Keynote & Faculty Speaker Biographies:

Theodore Wensel, Ph.D.
Department of Biochemistry & Molecular Biology, Baylor College of Medicine

Dr. Wensel received his Ph.D. in chemistry from the University of California, Davis. He then moved on to Stanford University as a post-doctoral fellow where he studied with Lubert Stryer. Currently, Dr. Wensel is professor of biochemistry & molecular biology at the Baylor College of Medicine, where his research focuses on signal transduction of G-protein coupled receptors in the retina and brain. Recent work in his laboratory has yielded considerable insight into the role that Regulator of G-Protein Signaling (RGS) proteins play in visual signal transduction. Using a vast array of techniques including X-ray crystallography, membrane biochemistry, and whole animal genetic manipulation, he aims to characterize the molecular mechanisms that are crucial for the proper regulation of GPCR signaling in the central nervous system.

Katrin Karbstein, Ph.D.
Departments of Chemistry & Chemical Biology, University of Michigan

Dr. Karbstein received her Ph.D. from Stanford University then moved on to the University of California, Berkeley where she was a Damon Runyon post-doctoral fellow. Dr. Karbstein joined the University of Michigan chemistry and chemical biology faculty in 2006. Her research group studies the complex biological process of eukaryotic ribosome assembly at the molecular level using multiple approaches including biochemistry, mechanistic enzymology, protein engineering and yeast genetics

Janet Smith, Ph.D.
Department of Biological Chemistry, University of Michigan

Dr. Smith received her Ph.D. from the University of Wisconsin, Madison and was a post-doctoral fellow at the Naval Research Laboratory. Currently she is a Professor of Biological Chemistry and the Margaret J. Hunter Collegiate Professor of Life Sciences at the University of Michigan. Her research is focused on studying protein structure and function using X-ray crystallography. Current projects include the study of glutamine amidotransferases and the replication machinery of alphaviruses and flaviviruses.

Student Poster Abstracts:

Development of a Time Resolved FRET High-Throughput Assay to Identify Inhibitors of the RGS4/Gαo Interaction

Levi L. Blazer, David L. Roman, Richard R. Neubig.

Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109

Regulator of G-protein Signaling (RGS) proteins are important regulatory molecules in the transduction of G-Protein Coupled Receptor (GPCR) signaling. By increasing the rate of intrinsic GTPase activity of the G alpha subunit, RGS proteins effectively shorten the duration and decrease the magnitude of signals from heterotrimeric G protein subunits. It has been proposed (Nat. Rev. Drug Disc. 1:187, 2002) that small molecule inhibitors of RGS proteins may provide a novel mechanism for therapeutic intervention in diseases that stem from deficiencies in GPCR signaling. To this end, we developed a high throughput time-resolved fluorescence resonance energy transfer (TR-FRET) assay to quantify the interaction between RGS4 and Gαo. The assay utilizes a lanthanide-chelate donor covalently attached to Gαo and an AlexaFluor-488 acceptor attached to RGS4. The Kd for binding in the presence of GDP:AlF4- is 35±4 nM with much lower affinity with GDP alone. Z' values of 0.50-0.88 were obtained in 384-well plate measurements. High throughput chemical screens using this approach are underway. Supported by NIH R01-GM39561 & T32-GM008597 (Chemical Biology Interface Training Program).

Design of Small Molecules for Contacting Protein Surfaces and Activating Transcription

