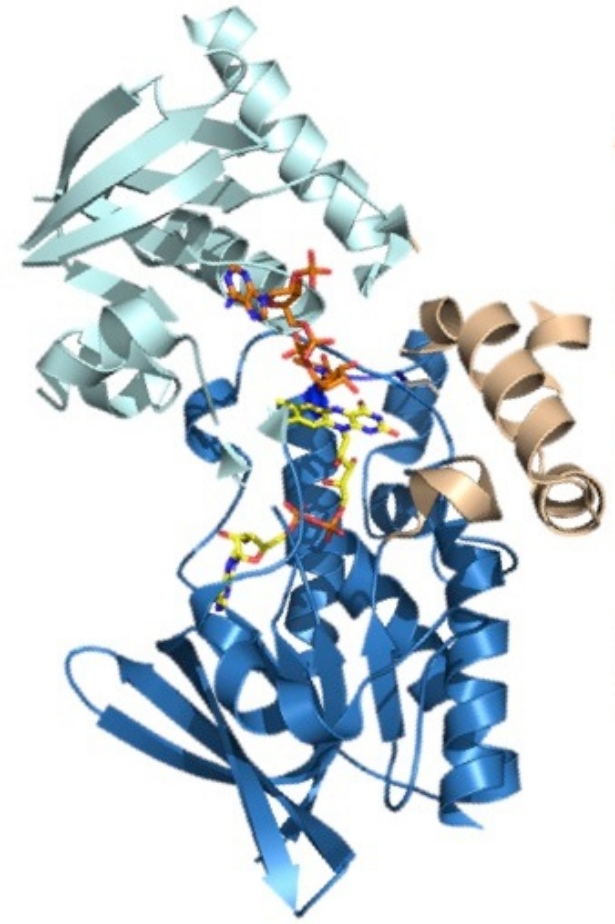
Seventh Southeast Enzyme Conference



Saturday, April 16, 2016

Georgia State University
Atlanta, GA

Urban Life Building
College of Law
140 Decatur Street
Room 220

Seventh Southeast Enzyme Conference

Saturday, April 16, 2016

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Southeast Enzyme Conference (SEC)

Meeting	Year	Program Chair	Site Chair	Site
I	2010	Giovanni Gadda	Will Lovett	GSU
II	2011	Nigel Richards	Giovanni Gadda / Will Lovett	GSU
III	2012	Robert Phillips	Giovanni Gadda / Will Lovett	GSU
IV	2013	Holly Ellis	Giovanni Gadda / Neil Renfroe / Will Lovett	GSU
V	2014	Liz Howell	Giovanni Gadda / Will Lovett / Neil Renfroe / Robert Daniel	GSU
VI	2015	Anne-Frances Miller	Giovanni Gadda / Will Lovett / Gwen Kenny / Robert Daniel	GSU
VII	2016	Pablo Sobrado	Giovanni Gadda / Will Lovett / Crystal Smitherman Rosenberg / Robert Daniel / Will Thacker	GSU
VIII	2017			

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Schedule:

Location: Urban Life Building, Room 220:

All Talks 15 min plus Q&A up to 20 min total!

8:00-8:30 Coffee
8:30-8:40 Welcome and Introductory Remarks: **Pablo Sobrado**, Virginia Tech, Blacksburg

Session 1 - *Discussion Leader*: **Bryan Knuckley**, University of North Florida, Jacksonville
8:40-9:00 **Siqi Guan**, University of Alabama, Tuscaloosa
Insights into Dynamics of SufBC2D Fe-S Scaffold Complex by Hydrogen Deuterium Exchange Mass Spectrometry
9:00-9:20 **John Hoben**, University of Kentucky, Lexington
An Approach for Generating and Characterizing Semiquinone Intermediates Proposed to be Critical to Electron Transfer in Flavin Dependent Nitroreductase
9:20-9:40 **Michael Casasanta**, Virginia Tech, Blacksburg
Characterization of a surface exposed phospholipase autotransporter from Fusobacterium nucleatum
9:40-10:00 **Shannon Rivera**, Emory University, Atlanta
Oligomerization-Dependent Changes to the Heme Pocket of Globin Coupled Sensors

10:00-11:30 **Poster Session 1** (Odd numbers presenting.)

Session 2 - *Discussion Leader*: **Dan Slade**, Virginia Tech, Blacksburg
11:30-11:50 **Kunhua Li**, University of Florida, Gainesville
Structure and function analysis of homocysteine methyltransferase involve in methionine biosynthesis and SAM repair
11:50-12:10 **Bharath Srinivasan**, Georgia Institute of Technology, Atlanta
A unique substrate-assisted mechanism of inhibition of Escherichia coli dihydrofolate reductase by a novel deoxybenzoin, ononetin
12:10-12:30 **Jacob Ball**, Georgia State University, Atlanta
Kinetic and Spectroscopic Characterization of an FMN-dependent Quinone Reductase from Pseudomonas aeruginosa PAO1
12:30-12:50 **Margaret Reck**, Washington University in St. Louis, St. Louis
Tabtoxinine-β-lactam bypasses antibiotic resistance mechanisms by evading β-lactamasemediated destruction

12:50-1:00 **Group photo**
1:00-2:00 **Lunch break**

2:00-3:30 **Poster Session 2** (Even numbers presenting.)

Keynote Presentation - *Discussion Leader*: **Pablo Sobrado**, Virginia Tech, Blacksburg

3:30-4:30 **Ruma Banerjee**, University of Michigan, Ann Arbor
Signaling Through Sulfide

Concluding Remarks: **Pablo Sobrado**, Virginia Tech, Blacksburg
5:00-8:00 **Conference Mixer** @ Meehan's Public House Downtown

Session 1:

Bryan Knuckley

Discussion Leader

Insights into Dynamics of $SufBC_2D$ Fe-S Scaffold Complex by Hydrogen Deuterium Exchange Mass Spectrometry

Siqi Guan[‡], Guangchao Dong[#], F. Wayne Outten[#] and Patrick A. Frantom[‡]

[‡]Department of Chemistry, University of Alabama, Tuscaloosa, AL 35401; [#]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208

Iron-sulfur (Fe-S) clusters, acting as cofactors of Fe-S proteins, are essential for maintaining fundamental biological processes. The Suf pathway is one Fe-S biosynthetic system and is a common system in eukaryotes, bacteria, and archaea. It is especially important since it synthesizes Fe-S when metabolism is disrupted by iron starvation or oxidative stress. The Suf pathway contains five proteins: SufA, SufB, SufC, SufD, SufE, and SufS. SufBCD usually work as a complex, assembling nascent Fe-S clusters. SufB and SufD are core domains for assembling the clusters. SufC is an ATPase that catalyzes the hydrolysis of ATP, providing free energy for stabilization of $SufBC_2D$ active conformation. Even though it has been reported that the addition of ATP/Mg^{2+} induces a structural change in $SufBC_2D$ complex, the details of the changes are still unclear. We used hydrogen deuterium exchange mass spectrometry (HDX-MS) to investigate the conformational changes of $SufBC_2D$ complex due to the binding of ADP/Mg^{2+}. Peptides 23-43 and 44-53 in SufC show a decrease in solvent accessibility when ADP/Mg^{2+} binds. This suggests that this region contains the ATP binding site, consistent with the structure of SufC with ATP modeled in. Peptides located at the SufC/SufC interface exhibit decreases in the rates of deuterium incorporation, suggesting ADP/Mg^{2+} binding triggers dimer formation of the two SufC modules. Changes in the rates of deuterium incorporation are also seen at the SufB/SufD interface with SufC. Interestingly, the changes are not symmetrical with more changes seen in SufB. Peptides 447-456, 457-461, and 462-471 located at the SufB/SufC interface show significant decreases in exchange rate. While only one peptide 374-388 located at the SufD/SufC interface shows lower deuterium incorporation. Unexpectedly, peptides 108-112 and 113-122 located at β-sheet domain 51.52Å from the interface of SufD/SufC also show decreases in deuterium incorporation. By application of HDX-MS, we detected structural and dynamic changes of $SufBC_2D$ complex on ADP/Mg^{2+} binding, which provides more information for investigation of Suf system.

An Approach for Generating and Characterizing Semiquinone Intermediates Proposed to be Critical to Electron Transfer in Flavin Dependent Nitroreductase

John Hoben[‡], Cara Lubner[#], Michael Ratzloff[#], Karl Hemple[‡], Zach Griffith[‡], Paul King[#] and Anne-Frances Miller[‡]

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Enterobacter cloacae nitroreductase (NR) and its many bacterial homologues catalyze the NADH- and NADPH-dependent reduction of a large variety of substituted nitroaromatic compounds as well as a smaller set of nitroalkenes. Much of the reported chemistry for the nitroreductase family of flavoenzymes has been limited to 2-electron reductions executed without accumulation of flavin semiquinone. Stopped-flow kinetics failed to detect a 1-electron reduced semiquinone state of NR, consistent with the oxygen insensitivity of NR's reaction. However ultrafast (ps) transient absorption spectroscopy (TAS) reveals a number of photoexcited flavin intermediates including anionic semiquinone. The lifetimes of transient species and the rate of recovery of oxidized flavin are significantly affected by the presence and concentration of substrate analogues, offering mechanistic insight. Therefore we are comparing the TAS of NR with that of flavodoxin (stabilizes neutral semiquinone) and *T. thermophilus* NADH oxidase (same fold as NR but more accessible semiquinone). The broad substrate repertoires of NR and NADH oxidase provide an opportunity to test a wide range of substrate analogues to identify those with the best rates and driving forces for reduction of photoexcited flavin and formation of sequiquinone. This capability will advance studies of electron transfer bifurcating enzymes in which a high-energy flavin semiquinone is proposed to play a pivotal role. Thus we are developing methodologies for the observation of very short-lived (ps to ns) electron transfer intermediates, as well as mechanistic determinants in flavoenzymes.

Characterization of a surface exposed phospholipase autotransporter from Fusobacterium nucleatum

Michael Casasanta[‡], Chris Yoo[‡], Hans Smith[‡], A. Cameron Varano[⌐] and Dan Slade[‡]

[‡]Department of Biochemistry, Virginia Tech, Blacksburg, VA 24060 and [⌐]Virginia Tech Carillion Research Institute, Virginia Tech, Roanoke, VA 24016

Fusobacterium nucleatum is a Gram-negative anaerobic bacterium of the human oral cavity that can disseminate throughout the body where it is associated with serious diseases including periodontitis, appendicitis, preterm birth, and abscesses of the brain and liver. Multiple studies have linked *F. nucleatum* to chronic inflammatory diseases of the gut, including the development of colorectal cancer by modulating the immune system[2–5]. While most Gram-negative pathogens utilize multiple protein secretion systems to facilitate host invasion and infection, *F. nucleatum* lacks these protein complexes (i.e. Types I, II, III, IV, and VI)[6], yet is still able to invade host cells to establish persistent infections. By contrast, *F. nucleatum* genomes are rich in Type V membrane-bound or secreted autotransporter proteins, which are virulence factors involved in binding and entry into host cells in Gram-negative pathogens. Here we present the first characterization of the *F. nucleatum* strain 25586 protein FN1704, which is a Type Vd autotransporter of the phospholipase A1 family, hereby renamed Fusobacterium phospholipase autotransporter (FplA). We show that FplA is embedded in the outer membrane of *F. nucleatum*, with the N-terminal passenger domain present and enzymatically active on the surface of the bacterium. Further enzymatic characterization of recombinant, truncated forms of FplA reveal robust lipid cleavage with a maximum catalytic efficiency (k_{cat}/K_m) of 375,000 s^{-1} M^{-1}. While the role of FplA has yet to be determined in *F. nucleatum* virulence, phospholipases from intracellular pathogens including *Listeria monocytogenes* are critical for vacuole escape and survival upon host invasion. Our enzymatic studies, in combination with a newly created *fplA* gene deletion strain, will allow for the analysis of FplA in virulence through testing of lipid sequestration, cellular entry, modulation of the host vacuole to facilitate survival, and a possible role in host inflammation and disease.

1. Kostic, A. D. et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 14, 207–215 (2013).

2. Castellarin, M. et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res. 22, 299–306 (2012).

3. Yusuf, E., Wybo, I. & Piérard, D. Case series of patients with Fusobacterium nucleatum bacteremia with emphasis on the presence of cancer. Anaerobe (2016). doi:10.1016/j.anaerobe.2016.02.001

4. Strauss, J. et al. Invasive potential of gut mucosa-derived Fusobacterium nucleatum positively correlates with IBD status of the host. Inflamm. Bowel Dis. 17, 1971–1978 (2011).

5. Desvaux, M., Khan, A., Beatson, S. A., Scott-Tucker, A. & Henderson, I. R. Protein secretion systems in Fusobacterium nucleatum: genomic identification of Type 4 piliation and complete Type V pathways brings new insight into mechanisms of pathogenesis. Biochim. Biophys. Acta 1713, 92–112 (2005).

Oligomerization-Dependent Changes to the Heme Pocket of Globin Coupled Sensors

Shannon Rivera[‡], *and Emily E. Weinert*[‡]
[‡]*Departments of Chemistry, 1515 Dickey Drive, Atlanta GA 30359*

Globin coupled sensors (GCS) are sensory proteins used by bacteria to determine the surrounding gaseous environment. The function of a GCS is determined by the output domain of the GCS, which include phosphodiesterases, kinases, and diguanylate cyclases (DGC). Diguanylate cyclase domains produce cyclic dimeric guanosine monophosphate (c-di-GMP) from guanosine triphosphate (GTP). C-di-GMP is a bacterial secondary messenger and a major regulator of biofilm formation. *Pectobacterium carotovorum* ssp. *carotovorum* and *Bordetella pertussis* both contain GCS proteins (*Pcc*GCS and *Bpe*GReg, respectively) with DGC output domains. Previous works has shown that oxygen binding in the globin domain regulates the output domain, but the signaling mechanism and structure of GCSs are not well characterized. Isolated globin domains from *Pcc*GCS (*Pcc*Globin) and *Bpe*GReg (*Bpe*Globin) have been characterized to assist in elucidating these gaps. The oligomeric state of *Pcc*Globin is dimeric while *Bpe*Globin is monomeric, indicating potential oligomer binding sites in the globin domain. As full length *Pcc*GCS and *Bpe*GReg exist as different oligomeric states (dimer-tetramer-octamer and monomer-dimer-tetramer, respectively), the globin domain appears to be a major determinant of oligomerization. The globin truncations also revealed altered oxygen dissociation kinetics, as compared to *Pcc*GCS and *Bpe*GReg. Furthermore, dimerization of the globin domain has been shown to correlates with biphasic dissociation kinetics. Site-directed mutagenesis has been used to interrogate the relative roles of distal pocket hydrogen-bonding residues in stabilizing bound O_2 and contributing to each dissociation rate.

Session 2:

Dan Slade

Discussion Leader

Structure and function analysis of homocysteine methyltransferase involve in methionine biosynthesis and SAM repair

Kunhua Li[‡], *Gengnan Li*[‡], *Louis M.T. Bradbury*[#], *Andrew D. Hanson*[#], *and Steven D. Bruner*[‡]

[‡]Departments of Chemistry; [#]Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, U.S.A.

Metabolites are usually restricted to small molecule natural products that are the intermediates and products of metabolism. Many important metabolites are found damaged *in vivo* by spontaneous or enzymatic side-reactions. This metabolite damage occurs on a large scale in all organisms and produces metabolites-like side products, which is useless and often toxic. Homocysteine *S*-methyltransferases (HMTs, EC 2.1.1.0) catalyze the conversion of homocysteine to methionine using *S*-methylmethionine or *S*-adenosylmethionine as the methyl donor. HMTs play an important role in methionine biosynthesis and are widely distributed among microorganisms, plants, and animals. Additionally, HMTs play a role in metabolite repair of *S*-adenosylmethionine (SAM) by removing an inactive diastereomer from the pool. The *mmuM* gene product from *Escherichia coli* is an archetypal HMT family protein and contains a predicted Zn-binding motif in the enzyme active site. Here we present X-ray structures for MmuM in oxidized, apo, and metallated forms, representing the first such structures for any member of the HMT family. The structures reveal a metal/substrate binding pocket distinct from those in related enzymes. The presented structure analysis and modelling of co-substrate interactions provide valuable insight into the function of MmuM in both methionine biosynthesis and cofactor repair.

1. Bradbury, L. M. T., Ziemak, M. J., Elbadawi-Sidhu, M., Fiehn, O. & Hanson, A. D. (2014) Plant-driven repurposing of the ancient *S*-adenosylmethionine repair enzyme homocysteine *S*-methyltransferase. *Biochem. J.* 286, 279–286
2. Li, K., Li, G., Bradbury, L. M. T., Hanson, A. D. & Bruner, S. D. (2016) Crystal structure of the homocysteine methyltransferase MmuM from *Escherichia coli*. *Biochem. J.* 473, 277–284

A unique substrate-assisted mechanism of inhibition of *Escherichia coli* dihydrofolate reductase by a novel deoxybenzoin, ononetin

Bharath Srinivasan[‡], Ambrish Roy[‡] and Jeffrey Skolnick[‡]

[§]Center for the Study of Systems Biology, School of Biology, 950 Atlantic Drive, Georgia Institute of Technology, Atlanta, GA 30332

Dihydrofolate reductase, DHFR, (E.C.1.5.1.3) is a critical enzyme involved in the biosynthesis of thymidine and purine nucleotides. Most reported inhibitors for DHFR are structural analogues of substrate dihydrofolate, competitively displacing the latter[1, 2]. Analogues of NADPH, on the other hand, could target the cofactor binding site in diverse enzymes making them unsuitable as inhibitors. This necessitates discovering novel scaffolds that not only overcome the excessive reliance on analogs of folate but should also inhibit the enzyme by binding to unique sites in the enzyme. In a previous study, employing our novel pocket-based VLS tool PoLi, we had identified ononetin as a unique scaffold binding to *Escherichia coli* DHFR[3]. The current study, employing the tool of detailed inhibition kinetics, shows that this small molecule inhibits the enzyme. Further, the study demonstrates that ononetin shows a unique uncompetitive mechanism of inhibition against both dihydrofolate and NADPH indicating that the small-molecules preferentially bind to either the cofactor-bound or the substrate-bound binary form of the enzyme. Furthermore, the substrate *vs* velocity plots show substantial deviation from their hyperbolic shape with marked similarity to substrate inhibition-like behavior possibly indicative of substrate-inhibitor complex forming an unproductive complex with the enzyme. The K_i values seem to be synergistic with both substrate and cofactor showing potent inhibition at high substrate/cofactor concentrations. This indicates non-overlapping binding vis-à-vis both substrate and cofactor and a unique substrate-assisted inhibition mechanism. This study is the first report on deoxybenzoin, ononetin, as a novel scaffold that inhibits *E. coli* DHFR inhibition and, to the best of our knowledge, the report of an inhibitor that neither binds to the substrate or cofactor binding sites.

1. Srinivasan, B., and Skolnick, J. (2015) Insights into the slow-onset tight-binding inhibition of Escherichia coli dihydrofolate reductase: detailed mechanistic characterization of pyrrolo [3,2-f] quinazoline-1,3-diamine and its derivatives as novel tight-binding inhibitors, *The FEBS journal 282*, 1922-1938.
2. Srinivasan, B., Tonddast-Navaei, S., and Skolnick, J. (2015) Ligand binding studies, preliminary structure-activity relationship and detailed mechanistic characterization of 1-phenyl-6,6-dimethyl-1,3,5-triazine-2,4-diamine derivatives as inhibitors of Escherichia coli dihydrofolate reductase, *European journal of medicinal chemistry 103*, 600-614.
3. Roy, A., Srinivasan, B., and Skolnick, J. (2015) PoLi: A Virtual Screening Pipeline Based on Template Pocket and Ligand Similarity, *Journal of chemical information and modeling 55*, 1757-1770.

Kinetic and Spectroscopic Characterization of an FMN-dependent Quinone Reductase from *Pseudomonas aeruginosa* PAO1

Jacob Ball [α], Francesca Salvi [α] and Giovanni Gadda [α,β,γ,ε]
Department of [α] Chemistry, [β] Biology, [γ] The Center for Biotechnology and Drug Design and [ε] Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, GA 30302-3965

Recent improvements in the efficiency and speed of gene sequencing coupled to a comparatively much slower experimental determination of protein function has given rise to a vast number of hypothetical proteins in the databases.[(1)] The nitronate monooxygenase (NMO) family of enzymes displays this large discrepancy, with over >5000 genes in the GenBank™, which consists mostly of hypothetical proteins. The gene product PA1024 from *Pseudomonas aeruginosa* PAO1 is an FMN-dependent protein currently classified as a 2-nitropropane dioxygenase,[(2)] the previous name for NMO.[(3)] The classification as an NMO was based on a gene function prediction and a qualitative enzymatic assay performed with 2-nitropropane (2NP). 2NP is an alternate substrate for a select number of NMOs (Class II), but not Class I NMO.[(4,5)] However, the physiological substrate for NMO, propionate 3-nitronate (P3N),[(4,5)] was unknown at the time of the study on PA1024 and therefore was not tested as substrate for the enzyme.

In the present study, we have purified PA1024 to high levels and demonstrated that PA1024 cannot act on P3N or other nitronates. The enzyme, instead, exhibited NADH:quinone and NADH:ferricyanide reductase activities. We showed that the enzyme is able to utilize a broad range of benzoquinones and naphthoquinones, including the ubiquinone analogue Coenzyme Q_0. The mode of reduction appears to be a two-electron reduction to the fully reduced hydroquinone. Thus, PA1024 is not an NMO, and a more appropriate classification would be as NADH:quinone reductase (NQR). These findings will amend the annotations of more than 1000 hypothetical proteins currently misannotated as NMO and highlight that gene prediction is not always reliable, with many of the genes predicted to be NMOs most likely being misannotated.

Support: NSF CHE-1506518 (G.G.), Molecular Basis of Disease Fellowship (F.S.)

References:

1. Anton, B. P. *et al.* (2013) The COMBREX Project: Design, Methodology, and Initial results. *PLoS Biol. 11*, e1001638.
2. Ha, J. Y. *et al.* (2006) Crystal Structure of 2-Nitropropane Dioxygenase Complexed with FMN and Substrate. Identification of the Catalytic Base. *J. Biol. Chem. 281*, 18660- 18667.
3. Gadda, G., Francis, K. (2010) Nitronate Monooxygenase, a Model for Anionic Flavin Semiquinone Intermediates in Oxidative Catalysis. *Arch. Biochem. Biophys. 493*, 53-61
4. Smitherman, C., Gadda, G. (2013) Evidence for a Transient Peroxynitro Acid in the Reaction Catalyzed by Nitronate Monooxygenase with Propionate 3-Nitronate. *Biochemistry 52,* 2694-2704
5. Salvi *et al.* (2014) The Combined Structural and Kinetic Characterization of a Bacterial Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1 Establishes NMO Class I and II. *J. Biol. Chem. 289*, 23764–23775

Tabtoxinine-β-lactam bypasses antibiotic resistance mechanisms by evading β-lactamase-mediated destruction.

