIC ORIGINS OF DISTINCT LIGAND SPECIFICITY IN HOMOLOGOUS PDZ DOMAINS FROM THE TIAM-FAMILY OF NUCLEOTIDE EXCHANGE FACTORS

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PSD-95/DlgA/ZO-1 (PDZ) domains are among the most abundant protein-protein interaction domains in the human proteome and typically bind the 4-10 most C-terminal residues of its interaction partner with exquisite specificity. To investigate the origin of this specificity, we used two homologous PDZ domains from the Tiam-family of GEFs that have distinct but overlapping ligand specificity. The Tiam1 PDZ domain binds 8-residue long C-terminal peptides derived from the proteins Syndecan1 and Caspr4 with micromolar affinity but does not bind Neurexin1. In contrast, the Tiam2 PDZ domain binds to peptides derived from Caspr4 and Neurexin1 with low micromolar affinity but does not bind Syndecan1. Analysis of the X-ray crystal structure of the Tiam1 PDZ domain bound to a "model" peptide shows two specificity pockets created by four residues in the Tiam1 PDZ domain. Moreover, comparison of nuclear magnetic resonance (NMR) titrations of the Tiam1 PDZ domain with the Syndecan1 and Caspr4 peptides showed substantial differences in the changes in chemical shift in these residues. Sequence comparison of Tiam-family PDZ domains revealed that these residues are not conserved, further suggesting that they play a role in establishing ligand specificity. Double mutant cycle analysis of residues in these two pockets revealed ligand-dependent cooperativity, supporting their role in specificity is ligand specific. Remarkably, substitution of all four residues in the Tiam1 PDZ domain with the amino acids found in the Tiam2 PDZ domain switched the specificity to that of Tiam2. Collectively, our data suggest that Tiam-family proteins have highly evolved PDZ-ligand interfaces with distinct specificities, and that they have disparate PDZ-dependent biological functions.

This work is supported by an AHA grant to E.J.F. (AHA 0835261N). T.R.S. was supported in part by an NIH Center for Biocatalysis and Bioprocessing Predoctoral Fellowship (GM08365-20).

Poster 37

LYOTROPIC LIQUID CRYSTALLINE NANOSTRUCTURE EFFECTS ON PHYSICAL PROPERTIES OF POLYMERIC DRUG DELIVERY SYSTEMS

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Polymeric biomaterials continue to be widely investigated for the treatment of a broad range of significant health issues. Drug delivery systems have seen improvement in blood circulation times, cellular uptake, and site-specific delivery through the incorporation of polymeric materials. Further improvement of polymeric biomaterials could be achieved by manipulating polymer nanostructure thereby altering delivery and degradation. Previous work has taken advantage of the inherent spatial and temporal control offered by photopolymerization to show that polymer nanostructures can be induced using lyotropic liquid crystals (LLCs). The nanostructure of LLC-templated polymers may control properties relevant to effective drug delivery such as drug release rate and cellular interaction. The goal of this research is to investigate the effect of induced submicron order, specifically the cubic phase, on the ability to effectively deliver therapeutic molecules. Small-angle x-ray scattering (SAXS) and polarized light microsocopy (PLM) were used to determine the submicron order of pre- and post-cross-linked poly(ethylene) diacrylate (PEGDA) polymers that were templated with various concentrations of the LLC polyoxyethylene (2) cetyl ether (Brij 52). Cubic nanostructures can be obtained at various concentrations of both PEGDA and Brij 52. Other nanostructures were also formed. To give an indication of any diffusion differences, the network swelling rate in distilled water was measured gravimetrically for select cross-linked, Brij 52-templated PEGDA samples. The differences found in the rate of water uptake and total network swelling across several of these samples indicates that drug diffusion rates from the polymer networks are affected by the nanostructure of the polymer. The control of the diffusion and other properties using submicron order may lead to a facile control and optimization of important drug delivery system physical properties.