Sara J. Buhrlage, Anna K. Mapp

Department of Chemistry, University of Michigan, Ann Arbor, Michigan, 48109-1055

Protein-protein interactions regulate diverse and complex biological processes such as transcription and apoptosis; it is thus not surprising that the misregulation of protein assembly is associated with a wide range of consequences including cancer, diabetes, and Alzherimer's disease. The ability to facilitate or inhibit protein-protein interactions with small molecules shows tremendous promise in the development of therapeutics for associated diseases. A class of protein surfaces that remains particularly difficult to recognize is transcriptional co-activator proteins. Co-activator proteins serve as a bridge between DNA bound transcription factors and components of the RNA polymerase holoenzyme and thus play an integral role in transcriptional activation. Small molecules that can contact the surfaces of co-activators have the potential to serves as transcriptional activators or inhibitors. Small molecule heterocyclic isoxazolidines have been designed to mimic general characteristics of peptides that contact co-activators. In a cell based system, the designed small molecules activate transcription in a dose dependent manner with a maximum activation of 80 fold at a concentration of 1 μM. Additionally, using NMR spectroscopy the interaction between isoxazolidines and a common transcriptional co-activator, the CBP KIX domain, has been characterized. These ligands target a site commonly bound by natural activators. The dissociation constant for this interaction was measured to by 30 μM; natural ligands for the CBP KIX domain also exhibit low micromolar dissociation constants. The ability to bind co-activator proteins and activate transcription with a small molecule the fraction of the size of natural activators represents a significant advance in the field of molecular recognition and a significant step towards the development of transcription based therapeutics.

Cooperative Binding by Jun-ATF2 and IRF3 at the PRDIV-III Recognition Element Alters Jun-ATF2 DNA-Binding Orientation

Veronica Burns[b] and Tom Kerppola[a,b]

[a]Howard Hughes Medical Institute and [b]Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109-0650

Regulation of the immune and inflammatory responses requires the expression of specific sets of genes, which is mediated by multi-protein complexes called enhanceosomes. Expression of beta interferon (IFN-β), which is secreted from cells in response to virus infection, requires the assembly of an enhanceosome that spans four positive regulatory domains (PRDs) within the promoter region. At the core of this enhanceosome are a heterodimer composed of Jun and ATF2 (Jun-ATF2) and interferon regulatory factor 3 (IRF3) that bind to PRDIV and PRDIII, respectively. Although Jun-ATF2 can bind to DNA in either one of two opposite orientations, only one orientation has been shown to correlate with more potent transcription activation from the IFN-β promoter in cells[1]. However, experimental studies using photo-crosslinking[1] and X-ray crystallography[2] have produced contradictory results regarding the orientation of Jun-ATF2 in functionally active Jun-ATF2-IRF3-DNA complexes. We have, therefore, sought to determine the orientation of Jun-ATF2 bound to PRDIV-III using a gel-based FRET assay. It was found that Jun-ATF2 is only slightly oriented at PRDIV, with Jun occupying the half-site proximal to PRDIII more often than ATF2. In complexes containing IRF3, however, Jun-ATF2 has the opposite orientation preference with ATF2 occupying the half-site proximal to PRDIII. Interestingly, manipulation of the intrinsic orientation preference of Jun-ATF2 at PRDIV did not affect the ability of IRF3 to alter the preferred orientation of Jun-ATF2. Experiments aimed at examining the mechanism of Jun-ATF2-IRF3 cooperativity as well as the effects of other IRF family proteins on the preferred orientation of Jun-ATF2 are currently underway.

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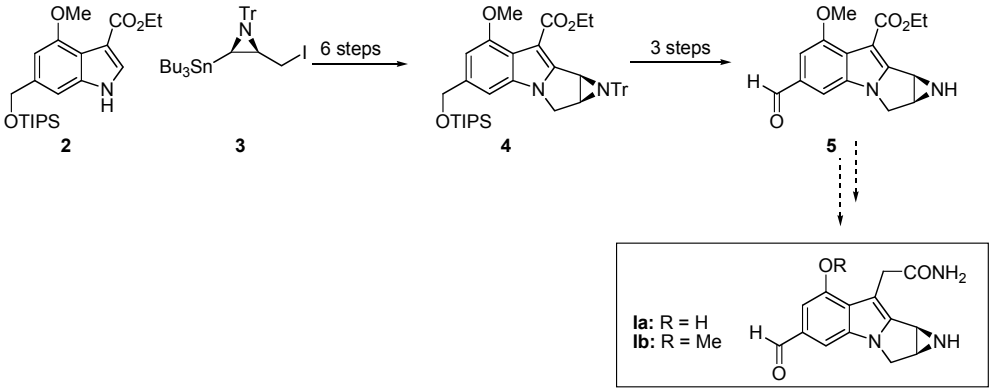
Synthetic Studies Toward the Aziridinomitosene of FK317

Susan Deeter, Edwin Vedejs, and Musong Kim

Department of Chemistry, University of Michigan

A synthetic approach to **1a**, proposed by Fukuyama to be the active form of FR900482, and **1b**, the proposed active form of semi-synthetic derivative FK317, will be described.[1,2] A key step is the early installation of the labile aziridine through coupling of **2** and **3**.[3] Subsequent tin-lithium exchange and intramolecular Michael addition provides tetracycle **4**. The difficult removal of the *N*-trityl group is accomplished without loss of the labile aziridine functionality to provide advanced intermediate **5**. Studies toward the completion of the synthesis will be discussed.



