Abstracts, Division of Biological Chemistry, 224th National Meeting of the American Chemical Society, August 18−22, 2002

John S. Blanchard, Program Chair

Sunday Morning: Frontiers of Enzymology

Tadgh Begley, Organizer

1. Comparative genomics of NAD, FAD and CoA cofactor biosynthesis: themes and variations. Andrei Osterman, Matthew Daugherty, Oleg Kurnasov, and Faika Mseeh. Integrated Genomics Inc., 2201 W. Campbell Park Dr., Chicago, IL 60612 (fax: 312-491-0856, andrei@IntegratedGenomics.com)

Comparative analysis of multiple and diverse genomes produces a remarkable impact on our ability to characterize cellular networks. It is now possible to associate thousands of genes with predicted enzymatic functions in a large number of pathways and subsystems forming a "core machinery of life". With a growing number of analyzed genomes, a remarkable conservation tendency in this machinery across the taxons is revealed at all levels, from the overall subsystem topology to individual genes. Biosynthesis of adenylate-derived cofactors NAD, FAD, and CoA, ubiquitous and indispensable in all forms of cellular life, provides a perfect illustration of this tendency. Until recently many genes involved with the biosynthesis of these cofactors remained unknown. We used various techniques of comparative genomics, such as metabolic reconstruction, chromosomal gene clustering, protein fusion events, and phylogenetic occurrence profiles, to produce conjectures for these "missing" genes. This integrated approach allowed us to identify and experimentally verify a number of genes encoding key enzymes in NAD, FAD, and CoA biosynthetic pathways in various species from archaea to human. Patterns of conservations and variations in these pathways reflect complex evolutionary relationships, including multiple lateral transfer, gene fusion, and rearrangement events.

2. Protein engineering by directed molecular evolution. SunAi Raillard. Department of Molecular biology, Maxygen Inc., 515 Galveston Dr., Redwood City, CA 94063 (SunAi.Raillard@maxygen.com)

Directed molecular evolution is a powerful, empirical approach to protein engineering to achieve optimized protein activity or stability, alteration of protein function or substrate specificity, and adaptation of enzymatic reactions to different environments. Unlike rational engineering where advantageous mutations are typically introduced in close proximity to the active site residues, molecular evolution often finds unexpected mutational solutions all over the entire structure of the protein. Key elements of molecular evolution are the same as found in natural Darwinian evolution and are based on creation of diversity at the genotypic level followed by selection for "survival of the fittest" at the phenotypic level. We utilize a combinatorial approach for creating our libraries, mimicking nature's way of creating diversity by sexual recombination. Our method allows the creation of vastly diverse libraries that contain high numbers of functional and improved offspring variants and thus allows for high-quality screens that are relevant for complex phenotypic measurements. A demonstration of the power of molecular evolution will be shown on selected examples of evolved enzymes for improved performance as well as altered functions.

3. Activity based proteome analysis. David A. Campbell. ActivX Bioscience, 11025 N. Torrey Pines Rd., La Jolla, CA 92037 (fax: 858-587-4878, davidc@activx.com)

At ActivX small molecule probes are developed to label specific protein families and are utilized to track the presence and/or activity of protein family members within different biological samples. The designs of these probes are based upon insights gained by numerous researchers in the chemical−biology field during the past half-century. The first probe that was synthesized targeted the catalytic triad of serine hydrolases by incorporating a fluorophosphonate residue coupled with a reporter group. This presentation will summarize applications of the fluorophosphonate probe including novel protein identification and inhibitor profiling. In addition, the development of probes targeting other protein families will also be described.

4. Small molecule protein kinase inhibitors: discovery and intracellular target identification. Nathanael Gray. Department of Chemistry, Genomics Institute of the Novartis Research Foundation, 3115 Merryfield Row, Suite 200, San Diego, CA 92121 (Gray@gnf.org)

From the discovery of Staurosporine as one of the first examples of a protein kinase inhibitor, to the successful development of Gleevec as an inhibitor of the transforming kinase Bcr-Abl in chronic mylogenous leukemia, there has been tremendous progress in our understanding of how to design and characterize small molecule kinase inhibitors. However, as we progress in the development of kinase inhibitors, what new tools are becoming available for the characterization of the cellular effects of these compounds? How can we study the intracellular targets of putative kinase inhibitors? This talk will provide examples from our work to develop combinatorial libraries of trisubstitued purine and phenylaminopyrimidine CDK inhibitors and to characterize their cellular effects by a combination of affinity chromatography, proteomics, and cellular assays.

5. Realizing the potential of high throughput screening technologies. Robert Hertzberg. Department of Molecular

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Screening, GlaxoSmithKline Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406 (robert.p.hertzberg@gsk.com)

High throughput screening (HTS) is at the center of lead discovery at most major pharmaceutical companies, and it has played an important role in driving the field of detection technologies and laboratory automation. Several new technologies have been applied to solving problems in the HTS lab, including: (a) novel detection applications such as fluorescence polarization and fluorescence correlation spectroscopy, which enable homogeneous assays with high sensitivity; (b) new liquid handling devices which enable high-density low-volume assays; and (c) dramatic improvements in the reliability and sophisication of laboratory robotics. The explosion of data produced by HTS activities has pushed the limits of todays informatics systems. This talk will focus on the strategy taken at GSK to marry the diverse disciplines of chemistry, biology, optics, robotics, and informatics to realize the potential of HTS technologies. Examples from successful screening campaigns will be used to illustrate this strategy.

Sunday Afternoon: Carbohydrate Enzymology

Martin Tanner, Organizer

6. Chemoenzymatic synthesis of glycosaminoglycan oligosaccharides. Paul L. DeAngelis. Department of Biochemistry and Molecular Biology, University of Oklahoma, Oklahoma Center for Medical Glycobiology, 940 Stanton L. Young Blvd., Oklahoma City, OK 73104 (fax: 405-271-3092, paul-deangelis@ouhsc.edu)

Glycosyltransferase enzymes have exquisite regiospecific and stereospecific synthetic control. The glycosaminoglycans hyaluronan, chondroitin, and heparin are sugar polymers that play many roles in mammals. Smaller fragments of these molecules are promising therapeutic candidates. We have used variants of glycosaminoglycan synthases, dual-action glycosyltransferases, from three types of *Pasteurella multocida* bacteria to synthesize GAG oligosaccharides. The hyaluronan synthase contains two independent active sites within one polypeptide: a beta-1,3-GlcNAc-transferase and a beta-1,4-GlcUA-transferase. Mutation of one site inactivates the hyaluronan polymerizing activity, but the enzyme can still catalyze the transfer of a single sugar. A pair of bioreactors with immobilized mutant enzymes can be utilized to synthesize defined hyaluronan molecules. A homologous chondroitin synthase also contains two sites and serves as a source of beta-1,3-GalNAc-transferase. The heparosan synthase promises to add an alpha-1,4-GlcNAc-transferase and a beta-1,4-GlcUA-transferase to our catalytic repertoire. (Supported by National Institutes of Health, National Science Foundation, and Hyalose, LLC.)

7. UDP-galactose 4-epimerase: catalysis of hydride transfer in the galactosyl-glucosyl interconversion. Perry A. Frey. Department of Biochemistry, University of Wisconsin, 1710 University Ave., Madison, WI 53705 (fax: 608-265-2904)

UDP-Galactose 4-epimerase, a member of the short chain dehydrogenase superfamily, uses NAD as a tightly bound coenzyme in catalyzing the interconversion of UDP-galactose and UDP-glucose, an essential step in galactose metabolism in all cells. Tyr 149, Ser 124, and Lys 153 in the active site are essential for enzymatic activity. Evidence from C-13 NMR, site-directed mutagenesis, absorption spectrophotometry, and steady-state kinetic analysis supports the following functional assignments to these residues. Lys 153 interacts electrostatically with the positively charged nicotinamide nitrogen in NAD, increasing its reactivity in hydride transfer. Tyr 149 displays a pK_a of 6.1 and plays a dual role. It serves as the base catalyst for hydride abstraction, and it mediates the uridine nucleotide induced activation of NAD through an electrostatic interaction with the nicotinamide ring. Ser 124 cooperates in the acid−base function of Tyr 149, either by mediating proton transfer or by aiding in positioning Tyr 149 and the glucopyranosyl ring of the substrate. (Supported by Grant GM 30480 from the NIGMS, USPHS.)

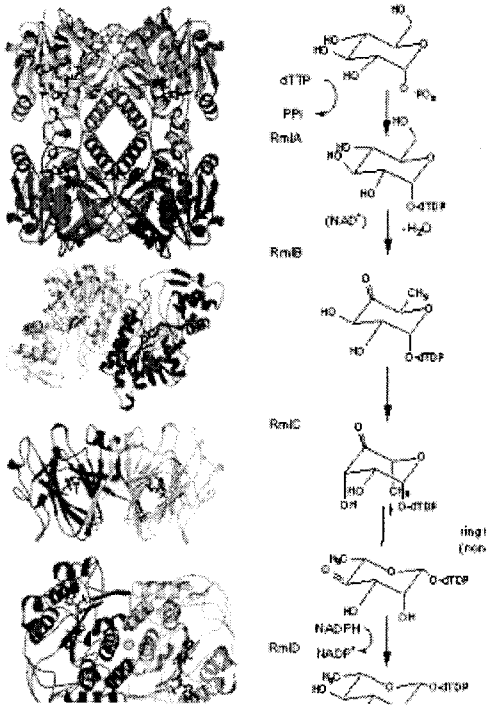
8. Origin of lipid A species modified with 4-amino-4-deoxy-L-arabinose in *Escherichia coli.* Christian R. H. Raetz. Department of Biochemistry, Duke University, Durham, NC 27710 (fax: 919-684-8885)

Addition of the 4-amino-4-deoxy-L-arabinose (L-Ara4N) moiety to the phosphate groups of Lipid A is implicated in bacterial resistance to polymyxin and cationic anti-microbial peptides of the innate immune system. The sequences of the products of the *Salmonella typhimurium* pmrE and pmrF loci, both of which are required for polymyxin resistance, recently led us to propose a pathway for L-Ara4N biosynthesis from UDP-glucuronic acid [Zhou et al. (1999) *J. Biol. Chem. 274*, 18503−18514]. We now report that extracts of a polymyxin-resistant mutant of *E. coli* catalyze the C-4 oxidation and C-6 decarboxylation of [alpha-32P]UDP-glucuronic acid, followed by transamination to generate [alpha-32P]UDP-L-Ara4N, when NAD and glutamate are added as cosubstrates. In addition, the [alpha-32P]-UDP-L-Ara4N is formylated when *N*-10-formyltetrahydrofolate is included. Interestingly, UDP-L-Ara4-formyl-N is an obligatory intermediate in the eventual transfer of the L-Ara4N moiety to Lipid A. All three activities are consistent with the proposed functions of two of the gene products (PmrI and PmrH) of the pmrF operon. PmrI (renamed AraA) was overexpressed using the T7 construct, and shown by itself to catalyze the unprecedented oxidative decarboxylation of UDP-glucuronic acid to form uridine-5-(beta-L-threo-pentapyranosyl-4-ulose diphosphate). A 6 mg sample of the latter was purified, and its structure was validated by NMR studies as the hydrate of the 4-ketone. AraN resembles UDP-galactose epimerase, dTDP-glucose-4,6-dehydratase, and UDP-xylose synthase in oxidizing the C-4 position of its substrates, but differs in that it releases the NADH product. In addition, AraN contains a formyl transferase domain that catalyzes the conversion of UDP-L-Ara4N to UdP-L-Ara4-formyl-N. (Supported by NIH Grants GM-51310 and GM-51796.)

9. Unusual carbohydrate chemistry in pathogenic bacteria. James H. Naismith. Centre for Biomolecular Science, University of St. Andrews, North Haugh, St. Andrews, Scotland KY16 9ST, United Kingdom (fax: 441334462595, naismith@st-andrews.ac.uk)

L-Rhamnose biosynthetic pathway has been studied for over 3 decades; however, it is only relatively recently that

interest in it as a potential therapeutic target has emerged. L-Rhamnose is a deoxy sugar, and immediate precursor dTDP-L-rhamnose is synthesized by bacteria from glucose-1-phosphate in four enzymatic steps (see figure below). The pathway is found in many pathogenic bacteria but is absent from humans. In bacteria, L-rhamnose plays a key role in cell wall structure. My laboratory has determined the structure of each of the enzymes. By obtaining co-complexes and performing site-directed mutagenesis, we have investigated the molecular mechanisms of the complex transformations they perform. For each enzyme, novel insights into carbohydrate chemistry have been obtained. The work is still very much in progress, and the lecture will focus on the current novel aspects of the pathway.



Monday Morning: Eli Lilly Symposium

Kevan Shokat, Organizer

10. Reprogramming cellular signaling using chemical dimerizers. Tim Clackson. ARIAD Pharmaceuticals, 26 Landsdowne St., Cambridge, MA 02139 (fax: 617-225-2589, clackson@ariad.com)

The use of chemical "dimerizers" to control protein−protein associations within cells has become well established. Cells are engineered to express chimeric proteins comprising a signaling domain fused to a drug-binding protein such as FKBP12; treatment with bivalent ligands cross-links the proteins and initiates signaling. The technique represents a general molecular interaction technology for creating inducible alleles of intracellular proteins, allowing function to be dissected inside cells and whole organisms. Dimerizers can also be used to regulate gene transcription through the controlled association of chimeric DNA binding and activation domains. To maximize the potency of regulated dimerization, we have used structure-based design and protein engineering to create highly potent and specific dimerizer−protein pairings. We are currently pursuing the clinical application of controlled dimerization to bring gene and cell therapies under small molecule control. A system for regulated apoptosis based on inducible aggregation of Fas is now in clinical development.

11. Activity-based protein profiling: chemical approaches for functional proteomics. Benjamin F. Cravatt. Departments of Chemistry and Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037 (fax: 858-784-2798, cravatt@scripps.edu)

The field of proteomics aims to characterize dynamics in protein function on a global scale. However, several classes of enzymes are regulated by posttranslational mechanisms, limiting the utility of conventional proteomics techniques for the characterization of these proteins. Our research group has initiated a program aimed at generating chemical probes that interrogate the state of enzyme active sites in whole proteomes, thereby facilitating the simultaneous activity-based profiling of many enzymes in samples of high complexity. Progress toward the generation and utilization of active site-directed chemical probes for the proteomic characterization of several enzyme classes will be described. These enzyme classes fall into two general categories: (1) enzymes for which active site-directed affinity agents have been well-defined, and (2) enzymes for which active site-directed affinity agents have been lacking. The application of activity-based protein profiling to the functional characterization of enzyme activities that vary in human cancer cell lines will be highlighted.

12. Proline isomerization—a molecular switch controlling substrate recognition and catalytic activity. Amy H. Andreotti. Department of Biochemistry, Iowa State University, 4208 Molecular Biology Building, Ames, IA 50011 (fax: 515-294-0453, amyand@iastate.edu)

Interleukin-2 tyrosine kinase (Itk) is a Tec family nonreceptor protein tyrosine kinase that participates in the intracellular signaling events leading to T cell activation. Itk contains the conserved SH3, SH2, and catalytic domains common to many kinase families, yet the molecular mechanisms by which Itk is regulated are not understood. NMR structural studies reveal a conformationally heterogeneous proline residue within the SH2 domain of Itk and show that this proline-dependent conformational switch regulates substrate recognition. Moreover, we show that the peptidyl prolyl cis/trans isomerase, cyclophilin A (CyPA), increases the rate of proline cis/trans isomerization within Itk. CyPA and Itk form a stable regulatory complex in Jurkat T cells, and we show that Itk catalytic activity is inhibited by CyPA. These findings support a novel mode of kinase regulation for a Tec family member and provide a molecular basis for understanding the cellular function of the ubiquitous peptidyl prolyl isomerase, CyPA.

13. Unnatural ligands for engineered receptors: new tools for chemical genetics. Kevan M. Shokat. Cellular & Molecular Pharmacology, University of California, San Francisco, 513 Parnassus Ave., Box 0450, San Francisco, CA 94143-0450 (fax: 415-514-0822, shokat@cmp.ucsf.edu)

Our laboratory focuses on the development of novel chemically based tools to decipher signal transduction

pathways on a genome-wide scale. We have developed a method for producing small molecules that are specific for any protein kinase of interest in a signaling cascade by combining protein design with chemical synthesis. Our highly specific inhibitors of individual kinases have revealed a number of new principles of signal transduction that have been missed by genetic studies of cell signaling. These studies suggest that new pathways and new functions can be revealed by small molecule inhibitors of protein kinases. These new functions are distinct from those suggested by genetic studies of the same kinases/pathways and thus represent new opportunities for drug discovery. Most importantly, these studies suggest that new drug targets identified via genetic means (comparative genomics) and validated by reverse genetics (gene knock-outs) may be difficult to convert into suitable small molecule drug targets. The reasons for the distinct responses of cells to small molecule inhibitors compared to genetic lesions will be discussed.

Monday Afternoon: Metalloenzymology

Stephen Ragsdale, Organizer

14. Metalloenzymes and microorganisms in the biogeochemical cycles of major elements. Edward I. Stiefel. Department of Chemistry, Princeton University, 101 Hoyt Laboratory, Princeton, NJ 08544-1009 (fax: 609-258-6746, estiefel@Princeton.EDU)

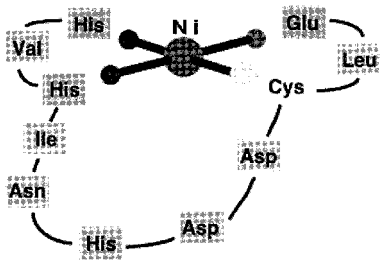
Life on Earth depends on and influences the biogeochemical cycling of the major elements: C, H, O, N, S, and P. Cycles for the first five elements involve redox reactions. Biological systems played major roles in the evolution of these cycles and play dominant roles in maintaining levels of carbon, hydrogen, nitrogen, oxygen, and sulfur compounds, locally and globally, in the atmosphere, lithosphere, and hydrosphere. Key steps in the cycles are catalyzed by metalloenzymes that contain Fe, Mn, Co, Ni, Cu, Zn, and Mo. This overview emphasizes the key redox role of metalloenzymes in the biogeochemical cycles. More briefly considered are: the co-evolution of the cycles and life; the interactions between the cycles; the dominant role of microorganisms in the cycles; and the perturbation of the natural cycles by human activities. Understanding metalloenzymes helps us apprehend the past history, current state, and ultimate fate of the biogeochemical cycles on Earth.