Poster 38

A BROAD-SPECIFICITY NON-HEME IRON *N*-DEMETHYLASE ENABLES *PSEUDOMONAS PUTIDA* CBB5 TO METABOLIZE SEVERAL PURINE ALKALOIDS

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N-Demethylation of many xenobiotics and naturally occurring purine alkaloids like caffeine and theobromine are primarily catalyzed in higher organisms, ranging from fungi to mammals, by the well-studied membrane-associated cytochrome P450s. In contrast, there is no well-characterized enzyme for *N*-demethylation of purine alkaloids from bacteria, despite several reports on their utilization as sole source of carbon and nitrogen. Here, we provide the first detailed characterization of a purified *N*-demethylase from *Pseudomonas putida* CBB5, which enables the organism to utilize caffeine and related methylxanthines as sole growth substrates. The soluble *N*-demethylase holoenzyme is composed of two components, a reductase component with cytochrome *c* reductase activity (Ccr) and a 2-subunit *N*-demethylase component (Ndm). Ndm, with a native molecular weight of 240,000 kDa, is composed of NdmA (40 kDa) and NdmB (35 kDa), probably in a $\alpha_3\beta_3$ structure. Ccr transfers reducing equivalents from NAD(P)H to Ndm, which catalyzes an oxygen-dependent *N*-demethylation of methylxanthines to xanthine, formaldehyde, and water. Paraxanthine and 7-methylxanthine were determined to be the best substrates, with apparent K_M and k_{cat} values of 50.4 ± 6.8 μM and 16.2 ± 0.6 min^{-1}, and 63.8 ± 7.5 μM and 94.8 ± 3.0 min^{-1}, respectively. Ndm also displayed activity towards caffeine, theobromine, theophylline, and 3-methylxanthine, all of which are sole growth substrates for this organism. Ndm was deduced as a Rieske [2Fe-2S] domain-containing non-heme iron oxygenase based on: (i) its distinct absorption spectrum and (ii) substantial identity of the N-terminal sequences of NdmA and NdmB with the gene product of an uncharacterized caffeine demethylase in *P. putida* IF-3 and a hypothetical protein in *Janthinobacterium* sp. Marseille, both predicted to be Rieske proteins.

Poster 39

STUDIES ON THE STABILITY OF N^5,N^{10}-METHYLENE-5,6,7,8-TETRAHYDROFOLATE

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N^5,N^{10}-Methylene-5,6,7,8-tetrahydrofolate (MTHF) is a biologically active form of vitamin B_9, also known as folic acid. *In vivo*, MTHF is synthesized by reduction of 7,8-dihydrofolate catalyzed by dihydrofolate reductase, followed by an oxidative methylene transfer from serine catalyzed by serine hydroxymethyltransferase. MTHF is a cofactor for thymidylate synthase to produce 2'-deoxythymidine-5'-monophosphate, dTMP (thymidylate). MTHF is also used by many other enzymes to generate multiple folate derivatives during metabolism, such as 5-methyltetrahydrofolate and 10-formyltetrahydrofolate.

Here we report studies on the stability of MTHF under various conditions. The methylene group of MTHF was labeled with carbon-14, and the rate of degradation of MTHF into formaldehyde was followed. We found that the half-life of MTHF increases exponentially with its concentration, while it is less dependent on the O_2 content in solution. The finding that MTHF decomposition is not unimolecular is surprising and under investigation. We also tested various conditions that may affect the stability of MTHF, such as temperature, pH, and presence of other folate derivatives.

Poster 40

STRUCTURAL REQUIREMENTS OF FLUOROQUINOLONES FOR KILLING NON REPLICATING BACTERIA

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Fluoroquinolones are broad spectrum antibiotics that target the DNA gyrase and topoisomerase IV enzymes of bacteria. DNA gyrase and topoisomerase IV introduce and relax supercoils in DNA, thereby allowing access to DNA helicase to form the replication fork. This action is carried out by nicking each strand of DNA, winding or unwinding the DNA, and then religating the strands. Upon binding the DNA-enzyme complex, fluoroquinolones prevent DNA gyrase (or topoisomerase IV) from ligating the DNA strands. This inhibits the growth and normal function of the cell. In addition, it has been shown that some fluoroquinolones are able to rapidly kill bacteria through a mechanism that results in chromosomal fragmentation. Some fluoroquinolones are able to kill bacteria even when protein synthesis is inhibited. While the mechanism for this unique lethality of some fluoroquinolones is unclear, it has profound implications for the treatment of diseases in which the bacteria grow very slowly or can lay dormant for indefinite periods of time (such as with *Mycobacterium tuberculosis* in tuberculosis disease). The goal of my research is to understand the structural requirements for fluoroquinolones to cause chromosomal fragmentation and kill bacteria in the absence of protein synthesis. It has already been shown that a C-8 methoxy group is important for rapid lethality and the killing of cells that are not undergoing protein synthesis. Presented here is work to develop efficient methods for synthesizing C-8 and N-1 analogs of fluoroquinolones in order to ascertain which functional groups at these positions will promote chromosomal fragmentation and rapid lethality. Optimization of a novel synthetic route to 8-methoxy fluoroquinolones in which a base induced ring opening can be performed on a ring-fused fluoroquinolone core will also be described.