Margaret Reck, *Timothy Wencewicz*
Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130

Antibiotic resistance is a growing threat to modern medicine.[1] To combat this problem, it is essential to understand the mechanisms by which resistance arises. The most effective resistance method bacteria have is the enzymatic destruction of antibiotic molecules to render them inactive. The β-lactamase family of enzymes evolved from transpeptidase enzymes, which are the target for β-lactam antibiotics.[2] β-Lactamase enzymes act by hydrolyzing open the β-lactam ring, granting resistance to the host bacteria. We have shown that a glutamine synthetase inhibitor, tabtoxinine-β-lactam, is resistant to destruction by β-lactamase enzymes despite containing the "enchanted" β-lactam ring.[3] This represents a novel approach to treating multi-drug resistant bacteria that express high levels of β-lactamase enzymes.

[1]K. Schwab, in Global Risks 2014, Ninth Edition, Insight Report, World Economic Forum, 2014.
[2]J. Knox, P. Moews, J. Frere. Molecular evolution of bacterial β-lactam resistance, *Chemistry & Biology*, 1996, **3**, 937-947.
[3]K. Hart, M. Reck, G. Bowman, and T. Wencewicz. Tabtoxinine-β-Lactam is a "Stealth" β-Lactam Antibiotic that Evades β-Lactamase mediated Antibiotic Resistance, *MedChemComm*., 2016, **7**, 118-127.

Keynote Presentation:

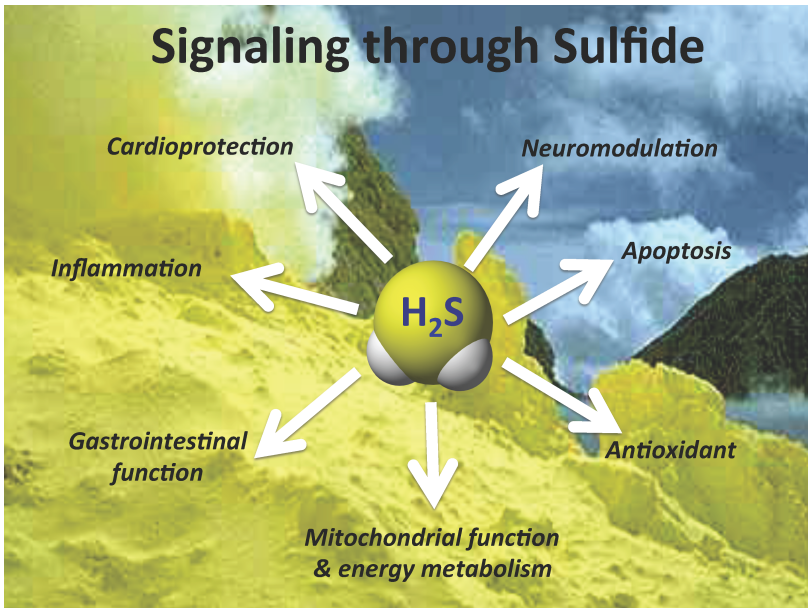
Pablo Sobrado

Discussion Leader

Signaling Through Sulfide

Ruma Banerjee
Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, USA

Despite the excitement about the varied physiological effects mediated by H_2S and the consequent profusion of literature on H_2S biology, there is a large gap in our understanding of how cells maintain very low steady-state levels of H_2S and amplify the signal as needed (*1*). Three enzymes in the sulfur network are important for its biogenesis. Two catalyze well-described non-H_2S producing reactions in the transsulfuration pathway and also synthesize cysteine persulfide from cystine (*2*), raising questions about how the decision between these competing reactions is made in the cell. The pathway for H_2S oxidation resides in the mitochondrion where the enzymes successively oxidize sulfide to sulfate. While sulfate is innocuous, a number of the intermediates in the sulfide oxidation pathway are reactive and their role in sulfide-based signaling remains to be assessed (*3*). We have recently discovered a noncanonical sulfide oxidation pathway in red blood cells, which lack mitochondria and will discuss the challenging heme-dependent oxidation chemistry that it uses (*4*).



1. O. Kabil, R. Banerjee, The redox biochemistry of hydrogen sulfide. *J Biol Chem* **285**, 21903-21907 (2010).
2. P. K. Yadav *et al.*, Biosynthesis and Reactivity of Cysteine Persulfides in Signaling. *J Am Chem Soc* **138**, 289-299 (2016).
3. T. V. Mishanina, M. Libiad, R. Banerjee, Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. *Nat Chem Biol* **11**, 457-464 (2015).
4. V. Vitvitsky, P. K. Yadav, A. Kurthen, R. Banerjee, Sulfide oxidation by a noncanonical pathway in red blood cells generates thiosulfate and polysulfides. *J Biol Chem* **290**, 8310-8320 (2015).

Abstracts for Poster Presentations:

1. Abdelwahab, Heba
2. Aguillon, Christopher
3. Anderson, Andrew
4. Ball, Jacob
5. Beattie, Nathan
6. Bhojane, Purva
7. Brown, Kierra
8. Buchholz, Martina
9. Carter, Kathleen
10. Casasanta, Michael
11. Chen, Wei-Hung
12. Chen, Wen
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26. Fuente, Gabriel
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31. Halalipour, Ali
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33. Han, Zhen
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40. Kneller, Daniel
41. Knuckley, Bryan
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46. LaMattina, Joseph
47. Li, Qiushi
48. Mahmoud, Sawsan
49. Marcus, Madeleine
50. Mariani, Victoria
51. Medrano, Mynor
52. Moomaw, Ellen
53. Morris, William
54. Musila, Jonathan
55. Nambiar, Deepika
56. Ngo, Liza
57. Ngo, Phong
58. Ouedraogo, Daniel
59. Owens, Victoria
60. Phillips, Robert
61. Pleinis, John
62. Rai, Sudhir
63. Rivera, Shannon
64. Rose, Harrison
65. S. Martin del Campo, Julia
66. Sanders, Justin
67. Schaffer, Jason
68. Scott, Bernard
69. Slaney, Trey
70. Solntsev, Kyril
71. Souffrant, Michael
72. Srinivasan, Bharath
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75. Thompson, Stephanie
76. Ting, Samuel
77. Tofighi, Hossein
78. Wong, Andres
79. Xiao, Mengchen
80. Yan, Chunli
81. Zhang, Jing
82. Zheng, Canna

New insights into L-lysine hydroxylation mechanism by *Nocardia farcinica* lysine monooxygenase (NbtG)

Heba Abdelwahab[#§‡¥], *Isabel Da Fonseca*[#§], *Sobhy El-sohaimy*[¥], *Camelia Adly*[‡] *and Pablo Sobrado*[#§⊥]

[#]Department of Biochemistry, [⊥]Enzyme Research and Drug Discovery Laboratory and [§]Fralin Life Science Institute, Virginia Tech, Blacksburg, Virginia, United States, 24061; [‡] Department of Chemistry, Damietta University, New Damietta, Egypt, 34517.; [¥]Department of Food Technology, Arid Land Cultivation Research Institute, City of Scientific Research and Technological Applications. Universities and Research Centers District, New Borg EL Arab, Alexandria, Egypt, 21934.

NbtG is lysine N-monooxygenase catalyzes the N^6-hydroxylation of L-lysine, which is a crucial step the biosynthesis nocobactin. Nocobactin is essential foriron acquisition during invasion [1, 2]. Similar enzymes such as L-ornithine monooxygenase (SidA), utilizes NADPH to reduce the flavin cofactor in order to react with molecular oxygen, forming a very stable C4a-hydroperoxyflavin intermediate. This intermediate should only have a rapid turnover when the substrate is present [2, 3]. In contrast to this behavior, NbtG is unable to form stable C4a-hydroperoxyflavin species; this limits the hydroxylation reaction and inverting the role of the enzyme from being a monooxygenase to be likely with higher oxidase activity. Thus, NbtG produces high levels of superoxide and hydrogen peroxide products due to the uncoupled reaction. This enzyme can also utilize NADH as, but with a lower efficiency than NADPH. Here, we present the results of the characterization of R301A and E216Q variats of NbtG. The kinetic and rapid reactions kinetic characterization of the recombinant mutant enzymes show that R301 confers NADPH selectivity, and E216 is crucial for controlling L-lysine binding and hydroxylation.

1. Krithika, R., *et al.* (2006). A genetic locus required for iron acquisition in Mycobacterium tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* 103:2069–2074.
2. Robinson, R., Franceschini, S., Fedkenheuer, M., Rodriguez, P. J., Ellerbrock, J., Romero, E., Sobrado, P. (2014). Arg279 is the key regulator of coenzyme selectivity in the flavin-dependent ornithine monooxygenase SidA. *Biochimica et Biophysica Acta*, *1844*(4), 778–84.
3. E. Romero, M. Fedkenheuer, S.W. Chocklett, J. Qi, M. Oppenheimer, P. Sobrado, Dual role of NADP(H) in the reaction of a flavin dependent N-hydroxylating monooxygenase (2012). *Biochim. Biophys. Acta*, 1824 850–857.

Elucidating the Role of Select Residues through Site-Directed Mutagenesis in Class I Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1

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Nitronates, such as propionate 3-nitronate (P3N), are toxic compounds found in fungi and plants as glycosides. Nitronate monooxygenases (NMOs; E.C. 1.13.12.16) are flavin-dependent enzymes that utilize oxygen to detoxify these toxins. P3N was recently discovered as the physiological substrate for *Pseudomonas aeruginosa* NMO (PaNMO). P3N irreversibly inhibits succinate dehydrogenase – an essential enzyme involved in energy production. Previous kinetic and spectroscopic studies have been carried out on both fungal and bacterial NMO demonstrating that P3N is oxidized with k_{cat} and k_{cat}/K_m values of 1000 s^{-1} and $\geq 10^7$ M^{-1} s^{-1}.[1] These high values for the kinetic parameters illustrate the importance of detoxifying the metabolic poison P3N. The crystal structures of both *Cyberlindnera saturnus* NMO (unpublished) and PaNMO show that these enzymes have conserved active sites.

Tyr254 is proposed here to modulate the polarity of the flavin due to its close proximity to the C7 methyl of FMN. Utilizing site-directed mutagenesis, variants of PaNMO with Tyr254 replaced with phenylalanine or histidine were prepared, expressed, and purified to high levels and characterized for their substrate specificity. A number of substrates were tested on variants of PaNMO with Phe254 or His254, which exhibited k_{cat} and k_{cat}/K_m values for P3N similar to the wild-type enzyme, and a two-fold reduction in the k_{cat}/K_m values with alkyl nitronates. Currently, the spectroscopic properties of both variants of PaNMO are being investigated. The goal of this study is to probe the role of the hydroxyl of Tyr254 using anaerobic substrate reduction, kinetic, and spectroscopic characterization of the variant enzymes.

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1. Salvi, F.; Agniswamy, J.; Yuan, H.; Vercammen, K.; Pelicaen, R.; Cornelis, P.; Spain, J. C.; Weber, I. T.; Gadda, G., The combined structural and kinetic characterization of a bacterial nitronate monooxygenase from *Pseudomonas aeruginosa* PAO1 establishes NMO class I and II. *J. Biol. Chem.* **2014,** *289* (34), 23764-75.
2. Gadda, G.; Francis, K., Nitronate monooxygenase, a model for anionic flavin semiquinone intermediates in oxidative catalysis. *Arch. Biochem. Biophys.* **2010,** *493* (1), 53-61.

Inhibition studies of Kynurenine 3-Monooxygenase with Potential Applications to Neurodegenerative Disorders

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Kynurenine 3-monooxygenase (KMO) is a committed step in the conversion of a tryptophan metabolite (kynurenine) into quinolinic acid. Quinolinic acid serves to stimulate glutamatergic receptors in astrocytes and can lead to hyperactivity if there are imbalances in the metabolic pathway of tryptophan. These imbalances have been proposed as possible mechanisms for the development of various neurodegenerative disorders including Alzheimer's disease. Inhibition of KMO in the bloodstream has recently been shown to ameliorate Alzheimer's disease in a mouse model of the disorder (1). A structure of KMO was recently obtained and a structural basis of its inhibitors was proposed (2). The compounds that were tested were modified variations of kynurenines and hippurates. The current study has been primarily concerned with identifying effective inhibitors and new competitive substrates of this enzyme. A bacterial model of KMO from *Cytophaga hutchisonii* was identified (3) and isolated from *Eschericha Coli*. Through utilizing strategies in enzyme kinetics, several efficient inhibitors and two new substrates of KMO were successfully identified.

1. Zwilling, D., Huang, S., Sathyasaikumar, K., Notarangelo, F., Guidetti, P., Wu, H., Lee, J., & Truong, J. […] (2011). Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell*, Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21640374.

2. Amaral, M., Levy, C., Heyes, D., Lafite, P., Outeiro, T., Giorgini, F., Leyes, D., & Scrutton, N. (2013). Structural basis of kynurenine 3-monooxygenase inhibition. *Nature*, Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3736096/.

3. Kurnasov, O., Goral, V., Colabroy, K., Gerdes, S., Anantha, S., Osterman, A., & Begley, T. (2003). Nad biosynthesis. *Chemistry & Biology*, *10*(12), 1195-1204. Retrieved from www.sciencedirect.com/science/article/pii/S1074552103002564.

Kinetic and Spectroscopic Characterization of an FMN-dependent Quinone Reductase from *Pseudomonas aeruginosa* PAO1

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Recent improvements in the efficiency and speed of gene sequencing coupled to a comparatively much slower experimental determination of protein function has given rise to a vast number of hypothetical proteins in the databases.[(1)] The nitronate monooxygenase (NMO) family of enzymes displays this large discrepancy, with over >5000 genes in the GenBank™, which consists mostly of hypothetical proteins. The gene product PA1024 from *Pseudomonas aeruginosa* PAO1 is an FMN-dependent protein currently classified as a 2-nitropropane dioxygenase,[(2)] the previous name for NMO.[(3)] The classification as an NMO was based on a gene function prediction and a qualitative enzymatic assay performed with 2-nitropropane (2NP). 2NP is an alternate substrate for a select number of NMOs (Class II), but not Class I NMO.[(4,5)] However, the physiological substrate for NMO, propionate 3-nitronate (P3N),[(4,5)] was unknown at the time of the study on PA1024 and therefore was not tested as substrate for the enzyme.

In the present study, we have purified PA1024 to high levels and demonstrated that PA1024 cannot act on P3N or other nitronates. The enzyme, instead, exhibited NADH:quinone and NADH:ferricyanide reductase activities. We showed that the enzyme is able to utilize a broad range of benzoquinones and naphthoquinones, including the ubiquinone analogue Coenzyme Q_0. The mode of reduction appears to be a two-electron reduction to the fully reduced hydroquinone. Thus, PA1024 is not an NMO, and a more appropriate classification would be as NADH:quinone reductase (NQR). These findings will amend the annotations of more than 1000 hypothetical proteins currently misannotated as NMO and highlight that gene prediction is not always reliable, with many of the genes predicted to be NMOs most likely being misannotated.

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References:

1. Anton, B. P. *et al.* (2013) The COMBREX Project: Design, Methodology, and Initial results. *PLoS Biol. 11*, e1001638.
2. Ha, J. Y. *et al.* (2006) Crystal Structure of 2-Nitropropane Dioxygenase Complexed with FMN and Substrate. Identification of the Catalytic Base. *J. Biol. Chem. 281*, 18660- 18667.
3. Gadda, G., Francis, K. (2010) Nitronate Monooxygenase, a Model for Anionic Flavin Semiquinone Intermediates in Oxidative Catalysis. *Arch. Biochem. Biophys. 493*, 53-61
4. Smitherman, C., Gadda, G. (2013) Evidence for a Transient Peroxynitro Acid in the Reaction Catalyzed by Nitronate Monooxygenase with Propionate 3-Nitronate. *Biochemistry 52,* 2694-2704
5. Salvi *et al.* (2014) The Combined Structural and Kinetic Characterization of a Bacterial Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1 Establishes NMO Class I and II. *J. Biol. Chem. 289*, 23764–23775

The Role of Protein Core Packing Defects in Allostery and Enzyme Hysteresis

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Packing defects (i.e. cavities) in the protein core are thermodynamically unfavorable. The fact that packing defects are commonly observed in protein cores suggests that a degree of instability is important for function. Here we show that packing defects can provide the conformational flexibility required for an allosteric transition. The enzyme human UDP-Glucose Dehydrogenase (hUDGH) exists as a mix of inactive (E*) and active (E) states. The allosteric inhibitor UDP-Xylose (UDX) stabilizes the E* state. Substrate binding induces hysteresis, which is observed as a slow isomerization to form the active E state. This isomerization involves a substantial repacking of the protein core in which eight buried residues change rotomeric states and a seven-residue loop shifts 5Å. We hypothesized that this remarkable transition is facilitated by large cavities in the protein core. To test this, we used the Rosetta design VIP protocol to selectively repack two of the cavities associated with the E state of the enzyme using amino acid substitutions: $hUDGH_{A104L}$ and $hUDGH_{S158Y\&S216A(DM)}$. Both $hUDGH_{A104L}$ and $hUDGH_{DM}$ no longer display hysteresis, suggesting they now favor the E state. Consistent with this interpretation, our kinetic analysis of $hUDGH_{A104L}$ shows that it does not display cooperativity in binding UDX. The crystal structure of $hUDGH_{A104L}$ shows the amino acid substitution is both well tolerated and fills the intended cavity. Our work suggests that these protein cavities could serve as allosteric sites for design of small molecule activators.

Quantification and Interpretation of Weak Preferential Interactions between Folate and Betaine

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In vitro studies with two different dihydrofolate reductases (EcDHFR, *E.coli* chromosomal and R67 DHFR, plasmid encoded) have shown that weak interactions between osmolytes and the substrate, dihydrofolate (DHF), decreases the affinity of DHF towards these enzymes. The unique changes in binding affinity with water activity for each osmolyte indicate preferential interactions between osmolyte and folate and its derivatives. Characterization of these interactions is essential for better understanding of *in vivo* effects of folate and its various redox states with available functional groups inside the cell. Quantitation of weak interactions using a vapor pressure osmometry method yields a preferential interaction coefficient, or μ_{23}/RT value. This provides a scale for measuring the preference of folate for betaine relative to water. Experimental measurements found a folate concentration dependence of the μ_{23}/RT values, consistent with dimerization of folate. Our results also indicate neutral folate preferentially interacts with betaine whereas the anionic form excludes betaine. Studies with other model compounds suggest aromatic rings prefer to interact with betaine as compared to water. The preferential interaction coefficients or μ_{23}/RT values obtained for additional nitrogen containing aromatic compounds were dissected into additive contributions from chemically distinct functional groups. The atomistic interaction potentials for each of the surface types (alpha values) were calculated. The calculated set of values coupled with the water-accessible surface areas (ASA) can be used to predict the μ_{23}/RT of any compound with betaine. Additionally, solubility assays were done to quantify the free energy of transfer of folate from water to 1 M betaine solution. Data indicate favorable interactions between betaine and folate at a lower pH with a negative free energy of transfer whereas at higher pH, the free energy of transfer is positive. These results are consistent with our μ_{23}/RT dependence of pH.

Can μ_{23}/RT values be used to predict osmotic stress effects on ligand binding? In some cases, yes. However, the caveat is whether all the ligand atoms are used in binding. As glutamate excludes betaine, calculation of the μ_{23}/RT value for polyglutamylated folates (pteroyltetra-γ-glutamate (PG4)) predicts an overall exclusion of betaine. This should translate into tighter binding of PG4 to DHFR. Our studies found betaine addition weakens binding of both folate and PG4 to R67 DHFR to similar extents. This result indicates the polyglutamylated tail does not contact the enzyme.

ChtB, a Novel Protein in *Corynebacterium diphtheriae*: Characterization using Optical Spectroscopy

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Corynebacterium diphtheriae is an aerobic gram-positive bacterial pathogen. *C. diphtheriae* primary iron sources are hemin and hemoglobin (Hb) when growing in iron-depleted environments. Iron utilization uses an iron-regulated *hmu* hemin uptake locus (1). Hemin is acquired from Hb by HtaA and then transferred to surface-anchored heme receptor proteins HtaB and HmuT; it is then transported into the cytosol by HmuUV. Deletion of HtaB does not result in reduced growth (2). This observation led to the discovery of a parallel pathway that uses the proteins ChtA and ChtB, which can each bind both hemin and hemoglobin (2).

A single conserved heme-binding 32.6 kDa (CR) domain is present in ChtB. Sequence alignment indicates three possible ligands, Y23, H108, and Y215. This domain, with an N-terminal Strep-tag, was expressed in *E. coli* BL21 (DE3). The protein was purified on a Strep-Tactin column. SDS PAGE showed pure protein of the correct molecular weight. The optical spectrum was consistent with a tyrosine-bound heme, specifically as indicated by the band at xxx nm. The protein as isolated was approximately 25% heme loaded. Overnight incubation with an approximate 10-fold excess of equine hemoglobin increased the ChtB heme loading to approximately 75%. The reconstituted apo/holo ChtB mixture shows subtle differences in the heme spectrum in comparison with the as-isolated protein.

1. Allen, C., Schmitt, M.P., 2011. Novel hemin binding domains in the *Corynebacterium diphtheriae* HtaA protein interact with hemoglobin and are critical for heme iron utilization by HtaA. J. Bacteriol. 193, 5374-5385.

2. Allen, C.E., Burgos, J.M., Schmitt, M.P., 2013. Analysis of novel iron-regulated, surface-anchored hemin-binding proteins in *Corynebacterium diphtheriae*. J. Bacteriol. 195, 2852-2863.

Stabilization of Alcohol Oxidase at High Hydrostatic Pressure

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Alcohol oxidase (AOX; E.C. 1.1.3.1), in the presence of oxygen, catalyzes the bioconversion of short-chained alcohols into hydrogen peroxide and the corresponding aroma active aldehydes and ketones. This enzyme has also been used for electrochemical biosensors and bioassays of alcohol. However, due to AOX poor stability its practical application in food processing and biosensors is very limited. The application of high hydrostatic pressure (HHP) to stabilize AOX at selected temperatures is reported. A heat-sealed plastic pouch with a 100-µL of AOX (*Pichia pastoris*) was submerged in a temperature-controlled HHP reactor. The kinetics of inactivation was studied at 0.1 – 300 MPa and 49.4 - 59.1 °C. Samples were treated at four processing times adjusted for each temperature to produce an approximate 80% reduction of the residual activity after the longest incubation time for each pressure-temperature combination. The plot of the logarithm of the residual activity *vs.* time revealed two linear regions of inactivation, the first corresponded to a presumed thermolabile isozyme "L" and the second to a thermoresistant "R" isozyme. Pseudo-first order kinetics of inactivation ($k_{(inact)}$) revealed a 202% increase in stability at 120 MPa and 52.6 °C for L. At 160 MPa and 49.4 °C, isozyme L's $k_{(inact)}$ was 13.75 times slower than at atmospheric pressure. The $k_{(inact)}$ for isozyme R was 34 times slower at 80 MPa and 55.8 °C as compared to isozyme L under the same conditions. Activation volume was greatest at 49.4 °C for both presumed isozymes at 23.8 cm^3/mol for L and 22.2 cm^3/mol for R. Temperature had a smaller effect on the $k_{(inact)}$ of R at all pressures as compared to L as indicated by a 23 – 110% decrease in apparent activation energy. Because we used a purified enzyme, this finding lets us hypothesize that the homooctameric structure of AOX dissociates into active subunits that are stabilized by pressure.