15. Proteins involved in the metalation of copper centers in cytochrome *c* oxidase. Dennis R. Winge, Hether Carr, Andrew Maxfield, and Keith McCall. Department of Biochemistry, University of Utah Health Sciences Center, 30 N. 1900 East, University of Utah Health Sciences Center, Salt Lake City, UT 84132 (fax: 801-585-5469, dennis.winge@hsc.utah.edu)

Assembly of cytochrome *c* oxidase in the inner mitochondrial membrane requires a number of accessory proteins. The delivery and insertion of copper ions in cytochrome *c* oxidase appear to require at least three proteins, Cox17, Sco1, and Cox11. Cox17 is the putative copper metallochaperone for delivery of copper ions to the mitochondrion. Insertion of Cu ions within the two mitochondrially encoded subunits Cox1 and Cox2 appears to involve co-metallochaperones Sco1 and Cox11 for metalation of CuA and CuB sites, respectively. Cox17 binds 3 Cu(I) ions per monomer in a polycopper cluster as shown by X-ray absorption spectroscopy. CuCox17 localized within the intermitochondrial membrane space appears to be predominantly tetrameric, whereas the cytosolic CuCox17 is primarily a dimeric species. Mutants of Cox17 that bind Cu(I) but fail to tetramerize are nonfunctional. Thus, the oligomeric state of Cox17 may be important to its physiological function. Sco1 and Cox11 are inner membrane proteins that each bind a single Cu(I) ion per subunit. Two cysteinyl residues present as a CxxxC motif and a distant His are essential for Cu binding and in vivo Sco1 function. Cox11 exists as a stable dimer with the Cu(I) ion in each monomer bridged in a binuclear cluster. The three Cys residues involved in Cu(I) ligation are essential for in vivo function in cytochrome oxidase assembly. Thus, the residues that ligate Cu(I) ions in the three proteins implicated in Cu ion metalation of the oxidase complex are also essential for activation of the oxidase. This correlation is consistent with Cox17, Sco1, and Cox11 functioning in the Cu ion metalation of cytochrome *c* oxidase.

16. Regulation of Ni uptake in *E. coli.* Michael J. Maroney,[1] Faizah Al-Mjeni,[1] Paul E. Carrington,[1] Peter T. Chivers,[2] and Robert T. Sauer.[2] [1]Department of Chemistry, University of Massachusetts, P.O. Box 34510, Lederle GRC Tower A, Room 701, Amherst, MA 01003-4510 (fax: 413-545-4490, mmaroney@chem.umass.edu), and [2]Department of Biology, MIT

Nickel uptake in *E. coli* is mediated by a Ni-specific permease, NikA-E. Expression of the permease is upregulated under anaerobic conditions under the control of FNR. The expression of the nik operon is repressed under conditions of excess Ni utilizing the repressor protein, NikR. NikR is a member of the β-α-α family of transcription factors and binds both Ni and DNA with high affinity (pM). A combination of results from XAS and site-specific mutagenesis indicates that the high-affinity Ni site has square planar geometry and has an N_2OS-donor ligand environment composed of His, Glu, and Cys ligands. XAS studies further reveal structural changes that occur upon DNA binding and as a result of mutations in the DNA- and Ni-binding domains. These results suggest possible mechanisms for the control of Ni concentration in the cell.



17. Assembly of nickel into the urease active site. Robert P. Hausinger,[1] Hyun Kyu Song,[2] Jason Kuchar,[1] Zhenzhan Chang,[1] and Scott B. Mulrooney.[1] [1]Department Microbiology & Molecular Genetics, Michigan State University, Biomedi-

cal Physical Sciences, East Lansing, MA 48824 (fax: 517-353-8957, hausinge@msu.edu), and [2]Harvard University

Klebsiella aerogenes urease is a heterotrimeric enzyme (UreA, UreB, and UreC) containing a binuclear nickel metallocenter bridged by a carbamylated lysine side chain. Three accessory proteins (UreD, UreF, and UreG) transiently bind to urease apoprotein and serve as a GTP-dependent protein chaperone during the enzyme activation process. Another accessory protein, UreE, functions as a metallochaperone by delivering nickel ions to the UreDFG−ABC complex. The properties of the UreDFG−ABC complex will be reviewed, and new information derived from protein cross-linking studies will be described. In addition, the two-domain structure of UreE will be presented, and site-directed mutagenesis studies involving each domain will be described.

18. Nickel sites in CO dehydrogenase and acetyl-CoA synthase. Tzanko I. Doukov,[1] Javier Seravalli,[2] Stephen W. Ragsdale,[2] and **Catherine L. Drennan**.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, 16-573, 77 Massachusetts Ave., Cambridge, MA 02139 (fax: 617-258-7847, cdrennan@mit.edu), and [2]Department of Biochemistry, University of Nebraska

The Ni-Fe-S containing carbon monoxide dehydrogenases/acetyl-CoA synthases (CODH/ACSs) convert carbon dioxide and carbon monoxide into cell carbon in a pathway involving interesting organometallic intermediates. The X-ray analyses of CODH from *Rhodospirillum rubrum* [Drennan et al. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 11973] and from *Carboxydothermus hydrogenoformans* [Dobbek et al. (2001) *Science 293*, 1281] have revealed that the structure of the C-cluster contains Ni as part of a (Fe-[NiFe3S4]) cubane. The positions of the metals in the both structures are approximately the same; differences are found in coordination geometries of the Ni and Fe and in the identity of the small molecule ligands. Comparison with a structure of the C-cluster from the bifunctional *Moorella thermoacetica* CODH/ACS will be valuable in understanding these coordination differences. A central question is the extent to which the A-cluster will resemble the C-cluster. Progress toward determining the entire CODH/ACS structure will be presented.

Monday Evening: Poster Session I

John Blanchard, Organizer

19. Characterization of site-directed mutants of serines 19 and 31 of tyrosine hydroxylase. **Monserrat Royo**, S. Colette Daubner, and Paul F. Fitzpatrick. Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843-2128 (fax: 979-845-4946, montse29@hotmail.com)

Tyrosine hydroxylase (TYH) catalyzes the initial step in the biosynthesis of catecholamine neurotransmitters. Serine residues at positions 8, 19, 31, and 40 in rat TYH are phosphorylated in vivo and in vitro. The details of the effects of phosphorylation and the physiological relevance of the different phosphorylation sites are not fully understood. To study the effects of phosphorylation at serines 19 and 31, the S19A, S19E, S31A, and S31E enzymes were expressed and purified. The kinetic parameters and the catecholamine binding affinities of the four mutants are similar to those of the wild type. This is in contrast to the large effects on catecholamine binding seen upon phosphorylation of serine 40 and in the S40E enzyme.

20. Ketopantoate hydroxymethyltransferase catalyzed enolization of ketoacids. **Michele Sugantino** and John S. Blanchard. Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 (fax: 718-430-8565)

The panB gene that encodes ketopantoate hydroxymethyltransferase has been cloned from *M. tuberculosis*, expressed, and purified to homogeneity. ^{1}H NMR spectroscopy was used to determine the rate of carbanion formation and deuterium exchange in the methylene tetrahydrofolate-independent enolization of α-ketoisovalerate catalyzed by PanB. These studies have demonstrated that substrate enolization by PanB is divalent metal dependent with the preference: $Mg^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+} > Ca^{2+}$. The rate of enolization is pH-dependent with optimal activity at 7.0. The pH profile was bell-shaped, demonstrating the presence of two ionizable groups with apparent p*K*s of 6.2 and 8.3. These studies have also revealed that a large number of α-keto acids are substrates for the enolization reaction. On the basis of these observations, we propose a stereochemical and chemical mechanism for this metalloenzyme.

21. Mechanism of the aromatic desulfinase, 2-(2′-hydroxyphenyl)-benzenesulfinate desulfinase. **Linette M. Watkins**, Melissa Cody, Rene C. Rodriguez, and Raychel Chambers. Department of Chemistry and Biochemistry, Southwest Texas State University, San Marcos, TX 78666 (fax: 512-245-2374, lw09@swt.edu)

2-(2′-Hydroxyphenyl)benzenesulfinate desulfinase (HPBS desulfinase) catalyzes the removal of sulfite from 2-(2′-hydroxyphenyl)benzenesulfinate to form 2-hydroxybiphenyl. It is the final and rate-limiting step in the biocatalytic desulfurization of dibenzothiophene by *Rhodococcus erythropolis* IGTS8. HPBS desulfinase does not require a cofactor for activity. It has a narrow substrate specificity, and the rate is altered by the presence of electron-withdrawing or electron-donating groups on the aromatic ring. There is an acidic and a basic group required for activity. Cysteine, tryptophan, and tyrosine modification agents are selective inactivators of the enzyme. A highly conserved and unique cysteine residue and a nearby tyrosine residue are proposed to be an essential active site base and acid, respectively, in the mechanism of this enzyme.

22. The lipoamide dehydrogenase from *Mycobacterium tuberculosis* permits the direct observation of flavin intermediates in catalysis. **Argyrides Argyrou**,[1] John S. Blanchard,[1] and Bruce A. Palfey.[2] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 (fax: 718-430-8565), and [2]Department of Biological Chemistry, University of Michigan

Lipoamide dehydrogenase catalyses the NAD^+-dependent oxidation of the dihydrolipoyl components of the pyruvate and α-ketoglutarate dehydrogenase multi-enzyme complexes. It contains a tightly bound FAD and a redox-active disulfide,

which cycle between the oxidized and reduced forms during catalysis. The mechanism of reduction of the *Mycobacterium tuberculosis* lipoamide dehydrogenase by NADH, and oxidation of the two-electron-reduced enzyme by NAD^+, was studied anaerobically at 4 °C and pH 7.5 by stopped-flow spectrophotometry. Reduction of the FAD cofactor by NADH is fast ($k_{for} = 1260\ s^{-1}$, $k_{rev} = 590\ s^{-1}$) and displays a primary deuterium kinetic isotope effect [$^D(k_{for} + k_{rev})$] of ~3.5. This is then followed by rate-limiting ($k_{obsd} = 200\ s^{-1}$) intramolecular transfer of electrons from FADH2 to the redox-active disulfide via an unstable intermediate, possibly an initial covalent adduct between the C4a-position of the FAD and Cys46, to generate the two-electron-reduced enzyme, EH2. Highly thermodynamically unfavorable reduction of the enzyme to the four-electron-reduced state (EH4) also occurs at high concentrations of NADH.

23. Investigating the unique G-1:C73 base pair of *E. coli* $tRNA^{His}$ via atomic group mutagenesis. Abbey E. Rosen and Karin Musier-Forsyth. Department of Chemistry, University of Minnesota, 207 Pleasant St. SE, Minneapolis, MN 55455 (rosen@chem.umn.edu)

Translation of the genetic code into proteins requires proper recognition of transfer RNA molecules by their cognate aminoacyl-tRNA synthetases. This work focuses on the RNA−protein interactions necessary for efficient aminoacylation of *Escherichia coli* $tRNA^{His}$. In the *E. coli* $tRNA^{His}$ acceptor stem, the major synthetase recognition element is the unique extra base pair G-1:C73. Previous in vitro studies showed that mutations and deletions at G-1 and C73 significantly affected aminoacylation kinetics. To further probe the role of the G-1:C73 base pair in specific aminoacylation by HisRS, we are carrying out atomic group "mutagenesis" studies. Systematic base analogue substitutions at the −1:73 position of chemically synthesized $microhelix^{His}$ substrates suggest that the G-1 base primarily serves to position the 5′-monophosphate, which has previously been shown to be critical for aminoacylation. In contrast, the C73 base appears to contain atomic groups essential to aminoacylation.

24. 2.4 Å structure of a binary mevalonate kinase−MgATP complex confirms active site functional assignments and suggests the mechanism of phosphoryl transfer. Zhuji Fu, Ming Wang, David Potter, **Henry M. Miziorko**, and Jung-Ja P. Kim. Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226

Mevalonate kinase catalyzes the ATP-dependent phosphorylation of mevalonic acid to form mevalonate 5-phosphate, a key intermediate in the pathway of isoprenoid and sterol biosynthesis. The crystal structure of rat mevalonate kinase, complexed with MgATP, has been determined at 2.4 Å resolution. Each monomer of this dimeric protein is composed of two domains; the active site is located at the domain interface. Enzyme-bound ATP adopts an anti conformation, in contrast with the syn conformation reported for ATP bound to the homologous protein, homoserine kinase. The Mg ion is coordinated to both beta- and gamma-phosphates of ATP as well as to the side chains of Glu193 and Ser146, as predicted based on our previous mutagenesis studies. Asp204 makes a salt bridge with Lys13, which in turn interacts with the gamma-phosphate; this observation confirms our previous hypothesis that Lys13 interacts with ATP. A model of mevalonic acid can be placed near the gamma-phosphoryl group of ATP; thus, the C5 hydroxyl is located within 4 Å of D204, K13, and the gamma-phosphoryl of ATP. This arrangement of residues and substrate functional groups strongly suggests that: (1) D204 abstracts the proton from the C5 hydroxyl of mevalonate, supporting our previous assignment of D204 as general base catalyst; (2) the pentacoordinated gamma-phosphoryl group may be stabilized by Mg, Lys13, and Glu193; and (3) Lys13 is likely to influence the pK_a of the C5 hydroxyl of the mevalonate substrate. (Supported by NIH Grant DK53226.)

25. 4-Oxalocrotonate tautomerase (4OT) has a low-level hydratase activity. William H. Johnson, Jr., Susan C. Wang, and **Christian P. Whitman**. College of Pharmacy, The University of Texas at Austin, Austin, TX 78712 (fax: 512-232-2606)

4-OT catalyzes the isomerization of β,γ-unsaturated enones to their α,β-isomers. Pro-1 abstracts the α-proton in conjunction with Arg-39, which functions as a general acid catalyst. Arg-11 has two roles—it binds the C-6 carboxylate group and serves as an electron sink. Phe-50 maintains the hydrophobic environment of the active site. We have now determined that 4-OT catalyzes the dehalogenation of *trans*-3-chloroacrylate, presumably by hydration, and the reversible hydration of fumarate to form (2*S*)-malate. The P1A mutant exhibits less hydratase activity while that of the R11A mutant is greatly diminished. Thus, 4-OT has at least two low-level activities, in addition to its well-known isomerase activity, and may have more activities if the appropriate substrates can be identified. These observations suggest that the active site of 4-OT is a versatile template with diverse catalytic capabilities and may serve as a starting point for the evolution of new enzymes.

26. β-Aminoalanine as C-terminus cap in helical peptides: an isotope-edited FTIR study. Aya Wakata and **Sean M. Decatur**. Department of Chemistry, Mount Holyoke College, Carr Laboratory, South Hadley, MA 01075 (fax: 413-538-2327, sdecatur@mtholyoke.edu)

In both proteins and model peptides, the ends of helices are stabilized by capping interactions which help to satisfy hydrogen bonds to terminal carbonyl and amino groups. The details of helix stabilization in model peptides by N-capping acetyl- and succinyl-groups have been well studied, but there are few details on the effects and mechanisms of C-capping groups. Recently, the amino acid β-aminoalanine (diaminopropionic acid) has been described by Kemp and co-workers as a strong helix terminator and C-terminus capping residue. In a previous study, our group applied the technique of isotope-edited FTIR spectroscopy to probe the capping efficiency of an N-terminal acetyl group. In this study, we use the same set of alanine-rich model peptides [based on the repeat $(AAKAA)_n$] to study the effect of β-aminoalanine as a C-terminus cap. We find that β-aminoalanine does stabilize the overall helix content of the peptide, but not as much as the *N*-acetyl group. Isotope-edited FTIR spectra are used to locate the regions of the peptide sequence most affected by the presence of the C-terminal cap.

27. Actions of *N*-arachidonylglycine on inflammation and regulation of anandamide degradation. Sumner Burstein,[1] William Pearson,[1] Ronald G. Rossetti,[1] and Robert B. Zurier.[2] [1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation St., Worcester, MA 01605 (fax: 508-856-6231, sumner.burstein@umassmed.edu), and [2]Department of Medicine, University of Massachusetts Medical School, 364 Plantation St., Worcester, MA 01605

The carboxylic analogue of anandamide, *N*-arachidonylglycine, occurs in rat and bovine brain and in peripheral sites and shows analgesic activity. It was also observed that it inhibits the hydrolytic activity of FAAH on anandamide. These and other data suggest that it may show efficacy in models of inflammation and can serve as an endogenous regulator of tissue anandamide concentrations. We now show findings that support both of these hypotheses. Arachidonic acid induced paw edema in mice was reduced by more than 50% by the prior oral administration of 4 mg/kg of *N*-arachidonylglycine. Blood levels of anandamide in rats given 10 mg/kg p.o. of *N*-arachidonylglycine were increased 10× when compared to vehicle controls. In vitro evidence demonstrated that *N*-arachidonylglycine was not a precursor for the increased anandamide, suggesting that the effect was due to the inhibition of FAAH, raising the possibility that *N*-arachidonylglycine may be an endogenous regulator of FAAH activity.

28. Aggregation: a common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. Susan L. McGovern,[1] Emilia Caselli,[1] Nikolaus Grigorieff,[2] and Brian K. Shoichet.[1] [1]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University, Searle 8-417, 303 E. Chicago Ave., Chicago, IL 60611 (fax: 312-503-5349, s-mcgovern@northwestern.edu), and [2]Howard Hughes Medical Institute, Rosenstiel Basic Medical Research Center, Brandeis University, Waltham, MA

High-throughput and virtual screening are widely used to discover novel leads for drug design. On examination, many screening hits appear non-drug-like: they act noncompetitively, show little relationship between structure and activity, and have poor selectivity. Despite the common occurrence of such inhibitors, no known mechanism explains their peculiar behavior. To investigate this problem, 45 diverse screening hits were studied. Of these, 35 were shown to inhibit several unrelated model enzymes. These 35 compounds showed time-dependent, reversible inhibition that was very sensitive to enzyme concentration and ionic strength. By light scattering and electron microscopy, these compounds formed aggregates 30−400 nm diameter. These large aggregates appear to be the active inhibitory species. Unexpectedly, aggregate formation may explain the activity of many nonspecific enzyme inhibitors, which are widespread in drug-discovery screening databases. Preliminary data suggest that this mechanism may also account for nonspecific inhibition by some widely used kinase inhibitors.