Poster 41

A HIGH RESOLUTION STRUCTURE OF K29Q MUTANT UBIQUITIN

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Ubiquitin is a small protein that is highly conserved and present in most types of living cells. It is involved in the regulation and modulation of a variety of cellular processes, including targeting proteins to the proteosome and the immune response. Ubiquitin binds to a remarkably large and diverse set of proteins and it does so with a high degree of specificity, even at often low affinities.While structures of ubiquitin in complex with cognate proteins have been determined, they represent only a fraction of the possible complexes ubiquitin likely forms. Here we report the crystal structure of a mutant K29Q form of ubiquitin at 1.2Å. To our knowledge, this is the highest resolution structure yet reported for ubiquitin. In addition, due to the small size of ubiquitin and the relatively high quality of the data collected, it was possible to determine the coordinate error for every atom in the structure. Thus, this structure may prove useful in docking and other simulations of ubiquitin alone and in complex with target proteins.

Poster 42

COMPARATIVE CROSS-REACTIVITY KINETIC STUDIES OF *LEISHMANIA MAJOR* PTERIDINE REDUCTASE 1 AND *E. COLI* DIHYDROFOLATE REDUCTASE

Atsushi Yahashiri, Robert Nixon III, Asad Hashmi, Nikhil Manjunath,
Michael J. Toraason, Arundhuti Sen and Amnon Kohen*
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Pteridine reductase 1 (PTR1) from *Leishmania major* primarily catalyzes the reduction of biopterin and dihydrobiopterin (H_2B) with NADPH and produces tetrahydrobiopterin (H_4B) and $NADP^+$. It has been suggested that PTR1 has some dihydrofolate reductase (DHFR) activity, reduction of dihydrofolate (H_2F) to tetrahydrofolate (H_4F). The enzyme DHFR from *E. coli* (ecDHFR) is an efficient enzyme in catalyzing the reduction of H_2F, however, its catalytic activity toward H_2B was not known. In order to understand their mechanistic differences, the steady state kinetics of ecDHFR and PTR1 catalyzed H_2B and H_2F reduction with NADPH was comparatively examined. The Michaelis-Menten parameters for H_2F and H_2B were very similar in the PTR1 catalyzed reaction. In the ecDHFR catalyzed reaction, however, k_{cat} for H_2B reduction was ~ 500 fold slower and and K_m was larger than for its natural substrate, H_2F. The findings indicate that the *p*-aminobenzoyl-glutamate (pAB-glu) moiety of H_2F contributes to both binding and product release rates (the rate limiting step on k_{cat}) for ecDHFR but has insignificant roles in PTR1 catalysis. To test whether the missing pAB-glu in the ecDHFR catalyzed H_2B reaction only affects binding or also affects the nature of the H-transfer reaction we examined the temperature dependence of its intrinsic kinetic isotope effects (KIEs). Interestingly, the ecDHFR reduction of H_2B showed inflated KIEs that were more temperature dependent than for H_2F reduction. In accordance with Marcus-like models, these findings suggest that the unnatural substrate reduction occurs from an ensemble of confirmations that is not as well tuned for H-tunneling as the natural substrate. Together, the findings support that for ecDHFR the lack of the interaction provided by the pAB-glu tail of the natural substrate reduced not only binding affinity, but also disturbed the reorganization of the donor and acceptor of the reactive conformations, from which H-tunneling can occur.