Molecular basis of sliding clamp diffusion along DNA

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DNA sliding clamps are toroid-shaped proteins that encircle DNA and slide along the DNA backbone. They play a crucial role serving as a scaffold for enzymes that act on DNA, specifically during replication by tethering DNA polymerase to the growing strand, increasing the processivity. The ring-shaped protein is able to diffuse along the DNA in a random walk, however the molecular nature of this process is poorly understood.

Whether sliding clamp diffusion along DNA is governed by simple Brownian motion or driven by internal motions of the DNA remains an elusive question. Here we investigate the DNA motion-driven diffusion hypothesis. We have employed molecular dynamics to sample the movement of sliding clamps in complex with a 30mer DNA duplex. Each sampled clamp presents a unique inner pore diameter and electrostatic environment, which is thought to influence the propagation. A smaller pore diameter presumably results in more protein-DNA contacts and may result in less DNA flexibility. If DNA motions contribute to sliding clamp diffusion, more rigid DNA would likely result in slower diffusion, restricting the speed at which a protein with a smaller pore diffuses. To further understand the driving force behind sliding clamp diffusion, we have quantified the protein-DNA interactions for each clamp. Additionally, we have analyzed the global motions of the protein by constructing a Markov state model that describes the kinetics of the clamp diffusion. By combining the description of the protein-DNA interactions with a kinetic model of the diffusion, new insight into the sliding clamp diffusional processes has been gained.

Characterization of a surface exposed phospholipase autotransporter from Fusobacterium nucleatum

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Fusobacterium nucleatum is a Gram-negative anaerobic bacterium of the human oral cavity that can disseminate throughout the body where it is associated with serious diseases including periodontitis, appendicitis, preterm birth, and abscesses of the brain and liver. Multiple studies have linked *F. nucleatum* to chronic inflammatory diseases of the gut, including the development of colorectal cancer by modulating the immune system[2–5]. While most Gram-negative pathogens utilize multiple protein secretion systems to facilitate host invasion and infection, *F. nucleatum* lacks these protein complexes (i.e. Types I, II, III, IV, and VI)[6], yet is still able to invade host cells to establish persistent infections. By contrast, *F. nucleatum* genomes are rich in Type V membrane-bound or secreted autotransporter proteins, which are virulence factors involved in binding and entry into host cells in Gram-negative pathogens. Here we present the first characterization of the *F. nucleatum* strain 25586 protein FN1704, which is a Type Vd autotransporter of the phospholipase A1 family, hereby renamed Fusobacterium phospholipase autotransporter (FplA). We show that FplA is embedded in the outer membrane of *F. nucleatum*, with the N-terminal passenger domain present and enzymatically active on the surface of the bacterium. Further enzymatic characterization of recombinant, truncated forms of FplA reveal robust lipid cleavage with a maximum catalytic efficiency (k_{cat}/K_m) of 375,000 s^{-1} M^{-1}. While the role of FplA has yet to be determined in *F. nucleatum* virulence, phospholipases from intracellular pathogens including *Listeria monocytogenes* are critical for vacuole escape and survival upon host invasion. Our enzymatic studies, in combination with a newly created *fplA* gene deletion strain, will allow for the analysis of FplA in virulence through testing of lipid sequestration, cellular entry, modulation of the host vacuole to facilitate survival, and a possible role in host inflammation and disease.

1. Kostic, A. D. et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 14, 207–215 (2013).

2. Castellarin, M. et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res. 22, 299–306 (2012).

3. Yusuf, E., Wybo, I. & Piérard, D. Case series of patients with Fusobacterium nucleatum bacteremia with emphasis on the presence of cancer. Anaerobe (2016). doi:10.1016/j.anaerobe.2016.02.001

4. Strauss, J. et al. Invasive potential of gut mucosa-derived Fusobacterium nucleatum positively correlates with IBD status of the host. Inflamm. Bowel Dis. 17, 1971–1978 (2011).

5. Desvaux, M., Khan, A., Beatson, S. A., Scott-Tucker, A. & Henderson, I. R. Protein secretion systems in Fusobacterium nucleatum: genomic identification of Type 4 piliation and complete Type V pathways brings new insight into mechanisms of pathogenesis. Biochim. Biophys. Acta 1713, 92–112 (2005).

Intradomain and intermodule organization in epimerization domain containing nonribosomal peptide synthetases

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Nonribosomal peptide synthetases are large, complex multi-domain enzymes responsible for the biosynthesis of a wide range of peptidyl natural products. Inherent to synthetase chemistry is the thioester templated mechanism that relies on protein/protein interactions and intra domain dynamics. Several questions related to structure and mechanism remain to be addressed, including the incorporation of accessory domains and inter-module interactions. The inclusion of nonproteinogenic D-amino acids into peptide frameworks is a common and important modification for bioactive nonribosomal peptides. Epimerization domains, embedded in nonribosomal peptide synthetases assembly lines, catalyze the L- to D- amino acid conversion. Here we present the structure of the epimerization domain/peptidyl carrier protein didomain construct from the first module of the cyclic peptide antibiotic gramicidin synthetase. Both holo- (phosphopantethiene post-translationally modified) and apo- structures were determined, each representing catalytically relevant conformation of the two domains. The structures provide insight into domain-domain recognition, substrate delivery during the assembly line process and guided us to carry out a structural based engineering with relaxed substrate specificity. In addition, the structures supply information into the structural organization of homologous condensation domains, canonical players in all synthetase modules.

1. Liu, Y., Zheng T., Bruner, S. D. (2011) Structural Basis for Phosphopantetheinyl Carrier Domain Interactions in the Terminal Module of Nonribosomal Peptide Synthetases, *Chemistry & Biology, 18,* 1482-1488.
2. Samel, S. A., Schoenafinger, G., Knappe, T. A., Marahiel, M. A., and Essen, Lars-Oliver (2007) Structural and Functional Insights into a Peptide Bond-Forming Bidomain from a Nonribosomal Peptide Synthetase, *J. Biol. Chem. 262*, 14428-14434.

Investigation of regulatory domain in citramalate synthase from *Methanococcus jannashii* for contributing to substrate specificity

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In the DRE-TIM metallolyase superfamily, enzyme members of the Claisen-Condensation-like (CC-like) subgroup exhibit three activities: isopropylmalate synthase (IPMS), citramalate synthase (CMS), and homocitrate synthase (HCS). Notably, CC-like subgroup members that share higher sequence similarity can exhibit diverse activities, while the ones with lower sequence similarity can exhibit similar activities. This means enzyme function can't be simply identified from local sequence alignments. Based on this point, investigating structure/function relationship of the residues that contribute to substrate selectivity may provide improvement in functional annotation for members of this superfamily. Previous research of substrate selectivity mechanisms of citramalate synthase (CMS) and α-isopropylmalate synthase (IPMS) from *Methanococcus jannashii* shows these two enzymes (sharing ~50% sequence identity) utilize different substrate selectivity mechanisms. While specific active site residues could be identified in *Mj*IPMS that contribute to select the native substrate ketoisovalerate (KIV) over pyruvate, analogous substitutions did not allow *Mj*CMS to use KIV as a substrate. We hypothesized that the regulatory domain of *Mj*CMS may contribute to substrate selectivity. After deleting the regulatory domain of *Mj*CMS, it accepts KIV as a substrate with the K_{KIV} value of 175 ± 20 μM, which is ~1.5-fold larger than the value of its native substrate pyruvate. This suggests the regulatory domain of *Mj*CMS contributes to substrate specificity. However, the same truncation of *Mj*IPMS didn't change the enzyme's preference for KIV over pyruvate, confirming that active site residues govern substrate selectivity. These results confirm that *Mj*CMS and *Mj*IPMS utilize diverse substrate selectivity mechanisms despite their high level of sequence identity.

Preliminary Kinetic Characterization of 2-Phosphinomethylmalic acid Synthase: A Member of the DRE-TIM Metallolyase Superfamily

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Investigation of evolutionary principles in functionally diverse enzyme superfamilies aids in identifying and understanding structure function relationships. Members of the Claisen-condensation like subgroup of the DRE-TIM metallolyase superfamily catalyzes a claisen-condensation like reaction between acetyl coenzymeA (AcCoA) and six different alpha keto acids. In order to investigate functional diversity in the CC-like subgroup a sequence similarity network (SSN) was used to analyze member enzymes. Analysis of this subgroup's SSN has prompted the investigation of a particular enzyme, 2-Phosphinomethylmalic acid synthase (PMMS) from *Streptomyces hygroscopicus*. While still possessing the conserved active site features of the superfamily including the DRE motif, TIM barrel fold and divalent metal binding, PMMS demonstrates an active site fingerprint of residues that differs from the rest of the subgroup. Studies conducted here are aimed at kinetically characterizing PMMS and exploring residues responsible for this particular activity. A basic characterization of the enzyme isolated from *S. hygroscopicus* has previously been reported. PMMS catalyzes the condensation reaction of phosphinopyruvic acid (PPA) and acetyl-CoA to form PMM. In the naturally occurring biosynthetic pathway, PMM is subsequently converted to bialaphos, a commonly used herbicide. Substrates for PMMS are PPA and its analog, oxaloacetate, as well as AcCoA. The structural difference between the two substrates is that PPA possesses a β-phosphenic acid in place of oxaloacetate's β-carboxylate group. Here we report the preliminary investigation of PMMS employing acetyl-CoA and oxaloacetate as substrates. Successful growth and purification were achieved using Bl21(DE3)pLysS *E. coli* cells and Ni^{2+} affinity chromatography. Preliminary K_m values were 0.64 mM for AcCoA and 1.5 mM for oxaloacetate, with a k_{cat} value of 506 min^{-1}. Additional experiments to determine conditions for optimal activity are underway.

Effects of Kynurenine and Benzoyl-L-alanine with S36A Pseudomonas Fluorescens Kynureninase

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Kynureninase [EC 3.7.1.3] is a pyridoxal-5'-phosphate (PLP)-dependent enzyme that is part of the major tryptophan metabolic pathway responsible for the hydrolytic cleavage of the tryptophan metabolite L-kynurenine to anthranilate and L-alanine. The kinetics and structure of *Pseudomonas fluorescens* kynureninase have been extensively studied *(*k_{cat}=16 s^{-1} and k_{cat}/K_m=6.0 x $10^5 M^{-1}s^{-1}$) (1). The proposed mechanism uses hydrogen bonding to assist in the formation of a ketimine intermediate, then undergoes hydration of the substrate carbonyl to form a *gem*-diol. The formation of the *gem*-diol is critical to the pathway because its deconstruction gives way to the retro-Claisen cleavage, assisted by nearby hydrogen bonding. We hypothesized that this intermediate is formed with the help of an oxyanion hole, using the hydroxyl groups on a nearby conserved tyrosine located 3.7 Å and a serine located 4.5 Å from the carbonyl oxygen of an inhibitor (1). We showed previously that Tyr-226 plays a critical role in hydrogen bonding and acid-base catalysis with the substrate carbonyl, since Y226F kynureninase has about 3000-fold lower activity (1). The role of this conserved serine residue was tested by culturing *P. fluorescens* kynureninase with a serine to alanine mutation (S36A) expressed in *Escherichia coli* cells. This S36A mutant enzyme was then purified and tested to determine changes in enzyme activity and catalytic efficiency when compared to the wild-type. Experimental results showed almost a 300-fold decrease in activity for the mutant enzyme, with k_{cat}=0.14 s^{-1} and k_{cat}/K_m=3800 M^{-1} s^{-1}, representing almost a 200-fold decrease in substrate efficiency. β-Benzoyl-L-alanine (kynurenine without the *o*-amino group) was also tested as a substrate or inhibitor to identify the reaction kinetics of the mutated enzyme. This substrate for wild-type enzyme showed no detectable activity with the mutant enzyme, suggesting the *gem*-diol forms at a much slower rate, and instead a side-reaction forms a vinylogous amide. β-Benzoyl-L-alanine was then tested as a possible competitive inhibitor for the S36A enzyme, with a K_i= 1.4 μM, showing that the mutation does not affect binding.

1. Phillips, Robert S. "Structure and Mechanism of Kynureninase." *Archives of Biochemistry and Biophysics* 544 (2014): 69-74. *Science Direct*. Web.

New Functions of Flavin Dependent Enzymes: the Mechanism of 2-Haloacrylate Hydratase

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The flavin-dependent 2-halocrylate hydratase (2-HAH) catalyzed conversion of 2-chlorocrylate, a major component in manufacturing of acrylic polymers, to a non-toxic pyruvate through a double bond hydration and subsequent chloride elimination. The enzyme from Pseudomonas sp. YL has been cloned, recombinantly expressed and purified from *E. coli*. The enzyme, which is monomer in solution, contains FAD and is active only in the reduced state, although the reaction is redox neutral. Enzyme activity was recorded after incubation of the enzyme with 2 mM NADH under light for 10 min (k_{cat} = 1.35 ± 0.02 s^{-1}; K_M = 2.01 ± 0.19). Interestingly, reaction with NADH is slow in the absence of light. When the enzyme is exposed to light, the reduction occurs ~ 30 fold faster, indicating the light activation is unique to 2-HAH. Mechanism of 2-HAH was studied, and the results indicated the catalysis of 2-HAH_YL may undergo the radical pathway, based on the observation of FAD radicals, both neutral and anionic semiquinones as well as the Flavin-adduct. This work was supported by NIH grant R01 GM094469.

Cloning and Soluble Expression of Bifurcating Electron Transfer Flavoprotein FixAB from Diazotroph *Rhodopseudomonas palustris*

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FixABs are a class of electron transfer flavoproteins (Etf) involved in bacterial nitrogen fixation. By a newly-discovered energy conservation mode, namely flavin-based electron bifurcation, FixAB couples NADH oxidation (-320 mV) to exergonic reduction of quinone (+10 mV) to drive endergonic reduction of ferredoxin (-420 mV), providing highly-reducing equivalents to nitrogenase for the demanding reduction of dinitrogen to ammonia. Detailed mechanistic studies of electron bifurcation in the Fix system have been hindered by the lack of an abundant stable soluble form of this protein, resulting from its propensity to form inclusion bodies upon overexpression. To solve this problem we assessed different expression strategies and compared the FixABs of two diazotrophs: *Rhodopseudomonas palustris and Rhodospirillum rubrum*. A dual-vector expression strategy produced a good yield of FixAB, however, little if any of the expressed proteins were soluble, regardless of the identity and position of polyhistidine and TEV recognition tags. The resulting inclusion bodies could be solubilized and refolded but failed to assume native flavin-binding structure. In contrast a dual-promoter expression system, especially with co-expression of molecular chaperone GroES/EL, produced the most abundant soluble FixAB. Thus we suggest the utilization of dual promoters along with co-expression of chaperone GroES/EL as an efficient method for soluble expression of aggregation-prone multisubunit protein complexes.

This work is supported as part of the Biological and Electron Transfer and Catalysis (BETCy) EFRC, an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science (DE-SC0012518).

Non-Specific Interactions of Dihydrofolate Reductase Ligands in Crowded Environments

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The reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (DHFR), using NADPH as a cofactor, is an essential part of the folate cycle. The product, THF, is necessary for the synthesis of methionine, purine nucleotides, thymidylate, and other compounds. Thus, the inhibition of DHFR leads to interruption of DNA synthesis and consequently cell death, making this enzyme a crucial target in the treatment of cancer and other diseases. Previous studies examined the effects of small molecule osmolytes on the substrate interactions with two non-homologous DHFRs, *E. coli* chromosomal DHFR (EcDHFR) and R67 DHFR, with vastly different active site structures. The results indicated that DHF weakly interacts with the osmolytes in solution, shifting the binding equilibrium from DHF bound to DHFR to unbound DHF. It is hypothesized that similar weak, nonspecific interactions may also occur between cellular proteins and DHF. Weak interactions between cellular proteins and DHF would have consequences *in vivo*, where the concentration of the cellular milieu is approximately 300 g/L. Under the crowded conditions in the cell, there is a higher propensity for intermolecular interaction.

Crowding effects of macromolecules in concentrations similar to those *in vivo* were examined. Isothermal titration calorimetry (ITC) and enzyme kinetic assays were used to detect effects of molecular crowders by monitoring activity of the (DHFR)-NADPH or DHF complex and the ternary DHFR-DHF-NADPH complex in the presence of these crowders. To recreate the conditions of molecular crowding *in vivo,* the binding of the enzyme-ligand complexes in the presence of molecular weight crowding agents was examined. Analysis of the K_d's and K_m's indicated a correlation between increased molecular crowding in the solution and weakened binding of the ligands to two structurally unrelated DHFRs. These findings indicate an importance of weak, transient interactions between molecular crowding and DHFR ligands.

Direct chemical control of PU.1, a hematopoietic regulator

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Direct chemical control of transcription factors at their sites of action, i.e. at the protein-DNA level, is currently unavailable for most transcription factors, including the ETS-family transcription factor PU.1, an essential regulator of hematopoiesis (1). Direct chemical control of transcriptional factors at their sites of action, i.e. at the protein-DNA level, is currently unavailable for most hematopoietic regulators, including PU.1. A major challenge in developing direct activators of PU.1 is the strong structural homology it shares with the ETS-family protein Ets-1 (and the ETS family in general) (2). This project aims to overcome the challenge of achieving specificity in targeting PU.1 over its structural homologs. Recent studies by our group on the mechanisms of DNA site recognition by PU.1 and Ets-1 show striking differences in molecular hydration accompanying DNA recognition, an exploitable feature that we use in this study to identify and characterize new compounds that directly activate the transcription factor PU.1 (3). Compounds that bind to the PU.1/DNA complex and retain PU.1's osmotic sensitivity are candidates for acting as direct activators of PU.1. We employ osmotically-directed phage display screening of 7- and 12-residue peptide libraries against the PU.1/DNA complex in our search for these compounds. An initial screening of the phage display library against a sequence-specific binding site of PU.1 has yielded eight peptide sequence candidates. Preliminary binding studies by ELISA and fluorescence anisotropy have shown potential differences in binding affinity of the peptide sequences to the PU.1/DNA complex. In optimizing the fluorescence anisotropy binding studies, we have characterized a rhodamine-based probe and determined that it is sufficiently sensitive to detect high-affinity DNA binding.

1. Scott EW, Simon MC, Anastasi J, Singh H. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science. 1994;265(5178):1573-7. PubMed PMID: 8079170.
2. Hollenhorst PC, McIntosh LP, Graves BJ. Genomic and biochemical insights into the specificity of ETS transcription factors. Annu Rev Biochem. 2011;80:437-71. doi: 10.1146/annurev.biochem.79.081507.103945. PubMed PMID: 21548782.
3. Poon GM. Sequence discrimination by DNA-binding domain of ETS family transcription factor PU.1 is linked to specific hydration of protein-DNA interface. J Biol Chem. 2012;287(22):18297-307. doi: 10.1074/jbc.M112.342345. PubMed PMID: 22474303; PMCID: PMC3365747.

Back to basics: another look at arginine pK_a and the effect of methylation

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Arginine methylation is a biologically relevant process, resulting in monomethylarginine (MMA), asymmetric dimethylarginine (aDMA) and symmetric dimethylarginine (sDMA) (1). Studies link the deregulation of protein arginine methyltransferases (PRMTs), the family of enzymes which can methylate arginine, with certain diseases including cancer (2). Methylation involves the removal of potential hydrogen bond donors, possibly inhibiting binding in addition to altering of the shape, hydrophobicity and affinity to aromatic rings, which can affect regulation of substrates. Methylation can also potentially perturb the pK_a of the arginine side chain and the charge of the guanidinium moiety. To assess the impact of methylation on interaction with other biomolecules, the pK_a of methylated arginine variants were determined using ^{1}H and ^{13}C NMR data. The pK_a of monomethylated (14.3), symmetrically dimethylated (14.7) and asymmetrically dimethylated arginine (14.3 ±0.4) are similar to the unmodified arginine (14.2) (3). While the pK_a has not been significantly affected by methylation, the functional consequences of methylation arise from other means, including changes in charge distribution and steric effects, suggesting alternative mechanisms for recognition (3).

1. Bedford, M. T. and Clarke, S. G. (2009) Protein arginine methylation in mammals: who, what, and why. *Molecular cell 33*, 1-13.

2. Yang, Y. and Bedford, M. T. (2013) Protein arginine methyltransferases and cancer. *Nature Reviews Cancer 13*, 37-50.

3. Evich, M., Stroeva, E., Zheng, Y. G., Germann, M. W. (2015) Effect of methylation on the side-chain pKa value of arginine. *Protein Sci. 25(2),* 479-86.

Purification and Characterization of a Putative NAD(P)H-Quinone Oxidoreductase in *Pseudomonas aeruginosa* Strain PAO1

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The product of gene *pa1225* in *Pseudomonas aeruginosa* PAO1 is currently annotated as a putative NAD(P)H-quinone oxidoreductase. A BLAST search revealed this gene does not have a match to any experimentally validated gene with an E value below 1e-5. This raises the possibility that the gene product PA1225 may be a novel enzyme. Interestingly, PA1225 in *P. aeruginosa* PAO1 is repressed 89 times in the presence of the LysR regulator PA4203. The latter also represses by 20 times nitronate monooxygenase (NMO), a detoxifying enzyme that oxidizes the mitochondrial toxin propionate 3-nitronate (1). Thus, PA1225 has potential as drug target against *P. aeruginosa,* an opportunistic gram-negative bacterium exhibiting multi-antibiotic resistance that thrives in water, immunocompromised humans, and hospital settings (2).

In this study, *pa1225* was amplified by PCR from the genomic DNA of *P. aeruginosa* PAO1 and ligated into vector pET20(b)+. The resulting recombinant plasmid was used to transform *Escherichia coli* strain Rosetta(DE3)pLysS for expression of PA1225. Optimization of recombinant protein expression, purification with ion-exchange chromatography, and kinetic characterization of the protein are currently ongoing and the results will be presented.

This study was supported in part by grant CHE-1506518 from the NSF (G.G.), a Molecular Basis of Disease Fellowship (D.S.) from GSU, and an LSAMP Fellowship (E.F.) from GSU.