29. Analysis of oncogenic protein tyrosine kinases expressed in recombinant yeast. Daniel D. Clark and Blake R. Peterson. Department of Chemistry, Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802 (fax: 814-863-8403)

Yeast two-hybrid systems are powerful proteomic tools for the discovery and characterization of protein−protein interactions. However, these systems are typically unable to detect interactions dependent on posttranslational modifications such as tyrosine phosphorylation. We report a novel yeast tribrid system that expresses a potentially universal protein tyrosine kinase (PTK) substrate to detect diverse PTKs. Validation with the oncogenic kinases v-Abl and v-Src, which exhibit divergent substrate specificities, demonstrated significant potential for cloning PTKs en masse from cDNA libraries. This approach also has potential for the discovery of small molecules that modulate PTK activity. Recent efforts to analyze synthetic and natural inhibitors of PTKs with recombinant yeast-based assays will be described.

30. Analysis of the ADAR2 RNA-editing reaction using substrate analogues modified at the atomic level. LaHoma M. Easterwood, Eduardo A. Véliz, Hye Young Yi-Brunozzi, and Peter A. Beal. Department of Chemistry, University of Utah, 315 S. 1400 East, Salt Lake City, UT 84112 (fax: 801-581-8433, leaster@chem.utah.edu)

ADARs are adenosine deaminases responsible for RNA-editing reactions that occur within double-stranded RNA. These editing events result in an adenosine to inosine conversion as seen in ADA (adenosine deaminase). Previously we have shown that ADAR2 and ADA share mechanistic similarities. Further understanding of this relationship has now been obtained through additional studies. We have synthesized atomic level modified structural analogues of a natural RNA editing substrate, based on analogues used in ADA studies, and compared editing reactions and binding affinities of these substrates by ADAR2. Deamination rates were shown to be sensitive to the bulkiness of the N^6 substituent and to the addition of a nitrogen within the purine ring. In addition, substrates proposed to contain tight-binding analogues did serve to increase the binding affinity observed between the enzyme and the substrate. Information gained from these studies furthers our understanding of the mechanistic relationship between ADARs and ADA.

31. Anionic substitutes for catalytically important aspartic acids in phosphoribulokinase. Jennifer A. Runquist and Henry M. Miziorko. Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226

Mutagenic substitution of D42 and D169 with the neutral residues alanine and asparagine identified these two aspartic acids as catalytically significant residues in PRK; rates diminished by 10^5- and 10^4-fold, respectively. X-ray structural studies confirmed the location of these residues in the catalytic site and suggested a role for D42 as the catalytic base which deprotonates the C1 hydroxyl of Ru5P; the role for D169 is less clear. To further explore the importance of anionic groups at these positions, substitutions with glutamic acid (D42E and D169E) and cysteine (D42C and D169C in an otherwise cysteine-free protein) were constructed, expressed, and evaluated. All mutant enzymes responded similarly to WT in binding studies with the fluorescent probe TNPATP and the allosteric effector NADH. For D42E and

D42C, V_{max} decreased by factors of 155 and 253, respectively, whereas for D169E and D169C the comparable factors were 30 and 45. Thus, under standard assay conditions, these anionic substitutions maintain the catalytic rate differential observed for the original neutral substitutions at these positions. Both cysteine mutant PRKs showed pK_a values altered from the WT value (pK_a 6.8); D42C shifted to 7.3 and D169C to 7.8, possibly indicating the need for the thiolate form of these residues. Upon $NaIO_4$ oxidation of the cysteine mutant enzymes to form sulfonic acids, the pK_a values decreased to 7.1 and 7.3, and catalytic rates increased by 5- and 7-fold, respectively. Thus, for PRK, the side chains of residues 42 and 169 require anionic character for reasonably efficient catalysis, although some structural and chemical variability in this anionic character is tolerated. (Supported by DOE Grant DE-FG02-OOER15100.)

32. Cloning, characterization, and crystallization of a transaminase involved in the biosynthesis of aminoglycoside antibiotics. Jonathan B. Spencer,[1] **Fanglu Huang**,[1] Xiaohong Tang,[1] Dimitri Chirgadze,[2] Yanyan Li,[1] and Tom L. Blundell.[2] [1]Cambridge Centre for Molecular Recognition, Department of Chemistry, and [2]Cambridge Centre for Molecular Recognition, Department of Biochemistry, Cambridge University, Lensfield Rd., Cambridge CB2 1EW, United Kingdom (fax: 0044-1223-336362, jbs20@cam.ac.uk, fh223@cam.ac.uk)

Butirosin is a member of the 2-deoxysteptamine (DOS)-containing family of aminoglycoside antibiotics. An important approach in fighting antibiotic resistance is modification of known antibiotics or even production of new antibiotics by manipulation of antibiotic biosynthesis. This requires an understanding of how antibiotics are synthesized in their producing strains. Recently part of the gene cluster involved in the biosynthesis of butirosin in *Bacillus circulans* has been sequenced. One of the genes, *btrC* that codes for 2-deoxy-*scyllo*-inosose synthase (DOI), was cloned and characterized. In our present study, we have carried out further sequencing of the cluster by gene-walking. An open reading frame, *btrR*, among the newly identified sequence, has been cloned and overexpressed in *Escherichia coli*. Functional studies have determined that BtrR is the enzyme responsible for the transamination of DOI using L-glutamine as amino donor to produce 2-deoxy-*scyllo*-inosamine (DOIA). BtrRs were also shown to transaminate DOS using pyruvate as cosubstrate. The crystal structure of the protein has been obtained and analyzed with a resolution at 2.1 Å.

33. Coenzyme binding by horse liver alcohol dehydrogenase: evaluating the role of charge at position 228. **Henry A. Charlier, Jr.**,[1] C. Mark Maupin,[1] and Bryce V. Plapp,[2] [1]Department of Chemistry, Boise State University, 1910 University Dr., Boise, ID 83725-1520 (fax: 208-426-3027, hcharlier@chem10.boisestate.edu), and [2]Department of Biochemistry, The University of Iowa

Horse liver alcohol dehydrogenase oxidizes alcohols using NAD^+, and coenzyme release is rate-limiting in catalysis. Chemical modification and X-ray crystallography studies suggest that the positive charge of lysine 228 (K228) is important for coenzyme binding. Site-directed mutagenesis was used to test this hypothesis by substituting lysine with arginine (previously reported), alanine, glutamine, or glutamate. K228R ADH binds NAD^+ and AMP with affinities similar to native enzyme. K228A and K228Q enzymes bind NAD^+ and AMP significantly less well than does native enzyme. K228E ADH binds NAD^+ with less affinity than K228A and K228Q enzymes. Preliminary molecular dynamics calculations show a high correlation between experimental changes in Gibbs free energy for AMP binding and computational electrostatic and H-bonding changes in energy. The results indicate that coenzyme binding to ADH is significantly decreased as the charge at position 228 goes from positive to negative. (Supported by USPHS Grants F32 AA05330 and T32 HL07344.)

34. Cold denaturation of RNA—implications for the thermodynamics of RNA folding. **Andrew L. Feig** and Peter J. Mikulecky. Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300, afeig@indiana.edu)

A long-held goal in RNA biochemistry and biophysics is to be able to predict the structure of a molecule from its sequence alone. Secondary structure prediction programs use thermodynamic calculations to compare the relative stabilities of "folded" species. These programs can only be as accurate as the calculation that lies at their heart. These calculations use a common assumption in RNA biochemistry that the heat capacity change of RNA folding (delta-C_p) is negligible. We recently showed that the hammerhead ribozyme unfolds at sufficiently low temperatures—the first direct observation of cold denaturation for any nucleic acid. This process only occurs if the delta-C_p of folding is large, however. We will present our studies on the hammerhead ribozyme, as well as work showing the generality of this phenomenon. We have compared the cold denaturation of the catalytic domain of P RNA from a thermophile (*B. stereothermophilus*) and a mesophile (*B. subtilis*) and shown that the thermophilic sequence is much more susceptible to cold denaturation than the mesophilic one—a prediction made by analogy to protein cold denaturation. One particularly exciting system wherein biology may have taken advantage of the temperature dependence of RNA structure is the cold shock response in *E. coli*, an adaptation mediated by a small, noncoding RNA called DsrA. By using a combination of biochemical and spectroscopic methods, we have been investigating the thermodynamics of DsrA, looking at its temperature-dependent folding and its interactions with the 5′-UTR of RpoS mRNA and the Sm-like protein Hfq.

35. Controlling protein activity by ligand-regulated RNA aptamers. **Momchilo Vuyisich** and Peter A. Beal. Department of Chemistry, University of Utah, 315 S. 1400 East, Salt Lake City, UT 84112 (fax: 801-581-8433, momo@chem.utah.edu)

Controlling the activity of a gene product can be useful in trying to understand its function. Using systematic evolution of ligands by exponential enrichment (SELEX), RNA ligands (aptamers) for many proteins and small molecules have been found. We wished to utilize this method to discover an RNA aptamer that would bind both a protein and a small molecule in a mutually exclusive fashion, such that the RNA's binding to the protein could be regulated by

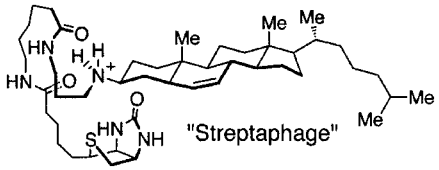
the small molecule (inducer). We used the SELEX protocol to raise RNA aptamers for a DNA repair enzyme, formamidopyrimidine glycosylase (Fpg), and employed neomycin in the elution step to dissociate Fpg-bound RNAs. After 23 rounds of selection, we identified an aptamer with the desired properties. This 91 nt RNA is able to completely inhibit Fpg at 100 nM concentration, but the enzymatic activity is nearly fully recovered in the presence of micromolar concentration of neomycin.

36. Crystal structure and potential applications of a rare earth binding antibody for biomedical imaging and therapy. Todd M. Corneillie,[1] Paul A. Whetstone,[1] Jeffrey H. Walton,[2] Andrew J. Fisher,[1] and Claude F. Meares,[1] [1]Department of Chemistry and [2]NMR Facility, University of California, One Shields Ave., Davis, CA 95616 (fax: 530-752-3386, tmcorneillie@ucdavis.edu)

The recently solved crystal structure of the monoclonal antibody 2D12.5 bound to its hapten, a derivative of yttrium-(III)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (Y-DOTA), is being used to engineer a new system for targeted cancer therapy. The nature of the binding interaction and similar chemical behavior of the trivalent rare earths has led to the discovery that 2D12.5 binds all of the rare earth DOTA chelates with high affinity. This expands the potential applications of antibody 2D12.5 in vitro and in vivo to include radiotherapy (^{90}Y, ^{177}Lu) as well as a variety of imaging techniques including gamma imaging (^{177}Lu), PET (^{86}Y), MRI (Gd), and 2D12.5 enhanced lanthanide luminescence (Eu, Tb, Dy, Yb). ^{177}Lu is complementary to ^{90}Y because of its lower energy γ-emission (0.50 MeV for ^{177}Lu compared to 2.2 MeV for ^{90}Y) and consequent decreased tissue penetration. Gd-chelates bound to macromolecules can enhance the relaxivity of surrounding solvent water molecules relative to unbound Gd-chelates and may be useful for targeted MRI. When Tb-DOTA binds to it, 2D12.5 greatly enhances terbium's luminescence intensity. Structural details and relative binding affinities between the various trivalent rare earths will be presented in addition to preliminary results describing how 2D12.5 may be useful for MR and luminescence imaging.

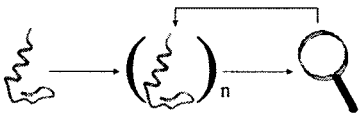
37. Delivery of streptavidin-linked toxins to cancer cells with Streptaphage: protein uptake regulated by a membrane-anchored synthetic ligand. Stephen L. Hussey and Blake R. Peterson. Department of Chemistry, The Pennsylvania State University, 152 Davey Lab, University Park, PA 16802 (fax: 814-863-8403)

The efficient delivery of macromolecules to tumor cells presents a formidable challenge to the development of effective macromolecular anticancer therapeutics. We describe here a novel synthetic ligand termed Streptaphage that promotes cellular uptake of the bacterial protein streptavidin by promoting noncovalent interactions with lipid raft subdomains of cellular plasma membranes. The ligand comprises an *N*-alkyl derivative of 3β-cholesterylamine linked to the carboxylate of biotin through an 11-atom tether. Molecular recognition between fluorescent conjugates of streptavidin and this ligand at plasma membranes promotes clathrin-mediated endocytosis, which renders streptavidin partially intracellular within 10 min and completely internalized within 4 h of protein addition. Recent efforts directed at ligand-regulated ablation of cancer cells with toxins conjugated to streptavidin will be described.



38. Design and characterization of oligomeric mini-protein motifs. Mayssam H. Ali, Kevin A. McDonnell, Adam R. Mezo, and Barbara Imperiali. Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (mhali@mit.edu)

De novo mini-protein design is a powerful tool for the elucidation of the physical forces that determine protein structure. Extension to an oligomeric system enables us to study the quaternary interactions found in many proteins. BBA5, a 23 amino acid $\beta\beta\alpha$ peptide based upon a zinc finger motif, was selected as the monomer unit for our oligomerization study. Shortening the linker between the BBA5 helix and hairpin regions exposes its hydrophobic core and produces a peptide prone to self-association. A BBA peptide that exists as a discrete trimer in aqueous solution has been identified from a small library. Biophysical and mutational studies have been used to characterize the trimer, and for the discovery of dimeric and tetrameric BBAs. Structural determination, including X-ray crystallographic and NMR analysis, is ongoing, and will be of significant value in the rational design of additional oligomers of defined aggregation state.



39. Determining how phosphorylation affects translational activator Vasa, a germline specific DEAD-box protein. Evelyn Jabri, Tara Lorenz, and Theresa Day. Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300, ejabri@indiana.edu)

Proper development of germ cells, precursors to egg and sperm, requires extensive regulation of translation to ensure fertility and prevent improper growth that could lead to ovarian or testicular cancers. Germ cell differentiation in many organisms shares a common RNA helicase, Vasa, which is a member of the DEAD-box protein family. We have generated *Drosophila* Vasa protein in quantities and purities sufficient for biochemical and structural studies. Furthermore, we have determined by MALDI-TOF-MS that Vasa, expressed in insect cells, is phosphorylated on at least two residues. These sites map to the C-terminal region of the DEAD-box motif of this protein. Comparative sequence and structure analyses suggests that phosphorylation in either region could affect the conformation, substrate (ATP and RNA) binding, turnover, or Vasa's ability to form protein:protein complexes. Progress toward identifying the exact

sites of modification as well as determining the effect of phosphorylation on Vasa activity will be presented.

40. Development of a safety-catch linker for the release of electrophiles. Peter J. Belshaw, **Christopher J. Ciolli**, Konstantin Levitsky, and Sean Kalagher. Departments of Chemistry and Biochemistry, University of Wisconsin−Madison, 1101 University Ave., Madison, WI 53706 (fax: 608-265-4534, belshaw@chem.wisc.edu, ciolli@chem.wisc.edu)

Development of a novel safety-catch linker for attachment of combinatorial library members to solid phase synthesis resins is presented. Cleavage of the linker generates an electrophilic reactive site providing each member of the library with the ability to covalently alkylate protein targets. Solid-phase synthesis and cleavage of the linker have been adapted to 500−600 μm polystyrene macrobeads, making the linker design amenable to the "one-bead, one-stock solution" approach of chemical genetics. The alkylation potential of the electrophilic reactive site has been assessed employing analogues of the immunosuppressant cyclosporine and mutants of its binding protein cyclophilin.

41. DFT calculations and NMR studies of α-D-Man*p*-[1→2]-α-D-Man*p* disaccharides: molecular geometries and trans-*O*-glycosidic $^3J_{COCH}$, $^3J_{COCC}$, and $^2J_{COC}$. Thomas E. Klepach,[1] Christophe Thibaudeau,[1] Shikai Zhao,[2] Ian Carmichael,[3] and Anthony S. Serianni.[1] [1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 (fax: 219-631-6924, Thomas.E.Klepach.1@nd.edu), [2]Omicron Biochemicals, Inc., and [3]Notre Dame Radiation Laboratory

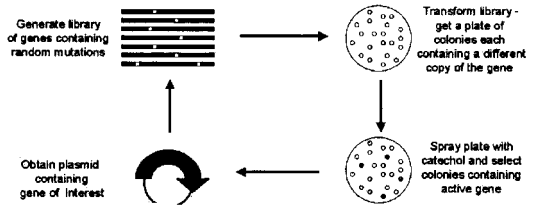
Solution NMR of enzymically prepared ^{13}C-labeled methyl α-Man*p*-[1→2]-α-Man*p* and DFT (B3LYP/6-31G*) calculations of α-D-Man*p*-[1→2]-α-D-Man*p* disaccharide mimics were used to investigate structure, conformations, and trans-*O*-glycoside Φ and Ψ torsion-dependent scalar couplings (*J*). Experimental $^3J_{C2,C2'}$ and $^2J_{C1,C2'}$ of 3.7 Hz and −1.7 Hz, respectively, interpreted using a theoretically determined Karplus relationship ($^3J_{CC}$) and the projection resultant rule ($^2J_{CC}$), indicated 60S as the predominant Φ rotamer. Interpretation of the small experimental $^3J_{C1,C1'}$ (<0.7 Hz) using a theoretical Karplus relationship ruled out the 60S orientation for Ψ while a similar interpretation of $^3J_{C1,H2'}$ suggested that the major Ψ torsional sampling occurs in the 60R regime. In the preferred exo-anomeric conformation ($\Phi = -60°$) where O2 lies approximately in the C2−C1−O1−C2′ coupling plane, an experimental ∼0.6 Hz enhancement in the $^3J_{CC}$ was observed over an analogous coupling pathway containing an equatorial O2, as predicted previously [(1998) *J. Am. Chem. Soc. 120*, 11158].