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FRET and CE assays for the detection of glucosamine-6-phosphate by the ligand-dependent glmS ribozyme

Jennifer R.W. Furchak[a], Colin Jennings[a], Peilin Yang[a],
Nils G. Walter[a], and Robert T. Kennedy[a,b]

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[b]*Department of Pharmacology, University of Michigan, Ann Arbor, Michigan, 48109-0632*

Biosensor development is an active and timely area of research at the interface of chemistry and biology. Molecular recognition of an analyte by a ribozyme has promise for use in a biosensor, where ligand binding transduces into catalytic cleavage of a fluorophore labeled external (rechargeable) substrate. We have developed glucosamine-6-phosphate (GlcN6P) sensitive assays that detect substrate cleavage by the naturally occurring bacterial *glmS* ribozyme via either fluorescence resonance energy transfer (FRET) or capillary electrophoresis with laser induced fluorescence (CE-LIF). We achieve a dynamic range extending from 0.5 to 500 μM GlcN6P when turning over a single substrate. Signal amplification and increased sensitivity are achieved under multiple-turnover reaction conditions. Our assays demonstrate the potential of ribozymes, in conjunction with FRET and CE-LIF detection, to serve as recognition elements for biosensors of small molecules.

Human Histone Deacetylase 8 Catalyzes Deacetylation using a Metalloprotease-like Mechanism

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Histone deacetylase 8 (HDAC8) is a member of the metal-dependent HDAC family, which catalyzes deacetylation of acetylated lysines to regulate transcription and other cellular processes. Based on crystallographic data, a general acid-base catalytic pair (H143/H142) mechanism has been proposed for HDAC8.[1] To test this hypothesis, we probed the catalytic mechanism of recombinant human HDAC8 using mutagenesis, pH dependence, and Co(II)-spectroscopy. The k_{cat}/K_M pH profile is bell-shaped with pK_a values of 7.7 and 9.1, indicating that two ionizable groups play a role in activity. Furthermore, the mutations H142A, H143A or Y306F decrease the catalytic activity by 220-fold, >10,000-fold, or 140-fold, respectively. These results suggest that H143 functions as both the general base and the general acid, while the positively charged H142 assists Y306 in stabilizing the tetrahedral intermediate in a mechanism that resembles a typical metalloprotease mechanism.

Additionally, two monovalent cations are observed in the HDAC8 crystal structure, one of which is near the active site. We have partially elucidated the function of these ions by demonstrating that one K^+ site activates catalytic activity, while the second inhibits activity. Furthermore, the inhibitory effect disappears in the H142A mutant suggesting that the inhibitory K^+ binds near the active site, leading to the deprotonation of H142. Thus, inhibition by K^+ provides further evidence to support the role of H142 as an electrostatic catalyst rather than as the general base.

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Incorporation of Fluorous Amino Acids into Antimicrobial Peptides as a Means for Modulating Antibacterial Activity

L. Gottler, A. Ramamoorthy. E. N. G. Marsh

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MSI-78 is an antimicrobial peptide derived from the naturally occurring Magainin 2 peptide. The peptide undergoes a structural transition from random coil to α-helix upon association with a lipid membrane[1]. The peptide has also been shown to form a homodimeric unit when associated with membranes[2]. These observations have lead to the hypothesis that the structure of the peptide is very important for its mechanism of antimicrobial activity. We have demonstrated that introducing highly fluorinated amino acids into peptides results in more structured and stable molecules[3]. We believe that by introducing highly fluorinated amino acid residues into MSI-78 that the antimicrobial activity of the molecule can be modulated to increase the potency and selectivity towards bacteria. Partial proteolysis experiments and circular dichroism studies suggest that the fluorinated analog of MSI-78 is α-helical and structurally more stable than the non-fluorinated peptide. In addition, the fluorinated peptide has shown as good or better antimicrobial activity against a variety of bacterial strains.