1. Vercammen, K., Wei, Q., Charlier, D., Dötsch, A., Haüssler, S., Schulz, S., Francesca, S., Giovanni, G., Spain, J., Morten, R., Nielsen-Tolker, T., Dingemans, J., Ye, L., Cornelis, P. (2015). *Pseudomonas aeruginosa* LysR PA4203 Regulator NmoR Acts as a Repressor of the PA4202 *nmoA* Gene, Encoding a Nitronate Monooxygenase. *J. Bacteriol.*, *197*(6), 1026-1039.
2. Winsor GL, Lam DK, Fleming L, Lo R, Whiteside MD, Yu NY, Hancock RE, Brinkman FS (2011). Pseudomonas Genome Database: Improved Comparative Analysis and Population Genomics Capability for Pseudomonas Genomes. *Nucleic Acids Res., 39, D596-600.*

Investigating Novel Bacterial mRNA Degradation Pathways and Nucleotide Pools

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Catabolism of messenger RNA is an important process in bacterial metabolism, and regulation of nucleotide pools has recently been implicated in additional functions, such as biofilm formation. Employing a LC-MS/MS-based assay recently developed within the group, this work reports the first quantification of 2',3'-cyclic nucleotide monophosphates (2',3'-cNMPs) in Escherichia coli. Previous reports have linked 2',3'-cNMPs to cellular stress and damage in eukaryotic systems, suggesting an intriguing connection with nucleotide pools. The present work demonstrates that 2',3'-cNMPs in E. coli arise from RNase I-catalyzed mRNA degradation, presumably as part of a previously unknown nucleotide salvage pathway. To validate the controversial role of RNase I in messenger RNA degradation, the mRNA-specific endoribonuclease YafQ was overexpressed, resulting in an RNase I-dependent increase in 2',3'-cNMP concentrations. This work also implicates phosphodiesterase CpdB in the physiological regulation of cytoplasmic 2',3'-cNMP levels. To identify potential biological processes regulated by 2',3'-cNMPs, experiments are underway to perturb 2',3'-cNMP pools using cell-permeable 2',3'-cNMP analogues and a recombinant 2',3'-cyclic-nucleotide phosphodiesterase. These experiments aim to elucidate potential roles of 2',3'-cNMPs in biofilm formation, growth phenotypes, and transcription/translation.

Ketosteroid isomerase catalyzed Kemp elimination

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Ketosteroid isomerase (KSI) catalyzes the isomerization of ketosteroids by using a catalytic aspartate in its active site (Asp 38 in KSI from *Comamonas testosteroni*). The Kemp elimination is a reaction promoted by abstraction of a proton from the relatively acidic C3 of benzisoxazoles. Because of the similarities between these two reactions, we investigated whether KSI could catalyze the Kemp elimination. We found that KSI catalyzes the Kemp elimination of 5-nitrobenzisoxazole and of other substituted benzisoxazoles. Modelling the substrate in the active site of KSI suggests that Asp 38 might not be the general base, and that another aspartate residue, Asp 99, might fulfill this role. Indeed mutation of Asp 38 does not slow down the reaction, suggesting that this residue is not involved in proton abstraction. Mutants version of KSI with the original Asp 38 mutated to either neutral (D38G, D38L) or positively-charge (D38K) residues produced variants significantly active than the wild-type enzyme, probably by relieving the unfavorable interaction between the negatively charged Asp 38 and the electron rich benzisoxazole ring. In contrast, mutation of Asp 99 resulted in ~200-fold decrease in catalytic efficiency, indicating that this residue could be the catalytic base. 4-Chlorophenol and equilenin, two competitive inhibitors of native KSI activity, also inhibit the KSI-catalyzed Kemp elimination, suggesting that this reaction takes place at the active site. Remarkably, D38N KSI catalyzes the Kemp elimination with a second-order rate constant greater than those measured for several computationally-designed Kemp eliminases, suggesting that the designed Kemp eliminases do not provide transition state stabilization beyond the one arising from non-specific effects.

Biochemical characterization of the two-component flavoproteins; Isobutylamine-N-hydroxylase (IBAH) and flavin reductase (FRED)

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Isobutylamine-N-hydroxylase (IBAH) and flavin reductase (FRED) from *Streptomyces viridifaciens* MG456-hF10 are part of a two-component flavin-dependent enzyme system that catalyzes the conversion of isobutylamine (IBA) to isobutylhydroxylamine (IBHA); a key step in the formation of valanimycin (1). Valanimycin is an azoxy antibiotic, which has also been shown to exhibit *in vitro* anti-tumorigenic activity against cell cultures of mouse leukemia L1210, P388/S (doxorubicin-sensitive) and P388/ADR (doxorubicin-resistant) (2). In this work, we present the over-expression and purification of this two-component enzyme system. IBAH and flavin reductase have been expressed and purified to homogeneity as separate proteins. FRED exhibited the oxido-reductase activity by catalyzing the oxidation of NADPH. Hydroxylation of IBA was confirmed using LC-MS. Steady state kinetic data from product formation assays showed the K_M value of IBA to be (0.21 ± 0.07) mM with a recorded k_{cat} value of (0.012 ± 0.001) s^{-1}, whilst the K_M of NADPH was determined as (0.26 ± 0.09) mM and with a k_{cat} value of (0.016 ± 0.003) s^{-1}. In pre-steady state kinetic characterization studies, FRED-catalyzed reduction of FAD by NADPH occurred at a rate of (10.0 ± 0.2) s^{-1}. The rate of reduction was slower (1.5-fold decrease) in the presence of substrate IBA, with a k_{red} of (6.6 ± 0.2) s^{-1}. NADH showed a markedly reduced rate of reduction with a k_{red} of (0.34 ± 0.03) s^{-1}. These results suggest that NADH is not a substrate for FRED and IBA does not affect the flavin reduction activity of flavin reductase. The availability of soluble and active monooxygenase complex will facilitate further mechanistic and structural studies of this enzyme system.

Reductive half:

$$FAD + NADPH \xrightarrow{E1} FAD_{red} + NADP^+ \ldots Flavin\ Reductase\ unit\ (E1)$$

Oxidative half:

$$FAD_{red} + IBA + O_2 \xrightarrow{E2} FAD_{ox} + H_2O + IBA\text{-}OH \ldots Monooxygenase\ unit\ (E2)$$

Scheme 1: Reductive and oxidative half reactions catalyzed by flavin reductase and isobutylamine-N-Hydroxylase. Fred oxidizes NADPH to generate reduced FAD, which is utilized by IBAH in the presence of molecular oxygen to catalyze the hydroxylation of isobutylamine (IBA) to isobutylhydroxylamine (IBHA).

1. Parry, R. J., Li, W. (1997) Purification and characterization of isobutylamine N-hydroxylase from the valanimycin producer *Streptomyces viridifaciens MG456-hF10*. *Arch Biochem and Biophys 339*, 47-54.
2. Yamato, M., Iinuma, H., Naganawa, H., Yamagishi, Y., Hamada, M., Masuda, T., Umezawa, H., Abe, V., Hori, M. (1986) Isolation and properties of valanimycin, a new azoxy antibiotic. *J Antibiot 39*, 184 -19

Extending the utility of the amine dehydrogenase: mechanistic insight and isolation of the *(S)*-amine

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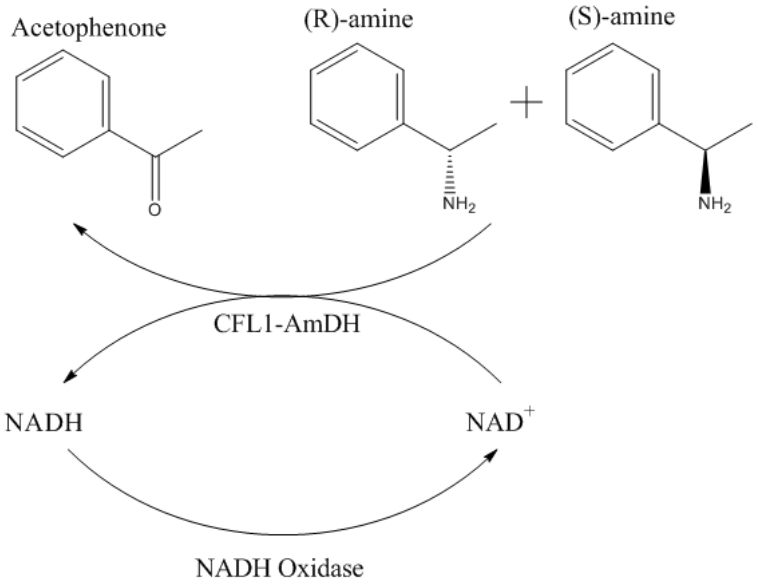
The novel amine dehydrogenase (AmDH) has been recently developed and further characterized. Through protein engineering of the amino acid dehydrogenase scaffold, the amine dehydrogenase now catalyzes the reduction of prochiral ketones to chiral amines. Further protein engineering to the phenylalanine amine dehydrogenase (F-AmDH) to expand the binding pocket has led to an increased specific activity toward its model substrate, para fluoro phenyl acetone (*p*FPA). In addition, F-AmDH exhibited a low affinity for ammonia (K_M: 550 mM), leading the enzyme to perform in unfavorable conditions. Detailed initial velocity studies are being conducted with F-AmDH and the wild type phenylalanine dehydrogenase from *B. badius* to gain mechanistic insight for these enzymes.

Previous work involving the AmDH included the development of a biphasic organic solvent system to allow for conversion of hydrophobic substrates. The F-AmDH naturally catalyzes formation of the *(R)*-amine. To further continue our reaction engineering work, oxidative deamination of racemic mixtures has led to a successful isolation to the *(S)*-amine. For proof of concept, we have produced *(S)*-1-phenylethylamine *((S)*-1-PEA) from the racemate, catalyzed by leucine amine dehydrogenase (L-AmDH). Additionally, four other compounds,1-(2-napthyl)ethylamine, 2-aminohexane, 1,3-dimethylbutylamine, and 3,3-dimythylbutylamine have been successfully deracemized by the amine dehydrogenases.



Targeted intrinsic protein fluorescence to facilitate inhibitor screening and mechanistic evaluation of TB shikimate kinase.

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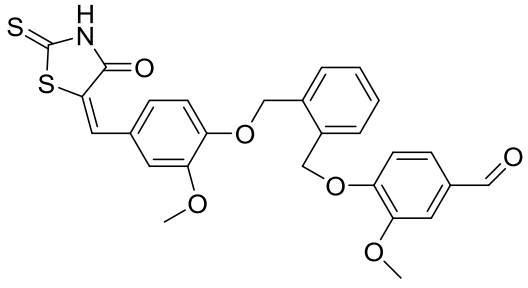
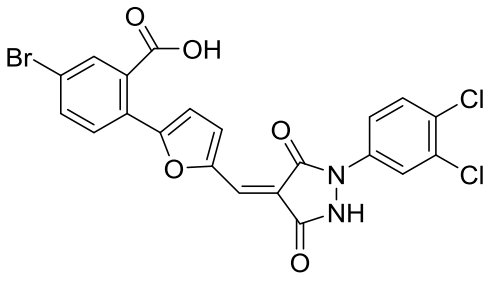
The shikimate pathway generates aromatic amino acids. Its enzymes, including shikimate kinase (SK), are essential for survival of pathogens like *M. tuberculosis*. Because the pathway has no mammalian analogue, its enzymes are also attractive targets for development of new antitubercular agents. Our objective is to develop means to more rapidly identify SK inhibitors and characterize their mechanisms of inhibition. Notably, *M. tuberculosis* SK (*Mt*SK) is devoid of Trp. We used SK sequence alignments and structures to guide Trp substitution to produce *Mt*SK variants, each with intrinsic protein fluorescence corresponding to key components of the enzyme. *Mt*SK variants were generated, expressed, and purified: N151W (nucleotide-binding domain), E54W (shikimate-binding domain) and V116W (Lid domain). Similar kinetic parameters with respect to ATP and shikimate were obtained for wild-type and all three variants. Only the three variants showed characteristic but individually distinct Trp fluorescence emission spectra. Titration of all three variants with ATP resulted in hyperbolic decreases in fluorescence emission intensity. The K_D values calculated for the variants ranged from 0.2 – 0.4 mM and were similar to apparent K_Ms for this substrate. In contrast, titration with shikimate produced no change in fluorescence emission by either E54W or N151W *Mt*SK, but there was a 30% decrease in V116W emission intensity in the presence of shikimate. V116 is part of the conformationally dynamic lid domain. Thus, this response may point toward shikimate-induced conformational changes in *Mt*SK. We also evaluated two inhibitors (see below). Both compounds produced a hyperbolic decrease in fluorescence intensity. Compound 1 produced K_Ds ranging from 16 to 33 mM depending on the variant evaluated. For each variant, K_Ds determined for compound 2 were about two fold lower than those of compound 1. Interestingly, emission spectra for the variants were differentially affected by inhibitor binding. E54W showed a 7 nm blue shift with compound 2 but no change with compound 1. Blue shifts were observed with both inhibitors with V116W. No shift in emission λ_{max} was observed with N151W with either inhibitor. Our data suggest that these variants will serve as valuable mechanistic probes of *Mt*SK catalysis and inhibition.



Compound 1



Compound 2

In vivo effects of macromolecular crowding on the binding affinity of dihydrofolate to dihydrofolate reductase.

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Escherichia coli is capable of growing in low and high osmolality environments, causing a rapid change in the movement of water across its cell membrane. To deal with the loss of water, many osmotically stressed cells either uptake or synthesize small molecules, called osmoprotectants. Examples of osmoprotectants include proline, betaine, trehalose, and glutamate. As a result, the water activity in the cells is decreased, leading to higher macromolecular crowding. We are interested in understanding how folate metabolism enzymes work under crowded conditions.

Dihydrofolate reductase is an enzyme that catalyzes the NADPH-dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF). DHFR is an important enzyme in folate metabolism as generation of THF is required for the synthesis of thymidylate, methionine and other metabolic intermediates. Two types of DHFR exist, the chromosomally encoded DHFR and the plasmid encoded R67 DHFR. Previous *in vitro* osmotic stress studies showed that upon addition of various osmolytes a tighter binding of the NADPH cofactor and weaker binding of DHF was observed in both enzymes. However, *in vitro* studies are far from representative of the heterogeneous and crowded intracellular environment. A crucial difference between *in vivo* and *in vitro* conditions is the high concentration of macromolecules, which can range in cytoplasm from 200 mg/mL in eukaryotes to >400 mg/mL in prokaryotes. In contrast, most biochemical studies are conducted under dilute (<10 mg/mL) macromolecular conditions. These arguments lead to the longstanding question as to what extent do the experiments observed *in vitro* reflect the behavior *in vivo*[6]? In this work we will address this question by determining the binding affinity of DHF to DHFR in the interior of *E. coli* cell by NMR. Here, an osmotic stress approach will be used to determine the *in vivo* effects of the ligand binding on the ^{19}F-labeled tryptophans of chromosomal DHFR and R67 DHFR. NMR will used to monitor the K_d for the DHF binding to DHFR as a function of folate concentration. Thus far, we have standardized the purification of R67 DHFR and we are in the process of labeling our protein.

Bicupin Oxalate Oxidase: Product Inhibition and a Novel Substrate Characterized Using Membrane Inlet Mass Spectrometry

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In membrane inlet mass spectrometry (MIMS), compounds are introduced to the mass spectrometer from solution through a semipermeable membrane. The ability to directly measure product formation and substrate consumption is an advantage over a coupled assay. Oxalate oxidase is a manganese dependent enzyme that catalyzes the oxygen-dependent oxidation of oxalate to carbon dioxide in a reaction that is coupled with the formation of hydrogen peroxide. Oxalate oxidase from *Ceriporiopsis subvermispora* (CsOxOx) is the first bicupin enzyme identified that catalyzes this reaction [1]. The MIMS method of measuring oxalate oxidase activity involves continuous, real-time direct detection of oxygen consumption and carbon dioxide production from the ion currents of their respective mass peaks. $^{13}C_2$-oxalate was used to allow for accurate detection of $^{13}CO_2$ (m/z 45) despite the presence of adventitious $^{12}CO_2$ [2]. Possible new applications of bicupin oxalate oxidase for use in pancreatic cancer treatment [3] and in biofuel cells [4] have highlighted the need to understand the extent of product inhibition of the CsOxOx catalyzed oxidation of oxalate and CsOxOx catalyzed the decarboxylation of the novel substrate mesoxalate (oxopropanedioate). In this work, we describe the inhibition of the CsOxOx catalyzed oxidation of oxalate by hydrogen peroxide, the preparation of $^{13}C_3$-mesoxalate from $^{13}C_3$-glycerol [5], and the characterization of the CsOxOx catalyzed decarboxylation of mesoxalate.

1. Escutia MR, Bowater L, Edwards A, Bottrill AR, Burrell MR, et al. (2005) Cloning and sequencing of two Ceriporiopsis subvermispora bicupin oxalate oxidase allelic isoforms: implications for the reaction specificity of oxalate oxidases and decarboxylases. Appl Environ Microbiol 71: 3608-3616.
2. Moomaw EW, Uberto R, Tu C (2014) Membrane inlet mass spectrometry reveals that Ceriporiopsis subvermispora bicupin oxalate oxidase is inhibited by nitric oxide. Biochem Biophys Res Commun 450: 750-754.
3. Chakrabarti G, Moore ZR, Luo X, Ilcheva M, Ali A, et al. (2015) Targeting glutamine metabolism sensitizes pancreatic cancer to PARP-driven metabolic catastrophe induced by ss-lapachone. Cancer Metab 3: 12.
4. Hickey DP, McCammant MS, Giroud F, Sigman MS, Minteer SD (2014) Hybrid enzymatic and organic electrocatalytic cascade for the complete oxidation of glycerol. J Am Chem Soc 136: 15917-15920.
5. Ciriminna R, Pagliaro M (2003) One-pot homogeneous and heterogeneous oxidation of glycerol to ketomalonic acid mediated by TEMPO. Advanced Synthesis & Catalysis 345: 383-388.

Probing the Role of a Rogue Cysteine Residue in Cysteine Dioxygenase

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Cysteine Dioygenase (CDO) is an iron-dependent enzyme that catalyzes the oxidation of L-cysteine to form L-cysteine sulfinic acid. Adjacent to the iron center is a thioether crosslink between Tyr157 and Cys93. Following purification, wild-type CDO has been shown to exist as a heterogeneous mixture of non-crosslinked and crosslinked isoforms. The thioether crosslink has been proposed to assist in stabilizing the coordination of the Cys substrate to the iron center. Located ~10Å away from the iron center is a cysteine (Cys164) residue positioned at the opening to the active site. Cys164 does not participate in a disulfide bond and exists as a free thiol. Previous studies have shown that Cys164 participates in a disulfide bond with free cysteine, and variants have shown abatement in catalytic activity.[1,2,3] However, the conflicting reports on the role of Cys164 have not been adequately probed.

Two variants, C164A and C164S CDO, were constructed and evaluated by steady-state kinetics and crosslink formation studies. Interestingly, the catalytic activity of C164A and C164S CDO displayed 65- and 35-fold decreases in activity, respectively, compared to wild-type CDO. Comparable to wild-type CDO, both variants appeared to exist as heterogeneous mixtures of crosslinked and non-crosslinked isoforms. However, crosslink formation studies showed that C164A CDO was able to generate the fully crosslinked species at lower concentrations of the Cys substrate compared to C164S and wild-type CDO. Although Cys164 is not in close proximity to the active site, these studies strongly suggest that Cys164 plays a key role in the mechanistic properties of CDO.

References

1. Jameson J.Biol. Inorg .Chem. (2009) 14, 913-921
2. Karplus J. Mol. Biol. (2013) 425, 3121-3136
3. Rao Biochem. (2007) 282, 3391-3402

The A225L substitution stabilizes the allosterically inhibited state of hUGDH

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Glucuronidation reduces the bioavailability of many drugs, rendering them ineffective. In fact, many drugs fail clinical trials due to glucuronidation Controlling glucuronidation is an important goal. Human UDP-α-D-glucose 6-dehydrogenase (hUGDH) catalyzes the NAD^+-dependent production of UDP-α-D-glucuronic acid. UDP-α-D-xylose (UDP-Xyl), a downstream metabolite of hUGDH, acts as a feedback inhibitor by competitively binding to the active site. UDP-Xyl binding also induces an allosteric isomerization to produce a catalytically inactive state. After the isomerization, the catalytic base (D280) is buried in a solvent inaccessible cavity with no neutralizing interaction. We designed the A225L substitution to fill the cavity and prevent the isomerization. Surprisingly, the crystal structure shows that $hUGDH_{A225L}$ in complex with UDP-Xyl adopts the allosterically inhibited state and still buries D280. The crystal structure is supported by kinetic analysis, which shows that the binding of the feedback inhibitor is still cooperative and the affinity for UDP-Xyl has increased. The most significant effect of the A225L substitution involves enzyme hysteresis (the slow isomerization of the enzyme from an inactive to an active state). Stopped-flow analysis shows that the hysteretic lag increases by 6-fold, suggesting that the A225L substitution favors the inactive state of the enzyme. The crystal structure of the active state of $hUGDH_{A225L}$ shows that helix α11 has shifted into the active site. We believe that it is this conformational change that favors the inactive state of the enzyme.

Insights into Dynamics of $SufBC_2D$ Fe-S Scaffold Complex by Hydrogen Deuterium Exchange Mass Spectrometry

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Iron-sulfur (Fe-S) clusters, acting as cofactors of Fe-S proteins, are essential for maintaining fundamental biological processes. The Suf pathway is one Fe-S biosynthetic system and is a common system in eukaryotes, bacteria, and archaea. It is especially important since it synthesizes Fe-S when metabolism is disrupted by iron starvation or oxidative stress. The Suf pathway contains five proteins: SufA, SufB, SufC, SufD, SufE, and SufS. SufBCD usually work as a complex, assembling nascent Fe-S clusters. SufB and SufD are core domains for assembling the clusters. SufC is an ATPase that catalyzes the hydrolysis of ATP, providing free energy for stabilization of $SufBC_2D$ active conformation. Even though it has been reported that the addition of ATP/Mg^{2+} induces a structural change in $SufBC_2D$ complex, the details of the changes are still unclear. We used hydrogen deuterium exchange mass spectrometry (HDX-MS) to investigate the conformational changes of $SufBC_2D$ complex due to the binding of ADP/Mg^{2+}. Peptides 23-43 and 44-53 in SufC show a decrease in solvent accessibility when ADP/Mg^{2+} binds. This suggests that this region contains the ATP binding site, consistent with the structure of SufC with ATP modeled in. Peptides located at the SufC/SufC interface exhibit decreases in the rates of deuterium incorporation, suggesting ADP/Mg^{2+} binding triggers dimer formation of the two SufC modules. Changes in the rates of deuterium incorporation are also seen at the SufB/SufD interface with SufC. Interestingly, the changes are not symmetrical with more changes seen in SufB. Peptides 447-456, 457-461, and 462-471 located at the SufB/SufC interface show significant decreases in exchange rate. While only one peptide 374-388 located at the SufD/SufC interface shows lower deuterium incorporation. Unexpectedly, peptides 108-112 and 113-122 located at β-sheet domain 51.52Å from the interface of SufD/SufC also show decreases in deuterium incorporation. By application of HDX-MS, we detected structural and dynamic changes of $SufBC_2D$ complex on ADP/Mg^{2+} binding, which provides more information for investigation of Suf system.