42. Dimerization of a pathogenic human mitochondrial tRNA. Lisa M. Wittenhagen and Shana O. Kelley. Department of Chemistry, Boston College, Merkert Chemistry Center, 2609 Beacon St., Chesnut Hill, MA 02467 (lisa.wittenhagen@bc.edu)

Human mitochondrial transfer RNA (tRNA) mutations are implicated in a variety of multisystemic diseases. The most prevalent pathogenic mitochondrial mutation is an A3243G substitution in the gene for $tRNA^{Leu(UUR)}$. Here, a dramatic structural change promoted by this mutation is described. The A3243G mutation promotes the formation of a tRNA dimer that strongly self-associates under physiological conditions. The dimerization interface in the mutant tRNA is a self-complementary hexanucleotide in the D-stem, a particularly weak structural element within $tRNA^{Leu(UUR)}$. Mutational studies demonstrate that dimerization attenuates aminoacylation of the mutant tRNA. The perturbation of a conserved tertiary structural contact also contributes to the loss of function observed. It is proposed that the pathogenic mutation interferes with the cellular function of human mitochondrial $tRNA^{Leu(UUR)}$ by promoting the formation of a structure with low biological activity.

43. Directed evolution of intradiol and extradiol catechol dioxygenases. Kirstin Eley,[1] Timothy D. H. Bugg,[1] and Patrick Crowley.[2] [1]Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom, and [2]Syngenta

Catechol dioxygenases are nonheme iron dependent enzymes that utilize O_2 and either Fe^{III} (intradiol) or Fe^{II} (extradiol) in the cleavage of catechol—a biodegradation product of aromatics. Directed evolution involves the high through-put screening of a library of mutants, and the bright yellow color of the extradiol cleavage product provides a convenient assay to screen for dioxygenase activity. The aim is to create dioxygenases with altered substrate specificity—from enzymes that cleave catechols containing electron-donating groups to ones that cleave catechols containing electron-withdrawing groups. The extradiol enzymes involved this study share sequence identity of around 40−50%, so the two methods of library generation used are error-prone PCR and primer-based family shuffling. Also, as intradiol and extradiol enzymes are believed to share a common reaction intermediate, it is envisaged that the conversion of an intradiol dioxygenase to an enzyme with extradiol dioxygenase activity will aid understanding of the mechanistic differences between the enzymes.



44. Effect of alkanols on cell growth, sporulation, and lipase production of *Bacillus licheniformis* S-86. Sebastián Torres, Mario D. Baigorí, and **Guillermo R. Castro**. Laboratorio de Biocatálisis, PROIMI−Biotechnology, Av. Belgrano y Caseros, Tucumán, 4000, Argentina (fax: 54-381-4344887, sebatk@hotmail.com, castroguillermor@hotmail.com)

Alcohol-tolerant *Bacillus licheniformis* S-86 produces stable lipases in organic solvents. The effect of seven alkanols on cell growth, sporulation, and lipase production was studied. Relationships between maximal alcohol concentration tolerated and log *P* were for 2,3-butanediol 6.0%:−0.86; ethanol 4.0%:−0.24; propan-2-ol 3.0%:0.28; butan-1-ol 0.9%:0.80; 3-methylbutan-1-ol 0.6%:1.30; hexan-1-ol 0.1%:

1.86; and heptan-1-ol 0.05%:2.39, respectively. Alkanol addition reduced biomass after exponential growth phase between 30%(0.4% 3-methylbutan-1-ol) to about 90%(0.1% hexan-1-ol). Specific activity of lipases was approximately 2-fold higher in cultures supplemented with C3−C5 alkanols than the control. Two different lipases activities were observed. One of them was inhibited by PMSF, and the other was not. In thepresence of 0.4% 3-methylbutan-1-ol, PMSF-sensitive and PMSF-resistant lipase specific activities were increased 2 and 4 times, respectively. Sporulation was strongly depleted compared to the control by 10^4-fold CFU/mL in the presence of 3-methylbutan-1-ol. Lipase production could not be linked to the sporulation process.

45. Effect of metals and pH on the stability and catalytic activity of *Chromobacterium violaveum* phenylalanine hydroxylase. Jerome Zoidakis, Kim Vu, and Mahdi M. Abu-Omar. Department of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Ave., Los Angeles, CA 90095 (zoidakis@chem.ucla.edu)

Phenylalanine hydroxylase from *Chromobacterium violaceum* catalyzes the conversion of phenylalanine to tyrosine. The enzyme is a monomer of 33.5 kDa and contains one non-heme iron. The effect of temperature and various metals on the stability and catalytic activity of the enzyme was determined by steady-state kinetics, circular dichroism (CD), and differential scanning calorimetry (DSC). Iron is required for catalytic activity, and other metals inhibit the enzyme, with cobalt and zinc having the most marked effect. The melting temperature of the protein is 53 ± 2 °C in the presence of EDTA and 63 ± 2 °C in the presence of iron or cobalt. The enzyme has optimal activity and stability at pH 7.4. The k_{cat} and K_m for phenylalanine were determined at different temperatures. The K_m remained constant between 20 and 40 °C but rapidly increased below 20 °C.

46. Engineering a protease to cleave phosphotyrosine substrates. Zachary A. Knight[1] and Kevan Shokat.[2] [1]Program in Chemistry and Chemical Biology and [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 513 Parnassus, San Francisco, CA 94143-0450 (knight@itsa.ucsf.edu)

The facile identification of protein phosphorylation sites remains a major challenge for proteomics. The availability of a protease that specifically cleaves its substrates at sites of phosphorylation would greatly simplify such efforts. To this end, we have designed and characterized a small library of 52 variants of the serine protease subtilisin BPN′ that have been rationally designed to cleave phosphotyrosine substrates. By combining single changes, we have identified double mutants that cleave a phosphotyrosine peptide in preference to the optimal wild-type substrate, and exhibit specificity switches of up to 10 000-fold. We further show that the optimal variants retain high specific activity, and that their specificity profile has been selectively altered to include phosphotyrosine; peptides containing a variety of P1 amino acids, including the acidic side chains of phosphoserine, phosphothreonine, aspartate, and glutamate, remain very poor substrates for the engineered enzyme. Additional experiments are described.

47. Evaluation of binding of important pharmaceutical compounds to separated alpha-1 acid glycoprotein (OMD). Elizabeth A. Shell and H. B. Halsall. Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221

The OMD protein within humans is very heterogeneous. The human protein has multiple gene products varying in amino acid sequences. The five N-linked glycan sites also vary with large changes in branching and sialic acid content occurring during disease. The genetic variants and the glycoforms of the protein can differentially bind drugs, causing drug dosage issues. While methods have been created to separate out the protein heterogeneity, the methods often do not leave enough protein for conventional drug binding studies. Immobilization on the BIAcore 2000 instrument, an optical biosensor using surface plasmon resonance to detect binding interactions, allows small amounts (μg−pg) of separated OMD protein to be evaluated for drug binding. Some common drugs were introduced to the different OMD variants for evaluation of binding. Results indicate that differential drug binding can be measured for the diverse OMD subclasses, allowing for high-throughput evaluations of drug binding.

48. Evidence for glutamate-79 as a ligand to the divalent cation activator of HMG-CoA lyase. Robbyn L. Tuinstra and Henry M. Miziorko. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (fax: 414-456-6570, rtuinstr@mcw.edu)

HMG-CoA lyase catalyzes the cleavage of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to form acetyl-CoA and acetoacetate. The enzyme requires a divalent cation (e.g., Mg^{2+}, Mn^{2+}) and sulfhydryl reagents (e.g., DTT) for activity. To identify amino acids that may support acid/base catalysis or ligation of the activator cation, a survey of invariant acidic residues was conducted using site-directed mutagenesis to eliminate side chain carboxyl residues. The survey focused on acidic residues that are invariant not only in HMG-CoA lyase but also in the entire family of HMG-CoA lyase related enzymes. Of several mutants characterized, E79A was the most notable. A reduction in V_m of almost 200-fold is observed ($V_{m\ WT}$ = 190 units/mg; $V_{m\ E79A}$ = 1 unit/mg). K_m for divalent cation substantially increases (for Mg^{2+}, $K_{m\ WT}$ = 233 μM; $K_{m\ E79A}$ = 5200 μM; for Mn^{2+}, $K_{m\ WT}$ = 0.3 μM; $K_{m\ E79A}$ = 43 μM). The possible effect on cation binding was more directly addressed by ESR measurements on binary Mn^{2+}−enzyme complexes. Scatchard analysis indicates that E79A stoichiometrically binds Mn^{2+} with a $K_{D\ Mn}$ (16 μM) that is elevated by an order of magnitude in comparison with the comparable $K_{D\ Mn}$ (1.5 μM) measured for wild-type enzyme. These data suggest a role for E79 in ligation of the divalent cation activator of HMG-CoA lyase. [Supported by NIH Grant DK21491 and by a fellowship (R.L.T.) from the American Heart Association.]

49. Fluorescence and two-photon excitation spectroscopy of peridinin in solution and in the peridinin−chlorophyll *a*−protein complex. Robielyn P. Ilagan,[1] **Sumie Shima**,[1] Nathan Gillespie,[1] Jesusa S. Josue,[1] Roger G. Hiller,[2] Frank P. Sharples,[2] Robert R. Birge,[1] and Harry A. Frank.[1] [1]Department of Chemistry, University of Connecticut, 55 N. Eagleville Rd., Storrs, CT 06269 (fax: 860-486-2981, robielyn.ilagan@uconn.edu, sumie.shima@uconn.edu), and [2]Department of Biological Sciences, Macquarie University

Peridinin in solution and in the peridinin−chlorophyll *a*−protein (PCP) complex from the dinoflagellate *Amphidinium carterae* have been investigated using fluorescence and two-photon excitation spectroscopy. Fluorescence from peridinin in solution is associated predominantly with the $S_1 \rightarrow S_0$ transition of the carotenoid and is substantially red-shifted relative to the strongly allowed $S_0 \rightarrow S_2$ absorption. The two-photon excitation spectrum of peridinin was obtained by monitoring the fluorescence spectrum at its maximum intensity. The spectrum is observed to have significant overlap with the one-photon absorption spectrum. In the isolated PCP complex, the two-photon excitation spectrum was detected using chlorophyll fluorescence. The spectrum was observed to be slightly red-shifted compared to the one-photon absorption profile. The two-photon data indicate strong mixing between the low-lying strongly allowed ionic state and a lower-lying forbidden covalent state. The mixing increases in the protein binding site and in polar solvent, and enhances the charge-transfer character of the strongly allowed state.

50. From two-state to three-state: effect of P61A mutation on the dynamics and stability of the factor for inversion stimulation results in an altered equilibrium denaturation mechanism. Sarah A. Hobart,[1] **Derrick W. Meinhold**,[1] Robert Osuna,[2] and Wilfredo Colón.[1] [1]Department of Chemistry, Rensselaer Polytechnic Institute, Cogswell Building, 110 Eighth St., Troy, NY 12180 (fax: 518-276-4887, hobars@rpi.edu, meinhd@rpi.edu), and [2]Department of Biology, State University of New York

Factor for inversion stimulation (FIS) is a 22.4 kDa homodimeric DNA binding protein found in enteric bacteria. It is involved in various cellular processes, including stimulation of DNA recombination events and transcription regulation of many genes. FIS has a central helix with a 20° kink, which is only reduced by 4° upon mutation of the proline at position 61 to alanine (P61A). As this mutation also appears to have no effect on FIS function, it is not clear why proline 61 is conserved in FIS. Therefore, we investigated the role of proline 61 on the stability and flexibility of FIS. The urea-induced equilibrium denaturation of P61A FIS was monitored by far- and near-UV circular dichroism, and fluorescence anisotropy. Despite the apparent two-state transition, the concentration dependence of the transition slope (*m* value) made it clear that a two-state denaturation mechanism, as seen for WT FIS, did not adequately describe the denaturation of P61A FIS. Global fitting of the data indicates that P61A FIS unfolds via a three-state mechanism involving a dimeric intermediate, and has an overall ΔG_{H2O} for unfolding of 18.5 kcal/mol, 4 kcal/mol higher than that for WT FIS. Limited trypsin proteolysis of WT and P61A FIS, analyzed by SDS−PAGE and mass spectrometry, demonstrated that P61A has increased flexibility in the C-terminal DNA binding region. Thus, our results suggest that despite decreasing the global stability of FIS, proline 61 may be important for modulating the dynamics and stability of the functionally critical C-terminus of FIS.

51. Functional modification of human serum albumin by lipid peroxidation by-products. Amy M. Pollock and David W. Seybert. Department of Chemistry & Biochemistry, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282 (fax: 412-396-5683, pollock958@duq.edu)

Lipid peroxidation is a free radical chain reaction that yields lipid hydroperoxides as major reaction products and also leads to the production of a variety of α,*β*-unsaturated aldehydes. These aldehydes can covalently modify proteins and result in fluorophore generation. Two α,*β*-unsaturated aldehydes, *E*-2-hexenal and *E*-2-octenal, were independently incubated with fatty acid free human serum albumin (HSA) at 37 °C. 11-(Dansylamino)undecanoic acid (DAUDA), a fluorescent fatty acid analogue, was used as a probe to monitor binding at the medium-chain fatty acid (bilirubin) binding sites of HSA. Differences in the rates of development of protein fluorescence and the loss of DAUDA binding of aldehyde-modified HSA vs unmodified HSA were observed. A comparison of the kinetics for development of protein fluorescence and functional modification will be presented.

52. Functional role of the prokaryotic proline-tRNA synthetase insertion domain in amino acid editing. Fai-Chu Wong,[1] Penny J. Beuning,[2] Maria Nagan,[3] and Karin Musier-Forsyth.[1] [1]Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN 55455 (fcwong@chem.umn.edu), [2]Department of Biology, Massachusetts Institute of Technology, and [3]Science Division, Truman State University

Faithful translation of genetic information is critical for the survival of all organisms. During tRNA aminoacylation by aminoacyl-tRNA synthetases, noncognate amino acids can be mis-activated during the first chemical step. The mis-activated noncognate aminoacyl adenylates can then be transferred to the wrong tRNA. Thus, to maintain high accuracy during protein translation, some synthetases have evolved an editing mechanism. In pre-transfer editing, the noncognate aminoacyl adenylate is hydrolyzed by the synthetase in either a tRNA-dependent or a tRNA-independent manner. In post-transfer editing, a tRNA aminoacylated with a noncognate amino acid is deacylated. Previously, our lab showed that class II *Escherichia coli* prolyl-tRNA synthetase (ProRS) is capable of hydrolyzing the mis-activated Ala-AMP and deacylating a mis-charged Ala-tRNAPro variant. Through alignment-guided mutagenesis and domain deletion study, we have determined catalytic amino acid residues involved in the editing function. In addition, we are also investigating species-specific differences in the editing activity of ProRS.

53. Glycopeptide antibiotics: insight into the biosynthesis of (*S*)-3,5-dihydroxyphenylglycine. Jonathan B. Spencer, **Tsung-Lin Li**, and Oliver W. Choroba. Cambridge Centre for Molecular Recognition, Department of Chemistry, Cambridge University, Lensfield Rd., Cambridge CB2 1EW, United Kingdom (jbs20@cam.ac.uk, tll24@cam.ac.uk)

The type I glycopeptide antibiotics vancomycin and teicoplanin are currently the drugs of last resort in the treatment of methicillin-resistant *Staphylococcus aureus* infections The knowledge of the biosynthetic pathway of glycopeptide antibiotics will enable us to generate novel antibacterial drugs in the fight against resistant bacteria. Therefore, we are undertaking research into the elucidation of the biosynthesis of this important class of compound. One of the rare amino acid constituents of type I antibiotics is (*S*)-3,5-dihydroxyphenylglycine (DHPG), **3**. The biosynthesis

of this nonproteinogenic amino acid is thought to be achieved by five enzymes, four of which are organized in an operon-like fashion. In the gene cluster of chloroeremomycin, open reading frames (Orf) 27−30 convert four molecules of malonyl-CoA **1** to 3,5-dihydroxyphenylglyoxylic acid **2** which is subsequently transaminated by an upstream transaminase (coded for by *orf*17) to DHPG **3**. Here we present stable isotope (^{2}H and ^{13}C) labeling experiments which prove that Orf 27 acts as a type III polyketide synthase (PKS). This is only the second characterized bacterial type III PKS, and we could show that malonyl-CoA acts both as the starter and as extender units.

54. Identification of protein−protein interactions mediated by interchain *β*-sheet formation. Pierre F. Baisnee, Gianluca Pollastri, Pierre F. Baldi, and **James S. Nowick**. Department of Information and Computer Science, University of California, Irvine, Irvine, CA 92697 (fax: 949-824-8571, pbaisnee@uci.edu, jsnowick@uci.edu)

Interactions between the edges of protein *β*-sheets occur widely in the formation of protein quaternary structures, in interactions between proteins, and in protein aggregation. In this under-appreciated mode of molecular recognition between proteins, hydrogen bonds form between the edges of protein *β*-sheets. The edges of each *β*-sheet partner are studded with an alternating array of hydrogen bond donors and acceptors with the pattern donor−acceptor−space, donor−acceptor−space, etc. These complementary edges hydrogen bond together and stabilize the partnership in conjunction with many other noncovalent forces (e.g., hydrophobic, van der Waals, salt-bridges, and hydrogen bonding). This presentation will describe and illustrate the structural role of interchain *β*-sheet contacts in protein−protein interactions that are central to healthy biological function and diseases ranging from AIDS and cancer to Alzheimer's and Huntington's diseases and will present a database that identifies these interactions within entries in the Protein DataBank and ranks their relative importance. This database will be made available at: http://www.igb.uci.edu/servers/icbs/.

55. Identification of the active site triad of homoserine transacetylase. Timothy L. Born and John Khuu. Department of Chemistry, George Mason University, MSN 4D7, 10900 University Blvd., Manassas, VA 20110 (fax: 703-993-4581, tborn@gmu.edu)

The first unique step in bacterial methionine biosynthesis is the acylation of homoserine by succinyl-CoA or acetyl-CoA to form *O*-acylhomoserine. We have cloned, expressed, and purified homoserine transacetylase (HTA) from *H. influenzae* and monitored enzyme activity using acetyl-CoA and homoserine. Initial velocity studies indicate a ping-pong kinetic mechanism involving covalent labeling of HTA with acetate. Sequence comparisons suggest that HTA is a member of the prokaryotic lipase superfamily, members of which utilize a catalytic triad of Ser-Asp-His. Site-directed mutagenesis identified serine-143 as the catalytic nucleophile. Mutation to alanine produced an enzyme with no measurable activity, while mutation to cysteine produced an enzyme retaining ∼5% of wild-type activity. Additional mutagenesis experiments suggest that histidine-337 is the second member of the catalytic triad. Mutation of aspartic acid-304 to alanine produced an enzyme that catalyzes the first half-reaction but is unable to catalyze the second half-reaction.