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Synthesis and Evaluation of Oligoisoxazolidines as Proteomimetics and Transcriptional Activators

Matt Leathen and Anna Mapp

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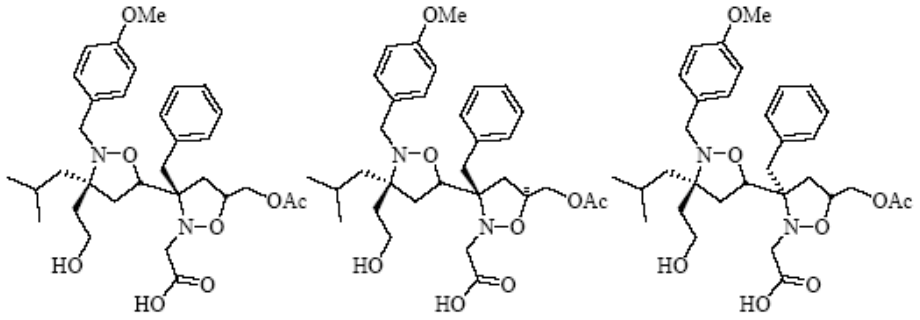
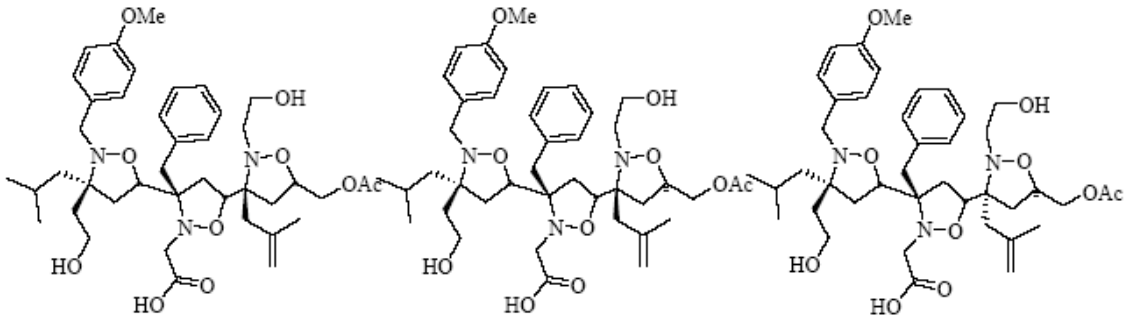
Protein function in virtually all biological processes requires a well-organized 3- dimensional structure. The development of artificial counterparts to proteins, proteomimetics, containing abiological building blocks that form similar 3-dimensional structures as proteins is a challenging goal. Isoxazolidines and bis-isoxazolidines radially display attached groups in 3-dimensional space with little dependence on functional group content. This project seeks to recapitulate the higher order structure of natural systems based on oligomers of the rigid five membered ring of isoxazolidines. A collection of bis- and tri-isoxazolidines are being synthesized for structural studies in order to better understand their conformational preferences and limitations. The synthesis uses a diastereoselective 1,3-dipolar cycloaddition of an oxime and allylic alcohol to build the heterocyclic scaffold, followed by a diastereoselective nucleophilic addition and N-alkylation. An alcohol side chain can then be converted to another oxime and the process is repeated to produce oligoisoxazolidines. The progress towards and synthesis of these molecules is presented. These compounds are also being evaluated as transcriptional activation domains. Initial results from a cell based transcription assay show a preference for compounds containing both hydrophobic and polar functionality.

Bis-Isoxazolidines



Tris-isoxazolidines



Buhrlage, S. J.; Brennan, B. B.; Minter, A. R.; Mapp, A. K. *J. Am. Chem. Soc*. **2005**, *127*, 12456-12457.