Increased Activity of Glucose Oxidase under High Hydrostatic Pressure for Food Processing Application

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In our previous study, the inactivation of glucose oxidase (GOx) at a high hydrostatic pressure (HHP) of 240 MPa at 74.5 °C, occurred at a rate (k_{inact}) 50 times slower than at atmospheric pressure at the same temperature. The objective of this study was to maximize GOx activity at HHP under non-inactivating conditions. Activity of GOx increased significantly ($p < 0.05$) using HHP up to 300 MPa compared to atmospheric pressure at 25 °C and at 63.8 °C. The apparent rate constant of the GOx-catalyzed oxidation of glucose (k_{cat}) increased 2-fold at 300 MPa and 25 °C and 9-fold at 300 MPa and 63.8 °C compared to reaction at atmospheric pressure and room temperature. The apparent activation volume of GOx catalysis was –11.8 ± 2.5 and –30.5 ± 0.3 cm^3mol^{-1} at 25 and 63.8 °C respectively. Negative apparent activation volumes confirmed that HPP favors GOx catalyzed oxidation of glucose. Hawley model described well the pressure-temperature dependence of the rate constant of GOx activation as indicated by a model adjusted-R^2 of 0.859. The model parameters were estimated using a nonlinear regression analysis based on the minimal sum of squares using SAS statistical software (Cary, NC). High hydrostatic pressure protected GOx against thermal inactivation, allowing carrying out enzymatic reaction at higher temperatures which resulted in greater catalytic rates. Using this approach can reduce process time and the operating costs for applications such as glucose and oxygen removal.

Preliminary purification and characterization of model erythromycin resistance methyltransferases.

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Modification of bacterial ribosomes by the erythromycin resistance methyltransferase (Erm) family of enzymes is a widespread mechanism used by bacteria to achieve multi-drug resistance to antibiotics, such as erythromycin and clindamycin, that exert their effect by binding to the bacterial ribosome and interfering with its role in protein synthesis (1, 2). Erm induced antibiotic resistance is found in both in soil dwelling bacteria that biosynthesize antibiotics (antibiotic producers) that are not a threat to humans and pathogenic bacteria that do pose a risk to humans. We are mechanistically characterizing two evolutionarily distant Erm family members, ErmC present in 'pathogens' and ErmE present in 'producers'. Biophysical assays with Erm enzymes will require large amounts of pure, soluble, and active protein. We report preliminary results of purification and characterization experiments with two model Erm enzymes.

1. Lai et al. (1973). Journal of Molecular Biology, 74(1): 67-72.
2. Dunkle et al. (2010). PNAS, 107(40): 17152-17157.

Probing Lysine acetyltransferase substrates with clickable acetyl-CoA analogs and engineered enzymes

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Lysine acetyltransferases (KATs) are posttranslational modifying enzymes that can transfer acetyl groups from acetyl Coenzyme A (acetyl-CoA) to specific lysines on both histone and non-histone proteins. KATs are implicated in many physiological processes by regulating acetylation level of different proteins and dysregulation of protein acetylation can induce many diseases. Although some KATs substrates have been identified and promoted our understanding of the mechanisms of KATs related physiologies and pathologies, the substrates of KATs, especially non-histone proteins, still remain largely unknown.

Our group has synthesized a suite of acetyl-CoA analogs with either azido or alkynyl functional groups that can act as chemical probes. Accordingly, we've generated different KATs mutants with expanded acetyl-CoA binding sites that can specifically react with the chemical probes and label their substrates with clickable tags. Protein labeling with this chemical biological approach enables us to visualize and identify KATs substrates which can largely prompt the study on KAT-related biologies.

Producing Reactive Intermediates via Photodriven Electron Transfer

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A significant portion of prokaryotic and eukaryotic genomes encode flavoenzymes which perform functions essential to all forms of life. *Enterobacter cloacae* nitroreductase (NR) and *Thermus thermophilus* NADH oxidase (NADOX) are two enzymes under study in order to elucidate protein determinants of flavin redox active properties. Understanding the mechanism by which flavin cofactors are photoexcited and transfer electrons is essential in harnessing flavoproteins for organic electronic applications. After the initial photoexcitation of flavin, the fluorescence lifetime can be anywhere from 1 femtosecond to 100 nanoseconds until the excited state decays or is quenched by electron transfer. To study rapid electron transfer events executed upon flavin photoexcitation, and probe for possible formation of semiquinone states, we are using sub-picosecond transient absorption spectroscopy (TAS). To optimize the yield of electron transfer intermediates, a donor molecule can be provided to supply an electron to the flavin π orbital vacancy that is formed upon photo-excitation of an electron to a π^* orbital. With this electron hole filled, the flavin is expected to adopt an excited semiquinone state. However, from initial TAS findings, the lifetime of the excited electronic state was shortened by addition of donor molecules. To understand the photochemical reactions between our donor dyes and the flavin, fluorescence quenching and UV-Vis Charge Transfer signatures are being used to probe the potential of candidate electron donors to participate in electron transfer to photoexcited flavin sites. An array of candidate electron donors were screened to identify those with optimal efficiency and favorable dissociation constants (K_d) in both NR and NADOX. With a possible way to produce flavin semiquinone, TAS could be utilized to understand the formation and decay of the semiquinone state.

An Approach for Generating and Characterizing Semiquinone Intermediates Proposed to be Critical to Electron Transfer in Flavin Dependent Nitroreductase

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Enterobacter cloacae nitroreductase (NR) and its many bacterial homologues catalyze the NADH- and NADPH-dependent reduction of a large variety of substituted nitroaromatic compounds as well as a smaller set of nitroalkenes. Much of the reported chemistry for the nitroreductase family of flavoenzymes has been limited to 2-electron reductions executed without accumulation of flavin semiquinone. Stopped-flow kinetics failed to detect a 1-electron reduced semiquinone state of NR, consistent with the oxygen insensitivity of NR's reaction. However ultrafast (ps) transient absorption spectroscopy (TAS) reveals a number of photoexcited flavin intermediates including anionic semiquinone. The lifetimes of transient species and the rate of recovery of oxidized flavin are significantly affected by the presence and concentration of substrate analogues, offering mechanistic insight. Therefore we are comparing the TAS of NR with that of flavodoxin (stabilizes neutral semiquinone) and *T. thermophilus* NADH oxidase (same fold as NR but more accessible semiquinone). The broad substrate repertoires of NR and NADH oxidase provide an opportunity to test a wide range of substrate analogues to identify those with the best rates and driving forces for reduction of photoexcited flavin and formation of sequiquinone. This capability will advance studies of electron transfer bifurcating enzymes in which a high-energy flavin semiquinone is proposed to play a pivotal role. Thus we are developing methodologies for the observation of very short-lived (ps to ns) electron transfer intermediates, as well as mechanistic determinants in flavoenzymes.

Investigation of Cobalamin-dependent Radical *S*-Adenosyl-L-methionine Enzymes Involved in Bacteriochlorophyll *c* Biosynthesis

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Green sulfur bacteria (GSB) produce a specialized photopigment, bacteriochlorophyll (Bchl) *c*, which enables them to grow phototrophically under extreme low-light intensity conditions. Bchl *c* has several unique structural features that allow it to self-assemble into the large nanostructures that comprise the highly efficient light-harvesting complex of GSB, the chlorosome. The biosynthetic pathway of Bchl *c* has yet to be fully characterized. Based on genetic information, three key reactions in Bchl *c* biosynthesis are thought to be catalyzed by homologous enzymes (BchE, BchQ, and BchR), which are annotated as cobalamin-dependent members of the radical *S*-adenosyl-L-methionine (SAM) superfamily. BchE catalyzes anaerobic O atom insertion and formation of the isocyclic ring of Bchl *c*. In contrast, BchQ and BchR are unusual methyltransferases capable of methylating unactivated C-H bonds. Specifically, BchQ transfers up to 3 methyl groups to the C8 ethyl group of Bchl *c*, while BchR catalyzes monomethylation of the C-12 methyl group. An investigation is underway to demonstrate the *in vitro* activities of these enzymes and to gain insight into the unprecedented enzymatic transformations they catalyze.

Characterization of the 6-Hydroxy-FAD of Y249F variant of D-Arginine Dehydrogenase from *Pseudomonas aeruginosa*

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Flavoenzymes catalyze a wide variety of reactions and play a central role in many important biological processes. The diversity in their function can be attributed to their ability to catalyze both 1 and 2 e- transfer reactions and the diversity of their active sites. Flavoenzymes are usually found to contain FMN or FAD as a yellow chromophore, either covalently or non-covalently bound to the apoprotein. D-Arginine dehydrogenase from *Pseudomonas aeruginosa* (PaDADH; E.C. 1.4.99.6) is an FAD-dependent enzyme that oxidizes D-arginine to iminoarginine, which is then non-enzymatically hydrolyzed to 2-ketoarginine and ammonia. Purification of a mutant variant of PaDADH in which tyrosine 249 is replaced with phenylalanine yielded two distinct protein fractions: one with FAD which was enzymatically active, and an unusual green fraction with a modified FAD, which was not reactive [1].

The green flavin extracted from the Tyr249Phe enzyme variant has been characterized by NMR, mass spectrometry, high-performance liquid chromatography, and UV−visible absorption spectroscopy [1].Using the techniques mentioned above, the green flavin was established as an FAD hydroxylated at the 6 position. Studies conducted on this modified flavin in other enzyme systems as well as in the Tyr249Phe variant of DADH have suggested that the 6-hydroxyflavin primarily exists in an anionic form at a pH above its pK_a ~7.05. Presence of 6-hydroxy FAD in the Tyr249Phe variant of DADH demonstrated that there is an intrinsic proclivity for 6-hydroxylation of flavin when the protein environment around the cofactor is changed through mutagenesis. These findings have highlighted the need of determining the mechanism of these modifications and also probe the physiological significance of these seemingly unreactive modified flavins [1].

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REFERENCES

[1] Gannavaram, S., Sirin, S., Sherman, W., and Gadda, G. (2014) Mechanistic and computational studies of the reductive half-reaction of tyrosine to phenylalanine active site variants of D-arginine dehydrogenase, *Biochemistry 53*, 6574-6583.

The Crystal Structures and Acceptor Specificities of Human Fucosyltransferases FUT5 and FUT9

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Glycan structures on cell surfaces and secreted glycoproteins play critical roles in biological recognition and macromolecule targeting events in animal systems. Fucosyltransferases (FUT) that belong to family GT10 of carbohydrate modifying enzymes are a family of glycosyltransferases that are involved in synthesizing terminal glycan linkages. In humans, the eight members of the GT10 family are divided into two sub-families based on sequence similarities, the glycan linkage synthesized, and acceptor specificities. All human GT10 fucosyltransferases (FUT3-7 and FUT9-11) synthesize α1,3-fucosyl linkages to GlcNAc residues in Type 2 LacNAc structures [R-Galβ(1,4)GlcNAc-R] and use GDP-fucose as the sugar donor. Different members of the family have unique specificities for using LacNAc acceptors with modifications to the Gal residue, including NeuAcα(2,3)Galβ(1,4)GlcNAc-R (sialyl-LacNAc), Fucα(1,2)Galβ(1,4)GlcNAc-R (H Type 2 chains), or extended polylactosamine structures. In contrast, FUT3 and FUT5 have the ability to also transfer Fucose to GlcNAc residues in an α1,4-linkage to Type 1 chains [R-Galβ(1,3)GlcNAc-R] as well as Type 1 chains containing modifications to the Gal residue, as specified above. FUT9 preferentially synthesizes α1,3-Fucose linkages on terminal Type 2 and H Type 2 acceptors. In contrast, FUT5 can add Fucose residues to unmodified or modified Type 1 or Type 2 acceptors. Here we present the first crystal structures of two human fucosyltransferases, FUT9 and FUT5. Structures of FUT9 were solved as apo, GDP, and GDP:lacto-N-neotetraose acceptor complexes, while FUT5 was solved as a GDP complex. Both enzymes adopt a metal-ion independent glycosyltransferases-B fold with two domains separated by an acceptor-binding pocket. In both structures a Glu sidechain is well positioned to act as the general base and Lys/Arg residues are positioned to stabilize the developing charge on the pyrophosphate during sugar transfer. Comparison of the FUT9 and FUT5 structures revealed potential residues that impact substrate specificity and catalysis. Based on our structural analysis, kinetic assays of catalytic mutants of FUT9 and FUT5 are underway. This poster presents models for the catalytic mechanism and acceptor specificity for the GT10 FUTs in the synthesis of terminal Fucose linkages in animal systems (supported by NIH grants P41GM103390 and P01GM107012).

The Role of Intrinsic Disorder in Human UDP-Glucose Dehydrogenase

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It is estimated that 33% of eukaryotic proteins contain at least one intrinsically disordered (ID) segment of 30 residues or longer.[1] Many of these ID-peptides are believed to be important for enzyme function or regulation, but only a few examples have been examined experimentally.[2] Here we show that the disordered C-terminus (ID-tail) of human UDP-α-D-glucose-6-dehydrogenase (hUGDH) contributes to the allosteric regulation of the enzyme. The crystal structures of hUGDH show that the ID-tail is disordered in both the active and allosterically inhibited conformations. Despite the disordered state, the deletion of the ID-tail (**Δ**C-term hUGDH) reduces the affinity for allosteric inhibitor UDP-xylose by more than an order of magnitude. The fact that the bound allosteric inhibitor is not solvent accessible suggests that any interaction between the ID-tail and the effector involves an indirect mechanism. Given that the hexameric assembly of hUGDH is important for the allosteric response[3], we examined the effect of the ID-tail on the structure of the enzyme. The crystal structures of **Δ**C-term and full-length hUGDH reveal the same hexameric complex. However, sedimentation velocity analysis shows that the loss of the ID-tail weakens the hexamer in solution. To decouple the contribution of the hexameric structure and the ID-tail from the binding of UDP-Xyl, we used a stabilized hUGDH dimer (M11-hUGDH). The M11-hUGDH dimer binds UDP-Xylose with a greater affinity than the hexamer. As observed in the full-length enzyme, deletion of the ID-tail in the M11-hUGDH dimer reduces the affinity for allosteric effector (6.4-fold). Thus, we show that the mechanism by which the ID-tail favors allosteric inhibition is independent of its role in stabilizing the hUGDH hexamer.

1. Tompa, P., Dosztanyi, Z., and Simon, I. (2006) Prevalent structural disorder in E. coli and S. cerevisiae proteomes, *Journal of proteome research 5*, 1996-2000.
2. van der Lee, R., Buljan, M., Lang, B., Weatheritt, R. J., Daughdrill, G. W., Dunker, A. K., Fuxreiter, M., Gough, J., Gsponer, J., Jones, D. T., Kim, P. M., Kriwacki, R. W., Oldfield, C. J., Pappu, R. V., Tompa, P., Uversky, V. N., Wright, P. E., and Babu, M. M. (2014) Classification of intrinsically disordered regions and proteins, *Chem Rev 114*, 6589-6631.
3. Kadirvelraj, R., Custer, G. S., Keul, N. D., Sennett, N. C., Sidlo, A. M., Walsh, R. M., Jr., and Wood, Z. A. (2014) Hysteresis in human UDP-glucose dehydrogenase is due to a restrained hexameric structure that favors feedback inhibition, *Biochemistry 53*, 8043-8051.

Detecting Hydrogens and Deuteriums in high resolution X-ray crystal structures of HIV-1 Protease

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Neutron crystallography of HIV protease in complex with the clinical inhibitor amprenavir has provided insights into the hydrogen bonding interactions and water structure (1). This analysis used perdeuterated protein due to the lower incoherent scattering of deuterium, however, previous studies have not determined the structural differences for hydrogenated vs perdeuterated HIV protease. We have solved, refined and analyzed the X-ray crystal structures of the same complex with perdeuterated and hydrogenated HIV protease and investigated the visibility of H/D atoms in X-ray diffraction data.

A perdeuterated HIV-1 protease complexed with amprenavir was refined to 1.08 Å resolution and compared with a 1.03 Å hydrogenated counterpart. No significant overall structural changes can be seen in the derdeuterated structure helping to confirm the validity of joint X-ray/Neutron refinement. Peaks in the difference density were observed at positions corresponding to H or D atoms. These peaks were enriched on main chain heavy atoms as well as in well-ordered motifs and hydrogen bond interactions. More hydrogen peaks were cataloged in the protonated structure than in the deuterated model and they are also associated with heavy atoms of relatively lower B-values.

1. Weber, Irene T., Mary Jo Waltman, Marat Mustyakimov, Matthew P. Blakeley, David A. Keen, Arun K. Ghosh, Paul Langan, and Andrey Y. Kovalevsky. (2013) Joint X-Ray/neutron Crystallographic Study of HIV-1 Protease with Clinical Inhibitor Amprenavir: Insights for Drug Design. *Journal of Medicinal Chemistry* 56 (13): 5631–35.

Development of a screening assay to evaluate the substrate specificity of PRMTs

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Protein Arginine Methyltransferases (PRMTs) convert protein arginine to monomethylarginine, asymmetric dimethylarginine, or symmetric dimethylarginine using *S*-adenosylmethionine as the methyl donor. Nine mammalian PRMT members (PRMTs 1-9) have been identified and characterized, however the factors contributing to the substrate specificity for individual isozymes has been limited by a lack of tools and methodologies to study these enzymes in a high-throughput fashion. We have developed a new, rapid, non-radioactive, and sensitive method for evaluating the substrate specificity of individual PRMT isozymes using a one-bead one compound (OBOC) peptide library. This methodology relies on synthesizing the peptides on resin in a 96-well plate. Subsequently, the library of peptides can be screened in the plate by incubating the PRMT of interest with the library. Library beads containing arginine residues that are methylated by PRMT can be visualized using an antibody-based system that results in blue beads. This screening methodology eliminates a need for purification of individual peptides and provides a timesaving, cost-effective alternative to the traditional PRMT assays.

Nguyen, H.C., Wang, M., Salsburg, A. & **Knuckley, B.*** Development of a Plate-Based Screening Assay to Investigate the Substrate Specificity of the PRMT Family of Enzymes. *ACS Comb Sci* 17, 500-5 (2015).

The role of solvent effect on the aminoglycoside-binding properties of thermophilic and mesophilic variants of Aminoglycoside Nucleotidyltransferase 4' (ANT4)

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Aminoglycosides are a group of antibiotics that bind to the 16s ribosome RNA of prokaryotes and thereby cause mistranslations and premature stops which interfere with protein translation. Some bacterial species developed resistance against aminoglycosides due to their aminoglycoside modifying enzymes (AGMEs). Aminoglycoside nucleotidyltransferase 4' (ANT4) is an AGME which transfers the AMP group from MgATP to the C4'-OH of aminoglycosides in order to detoxify them. Two thermostable variants (T130K and D80Y) of ANT4 have been created in our laboratory by introducing single point mutations to the wild type (WT) ANT4. Our group has shown earlier that; local flexibility and ligand binding properties of T130K resembles the mesophilic WT enzyme whereas D80Y, which has the highest melting temperature, presents distinct features representative of a true thermophilic enzyme. This work is based on the hypothesis that solvent reorganization upon ligand binding may be used to identify properties that lead to thermophilicity. Since the active sites of ANT4' are highly solvent exposed, binding of ligands will cause (or be affected by) differential solvent reorganization. Therefore, we are investigating the solvent reorganization on the aminoglycoside-binding properties of different ANT4 variants via performing isothermal titration calorimetry (ITC) both in light water (H_2O) and heavy water (D_2O).

1. Jing, X., and Serpersu, E.H. (2014) Solvent reorganization plays a temperature-dependent role in antibiotic selection by a thermostable Aminoglycoside Nucleotidyltransferase-4'. *Biochemistry 53,* 5544-5550.

Discovery of Selective Inhibitors of Tyrosyl-DNA Phosphodiesterase 2 by Targeting the Enzyme DNA-binding Cleft

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Tyrosyl-DNA phosphodiesterase 2 (TDP2) processes protein/DNA adducts resulting from abortive DNA topoisomerase II (Top2) activity. Selective TDP2 inhibition could, therefore, provide synergism with the established Top2 poison class of chemotherapeutics. By virtual screening of the NCI diversity small molecule database we identified selective TDP2 inhibitors and experimentally verified their selective inhibitory activity in whole cell extracts. Three selective inhibitors showed IC_{50} values in the low-micromolar range. Molecular docking and molecular dynamics (MD) simulations revealed a common binding mode for these inhibitors, which involved association to the TDP2 DNA-binding cleft. From MM-PBSA binding energy calculations and per-residue energy decomposition, we identified important interactions of the compounds with specific TDP2 residues. These interactions could provide new avenues for synthetic optimization of these scaffolds.

Improving Functional Annotation in the DRE-TIM Metallolyase Superfamily through Identification of Active Site Fingerprints

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Within the DRE-TIM metallolyase superfamily, members of the Claisen-condensation-like (CC-like) subgroup catalyze C-C bond-forming reactions between various α-ketoacids and acetyl-coenzyme A. These reactions are important in the metabolic pathways of many bacterial pathogens and serve as engineering scaffolds for the production of long-chain alcohol biofuels. To improve functional annotation and identify sequences that might use novel substrates in the CC-like subgroup, a combination of structural modeling and multiple-sequence alignments identified active site residues on the third, fourth, and fifth *β*-sheets of the TIM-barrel catalytic domain that are differentially conserved within the substrate-diverse enzyme families. Using *α*-isopropylmalate synthase and citramalate synthase from *Methanococcus jannaschii* (MjIPMS and MjCMS), site-directed mutagenesis was used to test the role of each identified position in substrate selectivity. Kinetic data suggest that residues at the β3-5 and β4-7 positions play a significant role in the selection of α-ketoisovalerate over pyruvate in MjIPMS. However, complementary substitutions in MjCMS failed to alter substrate specificity suggesting residues in these positions do not contribute to substrate selectivity in this enzyme. Analysis of the kinetic data with respect to a protein similarity network for the CC-like subgroup suggests that evolutionarily distinct forms of IPMS utilize residues at the β3-5 and β4-7 positions to affect substrate selectivity while the three versions of CMS use unique mechanisms. Importantly, mapping the identities of residues at the β3-5 and β4-7 positions onto the protein similarity network allows for rapid annotation of probable IPMS enzymes as well as several outlier sequences that may represent novel functions in the subgroup.

Characterization of an aminoglycoside modifying enzyme, aminoglycoside *N3* acetyltransferase-VIa

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Aminoglycoside (AG) antibiotics are bactericidal agents used to treat various bacterial diseases like tuberculosis, meningitis and hospital borne infections. However, the clinical effectiveness of these drugs has been drastically affected by the emergence of resistant bacterial strains, which is achieved by plasmid-encoded enzymes called aminoglycoside modifying enzymes (AGMEs). These enzymes can covalently modify their substrates, rendering them ineffective. AGMEs show variable levels of substrate promiscuity, but no correlation has been confirmatively observed between the sequence or structure of an AGME and its substrate profile. Our goal is to understand the molecular principles underlying this ligand selectivity by deciphering the thermodynamic and dynamic properties of enzyme-ligand complexes.

In this work, we describe kinetic, dynamic and thermodynamic properties of the aminoglycoside *N3* acetyltransferase-VIa (AAC-VIa). AAC-VIa catalyzes the modification of *N3* atom of the 2-deoxystreptamine (2-DOS) ring on aminoglycosides by transferring an acetyl group from acetyl CoA. Despite significant sequence similarity to other aminoglycoside acetyltransferases, unlike others, it has a very limited substrate profile and therefore it presents an excellent opportunity to study molecular basis of ligand promiscuity.