56. Insights into monoterpene cyclization reactions in biology: crystal structure of (+)-bornyl diphosphate synthase. Douglas A. Whittington,[1] Mitchell L. Wise,[2] Rodney Croteau,[2] and David W. Christianson.[1] [1]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 (dwhit@anchor.chem.upenn.edu), and [2]Institute of Biological Chemistry, Washington State University

Monoterpenes, the simplest of the terpenoid family of natural products, are formed by monoterpene synthase (cyclase) enzymes acting on the linear, achiral substrate geranyl diphosphate. Despite sequence and mechanistic similarities, these enzymes produce distinct products. To investigate the role of the protein in directing the reaction specificity, we have determined the crystal structure of (+)-bornyl diphosphate synthase (BPPS) from common sage (*Salvia officinalis*) to 2.0 Å resolution using MAD phasing of Se-Met containing protein. (+)-BPPS is a homodimer. Each monomer is structurally similar to the sesquiterpene cyclase 5-epi-aristolochene synthase, and the C-terminal catalytic domain bears the "terpenoid synthase fold." The active site consists of a 12 Å deep pocket on the surface of the cyclase domain flanked by sequence motifs known to be involved in binding of essential Mg^{2+} ions. Analysis of the active site structure with regard to the proposed mechanism of geranyl diphosphate cyclization to (+)-bornyl diphosphate is presented.

57. Investigation of the *Saccharomyces cerevisiae* oligosaccharyl transferase. R. E. Dempski, Jr., K. J. Glover, and Barbara Imperiali. Department of Chemistry, Massachusetts Institute of Technology, 400 Main St., 18-226, Cambridge, MA 02139 (fax: 617-452-2799)

The yeast oligosaccharyl transferase is a multimeric membrane-bound enzyme which catalyzes the transfer of a lipid-linked tetradecasaccharide to asparagine side chains of a nascent protein in the endoplasmic reticulum (ER), immediately after the protein is translocated through the ER membrane. The enzyme consists of at least nine integral membrane proteins, of which four are essential for in vitro activity [Nlt1p (Ost1p), Ost2p, Swp1p, and Wbp1p]. Three of the essential proteins have significant lumenal domains (Nlt1p, Swp1p, and Wbp1p), and two of the proteins contain N-linked glycosylation sites (Nlt1p and Wbp1p). The four essential proteins have been cloned, expressed, and purified in the baculovirus−insect cell system. We are currently working on the reconstitution of these subunits in model membranes with the ultimate goal of obtaining an active complex.

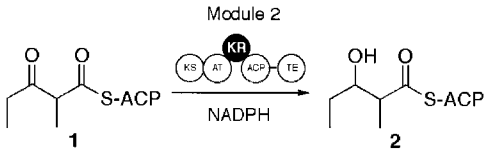
58. Investigation of the role of zinc(II) in mylein basic protein. Elisha D. Pendleton and Dr. Sonya J. Franklin. Department of Chemistry, University of Iowa, 337 Chem Building, Iowa City, IA 52242 (donshell@rocketmail.com)

The goal of this research is to determine the role of zinc(II) in the neuroprotein myelin basic protein (MBP). MBP is one of the autoimmune targets in the disease multiple sclerosis, and was only recently reported to be a Zn-binding

protein [Tsang (1997) *Neurochem. Res*.]. Elucidating the structure and function of zinc in MBP will give insight into the mechanism of nerve myelination, and this could lead to new therapeutic approaches for demylinating diseases such as MS. The Zn-binding affinity and coordination environment have been investigated by circular dichroism, equilibrium dialysis, and cloning of the different exons that are a part of the protein. Progress toward determining the number, location, and the affinities of the zinc-binding sites will be discussed.

59. Investigations into the activity of the KR domain of DEBS module 2. Lynne A. Collett and David E. Cane. Department of Chemistry, Brown University, Providence, RI 02912 (Lynne_Collett@Brown.edu)

The systematic study of the mechanism of catalysis of individual domains of modular polyketide synthases has remained a challenge in investigating the biosynthesis of polyketide macrolides. In view of the hypothesis that the homodimer is the true catalyst in the full DEBS complex, it is anticipated that β-ketoacyl-ACP analogue **1** will be a substrate for reduction by the ketoreductase (KR) domain in module 2, giving the diketide-ACP **2**. The chemical and enzymatic synthesis of substrate analogue **1** is described. Incubation of **1** with active DEBS module 2 in the presence of NADPH, but without the usual extender unit, methylmalonyl CoA, will allow an analysis of the activity of KR2 as the remaining domains in module 2 are not involved in the transformation, acting only as spectators.



60. Is DNA hairpin formation diffusion limited? Serguei V. Kuznetsov, Yiqing Shen, and **Anjum Ansari**. Department of Physics (M/C 273), University of Illinois at Chicago, 845 W. Taylor St., Room 2236, Chicago, IL 60607 (fax: 312-996-9016, skouznet@uic.edu, ansari@uic.edu)

The characteristic time for forming DNA hairpins ($\sim$10 μs at 25 °C) is more than 2 orders of magnitude slower than the diffusion-limited time ($\sim$40 ns) for forming a contact between the two ends of a single-stranded DNA $\sim$12 bases long. There are two possible explanations for this difference: (1) hairpin formation is reaction limited; (2) transient traps from misfolded loops impede hairpin formation. To address this issue, we have monitored the kinetics of unwinding, after temperature-jump, with varying solvent viscosity. The characteristic opening/closing times are nearly independent of the viscosity. These results contradict earlier measurements on the viscosity dependence of equilibrium fluctuations of DNA hairpins [Wallace et al. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 5584], where the opening/closing times were found to scale with the viscosity with an exponent of $\sim$0.8. Incorrect compensation of changes in stability from addition of viscogenic cosolvents is most likely the reason for the discrepancy between the two sets of measurements.

61. Kinetic investigations of the activation of soybean lipoxygenase-1 by 13-*S*-hydroperoxy-9-*cis*,11-*trans*-octadecadienoic acid and the influence of inhibitors. Viola C. Ruddat, Ted Holman, and Claude F. Bernasconi. Department of Chemistry and Biochemistry, University of California, Santa Cruz, 1156 Hightstreet, Santa Cruz, CA 95064 (fax: 831-459-2935, ruddat@chemistry.ucsc.edu)

Lipoxygenases are important due to their involvement in many inflammatory processes. They catalyze the conversion of unsaturated fatty acids to peroxides, which are then further modified by other enzymes. Lipoxygenases are present in many different organisms, such as plants and animals. Here we report on the kinetics of the activation of lipoxygenase-1 by 13-HPOD, which oxidizes the iron of this non-heme enzyme from Fe(II) to Fe(III). We found a strong dependence of the rates of activation on 13-HPOD concentration by stopped-flow fluorescence and calculated the K_D to be 2.59.E-05 $\pm$ 0.2 and k_2 to be 182 $\pm$ 4 s^{-1}. The reaction is shown in the scheme below. We used two inhibitors, one previously found to be a competitive inhibitor and one to be an allosteric inhibitor for lipoxygenase-1, in the reaction with linoleic acid and measured their effect on the rates of activation.

$$E + HPOD \underset{K_D}{\rightleftharpoons} E\cdot HPOD \xrightarrow{k_2} E_{OX}$$

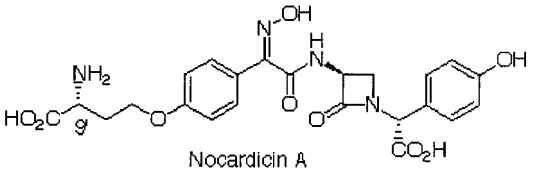
62. Kinetic isotope effects in soybean lipoxygenase-1 catalyzed oxidation of site-specifically deuterated arachidonic acids. Sheng Peng and Wilfred A. van der Donk. Department of Chemistry, University of Illinois at Urbana−Champaign, RAL 341 Box 32, 600 S. Mathew Ave., Urbana, IL 61801 (fax: 217-244-8068, peng@scs.uiuc.edu)

Lipoxygenases are a class of enzymes that catalyze the oxidation of the backbone of unsaturated fatty acids. Extremely large kinetic isotope effects have been found in this reaction using linoleic acid (LA) as substrate. In this study, both 13(*S*)-[^{2}H]-arachidonic acid (AA) and 13,13-[^{2}H]$_2$-arachidonic acid were synthesized efficiently through Wittig reactions or a combination of Grignard coupling and Wittig reactions. Competitive and noncompetitive kinetic isotope effect studies were carried out using both compounds as substrates for soybean lipoxygenase-1 (SLO). This work represents the first kinetic isotope effect study on soybean lipoxygenase-1 using deuterated arachidonic acids.

63. Late stages of nocardicin A biosynthesis. Craig A. Townsend and **Wendy L. Kelly**. Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218 (fax: 410-261-1233, Townsend@jhunix.hcf.jhu.edu, wlk1@jhunix.hcf.jhu.edu)

The nocardicins are monocyclic β-lactam antibiotics produced by the actinomycete *Nocardia uniformis* ATCC 21806, the most potent of which is nocardicin A. Among the structural features necessary for full biological activity are the oxime moiety and the D-configuration at the C-9′ center of the homoseryl side chain. A putative gene cluster for nocardicin A biosynthesis has been identified, and two of these gene products have been characterized. The cytochrome P450 NocL has been shown to effect oxidation of a primary amine to the oxime functionality. The second, NocJ, shows similarity to the PLP-dependent 1-aminocyclopropane-1-carboxylate deaminases. The role of NocJ as the epimerase

responsible for interconversion between the L and D isomers at C-9′ of the nocardicins has also been established.



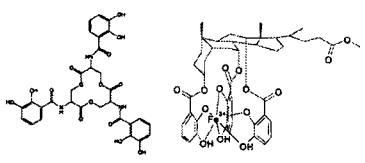
Nocardicin A

64. Ligand interactions and protein conformational changes of phosphopyridoxyl-labeled *Escherichia coli* phosphoenolpyruvate carboxykinase determined by fluorescence spectroscopy. Maria V. Encinas,[1] Fernando D. Gonzalez-Nilo,[1] Hughes Goldie,[2] and **Emilio Cardemil**.[1] [1]Departamento de Ciencias Quimicas, Universidad de Santiago de Chile, Casilla 40, Santiago 33, Chile (fax: +56-(2)681-2108, ecardemi@lauca.usach.cl), and [2]Department of Microbiology, University of Saskatchewan, Saskatoon, Canada

The interaction of substrates and ligands with *Escherichia coli* phosphoenolpyruvate (PEP) carboxykinase was studied in the phosphopyridoxyl (P-pyridoxyl)−enzyme adduct. The fluorescence decay of the P-pyridoxyl group fitted to lifetimes of 5.15 ns (34%) and 1.2 ns, and they were markedly altered in the presence of PEP/Mn(II). The binding affinity of PEP in the presence of Mn(II) was 3 kcal/mol higher than in the presence of Mg(II), and 2.4 kcal/mol more favorable than the binding affinities of ATP or ADP in the presence of either metal ion. Molecular models of the P-pyridoxyl−*E. coli* PEP carboxykinase showed different degrees of solvent-exposed surfaces for the P-pyridoxyl group in the open (substrate-free) and closed (substrate-bound) forms, which are consistent with acrylamide quenching experiments of the free and substrate-bound P-pyridoxyl−enzyme adducts. The results indicate that the P-pyridoxyl group is a useful fluorescent probe to sense ligand-induced protein conformational changes in *E. coli* PEP carboxykinase.

65. Metal complexes of enterobactin analogue ligands based on bile acids. Zahra Afrasiabi, Ekkehard Sinn, and Amilaprasadh Norbert. Department of Chemistry, University of Missouri, Rolla, MO 65409-0100 (fax: 573-341-6033, za347@umr.edu)

Bile acids are exciting drug delivery molecules. We have prepared new analogues of the siderophore ligand enterobactic (shown below), prepared by functionalizing the three OH groups in cholic acid, a trihydroxy-bile acid. The properties of the new ligand for metal recognition and sequestering are described (the Fe^{III} complex is shown).

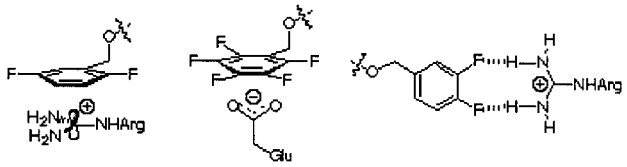


66. Nitrogen isotope effects and the OMP decarboxylase reaction: another look. Jeffrey A. Smiley, Christine Novicky, and Janet E. DelBene. Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH 44555 (fax: 330-742-1579, jasmiley@cc.ysu.edu)

Nitrogen isotope effects at N1 for the enzymatic reaction of orotidine-5′-phosphate decarboxylase (ODCase) have been addressed previously [Rishavy and Cleland (2000) *Biochemistry 39*, 4569−4574]. Using model reactions intended to mimic the proposed O2 protonation mechanism and the measured nitrogen isotope effect for the ODCase reaction, they concluded that O2 protonation does not occur with this enzyme. We address this issue using (1) determination of the equilibrium nitrogen isotope effect at N1 for the O2 protonation reaction of 1-methyluracil using ab initio calculations at the MP2/6-31+G(d,p) level, and (2) measurement of the kinetic nitrogen isotope effect at N1 for the nonenzymatic decarboxylation reaction of 1-methylorotate. The results from these approaches, designed to more closely resemble the proposed ODCase reaction, are fitted to a kinetic equation derived for the O2 protonation mechanism.

67. Novel paradigms for the interaction of fluoroaromatic inhibitors with neuraminidase. Ahamindra Jain, **Irene Wong**, Deborah Yang, Victor Wang, and Eric Q. Parkhill. Department of Chemistry, University of California, Berkeley, 325 Latimer Hall, Mail Code 1460, Berkeley, CA 94720-1460 (ahamindra@cchem.berkeley.edu, airiin@uclink.berkeley.edu)

Fluoroaromatic inhibitors of viral neuraminidase have the potential to interact with the active site by taking advantage of a wide range of intermolecular forces. We have previously shown that fluoraromatic inhibitors interact with carbonic anhydrase via hydrophobic forces, dipolar and quadrupolar forces, and even F·H "hydrogen bonds." We have prepared several fluoroaromatic inhibitors of viral neuraminidase and have measured their affinity for the protein in vitro. We propose that these molecules may interact with the protein via several of the forces mentioned above, as well as through specific, strong, F·H bonds.



68. Oxidized calmodulin mutants differentially regulate constitutive nitric oxide synthases. Heather J. Montgomery,[1] J. Guy Guillemette,[1] Ryan Bartlett,[2] and Thomas C. Squier.[3] [1]Department of Chemistry, University of Waterloo, 200 University Ave. W., Waterloo, ON N2L 3G1, Canada (hjmontgo@sciborg.uwaterloo.ca), [2]Molecular Biosciences, University of Kansas, and [3]Pacific Northwest National Laboratory

The oxidation of methionine residues in calmodulin (CaM) to methionine sulfoxides has been shown to inhibit the activity of a variety of calmodulin-regulated enzymes, including plasma membrane Ca-ATPase and neuronal nitric oxide synthase (nNOS). The specific methionine residues of CaM involved in the regulation of electron transfer within nNOS and endothelial nitric oxide synthase (eNOS) have not been determined. Mutation of seven or eight or all nine methionine residues in CaM to leucine and oxidation of the remaining methionines showed the importance of methionine

144 in the activation of nNOS. Cytochrome *c* reduction and NADPH oxidation by nNOS, in the presence of the different CaM mutants, indicated that electron transfer within the reductase domain of nNOS was affected more than electron transfer between the reductase and oxygenase domains of nNOS. Similar studies performed with eNOS revealed the opposite, with a more pronounced decrease in nitric oxide synthesis than cytochrome *c* reduction.

69. Pharmacophoric profiling of proteolytic enzymes using novel lanthanide-based fluorogenic substrates. Amy M. Barrios and Charles S. Craik. Department of Pharmaceutical Chemistry, University of California at San Francisco, 513 Parnassus Ave., Room S-907, San Francisco, CA 94143-0446 (fax: 415-502-8298, barrios@cgl.ucsf.edu)

Proteolytic enzymes have been implicated in a number of pathogenic states including hypertension, osteoarthritis, chronic degenerative disorders, and cancer. Although protease inhibitors have proven therapeutically useful in models of many of these disease states, a major barrier to their clinical use has been a lack of selectivity. A detailed knowledge of protease substrate specificity is necessary to aid in elucidating the roles played by proteases in vivo and to develop inhibitors capable of differentiating between similar proteases. To address this problem, we have designed a novel method for probing the substrate specificity of proteolytic enzymes utilizing a fluorescent lanthanide ion chelate. Fluorogenic peptide substrate libraries were developed to assay the substrate specificity of carboxypeptidases as well as endopeptidases. Specificity profiles for a number of proteolytic enzymes and the molecular basis behind the protease−substrate recognition event will be presented.

70. Preparation of a mutant myoglobin by intein mediated expressed protein ligation. Yushuan Lai and Sean M. Decatur. Department of Chemistry, Mount Holyoke College, Carr Laboratory, South Hadley, MA 01075

Apomyoglobin folding occurs via a well-characterized molten globule intermediate in which three (A, G, and H) of the eight native helices are formed. Both equilibrium and kinetic studies of folding can be observed by infrared spectroscopy, using the amide I band as a probe for secondary structure. Since the amide I band reports only on global secondary structure content, these experiments do not shed light on folding details at the residue level. In model peptides, residue-level information about local structure is obtained by the introduction of site-specific ^{13}C labels into the peptide backbone; the resulting ^{13}C amide I band serves as a local probe of backbone conformation. In larger proteins, introduction of site-specific labels into the sequence is a more challenging problem. Our strategy is to site specifically label individual helices in myoglobin using intein mediated protein ligation. We have prepared expression systems for the two fragments of the myoglobin polypeptide: one consisting of the A helix and the other helices B through H. These fragments can be expressed and purified independently from *E. coli*. In this paper, we report the results of this strategy as well as characterize the structure and folding properties of the fragments and religated protein.