Investigations into Amyloid-β Oligomerization: a Single Molecule Approach

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[a]Department of Biological Chemistry, [b]Biophysics Research Division, and [c]Department of Physics, University of Michigan, Ann Arbor, Michigan 48109-105

The deposition of intra- and extra-cellular amyloid is a hallmark of amyloidogenic diseases including Alzheimer's disease (AD), Parkinson's disease, type 2 diabetes, etc. However, recent evidence suggests that an oligomeric form of the amyloidogenic proteins, consisting of a few monomers may be the cause of cell degradation and death, rather than the fibrillar form (1,2). Consequently, identifying the toxic oligomeric species has become a primary focus in this research area. The ability to identify the toxic species has in the past been limited by the lack of existing techniques with the resolution and capability of determining the number of monomers that comprise an early oligomeric species within a heterogeneous mixture. We are developing single molecule spectroscopy using photobleaching and associated techniques to elucidate the dynamics of the oligomeric states of fluorescently labeled amyloid-beta (Aβ) as a model system. Understanding the aggregation process that creates these pore-forming intermediates opens the possibility of finding a way to prevent Aβ aggregates from developing and in treating Alzheimer's disease. We present evidence that single molecule spectroscopy is a valid approach to answering fundamental questions that have been particularly challenging to researchers studying AD as well as other amyloid diseases.

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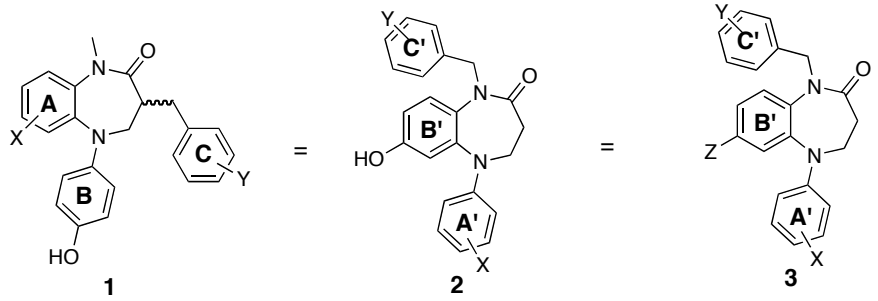
Molecular Recognition of Small Molecules by the Mitochondrial F_1F_0-ATPase

Gina M Ney[a], Joanne Cleary[a], Anthony Opipari[b], and Gary D Glick[a]

[a]*Department of Chemistry, University of Michigan, Ann Arbor, Michigan, 48109-1055*
[b]*Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, 48109-1055*

The mitochondrial F_1F_0-ATPase (Complex V) is a multi-subunit enzyme that plays a critical role in cellular metabolism. Consequently, mechanisms to control and/or help better understand this enzyme are of extreme interest. A series of benzodiazepines have been shown to be inhibitors of both ATP synthesis and hydrolysis through binding dependent on the oligomycin sensitivity conferring protein (OSCP) [1]. However, little structural and mechanistic information is currently known about the role of the OSCP in Complex V activity. Therefore, these molecules represent a tool able to further investigate both the structure and function of the OSCP.

Further structure activity studies were undertaken in an attempt to distill the minimal amount of structural information required to act in an ATPase inhibitory manner. Upon analysis, the phenol moiety required in **1** was not necessary for enzyme inhibition in the orientation of molecules such as **3**. While the hydroxyl group is optimal for Z, other substituents such as Cl, F, OCH_3, and H still demonstrate enzyme inhibition. The smaller, achiral compounds **2** and **3** represent a new set of molecules that are currently being used to better understand enzyme inhibition. The structural findings are currently being used to predict structural biases between selective hydrolysis inhibitors and hydrolysis/synthesis inhibitors [2].