Substrate profile of AAC-VIa was observed to be limited to four substrates; gentamicin, sisomicin, tobramycin and kanamycin B. Analytical ultracentrifugation (AUC) studies demonstrated that the proportion of dimeric enzyme increases with increasing concentrations of enzyme. AUC studies also showed that dimer formation was mainly due to polar interactions which become weaker in the presence of salt. Binding of ligands favor the monomeric form of AAC-VIa. Isothermal titration calorimetry (ITC) studies showed that ligand binding is enthalpically driven, entropically unfavorable and is accompanied by decreases in pKa's of titratable groups. Studies in D_2O and H_2O showed that solvent effect plays a role in the heat capacity exchange during the formation of binary complex, but no solvent effect is observed for ternary complex formation.

ChuY from *E. coli* O157:H7 is an Anaerobilin Reductase that Exhibits Kinetic Cooperativity

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Heme catabolism is an important biochemical process many bacterial pathogens utilize to acquire iron. However, tetrapyrrole catabolites often require further processing for their removal from the cell as a means to prevent toxic accumulation. In eukaryotes, the catabolic enzymes heme oxygenase and biliverdin reductase have been extensively characterized. Recent *in vitro* work has presented evidence for an anaerobic heme degradation pathway in *Escherichia coli O157:*H7. In this system a radical SAM methyltransferase, ChuW, provides the oxidizing power to break open the porphyrin macrocycle. The mechanism also involves the transfer of a methyl group from SAM to heme resulting in decyclization via an unstable intermediate, and the generation of a new tetrapyrrole termed "anaerobilin". Here we describe the function of ChuY, the enzyme expressed downstream from ChuW in the same heme utilization operon. ChuY has structural similarities to biliverdin reductases, and forms a dimeric complex that reduces anaerobilin to the product we have termed "anaerubin". Steady state analysis of ChuY suggests kinetic cooperativity, which is best explained by a random addition mechanism with a kinetically preferred path for NADPH binding.

Structural Studies of HIV-1 and HTLV-II Long Terminal Repeat substrates

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Retroviral Integrase (IN) recognizes different viral LTR sequences, allowing them to process a variety of substrates. Common to all viral DNA 3' processing substrates is a conserved CA dinucleotide motif and corresponding GT pairing nucleotides. Here we have systematically characterized the global and local features of different retroviral integrase substrates using identical procedures. Analysis of our DNA substrate structures reveals conserved but also different helical features of the CA motifs in these substrates. Our study suggests that the local DNA structure at the CA motif is not the main determinant for retroviral recognition, and we demonstrate removal of an exocyclic amide group from the substrate guanosine (base partner of the conserved cytosine) abolishes IN 3' processing. Based on our biophysical results and an analysis of previous literature we identify and confirm essential features of retroviral substrates that are required for IN activity.

Synthesis and Evaluation of 2'-Chloro,2'-Fluoro-7-Deazapurine Nucleosides and Prodrugs as Potential Inhibitors of Hepatitis C Virus Replication.

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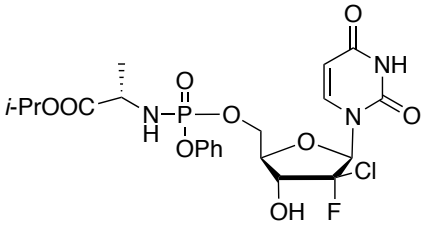
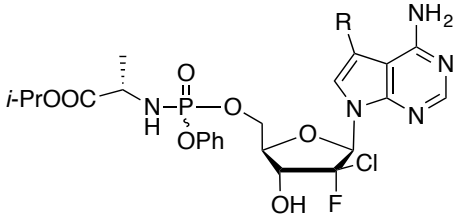
Pan-genotypic nucleoside and nucleotide hepatitis C virus (HCV) NS5B inhibitors display a high genetic barrier to drug resistance and are the preferred direct acting agents (DAA) in the pursuit to achieve 100% sustained virologic response (SVR). Despite the recent approval by the FDA of sofosbuvir (SOF), additional nucleoside analogs and direct acting agents (DAA) are required to achieve a faster SVR. We recently discovered a unique b-D-2'-Cl,2'-F-uridine phosphoramidate nucleotide (1) which has a desirable profile, including pan genotypic activity with no apparent cytotoxicity and an excellent preclinical profile. Based on this compound, we synthesized a new series of 7-deazapurine analogs along with their phosphoramidate prodrugs. All synthesized compounds were evaluated for inhibition of HCV RNA replication in Huh7 cells and their cytotoxicity was determined in various cell lines (HepG2, primary human lymphocyte, CEM and Vero cells). From this set, the 7-iododeazapurine displayed moderate activity against HCV (along with mild cytotoxicity in Huh7 cells). Molecular dynamics simulations on this set of nucleotides in the HCV NS5B active site suggest that Arg158 positioning and dynamics plays a critical role in analog recognition and activity. These studies provide chemical, biological, and structural advances to enable design of additional antiviral nucleotide analogs.



(1)



R= I, F, H

Exploring Novel Flavin-Dependent Chemistry: The Mechanism of Oleate Hydratase from *Elizabethkingia meningoseptica*

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Oleate hydratase (OhyA; E.C.4.2.1.53) from *Elizabethkingia meningitis,* is an flavin-dependent enzyme that catalyzes the conversion of oleic acid to 10-hydrostearic acid. OhyA has become the focus of studies due to its ability to introduce hydroxyl groups without wasted energy on cofactor recycling. By converting oleic acid to (R)-10-hydroxysteric acid, oleate hydratase provides a product that has potential use as a surfactant, a lubricant, in cosmetic applications, and a possible initiation to greater polymer chemistry. The hydration of unsaturated fatty acids is believed to be a detoxification mechanism and a survival strategy. The reaction catalyzed by OhyA does not involve a net redox changes, however the enzyme requires the flavin cofactor in the reduced form for activity. Our hypothesis is that the flavin cofactor in OhyA functions as an acid, which represents a novel role in nature. OhyA was expressed as a recombinant protein in *Escherichia coli* and purified with FAD bound (Figure 1). Overall purification resulted in 68.3 mg of purified OhyA per 1 liter of growth media, with FAD incorporation of 49 %. Activity assays were performed in the presence of oleic acid and the coenzymes: NADH, NADPH under both anaerobic and aerobic conditions. OhyA was active only under anaerobic and reduced conditions. We present the stopped-flow kinetic characterization of OhyA and propose a role for the reduced FAD in this non-redox reaction.

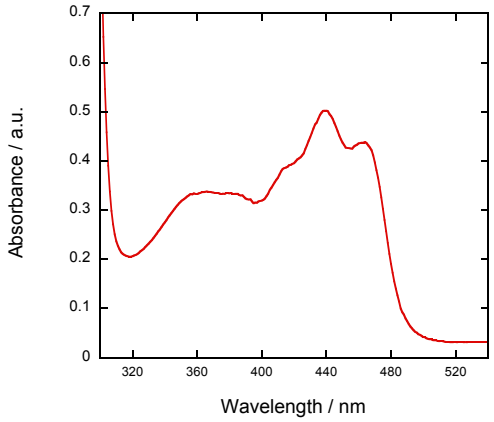

Figure 1. UV-Vis Spectra of Purified FAD-Bound OhyA

References

1. Joo, Y.C., Jeong, K.W., Yeom, S.J., Kim Y.S., Kim, Y., and Oh, D.K. (2012) Biochemical characterization and FAD-binding analysis of oleate hydratase from *Macrococcus caseolyticus,* Biochimie 94, 907-915.
2. Englender, M., Pavkov-Keller, T., Emmerstorfer, A., Hromic, A., Schrempf, S., Steinkellner, G., Wriessnegger, T., Leitner, E., Strohmeier, G.A., Kaluzna, I., Mink, D., Schürmann, M., Wallner, S., Macheroux, P., Gruber, K., and Pichler, H. (2015) Structure-Based Mechanism of Oleate Hydratase from *Elizabethkingia meningoseptica*, ChemBioChem 1730-1734.

New Research Based Laboratory Course in Biochemistry

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Recently, granting institutions such as the NSF have placed an emphasis in involving undergraduates to participate in research while pursuing their degree in a STEM discipline. Since spaces in research laboratories are often limited for undergraduates, developing research based courses as part of the undergraduate curriculum has become increasingly crucial. To address these needs in Georgia State University's Department of Chemistry, a course based on mutagenesis was designed. The lab is based on students designing mutants of research faculty existing protein targets. Students learn about the specific protein and the rationale behind the mutation. Each student carefully decides which mutation to attempt. Next, students will design primers and optimize the reaction. Ideally, these mutants can be passed on to be used in a participating research laboratory for further investigation.

Biochemical Characterization of *Amycolatopsis Alba* AlbA: A Flavin Dependent N-hydroxylating Monooxygenase

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Iron is essential for growth for many microbes, including several pathogenic bacteria and fungi. In order to scavenge iron from the host, many microbes synthetize and secrete iron binding molecules known as siderophores (1). The biosynthesis of siderophores that contain hydroxamate moieties is initiated by the action of N-hydroxylating monooxygenases (NMOs). These enzymes are flavin-dependent monooxygenases that have been shown to be essential for virulence for the opportunistic pathogens *Aspergillus fumigatus* and *Pseudomonas aeruginosa* (2). The purpose of this study was to characterize the recombinant form of a NMO from *Amycolatopsis Alba* (AlbA). AlbA is closely related to the NMOs from *A. fumigatus* and *P. aeruginosa*. We present the expression, purification, and partial characterization of this enzyme. The protein expresses in high levels in *E. coli* and was isolated using immobilized metal-affinity chromatography. The protein contains tightly bound FAD and is capable of hydroxylating ornithine in the presence of NADPH. AlbA is highly specific for its substrate and the binding of a similar substrate results in uncoupling. Results from oxygen consumption assays demonstrate that AlbA exhibits typical Michaelis-Menten kinetics with a k_{cat} value of $0.79 \pm 0.04 s^{-1}$ and a K_m value of 0.18 ± 0.04 mM for ornithine. For its cofactor, NADPH, a k_{cat} value of 0.46 ± 0.02 s^{-1} and a K_m value of 0.67 ± 0.12 mM were obtained. We are currently in the process of identifying crystallization conditions in order to solve the structure of AlbA. The results from our work will lead to a better understanding of the mechanism of action of NMOs. This information might help in drug discovery.

1. Fischbach, M.A., Lin, H., Liu, D.R., and Walsh, C.T. (2006) How pathogenic bacteria evade mammalian sabotage in the battle for iron. *Nat. Chem. Biol.* 2, 132-138.
2. Takase, H., Nitanai, H., Hoshino, K., and Otani, T. (2000) Impact of siderophore production on *Pseudomonas aeruginosa* infections in immunosuppressed mice. *Infect. Immun.* 68, 1834-1839.

Promiscuity of Bicupin Oxalate Oxidase

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Isothermal titration calorimetry (ITC) may be used to determine the kinetic parameters of enzyme-catalyzed reactions when neither products nor reactants are spectrophotometrically visible and when the reaction products are unknown. We report here the use of the multiple injection method of ITC to characterize the catalytic properties of oxalate oxidase from *Ceriporiopsis subvermispora* (CsOxOx), a manganese dependent enzyme that catalyzes the oxygen-dependent oxidation of oxalate to carbon dioxide in a reaction coupled with the formation of hydrogen peroxide. CsOxOx is the first bicupin enzyme identified that catalyzes this reaction. The multiple injection ITC method of measuring OxOx activity involves continuous, real-time detection of the amount of heat generated (dQ) during catalysis, which is equal to the number of moles of product produced times the enthalpy of the reaction (dH_{app}). Steady-state kinetic constants using oxalate as the substrate determined by multiple injection ITC are comparable to those obtained by a continuous spectrophotometric assay in which H_2O_2 production is coupled to the horseradish peroxidase-catalyzed oxidation of ABTS and by membrane inlet mass spectrometry. Additionally, we used multiple injection ITC to identify mesoxalate as a substrate for the CsOxOx-catalyzed reaction, with a kinetic parameters comparable to that of oxalate, and to identify a number of small molecule carboxylic acid compounds that also serve as substrates for the enzyme.

Novel α-(N)-heterocyclic thiosemicarbazones and their inhibition activity on human Topoisomerase IIα

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Anticancer research is one of the fastest growing fields in medical research and pharmacy. Topoisomerase IIα (TopoIIα) is a common target for chemotherapeutic drugs. It is required for tension release in supercoiled DNA and essential for proliferative eukaryotic cells. We are interested in thiosemicabazone and their ability to inhibit TopoIIα Our research uses *in vitro*assaysto analyze α-(N)-Heterocyclic thiosemicarbazone compounds inhibition on TopoIIα. Recent studies showed that Cu(II) acetylpyridine ethylthiosemicarbazone(Cu(APY-ETSC)Cl)is a noncompetitive inhibitor of TopoIIα. We examined benzoylpyridine, acteylthiazole, 2-acetyl-4-methylthiazole thiosemicabazones with various side chain substitutions and with ligand or metal complexes to analyze their inhibition on TopoIIα. Our results showed that almost all Cu(II) and Pd(II) thiosemicarbazone compounds inhibited TopoIIα-mediated relaxation. However, ligand thiosemicabazones without metal chelation do not inhibit TopoIIα. Selected metal thiosemicarbazone complexes indicated that the inhibition is dose-dependent. We also have preliminary data for two novel α-(N)-Heterocyclic thiosemicarbazone, MOX, and BZOX. Both metal MOX and BZOX thiosemicabazone complexes inhibited TopoIIα-mediated DNA relaxation and increase TopoIIα-mediated DNA cleavage.

FMN Reductase of Alkanesulfonate Monooxygenase System: Role of π-Helix and Flavin-Induced Oligomeric Switch

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Protein evolution confers enzymes with new functionalities in response to environmental pressures. The alkanesulfonate monooxygenase system enzymes are expressed during sulfur starvation in various bacteria to catalyze the desulfonation of a diverse range of alkanesulfonates (1). The mechanism of desulfonation is dependent on a reduced flavin transfer event from the FMN reductase (SsuE) to the monooxygenase (SsuD) enzyme. Many of the enzymes that comprise two-component systems utilize flavin as a substrate, but it is not clear what structural properties of the FMN reductase dictate flavin transfer. The SsuE enzyme undergoes an oligomeric switch from a tetramer to a dimer in the presence of FMN. It is unclear how changes in the oligomeric state regulate flavin reductase activity. A conserved π-helix is located at the tetramer interface of SsuE characterized by the insertion of Tyr118 in the α4-helix (2). Other FMN reductases in the flavodoxin superfamily that were part of a two-component system were also shown to possess a π-helix at the oligomeric interface. The π-helices often confer a gain of function, but the purpose of the π-helix in these enzymes has not been elucidated.

Several variants were constructed, in order to evaluate the functional role of the SsuE π-helix in flavin transfer and catalysis. Interestingly, a single residue substitution of Tyr118 to Ala (Y118A SsuE) or Ser (Y118S SsuE) transformed the apoflavin oxidoreductases into FMN-bound variants that were converted from a tetrameric to a dimeric oligomeric state. Mass spectrometric analysis of the flavin extracted from Y118A SsuE gave a mass of 457.11 similar to the FMN cofactor, suggesting the SsuE variant had retained flavin specificity. The variants containing the FMN-bound cofactor were reduced with one equivalent of NADPH in anaerobic titration experiments, but were not able to support continued flavin reduction. In addition, there was no detectable sulfite product in coupled assays with the Y118A and Y118S SsuE variants and SsuD, demonstrating that flavin transfer is no longer supported. The Y118F, Y118W, ΔY118 SsuE variants were flavin-free and existed as tetramers similar to wild-type SsuE, but desulfonation activity was only observed with the Y118F SsuE in coupled assays. These findings provide fundamental insights on the evolutionary role of the Tyr118 insertion resulting in the π-helix in SsuE. Additionally, the identification of a flavin-bound FMN-dependent reductase through a single residue mutation highlights the importance of the π-helix in maintaining the correct oligomeric state and effective flavin transfer.

1. Eichhorn, E., van der Ploeg, J. R., and Leisinger, T. (1999) Characterization of a two-component alkanesulfonate monooxygenase from Escherichia coli. *J. Biol. Chem.* 274, 26639-26646.
2. Driggers, C. M., Dayal, P. V., Ellis, H. R., and Karplus, P. A. (2014) Crystal structure of Escherichia coli SsuE: defining a general catalytic cycle for FMN reductases of the flavodoxin-like superfamily. *Biochem.* 53, 3509-3519.

Effect of osmotic stress on enzymes of the folate pathway

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Abstract:

Folate (Vitamin B9) is involved in one carbon transfer reactions required for the synthesis of important macromolecules such as DNA and amino acids. Since humans cannot synthesize folate, malfunctioning of the enzymes involved in folate metabolism can lead to various diseases like megaloblastic anemia, birth defects, increased risk of cardiovascular disease and cancer. In addition, enzymes in the folate biosynthesis pathway are potential drug targets in bacteria. Our current understanding of the folate pathway is mostly based on in vitro studies, which are very different from the crowded environment that exists in the cell. E. coli is known to produce osmoprotectants during times of osmotic stress. This leads to perturbation of water activity inside the cell, and an increase in macromolecular crowding. We have shown earlier that, in vitro, osmolytes weaken the binding of dihydrofolate to dihydrofolate reductase of the folate pathway. We hypothesize that an increased osmolyte concentration in the cell will also prevent the functioning of other folate pathway enzymes by interaction of osmolytes with the various folate redox states. Here we studied the effect of osmolytes on the enzymes methylenetetrahydrofolate reductase (metF) and serine hydroxymethyltransferase (glyA) required for methionine and glycine synthesis respectively in E. coli and also dihydropteroate synthase (folP) responsible for biosynthesis of dihydrofolate. Studies were done with knockout and rescued strains for the metF, glyA and folP genes. The knockout mutants were restored to prototrophy by addition of folate end products while the rescued strains contain a pKTS plasmid containing the knocked out gene under tetracycline control. The gene is fused with a sequence encoding a SsrA degradation tag to limit the amounts of protein produced in the cell. Osmotic stress studies for metF and glyA indicated that the rescued strain was unable to grow in higher osmolality conditions when compared to knockout strains. We predict this is due to an increase in osmolyte concentration in vivo which leads to interaction of osmolytes with folate intermediates in the pathway. This is turn decreases the efficiency of the folate pathway enzyme. These osmotic stress effects have also be observed in dihydropteroate synthase and we are currently working on understanding the effect of osmolytes on enzyme kinetics in vitro

Identifying Effective Quenchers to Eliminate the Substrate-Cofactor Interference in the Scintillation Proximity Assay of Histone Acetylation

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Histone acetyltransferases (HATs) mediate the transfer of an acetyl group from the cofactor, acetyl-CoA, to the ε- amino group of specific lysines in diverse protein substrates, most notably nuclear histones. The deregulation of HATs is connected to a number of disease states. Reliable and rapid biochemical assays for HATs are critical for understanding biological functions of protein acetylation, as well as for screening small-molecule inhibitors of HAT enzymes. In this report, we present a scintillation proximity assay for the measurement of HAT enzymatic activities. The acetyl donor was [^{3}H]Ac-CoA and a biotin-modified histone peptide served as the HAT substrate. Following the HAT reaction, streptavidin-coated beads were added to induce proximity of acetylated substrate to the scintillant molecules. However, we observed strong nonspecific binding between the cofactor and the histone peptide substrates, which adversely influenced the SPA performance. To prevent this problem, a set of chemical agents were evaluated to eliminate the cofactor-substrate interaction, thus providing reliable SPA readings. With optimization, the SPA showed consistent and robust performance for HAT activity measurement and HAT inhibitor evaluation. Overall, this mix-and-measure assay does not require any washing procedure, can be utilized in the microplate format, and is well suited for high-throughput screening of HAT chemical modulators.

A Computational Investigation of Dinoflagellate Bioluminescence: Evidence for an Unprecedented Biological Twisted Intra-molecular Charge Transfer Reaction

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Ubiquitous in the world's oceans, dinoflagellates are capable of fantastic displays of bright blue bioluminescence. This luminosity is a consequence of the oxidation of an open-chain tetrapyrrole, dinoflagellate luciferin, by the enzyme dinoflagellate luciferase. While many other bioluminescence systems have been thoroughly investigated (i.e., firefly, jellyfish, and bacterial), the mechanism of dinoflagellate bioluminescence remains enigmatic. A comprehensive time-dependent long-range corrected density functional theory (TDLCDFT) investigation was used to evaluate several competing reaction mechanisms of dinoflagellate luciferase catalysis employing distinct excited state luminophores. The results provide strong evidence in favor of a mechanism of dinoflagellate bioluminescence involving a biologically unprecedented twisted intramolecular charge transfer (TICT) reaction.

Insights on the Role of Ser/Ala Switch in Amine Oxidation in *Pseudomonas aeruginosa* D-Arginine Dehydrogenase

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D-Arginine dehydrogenase is a flavin-dependent enzyme from *Pseudomonas aeruginosa*, an opportunistic human pathogen.[1] *P. aeruginosa* D-arginine dehydrogenase (PaDADH) catalyzes the oxidative deamination of D-amino acids to their corresponding imino acids, which are hydrolyzed in solution to α-keto-acids and ammonia.[2] All D-amino acids except aspartate, glutamate and glycine are substrates for PaDADH, with the highest rate constants for substrate capture, k_{cat}/K_m, observed with D-arginine and D-lysine as substrates.[2] PaDADH participates in the racemization of arginine with L-arginine dehydrogenase in *P. aeruginosa*.[3] The active site residues S45 and A46, located at the *si* face of the flavin ring, adopt two alternate conformations corresponding to the ligand-free and product-bound conformations. In the ligand-free conformation, the side chain of A46 points away from the FAD and the hydroxyl group of S45 forms a hydrogen bond with the hydroxyl group of the C2' atom of the flavin ribityl group. In the product bound conformation, the side chain of S45 points away from the FAD ring and the side chain of A46 approaches closer to the FAD. Thus, S45 and A46 were hypothesized to play an important role in binding and catalysis in PaDADH.

In this study, site-directed mutagenesis was used to mutate S45 and A46 to alanine and glycine, respectively. pH profiles, rapid reaction kinetics and computational approaches were employed to investigate the roles of these residues in binding, catalysis, as well as their effect on the active site structural integrity. S45A and A46G variants showed a decrease of less than 10-fold in the k_{cat}/K_m value with D-arginine and k_{cat} was increased by ~2-fold compared to the wild-type enzyme. With the small substrate D-leucine, the k_{cat}/K_m values decreased by ~200-fold, whereas the k_{cat} value could not be determined due to increased K_m values. In agreement with these results, the reductive half-reaction of S45A and A46G variants with D-leucine as a substrate at pH 10.0 indicated that the rate of flavin reduction, k_{red}, could not be determined. The k_{red}/K_d values in both variants were ~150-fold lower compared to the wild-type enzyme.[4] In the S45A and A46G variants, the active site lid stays more in the open conformation as suggested by the computational studies. These data showed that S45 and A46 participate in the optimal orientation of the substrate α-amine group in PaDADH into a catalytically productive position.

Support: NSF CHE-1506518 (G.G.)