71. Probing human tRNA^{Pro} recognition by human prolyl-tRNA synthetase via site-specific atomic group backbone substitutions. Songon An and Karin Musier-Forsyth. Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN 55455 (san@chem.umn.edu)

We have previously shown that human prolyl-tRNA synthetase (ProRS) does not recognize tRNA^{Pro} acceptor stem nucleotides in a base-specific manner. Thus, we hypothesize that acceptor stem phosphate oxygens and ribose 2′-hydroxyls are important in recognition by the human enzyme. To test this hypothesis, single phosphorothioates, methyl phosphates, and 2′-deoxynucleotides were incorporated into the acceptor stem of semi-synthetic human tRNA^{Pro}. The 2′-OH of A66 located at the base of the acceptor stem contributes 1.7 kcal/mol to transition state stabilization. The phosphate linkage between C75 and A76 is an important functional contact as well. Indeed, the *pro-S* oxygen in the phosphate backbone at position A76 contributes 1.3 kcal/mol to catalysis. In addition, a strong hydrogen-bonding network through the phosphate backbone is observed in the middle of the acceptor stem. Taken together, these data support our hypothesis that human ProRS recognizes the acceptor stem of human tRNA^{Pro} in a backbone-specific fashion.

72. Probing the catalytic role played by copper ions in *S*-nitrosothiol degradation: a theoretical study. Celine Toubin, David Y.-H. Yeung, Ann M. English, and Gilles H. Peslherbe. CERMM and Department of Chemistry and Biochemistry, 1455 de Maisonneuve Blvd. W., Montreal, QC H3G1M8, Canada (fax: 514 848 2868, celine@cermm.concordia.ca)

The degradation of *S*-nitrosothiols (RSNOs) to release NO is believed to be catalyzed by CuI ions, but the mechanism by which trace metal ions catalyze the reaction remains unclear. Moreover, kinetic experiments have shown that decomposition rates vary significantly with the chemical nature of the RSNO considered. This preliminary study focuses on the possible CuI-RSNO intermediates involved in the decomposition pathway of *S*-nitrosocysteine, its *N*-acetylated and ethyl ester derivatives, and *S*-nitrosohomocysteine, on the basis of first-principle quantum chemistry calculations. The model chemistry is first validated by comparing computed gas-phase CuI-ligand binding energies and S−N bond homolysis energies with available experimental data. The present calculations show that the formation of stable CuI-RSNO intermediates results in weakening of the S−N bond and strengthening of the N−O bond, which promote S−N bond breaking and NO release from *S*-nitrosothiols. The investigation of different RSNOs provides an interpretation for the observed degradation rates. Finally, additional ligands and/or explicit solvent molecules are included in our model calculations of the possible intermediates, to quantify their influence on the CuI-catalyzed RSNO decomposition.

73. Probing the effects of glycosylation on the secondary structure and stability of mouse prion protein fragments. Carlos J. Bosques and Barbara Imperiali. Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (bosques@mit.edu)

Although it has been suggested that the carbohydrate moiety plays an important role in stabilizing the cellular form

of the prion protein (PrPc), the exact role of the sugars remains uncertain. To probe the effects of N-linked glycosylation on the secondary structure and stability of PrP, we have focused our studies on a simple model of helix 2 of the mouse prion protein (MoPrP). Using a combination of carbohydrate and solid-phase peptide synthesis (SPPS), a glycosylated and an unglycosylated fragment of helix 2 were generated. The glycosylated peptide was synthesized through the building block approach by attaching a Fmoc-Asn-[chitobiose$(TBDMS)_5$]-OH during the SPPS. Here, we report the results from CD, NMR, and FTIR studies that were used to investigate the structural differences exhibited by the glycosylated and unglycosylated peptides. Differences between the kinetics of fibril formation for the two peptides are also reported.

74. Probing the tertiary structure of GIR1, a novel group I intron. Evelyn Jabri, Jennifer Roberts, Melissa Thal, Peter Mikulecky, Paras Ramolia, and Eric Espinosa. Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300, ejabri@indiana.edu)

The GIR1 family of introns autohydrolyze at two internal processing sites. These two cleavage events are essential for in vivo expression of the associated open reading frame that encodes a homing endonuclease. Our laboratory is investigating the structure of these RNAs with the intent of identifying the mechanism by which the GIR1 hydrolytic activity is modulated in vivo. We have engineered RNAs with various crystallization modules to facilitate crystal formation. Nucleotide analogue modification interference (NAIM) has been used to show that the core of GIR1 is similar to that of other group I introns. Furthermore, an in vitro selection experiment identified RNAs that perform site-specific hydrolysis at faster rates. We are currently using these data, along with NAIM and hydroxyl radical footprinting of wild-type and selected RNAs, to understand how various nucleotides in GIR1 contribute to rate enhancement. Progress toward understanding the tertiary structure of GIR1 will be presented.

75. Progress toward the purification of iso-orotate decarboxylase by affinity chromatography. Daniel A. Landfried, **Vincient R. Barnes**, and Jeffrey A. Smiley. Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH 44555 (fax: 330-742-1579)

Iso-orotate decarboxylase (IDCase) completes the conversion of thymidine to uracil in the unusual thymidine salvage pathway identified to date only in certain fungi. We have developed assays for IDCase and found high specific activity in *Rhodotorula glutinis* when grown with thymine as the sole nitrogen source. We have found 5-nitrouracil to be a strong inhibitor of IDCase, and have embarked on affinity chromatography purification procedures based on the covalent attachment of 5-nitrouracil to activated agarose through either N1 or N3 of the pyrimidine. N1-modified analogues of 5-nitrouracil proved to be ineffective inhibitors of IDCase, but N3-modified analogues showed inhibition constants in the low micromolar range. We describe the syntheses of various N3-modified analogues of 5-nitrouracil, covalent attachments to activated agarose, and results of partial purification of IDCase using these modified agarose stationary phases in chromatography.

76. Proximity accelerated alkylation: a strategy for the irreversible inhibition of engineered receptor−ligand pairs. Peter J. Belshaw, Konstantin Levitsky, and Christopher J. Ciolli. Departments of Chemistry and Biochemistry, University of Wisconsin−Madison, 1101 University Ave., Madison, WI 53706 (fax: 608-265-4534, belshaw@chem.wisc.edu)

We have developed a new approach for the engineering of orthogonal receptor−ligand pairs based on the incorporation of weakly reactive groups in both the receptor and ligand. Upon ligand binding, a proximity accelerated reaction results in the formation of a covalent complex and irreversible modulation of receptor function. We demonstrate this approach in the cyclophilin−cyclosporin receptor−ligand system.

77. Shotgun scanning the streptavidin−biotin interaction. Gregory A. Weiss, Sara K. Avrantinis, and Ryan L. Stafford. Department of Chemistry, University of California, Irvine, 516 Rowland Hall, Irvine, CA 92697-2025 (fax: 949-824-8571, gweiss@uci.edu)

The streptavidin−biotin interaction is among the strongest naturally occurring noncovalent protein−ligand interactions. The combinatorial alanine-scanning method shotgun scanning was used to determine the functional contribution of the 38 C-terminal residues of streptavidin to biotin binding. Two phage-displayed protein libraries were constructed in which amino acids were mutated to alanine or conserved as wild-type. The library pools were subjected to three rounds of selection for functional streptavidin variants that bind biotin. Demonstrating that selection conditions identified streptavidin variants with high affinity for biotin, wild-type streptavidin accounted for approximately 8% of the selected variants. Shotgun scanning results were largely consistent with previous site-directed mutagenesis studies for the few residues probed by conventional mutagenesis. Results from shotgun scanning also demonstrate the importance of previously unreported hydrophobic residues contributing direct contacts with biotin, forming the beta barrel structure of streptavidin, and providing interactions at the tetramer interface.

78. Spectroscopic and photochemical properties of geometric isomers of spheroidene. Zeus D. Pendon,[1] Agnes Cua,[2] David Bocian,[2] Ineke van der Hoef,[3] Johan Lugtenberg,[3] and Harry A. Frank.[1] [1]Department of Chemistry, University of Connecticut, 55 N. Eagleville Rd., Storrs, CT 06269-3060 (fax: 860-486-2981, zeus.pendon@uconn.edu), [2]Department of Chemistry, University of California, and [3]Gorlaeus Laboratories, Leiden University

Spheroidene, a naturally occurring carotenoid, performs the dual functions of light harvesting and photoprotection. Because the all-trans configuration is found in the LH2 light-harvesting complex while the 15-cis configuration is found in the reaction center, it is important to ask whether there is any physiological significance to this difference. High-performance liquid chromatography (HPLC) has been used

to isolate various geometric isomers of naturally occurring spheroidene and synthetic locked 11-*cis*-spheroidene, 13-*cis*-spheroidene, and 15,15′-*cis*-spheroidene. The HPLC elution profiles show a number of peaks, the absorption spectra of some of which displayed strong intensity in the "cis-band" region around 350 nm in addition to the prominent absorption bands in the visible region. Resonance Raman spectroscopy is being used to deduce the configurations of the molecules. Semiempirical molecular orbital computations are being used to correlate the structures of the molecules with their spectral properties. The triplet−triplet absorption spectra and dynamics of both locked and unlocked spheroidene have been investigated.

79. Spontaneous transfer of phospholipid and cholesterol hydroperoxides between cell membranes and low-density lipoprotein. Andrew Vila and Albert W. Girotti. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (fax: 414-456-6510, avila@mcw.edu)

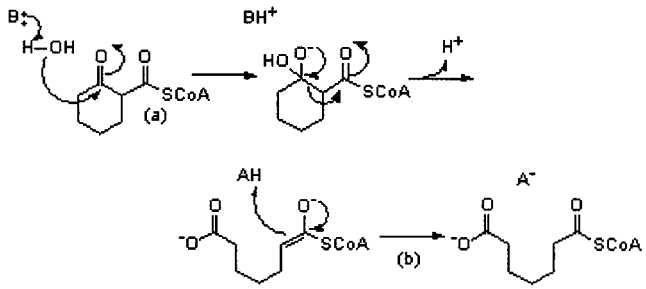
Translocation of lipid hydroperoxides (LOOHs) from circulating cells under high oxidative pressure (erythrocytes, phagocytes) to low density lipoprotein (LDL) may predispose it to oxidative modification, which has been implicated in atherogenesis. To address this hypothesis, we examined the transfer kinetics of cholesterol hydroperoxide species (7α/7*β*-OOH, 5α-OOH, 6α-OOH, and 6*β*-OOH) and phospholipid hydroperoxide (PLOOH) classes (PCOOH, PEOOH, PSOOH, and SMOOH) from photoperoxidized erythrocyte ghosts to LDL, using HPLC with mercury cathode electrochemical detection. Rate constants for ghost-to-LDL ChOOH transfer were similar to those previously measured with liposome acceptors, supporting the aqueous diffusion model for lipid transfer. Measured for the first time, rate constants for PLOOH transfer were ∼3−8-fold lower than those for overall ChOOH transfer: k(PCOOH) ∼ 0.45 h^{-1}; k(PEOOH) ∼ 0.41 h^{-1}; k(PSOOH) ∼ 0.39 h^{-1}; k(SMOOH) ∼ 0.17 h^{-1}. PLOOH transfer rates were similar when unilamellar liposomes replaced LDL as acceptors, in agreement with donor membrane desorption being the rate-limiting step. The susceptibility of LDL "seeded" with transfer-acquired LOOHs to chain peroxidation was examined by monitoring conjugated diene absorbance at 233 nm and formation of radiolabeled Ch oxidation products ([^{14}C]ChOX) by high-performance TLC with phosphorimaging radiodetection. In both cases, there was a significant decrease in the lag time for initiation of chain propagation relative to a control incubated with nonperoxidized ghosts. These results demonstrate a potential mechanism for LDL oxidation by way of translocation of priming LOOHs from neighboring cells, such as erythrocytes and activated neutrophils. (Supported by NIH Grant CA72630 and NRSA Predoctoral Fellowship F31-CA85171.)

80. Steady-state and pre-steady-state kinetic analysis of nucleotide insertion opposite the cis-syn photoproduct by yeast DNA polymerase eta. Hanshin Hwang and J. S. Taylor. Department of Chemistry, Washington University, Campus Box 1134, One Brookings Dr., St. Louis, MO 63130-4899 (fax: 314-935-4481, hanshin_h@yahoo.com)

Yeast DNA polymerase eta (Pol eta) is a member of the Y family of DNA polymerases which have been implicated in the bypass of DNA damage. Pol eta has been found to efficiently bypass the cis-syn thymine dimer, the major photoproduct of DNA, in a relatively error-free manner. The genetic disease *Xeroderma pigmentosum* variant, which results in an increased frequency of skin cancer, has been attributed to defects in human pol eta. We will describe the use of steady-state and pre-steady-state kinetics to investigate the kinetics and mechanism of nucleotide insertion opposite the cis-syn dipyrimidine TT dimer in comparison to an undamaged site. Pol h has recently been shown to utilize an induced-fit mechanism of nucleotide incorporation opposite undamaged DNA, in which the rate-limiting step is a conformational step that precedes the chemical step. We find that insertion opposite the 3′-T of the dimer is slower than opposite the 5′-T and both are slower than what is observed opposite the corresponding undamaged site. Furthermore, we find a greater elemental effect on the polymerization rate opposite the dimer, indicating that the chemistry step (the phosphodiester bond formation) becomes more rate limiting than it is for the undamaged site, possibly due to the more distorted geometry of the dimer. (This work is supported by NIH Grant CA40463.)

81. Stereochemical progress of the reaction catalyzed by BadI, a crotonase homologue. Ellen D. Eberhard[1] and John A. Gerlt.[2] [1]Department of Chemistry and [2]Department of Biochemistry, University of Illinois−Urbana Champaign, 600 S. Mathews, Box 76-5, Urbana, IL 61801 (fax: 217-244-7426, ehill@uiuc.edu)

2-Ketocyclohexanecarboxyl CoA hydrolase (BadI) catalyzes the last step in the anaerobic benzoate degradation pathway in the bacterium *Rhodopseudomonas palustris*. Sequence alignment indicates that BadI is a member of the crotonase superfamily, a group of enzymes which stabilize thioester enolate anion intermediates. A reverse Dieckmann condensation is proposed as the mechanism of the reaction catalyzed by BadI, representing a novel reaction pathway among crotonase superfamily members. A study toward the stereochemical progress of this mechanism has been divided into two parts: (a) absolute configuration of the substrate, and (b) stereospecificity of the delivery of the proton to the enolate anion intermediate. Potential active site residues are also being examined.



Proposed Mechanism of the Reaction Catalyzed by BadI

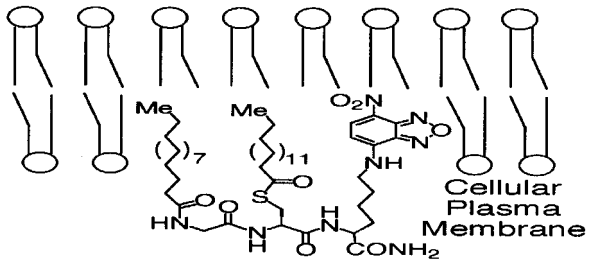
82. Stereochemistry and mechanism of the reaction catalyzed by the mammalian UDP-GlcNAc 2-epimerase. Martin E. Tanner,[1] Wayne K. Chou,[1] Werner Reutter,[2] and Stephan Hinderlich.[2] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (fax: 604-822-2847, mtanner@chem.ubc.ca),

and [2]Institut fur Molekularbiologie und Biochemie, Freie Universitat Berlin, Germany

The mammalian UDP-GlcNAc 2-epimerase catalyzes the epimerization and hydrolysis of UDP-GlcNAc to form ManNAc and UDP. This reaction is the rate-limiting step in the production of the sialic acids. The stereochemistry of the reaction has been determined using NMR spectroscopy. Studies on the mechanism of this reaction, focusing on a puatative 2-acetamidoglucal intermediate, will also be presented.

83. Strategies for the discovery of inhibitors of palmitoyl acyltransferase: assays based on fluorescent substrate mimics of Src oncoproteins. Steffen P. Creaser,[1] Amanda S. Varner,[2] Charles D. Smith,[2] and Blake R. Peterson.[1] [1]Department of Chemistry, Pennsylvania State University, 152 Davey Lab, University Park, PA 16802 (fax: 814-863-8403, brpeters@chem.psu.edu), and [2]Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA 17033 (cdsmith@psu.edu)

The localization of oncogenic Src and Ras proteins to cellular plasma membranes is critical for the proliferation of specific cancers. In addition to other lipid modifications, these proteins require posttranslational palmitoylation of specific cysteine residues by the enzyme palmitoyl acyltransferase (PAT) in order to be stably anchored at plasma membranes. To facilitate the identification of PAT inhibitors, we have developed a fluorescent cell-permeable PAT substrate that mimics the N-terminus of Src family proteins. Metabolic radiolabeling and epifluorescence microscopy of Jurkat lymphocytes treated with this Src-mimetic lipopeptide revealed that this compound is palmitoylated intracellularly, which confers localization at cellular plasma membranes. Analysis of inhibition of palmitoylation by flow cytometry with 2-bromopalmitic acid revealed that this fluorescent substrate represents a highly sensitive molecular probe of PAT activity. We report recent progress toward the rational design and combinatorial discovery of small molecules that affect PAT.



84. Structural determinants of inhibitor affinity to arginase I. Evis Cama and David W. Christianson. Department of Chemistry, University of Pennsylvania, S. 34th St., Philadelphia, PA 19104 (evis@anchor.chem.upenn.edu)

Arginase is a 105 kDa homotrimer containing a binuclear manganese cluster in each monomer that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. The crystal structure of liver arginase, also designated as type I arginase, has been determined to 2.1 Å resolution. Structure-based designing of the boronic acid analogue of L-arginine, 2(*S*)-amino-6-boronohexanoic acid (ABH), brought about the synthesis of this slow binding competitive inhibitor of arginase. The structure of the complex reveals the way in which ABH yields a tetrahedral boronate anion, mimicking the tetrahedral transition state of L-arginine. This transition state does not occur in NO synthase, explaining why ABH does not inhibit NO synthase. The intermediate in the NOS hydrolysis pathway of L-arginine to L-citrulline and NO, N^{ω}-hydroxy-L-arginine (NOHA), is a modest competitor of arginase. The structure of the arginase−NOHA complex provided further insight into the catalysis of arginase as well as a mechanistic link between arginase and NOS. Crystal structures of complexes of arginase with modified versions of these two inhibitors provide further insight into the role played by each of the functional groups of these compounds.