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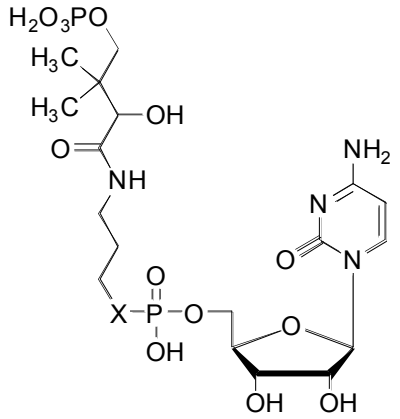
Synthesis and evaluation of catalytic intermediate-based inhibitors of phosphopantothenoylcysteine synthetase

James Patrone, Garry Dotson

Department of Medicinal Chemistry, University of Michigan, Ann Arbor MI 48109

Phosphopantothenoylcysteine synthetase (PPCS) is the second enzyme in coenzyme A biosynthesis from pantothenate. The enzyme catalyzes the amide bond formation between the carboxyl group of 4'-phosphopantothenate and the amino group of L-cysteine to yield phosphopantothenoylcysteine. Bacterial PPCS uses cytidine triphosphate (CTP) to activate 4'-phosphopantothenate as an acyl cytidylate intermediate in this conversion as opposed to adenosine triphosphate in mammalian PPCS. Molecules designed to mimic the bacterial cytidylate-activated intermediate, with the internal mixed-anhydride linkage replaced with either a phosphodiester or a phosphothioester, have been synthesized and characterized as to their inhibitory properties pertaining to bacterial PPCS. These molecules exploit the differences between the bacterial and mammalian PPCS intermediate and cofactor and should lead to selective inhibition of bacterial PPCS. These features make these molecules attractive as possible lead inhibitors for the development of novel antimicrobial agents.



PPCS inhibitor

X= O or S

Designed and Directed Assembly of Supramolecular Protein Structures

Dustin Patterson and Neil Marsh

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In Nature the assembly of individual protein subunits into larger quaternary structures allows new biological properties to emerge as consequence of the higher order structure. Inspired by Nature, our research seeks to develop novel biomaterials with useful properties by the directed assembly of proteins into larger protein structures. In particular, I am exploring the assembly of proteins into "cages", which may have useful applications in drug delivery by encapsulation of therapeutic agents. Our strategy for making such higher order protein structures in a specific and directed manner is to use protein building blocks (PBBs) with well defined symmetrical quaternary structures that can coordinate the assembly and to utilize novel noncovalent crosslinking agents that bring the PBBs together and link them to form the "cage" structures. For example, we are using the pentameric protein Cholera Toxin B (CTXB) as the PBB, whose geometry could coordinate the assembly of dodecahedron "cages". As linkers, we are exploiting peptides designed to form antiparallel heterodimeric coiled coils. This method of crosslinking is useful because the heterodimeric nature allows only subunits with complementary peptides to be joined, providing control over assembly. The antiparallel orientation maintains adequate spatial arrangement of the PBBs, and the peptide-peptide interactions can be easily modified. To date, genes encoding fusion proteins of CTXB and the peptides have been cloned, transformed into *E. coli*, expressed, and pentameric protein isolated.

Synthesis of 1,3-diols via Metal-free Oxygen-directed Hydroboration of Homoallylic Alcohols

Robert-André F. Rarig, Edwin Vedejs

Department of Chemistry, University of Michigan, Ann Arbor, Michigan, 48109-1055

1,3-diols are a common and crucial motif in biologicaly relevant compounds and diastereoselective methods for their construction have been targeted by many research efforts in organic synthesis. Selective methods exist for the syntheses of both *anti*-, and *syn*-1,3-diols, but, as each method is only ideal under certain conditions, a focus remains on new assemblies of the functionality. One approach on which there has been no groundbreaking in-depth study reported is that of hydroboration of alkenyl alcohols. This is due to complications with achieving good regioselectivity. There have been reports of metal-mediated oxygen-directed hydroborations but substrate scope is questionable and the presence of a metal is not ideal for use on a process scale. The goal of this research is the synthesis of *syn*-1,3-diols through a metal-free method of oxygen-directed hydroboration of homoallylic alcohols.