Poster 43

NOVEL ANTIMUTANT C-7 ARYL FLUOROQUINOLONES

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Antibiotic resistance eventually emerges in the treatment of most bacterial infections because current pharmaceutical practices place dosing levels directly within the mutant selection window (MSW). The MSW is a dosing range between the minimum inhibitory concentration (MIC) of wild-type bacteria and the mutant prevention concentration (MPC) of the least susceptible mutant populations. Dose levels below the MIC do not effectively lower bacterial populations to combat disease, and dose levels above the MPC are possibly toxic. Though doses within the MSW effectively treat infection, the resultant selection of mutant strains has given rise to highly resistant bacteria such as multi-drug resistant (MDR) and extensive drug resistant (XDR) *M. tuberculosis.* The goal of our work is the development of new antibiotics that restrict the emergence of resistant mutant strains of *M. tuberculosis* by having a very narrow MSW. In this study, computer modeling is employed to generate further understanding of the drug binding sites in topoisomerase IV and DNA gyrase A, the protein targets of ciprofloxacin and other antibiotics, to design new antimutant fluoroquinolones. The progress toward an investigative panel of ciprofloxacin analogues designed to further exploit the binding space around the C-7 position is presented.

PURIFICATION AND CHARACTERIZATION OF A NAD(P)H-DEPENDENT TRIMETHYLURIC ACID (TMU) OXIDOREDUCTASE FROM CAFFEINE-DEGRADING STRAIN *PSEUDOMONAS* sp. CBB1

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Pseudomonas sp. CBB1 degrades caffeine to 1,3,7-trimethyluric acid (TMU) (1). However, further metabolism of TMU is unknown. To elucidate the metabolic pathway of caffeine in CBB1, purification and characterization of the enzyme involved in further degradation of TMU and identification of downstream metabolites were studied in detail.

A novel NAD(P)H-dependent enzyme, TMU oxidoreductase, was isolated and purified from *Pseudomonas* sp. strain CBB1 to homogeneity using ion exchange, affinity, followed by hydrophobic interaction and gel filtration chromatography. The specific activity of trimethyluric acid degradation by the purified enzyme was found to be 3.51 μmol min^{-1} mg $protein^{-1}$ and required NAD(P)H as cofactor. The apparent molecular weight of the purified protein was estimated to be 43 KDa. The enzyme was yellow in color, showed UV-visible absorption maxima at 277, 315 and 415 nm, which suggested that it might be a flavoprotein.

The N-terminal amino acid sequence of the purified enzyme showed significant homology to FAD-binding monooxygenase, salicylate hydroxylase, and a novel FAD-dependent urate oxidase (HpxO) from *Klebsiella pneumonia*. HpxO catalyzes the hydroxylation of uric acid to 5-hydroxyisourate, which is converted spontaneously to allantoin (2).

TMU oxidoreductase also catalyzed the oxidation of various dimethyluric acids and monomethyluric acids, but had no activity on uric acid. LC-MS analysis of the products of TMU degradation detected an m/z 201.0 peak in the reaction mixture. This product most likely corresponds to the parent ion of 3,6,8-trimethylallantoin (TMA). Formation of TMA from TMU has also been reported by Madyastha (3). The same metabolite was also detected by LC-MS analysis in the caffeine-growth media of strain CBB1. Thin Layer Chromatography was used to purify this metabolite from growth media. NMR and High Resolution Mass Spectrometry analysis of this purified compound suggested that this metabolite is TMA.

TMU oxidoreductase is a NAD(P)H dependent flavoprotein specific for (tri)methyluric acids and is different from the FAD-dependent urate oxidase from *Klebsiella pneumonia*. This enzyme oxidizes TMU to TMA. The reaction stoichiometry needs to be confirmed by cloning the enzyme and determining direct formation of TMA with the pure product.

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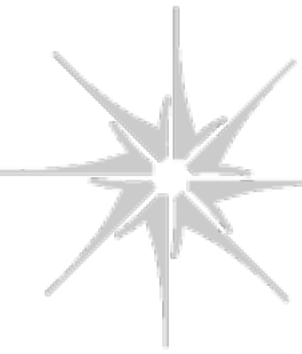
Sarah Kasperbauer	Research Assistant
Deanna Lettington	Research Assistant
Wensheng Liu	Research Assistant
Michelle Railsback	Research Assistant
Arsh Khare	Research Assistant
Jin Xu	Research Assistant
Hannah Cooley	Research Assistant
Jake Rashid	Research Assistant
Maggie Beer	Lab Assistant
Michael Johnson	Lab Assistant



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