85. Structural elucidation of calbindin D_{28k} using intramolecular cross-linkers, mass spectrometry, and molecular modeling. David Yong-Hoi Yeung, Ann English, and Gilles Peslherbe. Department of Chemistry & Biochemistry and Centre for Research in Molecular Modeling, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, QC H3G 1M8, Canada (fax: 514-848-2868, dave_yeung@canada.com)

Conventional methods for structural determination of proteins include NMR spectroscopy and X-ray crystallography. However, these methods have limitations. A novel approach for structural characterization of proteins involves the use of sequence alignment, chemical cross-linking, and mass spectrometry. The resulting experimental information can in turn be used to guide the protein structure determination with molecular modeling through structural constraints. Molecular modeling investigations may aid in the design of further experiments, and allow protein structure refinement in an iterative fashion. We are applying this approach to the characterization of the tertiary structure of a calcium-binding protein, human calbindin D_{28k}. Conformations that the protein may adopt in both its calcium-free and calcium-loaded forms are under investigation.

86. Structural energetics in a DNA triple helix probed by proton exchange. Lihong Jiang[1] and Irina M. Russu.[2] [1]Department of Molecular Biology and Biochemistry and [2]Department of Chemistry, Wesleyan University, Middletown, CT 06459

Nuclear magnetic resonance (NMR) spectroscopy has been used to characterize the energetics of the triple-helical structure formed by the 31-mer DNA oligonucleotide d(5′-AGAGAGAACCCCTTCTCTCTTTTTCTCTCTT-3′) at acidic pH. The structure belongs to the YRY family of triple helices, and contains three canonical C^{+}·GC and four canonical T·AT base triplets. We have measured the rates of solvent exchange of the imino protons in each protonated cytosine and in each thymine in this triple helix. The values of the exchange rates are found to span 7 orders of magnitude, in the range from 10^{-5} to 10^{2} s^{-1}. The exchange rates, and their temperature dependence, are used to define the stability of Watson−Crick and Hoogsteen base-pairing interactions in each triplet of the structure. The relationship between these results and the overall energetics of the triple helix formation will be discussed. (Supported by the National Science Foundation.)

87. Structural studies on the N-terminal domain of vitronectin: a disulfide knot. Anand Mayasundari, Engin H. Serpersu, and Cynthia B. Peterson. Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, M407 Walters Life Sciences Building, Knoxville, TN 37996 (fax: 865-974-6306, anand@utk.edu)

Human vitronectin is a circulating glycoprotein that interacts with a wide variety of macromolecules to control hemostasis. One of the most important interactions known for vitronectin is with the serine protease inhibitor PAI-1. The N-terminal domain of vitronectin has been identified as a primary binding site for PAI-1. This N-terminal domain contains 8 of the 14 cysteines present in vitronectin, arranged in 4 disulfide bonds. To determine the structure of the N-terminal domain, a 51-residue N-terminal fragment was produced from a CNBr digest of vitronectin and isolated using HPLC. Its size and monomeric state were confirmed by MALDI-TOF mass spectrometry (*m*/*z* 5762.26) and sedimentation equilibrium studies. Two-dimensional ^{1}H NMR experiments were performed on this polypeptide to obtain scalar and dipolar (NOE) connectivities. Molecular dynamics and simulated annealing using the observed NOEs as distance restraints will be used to arrive at the three-dimensional structure of the N-terminal domain.

88. Studies of the protonation and electophilic activation of methyltetrahydrofolate by the methyltransferase from *Moorella thermoacetica.* Joe J. Spicha and Stephen W. Ragsdale. Department of Biochemistry, University of Nebraska−Lincoln, Beadle Center, 19th and Vine St., Lincoln, NE 68588-0664 (fax: 402-472-7842, jspicha@unlserve.unl.edu)

The (6*S*)-methyltetrahydrofolate (methyl-THF) corrinoid iron−sulfur protein (CFeSP) methyltransferase (MeTr) catalyzes formation of the first of several enzyme-bound organometallic intermediates in the Wood−Ljungdahl pathway of anaerobic CO_2 fixation. Methyl-cob(III)amide is formed in a reaction catalyzed by MeTr involving SN2 attack by cob(I)amide on methyl-THF. These studies indicate that upon formation of the binary MeTr/methyl-THF complex, MeTr catalyzes electrophilic activation of methyl-THF by protonating N5 of the pterin ring. Upon binding, a 20 nm red shift, an increase in the molar absorption coefficient, and a clean isosbestic point are observed, similar to previous studies by Matthews et al. with methionine synthase (MS). However, fundamental differences exist between MeTr and MS. Despite sharing 4/5 conserved residues that contact the pterin ring, MeTr, unlike MS, protonates methyl-THF in the binary complex. Mutants (N199A, N199K, and N199D) of a conserved active site Asn199 residue within H-bonding distance of N5-pterin are compromised in catalysis and exhibit different temperature and pH optima.

89. Synthesis and spectroscopic studies of model red fluorescent protein chromophores. Xiang He, Alasdair Bell, and Peter J. Tonge. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400 (fax: 631-632-5797, xhe777@yahoo.com)

Here we describe the synthesis and spectroscopic characterization of two compounds designed to model the chromophore in DsRed, a red fluorescent protein. Comparison with model green fluorescent protein (GFP) chromophore indicates that the additional conjugation in the DsRed models can account, in part, for the red-shifted absorption and emission properties of DsRed compared to GFP. In contrast to the GFP models, the DsRed models are fluorescent with quantum yields of 0.002−0.01 in $CHCl_3$.

90. TATA box binding protein interactions with supercoiled minicircle DNA. Jung S. Byun and Jason D. Kahn. Department of Chemistry and Biochemistry, University of Maryland, College Park, College of Life Science, Chemistry Building 091, College Park, MD 20742-2021 (fax: 301-405-9376, jb379@umail.umd.edu)

In eukaryotic organisms, the binding of the TATA box binding protein (TBP) to its target DNA sequence is critical for the initiation of transcription. Previous work in our laboratory showed that binding of TBP induces a bend that is opposite in direction to an intrinsic bend of the TATA box, and this was proposed as a cause for the slow binding of TBP. TBP was also shown to induce negative supercoiling in small DNA circles, leading to the proposed accessible "flattened" form of the protein−DNA complex. To further explore the mechanism of TBP binding in a constrained DNA environment, we have constructed pre-bent, ^{32}P-labeled minicircle DNA topoisomers (203 bp); these may also mimic the constrained environment of DNA in chromatin. Quantitative DNase I footprinting has been used to derive the equilibrium binding constants and the Gibbs free energies of TBP binding to linear DNA and to minicircle topoisomers. We observed dissimilar footprinting patterns and sensitivity to DNase I cleavage in minicircles compared to the corresponding linear DNA fragments. This study is the first to employ quantitative footprinting experiments to examine the effects of pre-bending and pre-twisting of DNA on TBP binding.

91. Thermodynamic parameters for RNA bulge loop formation. Martin J. Serra, Sara B. Silvestri, Heather Volkman, Bob Boswell, and Brent M Znosko. Department of Chemistry, Allegheny College, 520 N. Main St., Meadville, PA 16335 (fax: 814-332-2789, mserra@allegheny.edu)

Thirty-four RNA duplexes containing single-nucleotide bulges were optically melted, and the thermodynamic parameters $H°$, $S°$, $G°_{37}$, and TM for each sequence were determined. Data from this study were combined with data from previous thermodynamic data [Longfellow, C. E., Kierzek, R., and Turner, D. H. (1990) *Biochemistry 29*, 278−285] to develop a model that will more accurately predict the free energy of an RNA duplex containing a single-nucleotide bulge. Differences between purine and pyrimidine bulges as well as differences between Group I duplexes, those in which the bulge is not identical to either neighboring nucleotide, and Group II duplexes, those in which the bulge is identical to at least one neighboring nucleotide, were considered. Length of the duplex, non-nearest-neighbor effects, and bulge location were also examined. A model was developed which divides sequences into two groups: those with pyrimidine bulges and those with purine bulges. The proposed model for pyrimidine bulges predicts $G°_{37,\mathrm{bulge}} = 3.9$ kcal/mol + (0.10) ($G°_{37,\mathrm{nn}}$) + β, while the model for purine bulges predicts $G°_{37,\mathrm{bulge}} = 3.3$ kcal/mol − (0.30)

($G°_{37,nn}$) + β, where β has a value of 0.0 and −0.8 kcal/mol for Group I and Group II sequences, respectively, and $G°_{37,nn}$ is the nearest-neighbor free energy of the base pairs surrounding the bulge. The conformation of bulge loops present in rRNA was examined. Three distinct families of structures were identified. The bulge loop was either extrahelical, intercalated, or in a "side step" conformation.

92. Thiazole-orange/peptide conjugates: application to DNA damage detection. Kerry P. Mahon, Jr., and Shana O. Kelley. Department of Chemistry, Boston College, Merkert Chemistry Center, 2609 Beacon St., Chestnut Hill, MA 02467 (mahonke@bc.edu)

To generate fluorescent sensors for the quantitation and study of DNA damage in the human genome, we have prepared a family of DNA-binding intercalator/peptide conjugates. A derivative of thiazole orange amenable to functionalization was prepared and appended to series of small peptides using solid-phase chemistry. Thiazole orange serves as an ideal scaffold for these conjugates because it is highly fluorescent when bound to DNA, but displays little sequence specificity and modest DNA-binding affinity in its unfunctionalized form. We have characterized the DNA-binding properties of the conjugates using spectroscopic methods, and have observed that the intercalative binding mode of thiazole orange is sensitive to the identity of adjacent amino acids. The binding specificity of the intercalator conjugates in the presence of damaged sites has also been investigated. These molecular sensors are useful for the detection of DNA damage in complex biological samples.

93. Toward the de novo design of a catalytically active helix bundle. Herschel Wade,[1] Luigi Di Constanzo,[2] Vincenzo Pavone,[2] Angela Lombardi,[3] and William F. DeGrado.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania, 1111 Spruce St., #100, Philadelphia, PA 19107 (fax: 215-573-7229, hwade@mail.med.upenn.edu), [2]Department of Chemical Sciences, University of Trieste, and [3]Department of Chemistry, University of Napoli

The design of metalloproteins provides an attractive approach to test the essential features for electron transfer and catalysis. We have approached this problem through the introduction of metal binding sites into de novo designed proteins. Through an iterative design process, we have designed four helical bundles that contain dicarboxylate bridged dinuclear metal centers that are patterned after the diiron−dimanganese class of redox-active protein. Here we report the three-dimensional structure of the dimanganese form of L13A-DF1 and solution studies of a variant that indicate that these model proteins can bind small molecules at their metal centers. The spectral properties of several metal and small molecule complexes indicate that these proteins form complexes that are similar to their natural counterparts. We also show that the metal centers can undergo metal exchange reactions that yield proteins with mixed metal dinuclear sites.

94. Toward the redesign of WW domains. Jennifer R. Rosenberg,[1] Ronald H. Hoess,[2] and William F. DeGrado.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania, 422 Curie Blvd., Philadelphia, PA 19104 (jrose2@mail.sas.upenn.edu), and [2]Bristol Myers Squibb

WW domains are small peptide-binding modules involved in mediating a variety of cellular processes through protein interactions. These monomeric domains of 38−40 residues consist of a three-stranded antiparallel β-sheet. Their compact nature makes them a desirable scaffold for incorporating functional diversity. We are interested in incorporating catalytic activity into these small proteins by evolving the motif for transition state analogue binding. One difficult challenge with WW domains is they have a relatively low affinity (>50 mM) for their cognate substrates. To overcome this challenge, we will generate a WW domain that binds substrates with higher affinity using phage display. A library of 6 randomized residues was generated to identify novel sequences for the hPin1 WW domain that bind to the pSP consensus sequence. Characterization of ligand binding using surface plasmon resonance and isothermal titration calorimetry is presented as well as novel WW sequences that bind to the consensus sequence pSP.

95. *trans-N*-Glycoside *J*-couplings in nucleosides: Karplus dependencies of $^3J_{CCNC}$ and $^3J_{HCNC}$ derived from DFT calculations. Christophe Thibaudeau,[1] Yuping Zhu,[1] Ian Carmichael,[2] and Anthony S. Serianni.[1] [1]Department of Chemistry and Biochemistry and [2]Notre Dame Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556-5670 (fax: 219-631-6924, Christophe.Thibaudeau.2@nd.edu)

Base conformation in nucleosides is described by syn/anti equilibria determined from nOes or δ changes, assuming representative geometries for the χ rotamers. ^3Js in principle allow more reliable determination of χ when Karplus relationships are known. Prior efforts to exploit $^3J_{HCNC}$ and $^3J_{CCNC}$ have remained qualitative owing to restricted sets of model compounds. We report here the dependencies of $^3J_{H1',C2/C6}$ and $^3J_{C2',C2/C6}$ in 2′/3′-dU and of $^3J_{H1',C4/C8}$ and $^3J_{C2',C4/C8}$ in 2′/3′-dA on χ based on DFT calculations. $^3J_{HCNC}$ Karplus curves confirm experimental trends observed previously in cyclouridines. $^3J_{HCNC}$ are sensitive to electronegativities of substituents on the coupling pathway. $^3J_{CCNC}$ values exhibit Karplus-like dependencies on θ_{CCNC}. $^3J_{C2',C2/C4}$ is largest for $\theta = 180°$ and small for $-70° < \theta < 80°$. A more typical relationship was found for $^3J_{C2',C6/C8}$, with larger Js for $\theta = 0°$ than 180°. Using newly derived Karplus equations, we analyzed experimental $^3J_{CCNH}$ and $^3J_{CCNC}$ in ^{13}C-labeled uridine and adenosine to determine the populations of χ rotamers.

96. Unique copper chelators for angiosuppression. Chandrika P. Kulatilleke,[1] David B. Rorabacher,[2] and and Leo A. Ochrymowycz.[3] [1]Department of Natural Sciences, Baruch College, CUNY, 17 Lexington Ave., Box A-0506, New York, NY 10010 (fax: 212-802-3082, chandrika_kulatilleke@baruch.cuny.edu), [2]Department of Chemistry, Wayne State University, and [3]Department of Chemistry, University of Wisconsin

The search for selective copper chelating agents has recently received renewed attention as a result of mounting evidence that copper is essential to the growth of cancerous tumors. It has long been recognized that copper levels are

abnormally high in solid tumors. It is now suspected that the increased copper level is associated with the development of a capillary network (angiogenesis) that is essential in sustaining tumor growth. Of the several compounds identified as angiogenic promoters, all bind copper strongly, suggesting that copper may be essential to their function. Furthermore, copper depletion therapy has shown encouraging results in inhibiting angiogenesis and/or tumor growth. The copper chelation drugs tested so far have poor selectivity for copper and have posed serious side effects. We have developed a series of tetrathiaether ligands, which show promise as selective copper chelators. Our preliminary studies on chelation of these ligands with copper(2+) and nickel(2+) showed that they bind copper(2+) 10^9 times stronger than nickel(2+). Studies are underway to estimate the selectivity of these ligands with respect to other essential metal atoms such as iron and zinc.

97. Zinc switch in pig heart lipoamide dehydrogenase: steady-state and transient kinetic studies. Irina G. Gazaryan,[1] Valentina Schedrina,[2] Natalia Klyachko,[2] Alexander Efimov,[3] Roger N. F. Thorneley,[4] and Abraham M. Brown.[1] [1]Burke Medical Research Institute, 785 Mamaroneck Ave., White Plains, NY 10605 (fax: 914-597-2757, igazarya@burke.org), [2]Department of Chemical Enzymology, Moscow State University, [3]Institute of Protein Research of Russian Academy of Sciences, and [4]Biochemistry Department, John Innes Centre

Lipoamide dehydrogenase, a key component of mitochondrial dehydrogenase complexes, catalyzes the transfer of reducing equivalents from the bound dihydrolipoate of the neighboring dihydrolipoamide acyl transferase subunit to NAD^+. This reversible reaction involves two reaction centers: a thiol pair, which accepts electrons from dihydrolipoate, and a noncovalently bound FAD moiety, which transfers electrons to NAD^+. The lipoamide dehydrogenase reaction catalyzed by the purified pig heart enzyme is strongly inhibited by Zn^{2+} (K_i ca. 0.15 μM) in both directions. Binding of Zn^{2+} with the two-electron-reduced enzyme was directly monitored in anaerobic stopped-flow experiments. The value of the binding constant determined from stopped-flow data agrees with the inhibition constant estimated from steady-state kinetics. Submicromolar zinc binding to the catalytic disulfide of this enzyme induced a switch from lipoamide dehydrogenase activity to a diaphorase activity measured with 2,6-dichrolophenol−indophenol as a substrate. The steady-state kinetic measurements demonstrated the switch from the apparent ternary complex mechanism to a common ping-pong mechanism upon zinc addition to the reaction medium. Anaerobic stopped-flow experiments on the diaphorase reaction demonstrated that only the enzyme forms with reduced FAD (preferably four-electron-reduced enzyme) were able to react with the electron acceptor. The enhancement of the enzyme diaphorase activity in the presence of zinc, thus, originates from blocking the thiol active center and keeping the reducing equivalents on FAD. Structural modeling indicates significant changes in intersubunit contact in lipoamide hydrogenase upon zinc binding to the catalytic thiols, which may cause dissociation of the dimer to monomers. One can speculate that this may lead to structural changes in the mitochondrial dehydrogenase complexes as a whole, i.e., to the release of lipoamide dehydrogenase component. Since lipoamide dehydrogenase is representative of the class of thiol−disulfide flavin−oxidoreductases that includes glutathione reductase and thioredoxin reductase, the similar effects of zinc can be expected for these enzymes. The inhibition of activity with respect to the physiological substrate and activation of diaphorase activity have been shown for glutathione reductase. The possible physiological implications of the discovered zinc-induced switch in redox enzymes controlling mitochondrial energy complexes and redox status are discussed. [Supported by NIH Grant NS38741 (to A.M.B.) and by NATO Linkage Grants to R.N.F.T. (LST.97 5772) and A.M.B. (LST.CLG.978592).]

Tuesday Morning: Pfizer Award Symposium

Karin Musier-Forsyth, Organizer

98. Molecular delineation of a multi-functional tRNA synthetase. Susan A. Martinis. Department of Biology and Biochemistry, University of Houston, 3201 Cullen, Houston, TX 77204-5001 (fax: 713-743-8351, smartinis@uh.edu)

The tRNA synthetases' role in protein synthesis requires that each enzyme accurately links a specific amino acid to its cognate tRNA via an aminoacylation reaction. To ensure fidelity and also to perform additional duties within the cell, the tRNA synthetases have adapted in a very idiosyncratic manner. In a separate site from the aminoacylation active site, leucyl-tRNA synthetase carries out enzymatic editing to enhance discrimination of its substrate leucine from other structurally related aliphatic amino acids. The hydrolytic editing active site has been localized within a small discrete protein domain called CP1. This domain was also determined to be a critical factor that aids certain mitochondrial RNA splicing reactions. Extensive mutagenesis efforts have identified the amino acid binding pocket of the editing active site, key residues that are involved in the overall editing mechanism, and also characterization of CP1 as a group I intron splicing factor.