Characterization of Dihydrouridine Synthases

Lance Rider, Bruce Palfey

Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan, 48109-1055

Dihydrouridine is a modified nucleoside present in the tRNAs of eubacteria, eukaryotes, and some archea. This modified nucleoside is formed by the reduction of the double bond in uridine at specific sites in tRNAs, most commonly the D-loop region. The family of proteins catalyzing this reaction, dihydrouridine synthases, have only recently been identified in *E.coli* and *S. cerivesiae*. We have cloned, expressed, and partially characterized a members of this family of proteins from the thermophilic bacterium *Thermotoga maritima* and the DUS2 enzyme from *S. cerivesiae*. This both proteins are FMN utilizing enzymes where catalysis is comprised of a reductive half reaction, in which NADPH reduces the flavin, and an oxidative half-reaction whereby the flavin reduces the double bond of uridine. The reductive half-reactions at 35 ^{o}C shows an apparent limiting rate constant of 2.3 x 10^{-4} s^{-1} with a K_D of 600 μM for *T. maritima* and at 25 ^{o}C the reductive half reaction of the *S. cerivesiae* enzyme the reductive rate is 2.5 ± 0.3 s^{-1} with a K_D of 350 μM. Oxidation of the enzymes by a *in vitro* transcribed tRNA substrates reoxidizes the flavin with a rates of 3.2 x 10^{-5} s^{-1} and 2.4 x 10^{-5} for the *T. maritima* and *S. cerivesiae* enzymes. This slow catalytic rate when using *in vitro* transcribed substrates for the oxidative half-reactions of the enzymes seems to be a ubquitous phenomenon indicating a potential need for either prior substrate modification or other unknown protein partners.

Peptide Ligands of Regulators of G-Protein Signaling 4 (RGS4) Identified by Screening of a Focused One-Bead, One-Compound Peptide Library

Rebecca A. Roof[1], David L. Roman[1], Katarzyna Sobczyk-Kojiro[2], Anjanette Turbiak[2] Richard R. Neubig[1], and Henry I. Mosberg[2]

Departments of Pharmacology[1] and Medicinal Chemistry[2], University of Michigan, Ann Arbor, Michigan 48109

The objective of this study is to identify peptide inhibitors of Regulator of G-Protein Signaling 4 (RGS4) that are more potent than our lead compound, YJ34 (Ac-Val-Lys-[Cys-Thr-Gly-Ile-Cys]-Glu-NH_2, S-S). YJ34 was rationally designed to inhibit RGS activity by mimicking the switch 1 region of Gαi, based on the RGS4-Gαi crystal structure. We synthesized a focused One-Bead, One-Compound peptide library which retains structural features known to be necessary for YJ34 activity. The library of 2.5 million peptides was screened and peptide-beads with increased binding to a fluorescently labelled RGS4 were isolated. The hit sequences identified were resynthesized and verified for binding fluorescent RGS4 in a flow cytometry assay. One of the verified hits decreases the intrinsic fluorescence of RGS4 with Kd = 50 nM, and another blocks the interaction of RGS4 with Gαo with IC50 = 40 μM. In conclusion, even though the library was constrained to have structural similarities to our lead compound, we have identified peptides that have different functions and possibly different modes of binding RGS4. Future directions include more complete functional evaluation and determining if peptides of similar structures can compete for binding.

Development of oxovanadium biomimetic oxidation catalysts as asymmetric oxidants

Curtis J. Schneider [a], Jeff Kampf [a], Luca DeGioia[c] and Vincent L. Pecoraro [a,b]

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[b]*Biophysics Research Division, University of Michigan, Ann Arbor, MI, USA*
[c]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milano, Italy*

Asymmetric oxidations can provide a convenient and potentially environmentally benign synthetic pathway towards a number of important chiral auxiliaries including chiral sulfoxides. Tripodal amine complexes of peroxo-oxovanadium(V) complexes have been shown to be effective thioether[1] and halide oxidation[2] catalysts. Using the tripodal amine architecture based on N-[2-hydroxylethyl iminodiacetic acid], we have developed sterically hindered coordination complexes capable of oxidizing halides and thioethers. Modern computational methods[3,4] have been employed to determine the relevant solution speciation, protonation states, and transition states to guide the rational design of sterically hindered ligands based on the Hheida architecture.