References:

1. Fu, G., et al. (2010). Conformational changes and substrate recognition in *Pseudomonas aeruginosa* D-arginine dehydrogenase, Biochemistry 49, 8535-8545.
2. Yuan, H., et al. (2010) Steady-state kinetic mechanism and reductive half-reaction of D-arginine dehydrogenase from *Pseudomonas aeruginosa*, *Biochemistry 49*, 9542-9550.
3. Li, C. and C. D. Lu. (2009) Arginine racemization by coupled catabolic and anabolic dehydrogenases, *Proc. Natl. Acad. Sci. U S A 106*, 906-911.
4. Yuan, H., et al. (2011) Insights on the mechanism of amine oxidation catalyzed by D-arginine dehydrogenase through pH and kinetic isotope effects, *J. Am. Chem. Soc. 133*, 18957-18965.

An Investigation into the Potential Dual Role of SirC in the Alternative Heme Biosynthetic Pathway of *Methanosarcina acetivorans* C2A

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The methanogenic archaeon, *Methanosarcina acetivorans* C2A, utilizes tetrapyrroles such as heme, factor III, and coenzyme F430 for energy production. Each of these tetrapyrroles contain different metal ions (iron, cobalt, and nickel, respectively), which are generally thought to be inserted into sirohydrochlorin, a common biosynthetic intermediate of each pathway, by a unique chelatase. Interestingly, the genome of *M. acetivorans* C2A encodes only two class II chelatase (CbiX) homologs, and one precorrin 2 dehydrogenase (SirC). SirC is likely responsible for the production of sirohydrochlorin for each of the aforementioned tetrapyrrole biosynthetic pathways, and is homologous to the N-terminal dehydrogenase/ferrochelatase domain of the trifunctional enzyme siroheme synthase (CysG), which converts uroporphyrinogen III to siroheme. Since SirC is found within the alternative heme biosynthetic (*ahb*) gene cluster, which does not contain a chelatase homolog, it is possible that SirC is responsible for both the formation and ferrochelation of sirohydrochlorin in *M. acetivorans* C2A. Here, the ability of SirC to carry our both of these reactions is examined and the implications on the biosynthesis of tetrapyrroles in *M. acetivorans* C2A is discussed.

Ground-state Destabilization by Phe-448 and Phe-449 in Tyrosine Phenol-lyase Catalysis

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The role of transition state stabilization in enzyme catalysis, as proposed by Pauling, has been clearly demonstrated by extensive studies. In contrast, ground-state destabilization can also contribute to enzyme catalysis, but experimental evidence has been more limited. In recent years, high resolution x-ray crystal structures of enzyme-substrate complexes have been obtained which show evidence for ground-state strain. We found that Y71F and F448H mutant tyrosine phenol-lyase (TPL) form complexes with 3-fluoro-L-tyrosine, a substrate, which shows a bending of the substrate aromatic ring about 20º out of plane, and we suggested that this was evidence for ground-state destabilization in TPL catalysis (1). We have now performed additional experiments to evaluate the role of ground-state strain in TPL catalysis. Phe-448 and Phe-449 are in close contact with the substrate side chain in the active site of TPL, and thus we have now mutated Phe-448 and Phe-449 to alanine and leucine. F448A, F448L and F449A TPL have activity for elimination of phenol from L-tyrosine reduced by a factor of 10^4, 10^3 and 10^4, respectively, but they have near normal activity with alternate substrates, S-(*o*-nitrophenyl)-L-cysteine and S-ethyl-L-cysteine. F448A TPL forms quinonoid intermediates from L-tyrosine and S-ethyl-L-cysteine with rate constants similar to wild-type TPL. In addition, F448A TPL can form an aminoacrylate intermediate from S-ethyl-L-cysteine with a rate constant similar to wild-type TPL. We also examined the effect of hydrostatic pressure on the rates and equilibria of formation of the quinonoid intermediates from F448H and F448A TPL and 3-fluoro-L-tyrosine. Although the fastest phase shows only a small effect of pressure, the three slower phases have significant pressure dependences, suggesting that they may be associated with a conformational change. These results demonstrate that Phe-448 and Phe-449 contribute a total of about 10^8 to catalysis in TPL, about 50% of the estimated rate acceleration, by introducing ground-state destabilization into the L-tyrosine substrate.

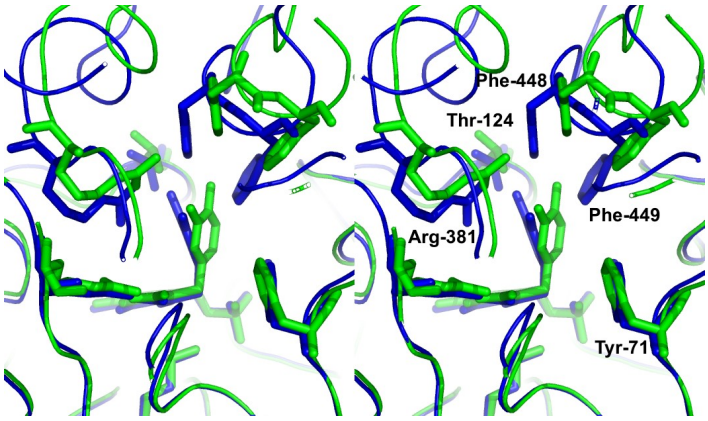

Figure 1. Crossed-eye stereo view overlay of relaxed (green) structure of Y71F TPL and tense (blue) structure of F448H TPL (blue) complexed with 3-fluoro-L-tyrosine.

1. Dalibor, M., Demidkina, T. V., Faleev, N. G., Phillips, R. S., Matkovic-Calogovic, D. and Antson, A. A. (2011) *J. Am. Chem. Soc. 133*, 16468-16476.

Enzymatic Characters of Apoptosis Signal-Regulating Kinase I

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Apoptosis signal-regulating kinase I (ASK1) is a mitogen-activated kinase kinase kinase (MAP3K) that actives the downstream MAP kinase kinases (MKKs) from c-Jun N-terminal kinase (JNK) and p38 cascades. The activity of ASK1 is precisely regulated in response to various stimulations, and has pivotal roles in a wide variety of cellular responses, including apoptosis, differentiation and inflammation. The dysfunctional regulation of ASK1 causes various diseases such as: cancer, diabetes, Parkinson's disease, and Alzheimer's disease. The essential physiological function of ASK1 has garnered extensive attentions. However, there are still some serious gaps in our understanding of the molecular mechanisms of ASK1 activation and catalytic features. The lack of purified functional ASK1 protein has prevented us from elucidating the mechanistic insights of ASK1-mediated signal transduction. Here, we report a simple and efficient method for the expression and purification of wild-type ASK1 in *Escherichia coli*. The purified ASK1 demonstrates self-phosphorylation activities. The kinase activities of self-phosphorylated ASK1 (pASK1) were also evaluated on two MKKs, MKK4 and 7, from JNK cascades. MKK7 can be effectively activated by pASK1, which in turn phosphorylates the downstream JNK3. We also found that pASK1 alone cannot effectively activate MKK4 as reported, even though the unexpected phosphotyrosine signal was detected on MKK4. Interestingly, robust MKK4 activation catalyzed by pASK1 was observed in the presence of MKK7. The kinetic analysis also showed that MKK7 is a much better substrate of pASK1 than MKK4.

BACTERIAL KERATINASE: A TOOL FOR DEVELOPMENT OF FEEDSTUFF FROM POULTRY WASTE (CHICKEN FEATHER)

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Key words: biodegradation, waste management, waste to wealth, green chemistry, feather degradation, *Bacillus subtilis*, keratinase

Abstract

Worldwide, several million tons of feathers are generated annually as waste by poultry-processing industries. However, because of the insoluble nature of keratin and its resistance to enzymatic digestion by animal, plant and many known microbial keratinases; use of feather as a source of value-added products has been very limited. Present day, hydrothermal treatment which is used to make keratin wastes more digestible, are expensive and destroy certain amino acids, yielding a product with poor digestibility and variable nutrient quality. As such the large amount of feather produced and its localised accumulation around poultry-processing sites create a serious disposal problem leading to environmental pollution.

North-eastern India is considered as one of the mega biodiversity zones of the world and by screening the soil samples of this region, we have isolated a potent chicken feather degrading bacterial strain capable of secreting significant amount of alkaline keratinase in the culture medium at 50ºC. By polyphasic taxonomy approach, the isolate was identified as *Bacillus subtilis* RM-01. Production of keratinolytic enzyme by this isolate was monitored during cultivation of the bacterium on raw chicken-feathers. Maximum growth and feather-degrading activity were observed at 45-50°C pH 8.0 post 96h of incubation. It was observed that feather degradation led to an increase in free amino acids such as alanine, leucine, valine and isoleucine in feather -hydrolyzate. Moreover, methionine and phenylalanine were also produced as microbial metabolites. Development of enzymatic and/or microbiological methods for the hydrolysis of feather to soluble proteins and amino acids for feed formulation is extremely attractive, as it offers a cheap and eco-friendly technology for the production of valuable products from waste materials.

Oligomerization-Dependent Changes to the Heme Pocket of Globin Coupled Sensors

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Globin coupled sensors (GCS) are sensory proteins used by bacteria to determine the surrounding gaseous environment. The function of a GCS is determined by the output domain of the GCS, which include phosphodiesterases, kinases, and diguanylate cyclases (DGC). Diguanylate cyclase domains produce cyclic dimeric guanosine monophosphate (c-di-GMP) from guanosine triphosphate (GTP). C-di-GMP is a bacterial secondary messenger and a major regulator of biofilm formation. *Pectobacterium carotovorum* ssp. *carotovorum* and *Bordetella pertussis* both contain GCS proteins (*Pcc*GCS and *Bpe*GReg, respectively) with DGC output domains. Previous works has shown that oxygen binding in the globin domain regulates the output domain, but the signaling mechanism and structure of GCSs are not well characterized. Isolated globin domains from *Pcc*GCS (*Pcc*Globin) and *Bpe*GReg (*Bpe*Globin) have been characterized to assist in elucidating these gaps. The oligomeric state of *Pcc*Globin is dimeric while *Bpe*Globin is monomeric, indicating potential oligomer binding sites in the globin domain. As full length *Pcc*GCS and *Bpe*GReg exist as different oligomeric states (dimer-tetramer-octamer and monomer-dimer-tetramer, respectively), the globin domain appears to be a major determinant of oligomerization. The globin truncations also revealed altered oxygen dissociation kinetics, as compared to *Pcc*GCS and *Bpe*GReg. Furthermore, dimerization of the globin domain has been shown to correlates with biphasic dissociation kinetics. Site-directed mutagenesis has been used to interrogate the relative roles of distal pocket hydrogen-bonding residues in stabilizing bound O_2 and contributing to each dissociation rate.

New Enzymes with New Flavin Chemistry: Flavin-Mediated Aromatic Decarboxylation in a Two-Enzyme System

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Flavins, including flavin mononucleotide (FMN), are fundamental catalytic cofactors responsible for the redox functionality of diverse set of proteins. In general, these flavoenzymes have been shown to catalyze redox and oxygenation reactions almost exclusively. Alternatively, modified flavin analogues are rarely found in nature as their incorporation typically results in inactivation of flavoproteins, thus leading to the disruption of important cellular pathways. Recently however, a new class of flavoenzymes have been identified in the ubiquinone (coenzyme Q) biosynthesis pathway, in which a prenylated FMN (prFMN) is required to catalyze the reversible decarboxylation of an aromatic substrate: this previous study details the characterization of a aromatic decarboxylase (FDC1) which is dependent on a separate flavin prenyltransferase (UBIX) to provide prenylated flavin for activity (1,2). Here, we report the cloning, expression, and purification of a putative flavin prenyltransferase (HMFG) and carboxylase (HMFF) from *Cupriavidus basilensis* (3). The existence of this new class of flavoenzymes opens the door to a new field of flavin chemistry, one in which flavins perform decarboxylations as opposed to the traditional roles as redox catalysts. A proper understanding of the mechanisms governing these reactions will serve in the development of new biocatalysts capable of diverse functionality for industrial applications.

1. Payne KAP, et al. New cofactor supports α,β-unsaturated acid decarboxylation via 1,3-dipolar cycloaddition. *Nature* **2015,** *522* (7557), 497-501.

2. White MD, et al. UbiX is a flavin prenyltransferase required for bacterial ubiquinone biosynthesis. *Nature* **2015,** *522* (7557), 502-506.

3. Koopman, F, et al. Identification and characterization of the furfural and 5-(hydroxymethyl)furfural degradation pathways of Cupriavidus basilensis HMF14.

Targeting Iron acquisition in *Aspergillus fumigatus*: Inhibition of Siderophore biosynthesis by Celastrol

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Aspergillus fumigatus is an opportunistic fungal pathogen and the most common causative agent of fatal invasive mycoses and it is responsible for 90 % of systemic invasive aspergillosis. With treatment using currently available drugs, mortality rates averages 26-65% for invasive aspergillosis (1). *A. fumigatus* is capable of growing in environments with low iron concentrations (e.g. human serum), because of its ability to produced Fe-chelating agents called siderophores (2). *A. fumigatus* produce several hydroxamate containing siderophores with ferrichoricin and N′,N′′,N′′′N-triacetylfusarinine C (TAFC) being the most abundant. The hydroxamate containing siderophores biosynthetic pathway is initiated by the action of the flavin-dependent monooxygenase Siderophore A (SidA). SidA catalyzes the NADPH and oxygen dependent hydroxylation of L-ornithine to N5- L-hydroxyornithine. *A. fumigatus* strains with a deletion of the SidA gene are unable of establishing infection (3), suggesting that SidA is an attractive drug target. Previously, our group reported the synthesis of a fluorescence probe (ADP-TAMRA) for high throughput screening (HTS) of inhibitors of AfSidA (4) as well the crystals structure of the SidA with ornithine and $NADP^+$ (5). In this work we present the results of the HTS of a 2320 compound library using the probe ADP-TAMRA to identify inhibitors against SidA. Celastrol, a natural quinone methide extracted from *Tripterygium wilfordii*, was identified as a non competitive inhibitor of AfSidA with a K_i value of 3 μM. In cell-based assay the presence of celastrol causes a significant *A. fumigatus* growth inhibition in minimum media with no Fe supplemented (MM-Fe) and a complete growth inhibition in blood agar after 24 h of incubation (MIC=2 μM). Addition of Fe(III)-TAFC to MM-Fe and blood agar recovered the growth to the level of the control. This confirms that celastrol is inhibiting the siderophore biosynthesis by binding to SidA.

1. Patterson, T. F., Kirkpatrick, W. R., White, M., Hiemenz, J. W., Wingard, J. R., Dupont, B., Rinaldi, M. G., Stevens, D. A., and Graybill, J. R. (2000) Invasive Aspergillosis Disease Spectrum, Treatment Practices, and Outcomes. *Medicine 79*, 250-260.
2. Hissen, A. H. T., Chow, J. M. T., Pinto, L. J., and Moore, M. M. (2004) Survival of *Aspergillus fumigatus* in Serum Involves Removal of Iron from Transferrin: the Role of Siderophores. *Infection and Immunity 72*, 1402-1408.
3. Hissen, A. H. T., Wan, A. N. C., Warwas, M. L., Pinto, L. J., and Moore, M. M. (2005) The *Aspergillus fumigatus* Siderophore Biosynthetic Gene sidA, Encoding l-Ornithine N5-Oxygenase, Is Required for Virulence. *Infection and Immunity 73*, 5493-5503.
4. Qi, J., Kizjakina, K., Robinson, R., Tolani, K., and Sobrado, P. (2012) A fluorescence polarization binding assay to identify inhibitors of flavin-dependent monooxygenases. *Analytical Biochemistry 425*, 80-87.
5. Franceschini, S., Fedkenheuer, M., Vogelaar, N. J., Robinson, H. H., Sobrado, P., and Mattevi, A. (2012) Structural Insight into the Mechanism of Oxygen Activation and Substrate Selectivity of Flavin-Dependent N-Hydroxylating Monooxygenases. *Biochemistry 51*, 7043-7045

Structure and analysis of the human Mgat2 catalytic domain, a key enzyme in the initiation of complex type N-glycan synthesis on animal glycoproteins

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The biosynthesis of N-glycan structures in animal cells is initiated in the endoplasmic reticulum (ER) with the co-translational transfer of an oligomannose glycans from lipid-linked precursors to nascent polypeptide chains. Maturation of the glycan structures occurs during transit through the secretory pathway by trimming to a Man5GlcNAc2-Asn structure prior to the addition of a single GlcNAc residue on the α1,3-linked core mannose residue by Mgat1. Further trimming by Golgi a-mannosidase II leads to the formation of a GlcNAcMan3GlcNAc2-Asn glycan that is a critical transition structure between oligomannose and mono-antennary hybrid N-glycans versus the branched and extended complex type glycan structures found on cell surface and secreted glycoproteins. Initiation of complex type glycan structures requires the addition of a single β1,2-GlcNAc linkage to the core α1,6-linked mannose residue by mannosyl(α-1,6-)-glycoprotein β1,2-N-acetylglucosaminyltransferase (Mgat2). This structure is then further branched and extended with terminal glycan capping modifications to create the diverse collection of complex type N-glycan structures in animal systems. A mouse gene disruption in Mgat2 results in small size pups and perinatal lethality with numerous dysmorphic features. Similar phenotypic effects were observed in human patients with carbohydrate-deficient glycoprotein syndrome (CDG) type IIa characterized by defects in Mgat2 function. Conditional mouse knockouts that target neural tissues later in life led to a normal phenotype indicating a critical role for Mgat2 early in development.

Acceptor specificity studies previously indicated that Mgat2 requires the prior action of Mgat1 to produce the minimal GlcNAcβ(1,2)Manα(1,3)(Manα(1,6))Manβ1,4-R structure necessary for substrate recognition and catalysis. To identify the structural basis for Mgat2 action, a recombinant form of the human Mgat2 catalytic domain was produced in suspension culture HEK293S cells as a secreted fusion protein. The recombinant product was purified and the glycans were trimmed by endoglycosidase F. The crystal structure of apo-Mgat2 was solved with SAD phasing using a uranium derivative. The addition of uranium to the crystals induced a space group change from $P2_1$ to $P4_32_12$ which benefited phasing by increasing data redundancy. The binary Mgat2:UDP:Mn^{2+} and tertiary Mgat2:UDP:Mn^{2+}:GlcNAcMan3GlcNAc2 complexes were solved by molecular replacement with apo- Mgat2 as a search model. The addition of uranium to the crystals induced a space group change from $P2_1$ to $P4_32_12$ which benefited phasing by increasing data redundancy. The structures display a GT-A fold comprised of a single extended Rossmann domain with one side harboring the UDP-GlcNAc:Mn^{2+} donor binding site and an adjacent portion of the domain containing the GlcNAcMan3GlcNAc2 acceptor binding site. Interactions of the UDP:Mn^{2+} donor analog was facilitated by coordination of the Mn^{2+} ion with Asp233 in the enzyme-associated DXD motif and an adjacent His346 residue. The charge on the pyrophosphate in the UDP-GlcNAc donor is stabilized by the bound Mn^{2+}. An analysis of the Mgat2:UDP:Mn2+ and Mgat2:UDP:Mn^{2+}:GlcNAc2-Man3-GlcNAc structures details the conformational changes that accompany the binding of the acceptor moiety. This poster will present the first structure of hMgat2 and discuss the structural basis for substrate specificity, the proposed catalytic mechanism for GlcNAc addition, and the characteristics of inactivating CDG type IIa mutations (supported by NIH grants P41GM103390 and P01GM107012).

The Biosynthesis and Biological Activity of Obafluorin, a novel β-lactone Antibiotic

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Strained heterocycles have fascinated chemists for decades due to their synthetic challenges, unique reactivity, and biological relevance. Starting with the discovery of the penicillins in the mid-20th century, β-lactam antibiotics have been the cornerstone of the fight against bacterial pathogens for more than 75 years; however, the rise of resistant bacteria have created a dire need for new antibiotic scaffolds and novel cellular targets to combat this problem[1]. A similar class to the β-lactams, β-lactones share the strained heterocyclic scaffold, but unlike the β-lactams, the biological properties and biosynthesis of these molecules is relatively unknown. Obafluorin (Obi) is a secondary metabolite, β-lactone produced by the soil pathogen *Pseudomonas fluorescens* ATCC 39502. The work highlighted here elucidates the annotated biosynthetic gene cluster of Obi, by the *in vitro* reconstitution and functional characterization of five key biosynthetic enzymes (ObiD,F,G,H,L), enabling the cell free, biocatalytic total synthesis of obi from the readily available starting materials of benzoic acids, phenylpyrvates, and amino acids. We also show that the key ring-closing step of the biosynthesis is mediated by the NRPS ObiF, showing the first enzyme dependent cyclization of a β-lactone. Through growth monitoring assays we demonstrate the dose dependent biological activity of obi is both β-lactone dependent and bacteriostatic. Microscope studies with obi shows a unique "cell-clumping" phenotype in pathogenic *E. coli*, but the unique cellular mechanism of obi is the subject of ongoing research.

1. Walsh, C. T. and Wencewicz, T. A. (2014) Prospects for new antibiotics: a molecule-centered perspective, *J. Antibiot.* **67**, 7-22.

Computational Discovery of Novel p300 Histone Acetyltransferase Inhibitors

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Histone acetyltransferases (HATs) regulate gene expression by acetylating the lysine tails of histones. Dysregulation of HATs often leads to disease states including various types of cancer. While the p300 HAT has been studied extensively with regard to mechanism (1) and structure (2), efforts to discover and develop novel p300-inhibitors is challenging due to the low selectivity or high reactivity of many drug candidates. P300 inhibitors are useful for disease treatment and can also be used to study p300's role in transcriptional regulation. We have discovered several potential p300 inhibitors through the use of virtual screening. These inhibitors could be beneficial to future studies of p300 activity. Docking, molecular dynamics and Molecular Mechanics Poisson Boltzmann Surface Area (MM-PBSA) calculations suggest a binding mode whereby the p300 L1 Loop contracts over the active site to form favorable contacts with the inhibitor candidates. MM-PBSA calculations reveal that this loop contraction stabilizes ligand binding by approximately 7 kcal/mol, likely due in part to Gln1455 reorienting to form a hydrogen bond with the ligand.

1. Liu, Xin, Ling Wang, Kehao Zhao, Paul R. Thompson, Yousang Hwang, Ronen Marmorstein, and Philip A. Cole. "The Structural Basis of Protein Acetylation by the P300/CBP Transcriptional Coactivator. " Nature 451.7180(2008): 846-50.
2. Manzo, Fabio, Francesco Paolo Tambaro, Antonello Mai, and Lucia Altucci. "Histone Acetyltransferase Inhibitors and Preclinical Studies." Expert Opinion on Therapeutic Patents 19.6 (2009): 761-74
3. Manzo, Fabio, Francesco Paolo Tambaro, Antonello Mai, and Lucia Altucci. "Histone Acetyltransferase Inhibitors and Preclinical Studies." Expert Opinion on Therapeutic Patents 19.6 (2009): 761-74

Bioluminescence and Formation of the Lower Axial Ligand of Adenosylcobalamin in *Acidiphilium multivorum*

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Acidiphilium multivorum is a rod-shaped gram-negative bacterium found in extreme acidic environments. It is a member of the group of aerobic anoxygenic photosynthetic bacteria and has eight naturally occurring plasmids, the largest of which contains genes homologous to the *lux* operon of *Vibrio fischeri.* The *lux* operon encodes the enzymes necessary for the oxygen-dependent production of light via the bacterial bioluminescence system. The large plasmid of *A. multivorum* also contains a nearly complete adenosylcobalamin (coenzyme B_{12}) biosynthetic gene cluster. Included in this cluster is a gene encoding a putative multifunctional CobC-BluB enzyme. In other organisms, CobC has been shown to function as a pyridoxal phosphate (PLP)-dependent L-threonine 3-*O*-phosphate decarboxylase, while the 'flavin destructase' BluB is responsible for the formation of 5,6-dimethylbenzimidazole (DMB) from reduced flavin mononucleotide ($FMNH_2$). Thus, this unusual multifunctional enzyme is predicted to catalyze non-sequential steps in the adenosylcobalamin biosynthetic pathway. Current work is focused on determining whether the CobC-BluB enzyme catalyzes both of the predicted reactions, if the LuxAB homolog is a functional bacterial luciferase, and if the flavin reductase (LuxG) of the bioluminescence system is able to efficiently supply $FMNH_2$ for the BluB reaction.