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Activation of the Proapoptotic Protein Bak Links the Inhibition of Mitochondrial Respiration to Apoptosis

Thomas B. Sundberg[a], Anthony W. Opipari, Jr.[b] , Gary D. Glick[a]

[a]*Department of Chemistry and* [b]*Obstetrics & Gynecology, University of Michigan, Ann Arbor, Michigan, 48109-1055*

Cancer and autoimmune cells harbor bioenergetic abnormalities (elevated reactive oxygen species (ROS) levels and diminished anti-oxidant stores) due to an increase in oxidative phosphorylation required to meet the energy demands of uncontrolled proliferation. These metabolic traits may allow for selective treatment of autoimmunity and cancer with cytotoxic, pro-oxidant agents. Bz-423 is a 1,4-benzodiazepine that binds to the oligomycin sensitivityconferring protein subunit of the mitochondrial F_1F_o-ATPase. This binding inhibits the enzyme causing a transition from active to resting (state 3-to-4) mitochondrial respiration resulting in superoxide ($O_2^{\cdot -}$) production and redox-regulated apoptosis. Despite targeting an enzyme expressed in all cells with mitochondria, administering Bz-423 to the MRL/*MpJ-Fas^{lpr}* (MRL*lpr*) murine model of lupus results in selective depletion of splenic CD4+ T lymphocytes, an effect coupled to a significant therapeutic response. This in vivo selectivity prompted interest in studying the in vitro response of T cell-derived lines to Bz-423 to provide further insight into the cell type selective effects of this agent. Unlike other pro-oxidant drugs that target the mitochondria, Bz-423-induced cell death depends on cytosolic factors that translate the initial $O_2^{\cdot -}$ signal into release of mitochondrial proteins that trigger the execution phase of apoptosis. Specifically, the proapoptotic Bcl-2 protein family member Bak is critical for coupling ROS production to loss of mitochondrial outer membrane integrity. These findings form the basis for a model explaining the selectivity of Bz-423 in the MRL-*lpr* mice accounting for redox balance abnormalities and protein tyrosine kinase defects present in lupus CD4+ T lymphocytes.

Designed Coiled Coil Peptides that bind Cd(II) and Pb(II) Site Selectively

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Metalloproteins often display exquisite metal ion specificity. We have designed peptides that are capable of mimicking nature's ability to bind two different metals site selectively in nearly identical binding sites, such as is observed in CuZn SOD. We have used the TRI family of *de novo* designed peptides (Ac-G $(LKALEEK)_4G\text{-}NH_2$) which has been shown to aggregate into a three-stranded coiled coil. Replacement of two interior leucine groups with cysteine residues creates two trigonal thiolate sites ideal for binding heavy metals. TRI L9CL19C was previously shown to bind Cd(II) with a 10 fold higher affinity to the "a" (9) site than the "d" (19) site. Simultaneous Pb(II) and Cd(II) binding studies demonstrate the remarkable ability of both L9CL19C and L12CL19C to site selectively bind Cd(II) and Pb(II), providing insight into how site selective metal binding to peptides can be achieved and elucidating the binding preferences of these toxic metals to metalloproteins.

Mechanistic Studies of Alkyladenine DNA Glycosylase Base Flipping

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Alkyladenine DNA Glycosylase (AAG) is a human base excision repair enzyme that is responsible for the recognition and excision of various damaged purine bases. AAG utilizes a base flipping mechanism, whereby the damaged nucleotide is rotated ~180° out of the duplex and into the active site. We want to understand how AAG accomplishes this remarkable conformational change and seek to determine how base flipping contributes to specificity for damaged DNA, despite a vast excess of normal DNA within the cell. Based on the crystal structure of AAG bound to its DNA substrate, several conformational changes in the complex appear to be necessary for the base to be flipped. These are DNA bending, intercalation of Y162 into the DNA duplex and insertion of the damaged base into the active site pocket. The order of these steps has not yet been determined, but will be established by performing fluorescence based rapid reaction kinetic studies. These studies utilize naturally fluorescent substrates, such as 1-N^6-ethenoadenine in the DNA, as well as active site tryptophan residues inserted in place of native tyrosines. Using these fluorescent probes allows an understanding of movements that are happening at different points in the active site in real time. Once the reaction framework has been established, we will investigate the contribution of individual amino acids to base flipping by looking at the kinetic differences between certain active site mutants. Additionally, a comparison of damaged to undamaged DNA will provide insight into how base flipping plays a role in AAG specificity.