Photochemical/spectroscopic Studies in Turbid and Scattering Media

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The talk is dedicated to the scientific research in business environment. The research at OLIS involves the development and utilization of various state or the art optical spectrometers. This privately-held American company continues to serve the biophysical, biochemical, and bioenergetics fields with absorbance, fluorescence, and circular dichroism spectroscopy for sophisticated research and routine measurements. Our newest product line – the CLARiTY series – has eliminated the previously insurmountable barrier to accurate absorbance spectroscopy of live, whole, particulate, aggregated, turbid samples. Few sample cases will be discussed. They involve steady-state and time-resolved measurements of redox reaction in cell suspension, photoswitching of MOFs, monitoring vitamin A delivery & uptake in intact frog eye retina, and many more. It is obvious that this analytical technique offers a new and powerful approach to examine the extents and rates of biological events in situ without disrupting the complexity of the live cellular environment. Finally, an active CLARiTY spectrophotometer will be demonstrated and the audience will have to chance to take the absorbance spectra of their own samples. Bring your own turbid sample!

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Establishing the Relationship Between Function and Dynamics Within the Gated Mechanism of D-Arginine Dehydrogenase

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Enzymes are ubiquitous in biological systems. They catalyze chemical reactions and are involved in many biochemical processes. Molecular dynamics is a statistical mechanics approach that considers the change in position of each and every atom in a system over time. The enzyme of interest is D-Arginine dehydrogenase (DADH). This enzyme is composed of approximately 375 residues and a proposed catalytic site with broad substrate specificity (1). A water recognition motif, observed in roughly 1200 non-redundant protein structures (2), was revealed within the secondary properties of DADH. This motif coincides with the conformational changes of the enzyme's gated mechanism proposed by Fu et al. (1). Single point mutations were undertaken to further understand the dynamics of this gated mechanism in catalysis.

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1. Fu, G., et al. (2010) Conformational Changes and Substrate Recognition in *Pseudomonas Aeruginosa* D-Arginine Dehydrogenase, *Biochemistry* 49, 8535-545.
2. Barman, A., et al. (2016) Conserved Hydration Sites in Pin1 Reveal a Distinctive Water Recognition Motif in Proteins, *J. Chem. Inf. Model* 56.1, 139-47.

A unique substrate-assisted mechanism of inhibition of *Escherichia coli* dihydrofolate reductase by a novel deoxybenzoin, ononetin

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Dihydrofolate reductase, DHFR, (E.C.1.5.1.3) is a critical enzyme involved in the biosynthesis of thymidine and purine nucleotides. Most reported inhibitors for DHFR are structural analogues of substrate dihydrofolate, competitively displacing the latter[1, 2]. Analogues of NADPH, on the other hand, could target the cofactor binding site in diverse enzymes making them unsuitable as inhibitors. This necessitates discovering novel scaffolds that not only overcome the excessive reliance on analogs of folate but should also inhibit the enzyme by binding to unique sites in the enzyme. In a previous study, employing our novel pocket-based VLS tool PoLi, we had identified ononetin as a unique scaffold binding to *Escherichia coli* DHFR[3]. The current study, employing the tool of detailed inhibition kinetics, shows that this small molecule inhibits the enzyme. Further, the study demonstrates that ononetin shows a unique uncompetitive mechanism of inhibition against both dihydrofolate and NADPH indicating that the small-molecules preferentially bind to either the cofactor-bound or the substrate-bound binary form of the enzyme. Furthermore, the substrate *vs* velocity plots show substantial deviation from their hyperbolic shape with marked similarity to substrate inhibition-like behavior possibly indicative of substrate-inhibitor complex forming an unproductive complex with the enzyme. The K_i values seem to be synergistic with both substrate and cofactor showing potent inhibition at high substrate/cofactor concentrations. This indicates non-overlapping binding vis-à-vis both substrate and cofactor and a unique substrate-assisted inhibition mechanism. This study is the first report on deoxybenzoin, ononetin, as a novel scaffold that inhibits *E. coli* DHFR inhibition and, to the best of our knowledge, the report of an inhibitor that neither binds to the substrate or cofactor binding sites.

1. Srinivasan, B., and Skolnick, J. (2015) Insights into the slow-onset tight-binding inhibition of Escherichia coli dihydrofolate reductase: detailed mechanistic characterization of pyrrolo [3,2-f] quinazoline-1,3-diamine and its derivatives as novel tight-binding inhibitors, *The FEBS journal 282*, 1922-1938.
2. Srinivasan, B., Tonddast-Navaei, S., and Skolnick, J. (2015) Ligand binding studies, preliminary structure-activity relationship and detailed mechanistic characterization of 1-phenyl-6,6-dimethyl-1,3,5-triazine-2,4-diamine derivatives as inhibitors of Escherichia coli dihydrofolate reductase, *European journal of medicinal chemistry 103*, 600-614.
3. Roy, A., Srinivasan, B., and Skolnick, J. (2015) PoLi: A Virtual Screening Pipeline Based on Template Pocket and Ligand Similarity, *Journal of chemical information and modeling 55*, 1757-1770.

Exploring Possible Metabolic Mechanisms of Bacterial CDO

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Putative cysteine dioxygenase homologs have been identified in various bacteria. The amino acid sequence identity of bacterial CDO and mammalian CDO is less than 30%, and the Cys residue that plays a role in crosslink formation in mammalian CDO is substituted with a Gly residue. Bacterial CDO are divided into two types known as "Arg-type" and "Gln-type", depending on the identity of a conserved active site residue. The "Arg-type" resembles mammalian CDO by having a conserved arginine residue that is proposed to be involved in stabilization of the cysteine substrate carboxylate group.[1] However, the arginine is substituted with glutamine in the "Gln-type" and shows an apparent specificity for 3-mercaptopropionate.[1] Although 3 mercaptopropionate is the proposed substrate, the environmental prevalence of 3-mercaptopropionate is unclear.

Mammalian CDO was modified to resemble the bacterial enzyme by substituting Cys93 with Gly (C93G CDO), and *Bacillus subtilis* (*Bs*CDO) was used to investigate the mechanistic properties of the "Arg-type" bacterial CDO. The amount of iron bound to C93G CDO was 100%, while *Bs*CDO had 70% iron bound. Results from circular dichroism studies showed there were no significant alterations in the secondary structure of C93G CDO and *Bs*CDO compared to wild-type. There was a 400-fold decrease in the k_{cat}/K_m value for C93G CDO compared to wild type CDO when measuring dioxygen utilization, but *Bs*CDO demonstrated only nominal activity with the cysteine substrate. Due to the limited activity of *Bs*CDO with cysteine, cysteamine was also evaluated as a potential substrate for the enzyme. Similar to the results obtained with cysteine, there was no significant activity observed for *Bs*CDO with cysteamine. These studies suggest that L-cysteine or cysteamine is not the ideal substrate for the "Arg-type" bacterial CDO as was previously reported. Therefore, the functional role of CDO in *Bacillus subtilis* has not been sufficiently elucidated.

1. Fellner Matthias, Aloi Sekotilani, Tchesnokov E.P, Wilbank S.M, Jameson N.L, (2016) Substrate and pH-Dependent Kinetic Profile of 3-Mercaptopropionate Dioxygenase from *Pseudomonas aeruginosa*. Biochemistry 55: 1362-1371.

Mechanistic and Spectroscopic Study of Bacterial Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1

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Propionate 3-nitronate (P3N) is a natural toxin that irreversibly inhibits mitochondrial succinate dehydrogenase. P3N poisoning leads to a variety of neurological disorders and even death at sufficiently high concentrations (1). Up to date, no antidote has been discovered for P3N poisoning. Nitronate monooxygenase from *Pseudomonas aeruginosa* PAO1 (PaNMO) was the first NMO identified and characterized in bacteria, which was shown to oxidize P3N to malonic semialdehyde for energy production with FMN as cofactor (2).

In this study, a mechanistic characterization of PaNMO was carried out using steady-state kinetics, pH effects, and UV-visible and time-resolved absorption spectroscopy to probe the species that are relevant for catalysis. Varied relative amounts of neutral and anionic flavin semiquinones were observed after anaerobic reduction of PaNMO at different pH values, displaying a pK_a value of 6.3 for semiquinone ionization in the enzyme active site. The presence of both flavin semiquinones was detected also during enzymatic turnover with P3N using a stopped-flow spectrophotometer equipped with a photodiode array detector. The pH profile of the k_{cat}/K_{P3N} value with P3N as a substrate reveals that protonation of two ionizable groups of pK_a value of 10.0 is required for efficient substrate binding, while the k_{cat} value remains pH-independent from pH 6.0 to 11.0. pH titration of the UV-visible absorption spectrum of oxidized PaNMO shows a pK_a value of 9.5 for a tyrosine. Given that four out of five total tyrosines in PaNMO are located in the active site, this indicates one tyrosine in the active site may be responsible for the pK_a observed in the steady-state kinetics.

1. Hipkin, C. R., Simpson, D. J., Wainwright, S. J., Salem, M. A. (2004) Nitrification by plants that also fix nitrogen, *Nature* 430, 98–101.

2. Salvi, F., Angniswamy, J., Yuan, H. L., Vervammen, K., Pelicaen, R., Cornelis, P., Spain, J. C., Weber, I. T., Gadda, G. (2014) The combined structural and kinetic characterization of a bacterial nitronate monooxygenase from *Pseudomonas aeruginosa* PAO1 establishes NMO class I and II, *J Biol Chem 289, 23764-75.*

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Auto-reduction Studies of Shr-NEAT2 Domain in *Streptococcus pyogenes*

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Streptococcus pyogenes is a Gram-positive pathogenic bacterium. It belongs to group A β-hemolytic streptococcus (GAS) bacteria. This bacterium is responsible for a wide range of infections and diseases including streptococcal pharyngitis, rheumatic heart disease, impetigo, and many more diseases. For growth, GAS obtains iron from host proteins that contain heme, e.g., hemoglobin. *S. pyogenes* utilizes a direct uptake system where a donor transfers the heme to proteins expressed on the surface of the cell. Shr is one protein expressed in this system and contains two NEAT domains, Shr-N1 and Shr-N2 [1]. A combination of UV-visible, Raman, and magnetic circular dichroism spectroscopies indicate that the protein is Shr-N2 is hexacoordinate with two axial methionines. Sequence alignment and homology modeling indicate that M26 and M136 are the axial ligands. Shr-N2 is isolated as a mixture of the Fe(II) and Fe(III) forms of the protein. It is prone to autoreduction, autoreducing as the pH increases and autoxidizing as the pH decreases. Lysines are the most likely source of electrons in heme autoreduction. I-TASSER modeling shows that each of the proposed axial methionine ligands has a nearby lysine. Therefore, we have mutated each of these lysines to alanines. The proteins have been expressed with a Strep-tag and purified on a Streptactin column.

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1. Ouattara, M., Cunha, E.B., Li, X., Huang, Y.S., Dixon, D.W., Eichenbaum, Z., 2010. Shr of Group A streptococcus is a new type of composite NEAT protein involved in sequestering haem from methaemoglobin. Mol. Microbiol. 78, 739-756.

Glucosaminate 6-phosphate ammonia lyase from Salmonella typhimurium: A novel Pyridoxal-5'-phosphate dependent enzyme

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Salmonella is a foodborne pathogen that affects many mammals, particularly humans; however, it has few treatments other than time, and the few treatments may be ineffective and prolong symptoms. However, S. typhimurium shows a dependence on rare substrates such as D-glucosaminic acid for efficient colonization of the gut. For this reason, the structure and metabolism of enzymes in S. typhimurium, specifically D-glucosaminate PTS permease components EIIE (D-glucosaminic acid 6-phosphate dehydratase - DgaE), is under study in order to further understand alternative treatments to Salmonella. We have expressed and purified DgaE, and determined its properties. The structure of DgaE is proposed to be a dimer from gel filtration results. DgaE activity is easily followed by a spectrophotometric assay including 2-keto-3-deoxygluconate aldolase (DgaF) and lactate dehydrogenase, with NADH. We have screened for possible inhibitors of DgaE. We have found that amino acids such as D-serine, phosphate, and potassium ion are inhibitors of DgaE. We have obtained crystals of DgaE that diffract to 3 A and have collected a complete data set. A better understanding of the structure and metabolic factors for DgaE may open new medicinal approaches to dealing with Salmonella.

1. Miller, Katherine A., Robert S. Phillips, Jan Mrázek, and Timothy R. Hoover. "Salmonella utilizes D-glucosaminate via a mannose family phosphotransferase system permease and associated enzymes." Journal of bacteriology 195, no. 18 (2013): 4057-4066.

Combined Mutagenesis and Kinetic Characterization of Bacterial Nitronate Monooxygenase from *Pseudomonas aeruginosa* PAO1

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Nitronate monooxygenases (NMO; E.C. 1.13.12.16) are flavin-dependent enzymes that oxidize nitronates to aldehydes, nitrite and nitrate using molecular oxygen as co-substrate. The physiological substrate of these enzymes, propionate 3-nitronate (P3N), is a highly toxic nitro-compound that irreversibly inhibits an important enzyme in Krebs cycle, succinate dehydrogenase. The crystal structure of NMO from *Pseudomonas aeruginosa* PAO1 shows an (βα) 8-barrel fold.[1] This fold consists of a closed eight-stranded parallel β-sheets forming a central barrel, surrounded by eight α-helices. The internal core of parallel β-sheets is arranged in three layers. Each layer consists of four side chains from alternating β-strands part of the central barrel. In the (βα) 8-barrel fold, active site residues are commonly located at the end of the barrel associated with the C-terminal ends of β-strands and the loops connecting these to α-helices.[2]

Substrate interactions in PaNMO were investigated using flexible molecular docking studies with AutoDock Vina. The conserved residues Tyr109, His183, Tyr299, Tyr303 and Trp325 were identified as important for maintaining structure and activity in the PaNMO. These residues are located at the end of the barrel associated with the C-terminal ends of β-strands. To further probe the role of Tyr109, Tyr299, and Tyr303, these positions were replaced with phenylalanine through site-directed mutagenesis. Kinetic and spectroscopic investigations are currently undergoing to elucidate the roles of these tyrosine residues in the active site of the enzyme, with particular focus on whether they contribute to substrate specificity in the reaction catalyzed by PaNMO.

Keywords: Mutagenesis, FMN, Nitronate Monooxygenase

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1. Agniswamy J, Yuan H, Vercammen K, Pelicaen R, Cornelis P, Spain JC, Weber IT, Gadda G. The combined structural and kinetic characterization of a bacterial nitronate monooxygenase from *Pseudomonas aeruginosa* PAO1 establishes NMO class I and II. *J. Biol. Chem.* **2014** 22;289 (34): 23764-75.

2. Höcker B, Jürgens C, Wilmanns M, Sterner R. Stability, catalytic versatility and evolution of the (βα) 8-barrel fold. *Curr. Opin. Biotechnol.* **2001**, 12; 376–381.

Highly resistant HIV-1 proteases and strategies for their inhibition

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The virally encoded protease is an important drug target for AIDS therapy. Despite the potency of current drugs, infections with resistant viral strains limit the long-term effectiveness of therapy. Highly resistant variants of HIV protease from clinical isolates have different combinations of about 20 mutations and several orders of magnitude worse binding affinity for clinical inhibitors. Strategies are being explored to inhibit these highly resistant mutants. The existing inhibitors can be modified by introducing groups with the potential to form new interactions with conserved protease residues, and the flexible flaps. Alternative strategies are discussed, including designing inhibitors to bind to the open conformation of the protease dimer, and inhibition of the protease-catalyzed processing of the Gag-Pol precursor.

1. Weber, I. T., Kneller, D. W., and Wong-Sam, A. (2015) Highly resistant HIV-1 proteases and strategies for their inhibition, *Future Med. Chem. 7(8)*, 1023-1038.

Anaerobic aromatic degradation mechanisms of benzylsuccinate synthase and m-hydroxybenzoate synthase in *Geobacter daltonii* strain FRC-32

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Fe(III) and U(VI)-Reducing bacterium *Geobacter daltonii* strain FRC-32 is capable of oxidizing carcinogenic petroleum hydrocarbons anaerobically using a host of unique enzymatic mechanisms. Annotation of the *G. daltonii* genome revealed many duplicates and homologs of known genes from various anaerobic aromatic degradation pathways. One of these genes, *mbs*, encodes for m-hydroxybenzylsuccinyl-CoA synthase, while a homologous gene, *bss*, encodes for the radical forming, toluene activating enzyme benzylsuccinate synthase. Our findings suggest that while *bss* is controlled by a single promotor, *mbs* utilizes a unique promoter for control of the beta subunit, which appears to be the standard organization across other *bss*-utilizing microorganisms. The active site of Bss, Mbs and other Bss family proteins is highly conserved, and predictive 3D-models of both Mbs and Bss demonstrated conservation of the tertiary structure of the substrate binding pocket. Similar models of the activating subunit of both enzymes elucidate the presence of SAM sites on each, as well as an extra lobe when compared to other Bss-utilizing microorganisms. We hypothesize that Mbs is utilized during benzene degradation while Bss is utilized during toluene degradation, and that the the SAM site utilized by MbsD has the potential to methylate benzene to toluene for continued oxidation via the toluene degradation pathway. The findings from this study demonstrates *G. daltonii*'s unique ability to degrade these carcinogenic and highly stable compounds, and will contribute to new strategies of oil spill bioremediation otherwise unfeasible in anaerobic environments.

Integrative modeling of pre-initiation complex (PIC) assembles at the core promoter

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In eukaryotic transcription initiation, a large multi-subunit pre-initiation complex (PIC) assembles at the core promoter that is required for the opening of the duplex DNA and identification of the start site for transcription by RNA polymerase II. We have used integrative modeling by combining cryo-EM to determine near-atomic resolution structures of the human PIC in a closed state (engaged with duplex DNA), an open state (engaged with a transcription bubble), and an initially transcribing complex (containing six base pairs of DNA-RNA hybrid). Comparison of the different structures has revealed the sequential conformational changes that accompany the transitions from one state to the other throughout the transcription initiation process. This analysis illustrates the key role of TFIIB in transcription bubble stabilization and provides strong structural support for a translocase activity of XPB.

1. Boeger, H., Bushnell, D.A., Davis, R., Griesenbeck, J., Lorch, Y., Strattan, J.S., Westover, K.D., Kornberg, R.D. (2005) Structural basis of eukaryotic gene transcription, *FEBS Lett. 579, 899-903.*

Exploring the Effects of Histone H4 Lysine Modifications on Arginine-3 Methylation

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It is estimated that there are at least 160 different histone modifications. Understanding their interactions and the effect thereof on gene transcription and cellular activity are of great value and yet to be realized. Lysine and arginine modifications are two important post-translational modifications of histone proteins, and their interaction accounts for a crucial part of this so-called "histone code". Here we examine the how the lysine modifications on histone H4 1-20 peptide affect the methylation of arginine-3 using two different protein arginine methyltransferases PRMT1 and PRMT5. Our studies found that lysine-5 acetylation decreased the methylation of arginine-3 by PRMT1 but increased that of PRMT5. This result shields light on the complex interplay of histone modifications and provides new insights into the distinctive characteristics of PRMT1 and PRMT5.

Sulfur transfer reactions involving *B. subtilis* ThiI and their role in tRNA modification

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4-Thiouridine (s^4U) is a conserved tRNA modification throughout bacteria and archaea. It serves as a near-UV sensor and protects the cell from environmental stress. In *Escherichia coli* and other Gram-negative bacteria, the master cysteine desulfurase IscS is the initial enzyme to mobilize the sulfur from the amino acid cysteine and to subsequently transfer it to ThiI via persulfide enzyme intermediate (1,2). The site of sulfur transfer within ThiI is located at its C-terminal rhodanese domain and it serves a sulfur intermediate in the biosynthesis of s^4U and thiamin. In addition, ThiI contains a THUMP/PP-loop ATPase domain involved in tRNA binding and adenylation of C4 of U8 of tRNA, a step required prior tRNA thiolation (3, 4).

In a previous study, we demonstrated that despite the lack of the essential rhodanese domain, the *Bacillus subtilis* ThiI is able to recruit a specific cysteine desulfurase NifZ and complete the biosynthesis of s^4U using an alternate site of sulfur transfer (5). In this study, a survey of bacterial and archaeal genomes showed that most ThiI sequences lacking the rhodanese domains contain a double cysteine motif near the C-terminus, and in the chromosomal location these truncated *thiI* genes tend to be adjacent to cysteine desulfurase genes. To gain further insight into the alternate mechanism of sulfur transfer in s^4U biosynthesis, we determined the effect of single cysteine to alanine substitutions within ThiI, and showed that only the last Cys of the double CC motif (C345) is essential for the *in vivo* activity of ThiI-NiZ enzyme pair. Our current efforts are geared towards understanding the sequence of mechanistic events associated with the final step of thiolation and the possible occurrence of a ternary complex involving tRNA-ThiI-NifZ.

1. Lauhon, C. T. (2002) Requirement for IscS in Biosynthesis of All Thionucleosides in *Escherichia coli*, *J. Bacteriology. 184*, 24, 6820-6829.
2. Kambampati, R., and Lauhon, C. T. (1999) IscS Is a Sulfurtransferase for the *in vitro* Biosynthesis of 4-thiouridine in *Escherichia coli* tRNA, *Biochem. 38*, 16561-16568.
3. You, D., Xu, T., Yao, F., Zhou, X., and Deng, Z. (2008) Direct Evidence that ThiI is an ATP Pyrophosphatase for the Adenylation of Uridine in 4-Thiouridine Biosynthesis. *ChemBioChem. 9*, 1879-1882.
4. Mueller, E. G., Palenchar, P. M., and Buck, C. J. (2001) The Role of the Cysteine Residues of ThiI in the Generation of 4-Thiopuridine in tRNA. *J. Biol Chem. 276*, 36, 33588-33595.
5. Rajakovich, L. J., Tomlinson, J., and Dos Santos, C. P. (2012) Fuctional Analysis of *Bacillus subtilis* Genes Involved in the Biosynthesis of 4-Thiouridine in tRNA. *J. Bacteriology. 194*, 18, 4933-4940.

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