99. Initiator transfer RNAs and initiation of protein synthesis. Uttam L. RajBhandary. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 (fax: 617-252-1556, bhandary@mit.edu)

Initiator tRNAs are used exclusively at the initiation step of protein synthesis and not at the elongation step. Because of their special role, initiator tRNAs possess a number of properties distinct from those of elongator tRNAs. We are investigating the relationship between the sequence and/or the structural features of the initiator tRNA and their distinctive properties and the molecular mechanism by which the various components of the protein synthesis machinery distinguish the initiator tRNA from the elongator tRNAs. The structural features in the *E. coli* initiator tRNA important for specifying its distinctive properties have been identified. Work from our laboratory and that of Blanquet has also defined the molecular mechanism of recognition of the initiator tRNA by *E. coli* methionyl-tRNA formyltransferase. Mutant tRNAs generated during this work are being used in studies aimed at the development of methods for site-specific insertion of amino acid analogues into proteins in vivo.

100. Dissecting the genetic code. Paul Schimmel. Departments of Molecular Biology and Chemistry, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., BCC 379, La Jolla, CA 92037 (fax: 858-784-8990)

The genetic code relates specific amino acids to nucleotide triplets that are encoded in genes and messenger RNAs. The code is established by the specific aminoacylations of transfer RNAs by aminoacyl tRNA synthetases. Not understood is the format of the table of codons, where groups of triplets are arranged according to specific nucleotides (U, C, A, and G) and their associated amino acids. The same format is found in all three domains of the tree of life, but the reason for this particular organization of the table is not known. Recent investigations suggest that this organization is a result of structural features of aminoacyl tRNA synthetases and of the way that they interact with tRNAs. These interactions include complexes where two synthetases bind to a single tRNA according to specific structural constraints. In addition, the early code was sufficiently ambiguous that the first proteins were doubtless statistical. Under strong selective pressure, the true chemical entities (i.e., proteins with specific sequences) of modern cells were generated. This step required the acquisition of editing functions that remove ambiguities from the code. Several lines of investigations with contemporary synthetases support these interpretations, and give a picture of how the code was assembled into its present-day format.

101. Functional diversity in class II aminoacyl-tRNA synthetases. Karin Musier-Forsyth. Department of Chemistry, University of Minnesota, 207 Pleasant St. SE, Minneapolis, MN 55455 (fax: 612-626-7541, musier@chem.umn.edu)

To maintain high accuracy during protein translation, some aminoacyl-tRNA synthetases have evolved an editing mechanism. Class II *Escherichia coli* proline-tRNA synthetase (ProRS) is capable of hydrolyzing a misactivated Ala-AMP in a reaction known as pre-transfer editing, and deacylating mischarged Ala-$tRNA^{Pro}$ via post-transfer editing. We have identified residues in a novel domain inserted within the aminoacylation catalytic site in prokaryotic ProRS that play a critical role in editing. Thus, the editing activity resides in a domain that is functionally and structurally distinct from the aminoacylation active site. Aside from their role in translation of the genetic code, tRNAs play a critical role as primers for initiation of reverse transcription in retroviruses. Human $tRNA^{Lys,3}$ is the primer for HIV, and class II human lysyl-tRNA synthetase is also packaged into the virus. Efforts are underway to further characterize the molecular interactions that dictate specific tRNA packaging and other nucleic acid−protein interactions in HIV.

Tuesday Afternoon: Approaches to Novel Antibiotic Design

Chris Walsh, Organizer

102. Turning virulence on and off in *Staphylococci.* Tom W. Muir. Laboratory of Synthetic Protein Chemistry, Rockefeller University, 1230 York Ave., New York, NY 10021 (fax: 212 327 7358, muirt@mail.rockefeller.edu)

The emergence of methicillin-resistant and, more recently, vancomycin-resistant strains of *Staphylococcus aureus* represents an enormous threat to public health. Consequently, there is a pressing need to identify new types of antibacterial agents, and it has been suggested that interference with the expression of virulence may represent a promising antibacterial modality. Staphylococcal virulence is regulated by a two-component quorum sensing system, agr, activated by a self-coded autoinducing peptide (AIP). The agr system is widely divergent and is unique in that variant AIPs cross-inhibit agr activation in heterologous combinations. Cross-inhibition, but not self-activation, is widely tolerant of structural diversity in the AIPs so that these two processes must involve different mechanisms of interaction with the respective receptors. We have used a combination of molecular genetics, protein chemistry, and chemical synthesis to establish that these AIPs from *S. aureus* contain a thiolactone structure, and that this feature is absolutely necessary for full biological activity. Moreover, structure−activity studies have allowed key aspects within the AIP and its histidine-kinase receptor, AgrC, involved in the differential activation and inhibition functions to be identified. This has led to the rational design of global inhibitors of virulence within the *Staphylococci* as well as the development of a model for receptor agonism and antagonism.

103. Substrate-binding antibiotics and their targets: ramoplanin. Suzanne Walker. Department of Chemistry, Princeton University, 169 Frick Laboratory, Washington Road & Williams St., Princeton, NJ 08540 (swalker@princeton.edu)

Microorganisms produce a wide range of antimicrobial agents that bind to biosynthetic intermediates involved in cell wall biosynthesis. The best known of these substrate-binding antibiotics is vancomycin, which recognizes the terminal dipeptide of the pentapeptide attached to *N*-acetylmuramic acid, a key building block of bacterial peptidoglycan. However, there are many other natural products that bind to peptidoglycan precursors, and a significant number of them recognize Lipid II, the lipid-linked disaccharide that is polymerized by bacterial transglycosylases to form the carbohydrate chains of peptidoglycan. Despite their potential both as antibacterial agents and as model systems for understanding how to design small molecules that recognize cell surface ligands, little is known about most Lipid II-binding antibiotics. We will discuss work we have been doing to understand ramoplanin, a cyclic lipoglycodepsipeptide antibiotic that was originally proposed to kill bacterial cells by inhibiting MurG, the glycosyltransferase that converts Lipid I to Lipid II. Work in our lab has suggested instead that ramoplanin inhibits the transglycosylation step of peptidoglycan synthesis by binding to Lipid II. We will discuss the basis for this new mechanism and present what we know about how ramoplanin recognizes peptidoglycan intermediates. We will also discuss the crystal structure of MurG and how it could be used to design inhibitors that do block the conversion of Lipid I to Lipid II.

104. Macrocyclizations catalyzed by TE domains of nonribosomal peptide synthetaseses. Christopher Walsh. Department of Biological Chemistry and Molecular Phar-

macology, Harvard Medical School, Boston, MA 02115 (fax: 617-432-0438)

Nonribosomal peptide antibiotics grow as a series of elongating peptidyl-*S*-enzyme intermediates covalently tethered to carier protein domains in nonribosomal peptide synthetase assembly lines. Release of the full-length peptide involves chain transfer to the most downstream protein domain, the thioesterase (TE) domain of the assembly line, as a covalent peptidyl-*O*-seryl enzyme. TE-mediated release can be hydrolytic, as in vancomycin and the ACV precursor of penicillins, or can be cyclizing to create macrolactone and macrolactam rings, as in tyrocidine, bacitracin, and surfactin. We have studied the TE domains excised from tyrocidine synthetase and surfactin synthetase assembly lines for their autonomous ability to macrocyclize peptidyl-*S*-nacetylcysteamines (SNACs) and peptidyl-ONACs to probe structure, mechanism, and selectivity of product formation and to create libraries of cyclic macrolactones and macrolactams.

105. The structural basis for glycopeptide resistance. Daniel Kahne. Department of Chemistry, Princeton University, 160 Frick Lab, Princeton, NJ 08544 (fax: 609-258-2617, dkahne@princeton.edu)

Vancomycin and teicoplanin are the two glycopeptide antibiotics currently in clinical use for the treatment of methicillin-resistant Gram-positive infections. Both compounds bind the D-Ala-D-Ala dipeptide terminus of peptidoglycan precursors and prevent further processing of these precursors into mature peptidoglycan. The most common form of resistance to glycopeptide antibiotics arises when bacteria express genes that enable them to incorporate D-Ala-D-Lac instead of D-Ala-D-Ala into peptidoglycan precursors. Precursors terminating in D-Ala-D-Lac do not bind vancomycin or teicoplanin well, but still serve as substrates for the enzymes that make peptidoglycan. Given the similarities in their structures and mechanisms of action, there are some surprising differences in the activity of vancomycin and teicoplanin against bacterial strains containing genes involved in remodeling peptidoglycan precursors. For example, vancomycin has no activity against vanB strains whereas teicoplanin has full activity. Some analogues of vancomycin containing lipid substituents attached to the vancosamine sugar also have activity against resistant strains. The difference is evidently due to the fact that vancomycin induces expression of the genes that remodel peptidoglycan precursors whereas teicoplanin and the vancomycin analogues do not. We are interested in understanding the structural basis for induction of resistance and have made several derivatives of teicoplanin and vancomycin in order to delineate the structural features required to induce (or circumvent) resistance. The compounds were evaluated in a range of cell biological and biochemical assays. We will discuss the work, our conclusions about how resistance is induced, and the ramifications of our results for the design of glycopeptide antibiotics that circumvent resistance.

Wednesday Morning: Repligen Award Symposium

Dale Poulter, Organizer

106. Isoprenoid biosynthesis: structure−mechanism relationships in sesquiterpene and monoterpene cyclases. David W. Christianson. Department of Chemistry, University of Pennsylvania, 231 S. 34 St., Philadelphia, PA 19104-6323 (fax: 215-573-2201, chris@xtal.chem.upenn.edu)

Terpenoid cyclases catalyze the most complex reactions in biology, converting achiral isoprenoid substrates into hydrocarbon products containing multiple fused rings and stereocenters. Sesquiterpene cyclases utilize the universal C15-isoprenoid substrate farnesyl diphosphate. Crystal structures of sesquiterpene cyclases such as trichodiene synthase allow for the analysis of detailed structure−mechanism relationships. Surprisingly, despite substantial evolutionary drift in their amino acid sequences, these enzymes share a core terpenoid synthase fold. The active site of each cyclase serves as a template to fix the flexible substrate in the productive conformation for catalysis. Monoterpene cyclases utilize the universal C10-isoprenoid substrate geranyl diphosphate. The structure of (+)-bornyl diphosphate synthase reveals a terpenoid synthase fold with a shallower active site that accommodates the smaller isoprenoid substrate, as well as quaternary structural features contrasting with those of trichodiene synthase. Comparisons of monoterpene and sesquiterpene cyclases reveal additional contrasts in the cyclization of C10- and C15-isoprenoid substrates.

107. RNA-editing by adenosine deamination. Peter A. Beal. Department of Chemistry, University of Utah, 315 S. 1400 East, Salt Lake City, UT 84102 (fax: 801-581-8433, beal@chem.utah.edu)

RNA-editing is a process by which structural diversity is created in the proteomes of higher organisms via base modification reactions of pre-mRNA molecules. ADARs are adenosine deaminases that act on RNA and are responsible for RNA-editing reactions that occur in the pre-mRNAs of glutamate and serotonin receptors. ADARs capable of editing biologically relevant RNA substrates have been identified. However, our understanding of the mechanism of the ADAR-catalyzed adenosine deamination is limited. Here we describe an experimental approach to the study of the chemistry of ADAR-mediated RNA-editing reactions. A variety of analogues of a naturally occurring editing substrate have been prepared and characterized kinetically. These results have guided the design of active site-directed inhibitors of the editing reaction, including 8-azanebularine- and 6-trifluoropurine-containing RNA. Studies of the isolated RNA-binding domain of ADAR2 indicate it binds selectively adjacent to naturally occurring editing sites, demonstrating a critical role for this domain in controlling selectivity. In addition, conformational changes in the RNA substrate, which are consistent with base flipping, have been detected using 2-aminopurine (2-AP) fluorescence experiments. These experiments indicate that, although the RNA-binding domain of ADAR2 binds with high affinity and selectivity, the catalytic domain is required for the base flipping step.

108. Modular organization and ammonia transfer mechanisms in glutamine amidotransfereases. Vincent J. Davisson[1] and Janet L. Smith.[2] [1]Department of Medicinal Chemistry and Molecular Pharmacology and [2]Department of Biological Sciences, Purdue University, 1333 Robert Heine Pharmacy Building, West Lafayette, IN 47906 (fax: 765-494-1414, vjd@pharmacy.purdue.edu)

In primary metabolism, ammonia is captured as glutamine and used in the de novo biosynthesis of nitrogenous metabolites including amino acids, purines, and pyrimidines. The glutamine amidotransferases (GATs) catalyze the hydrolysis of glutamine to glutamate and transfer ammonia through molecular tunnels over distances of 20−100 Å to an acceptor site for incorporation into activated carbon substrates. One striking feature of these enzymes is their capacity to both regulate the production of ammonia and mediate the efficient transfer to a distinct acceptor active site. Two GAT subclasses exist with seven proteins assigned to the Triad subgroup. The three-dimensional structures from several laboratories have revealed wide variations in the evolution and mechanisms for the transfer of ammonia within the Triad GATs. Our efforts have focused on the structural and dynamic properties of GMP synthetase and IGP synthase to reveal the molecular features relating the glutaminase and acceptor sites.

109. New tricks for old enzymes. C. Dale Poulter. Department of Chemistry, University of Utah, 315 S. 1400 East, Salt Lake City, UT 84112 (fax: 801-581-4391, poulter@chem.utah.edu)

There are two major building reactions for constructing complex hydrocarbon chains from simpler precursors in the isoprenoid biosynthetic pathway. The most common reaction is the 1′−4 (head-to-tail) condensation, where the hydrocarbon moiety of an allylic diphosphate is attached to isopentenyl diphosphate to add an isoprene unit to the growing chain. The second reaction is the c1′−2−3 (cyclopropanation) condensation encountered in the sterol and carotenoid pathways, where two allylic diphosphates are condensed to double the size of the hydrocarbon moiety. A similar cyclopropanation joins two molecules of dimethylallyl diphosphate during biosynthesis of chrysanthemic acid. Phylogenetic correlations and X-ray structures indicate a common structural motif for the elongation and cyclopropanation enzymes. Experiments that demonstrate a structural and mechanistic similarity between farnesyl diphosphate synthase (chain elongation) and chrysanthemyl diphosphate synthase (cyclopropanation) from the sagebrush *Artemisia tridentata* speciformis will be presented.

Wednesday Afternoon: Probing Reactivity at Enzyme Active Sites

Peter Tipton, Organizer

110. *trans*-Sialidase, the case for SN_2-like behavior in a glycosyltransferase active site. Ben Horenstein,[1] Jingsong Yang,[1] and Sergio Schenkman.[2] [1]Department of Chemistry, University of Florida, Gainesville, FL 32611 (horen@chem.ufl.edu), and [2]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, Brazil

Trypanosoma cruzi trans-sialidase catalyzes the transfer of sialic acid between host and parasite glycoconjugates with retention of configuration. Kinetic isotope effect studies on recombinant *trans*-sialidase were utilized to determine transition state structural features for this glycosyltransferase. The enzymatic transfer reactions featured b-dideuterium and primary C-13 KIE's for sialyl-galactose of 1.060 ± 0.008 and 1.032 ± 0.008, respectively. The low deuterium isotope effect describes a transition state with a small degree of oxocarbenium ion character, and the large C-13 primary isotope effect is indicative of nucleophilic participation. A small amount of presumed covalent intermediate was detected by quench of reaction mixtures. Work toward chemical rescue of inactive *trans*-sialidase mutants will be described. The results for *trans*-sialidase will be considered in light of other glycosyl transfer reactions.

111. Thermodynamic bases for the obligate two-electron, oxygen-insensitive reactivity of enteric nitroreductase. Anne-Frances Miller,[1] Ronald L. Koder, Jr.,[2] Michael E. Rodgers,[3] Chad A. Haynes,[4] and David W. Rodgers.[4] [1]Department of Chemistry and [4]Department of Molecular and Cellular Biochemistry, University of Kentucky, Rose St., Lexington, KY 40506-0055 (fax: 859-323-1069, afm@pop.uky.edu), [2]The Johnson Research Foundation, University of Pennsylvania, and [3]Department of Biology, The Johns Hopkins University

The nitroreductase of *Enterobacter cloacae* metabolizes TNT and other nitrated aromatic substrates. Thus, it is relatively nonspecific. However, the FMN cofactor undergoes exclusively two-electron reduction, corresponding to reduction potentials which are below −380 mV for acquisition of the first electron but above 0 mV for acquisition of the second (E_m = −190 mV vs NHE). Our crystal structures of nitroreductase determined to 1.8 Å in the reduced and oxidized states reveal very large flavin butterfly bends of 25° and 16° from planar, respectively, and our ab initio calculations demonstrate that this should favor 2-electron reduction to the hydroquinone over 1-electron reduction. In addition, hydrogen bond donation by the protein to the flavin N5 position is expected to disfavor formation of the neutral semiquinone. Thus, we are able to rationalize the reduction potentials of nitroreductase and propose an explanation for its exclusive use of two-electron chemistry.

112. Quantitation of electronic strain in substrates bound at active sites. Vernon E. Anderson. Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4935 (fax: 216-368-3419, vea@po.cwru.edu)

Various spectroscopic changes can be detected when a substrate or inhibitor binds to the active site of an enzyme. The interpretation of the observed spectroscopic changes can reflect alterations in the conformation, dynamics, and electronic polarization of the bound molecule. Interpretation of observed spectral changes requires the consideration of all three factors. Changes in the UV−vis, ^{13}C NMR, and vibrational spectra of α,β-unsaturated CoA thiolesters binding to the active site of enoyl-CoA hydratase will be considered, and the role of each of the three factors considered. Under special circumstances, changes in the ^{13}C NMR chemical shifts can be interpreted as in terms of electronic rearrangement. A more direct measurement can arise from changes in the observed vibrational frequencies. The large spectral changes observed when α,β-unsaturated thiolesters bind to the active sites of enzymes in the enoyl-CoA hydratase family have been interpreted as supporting electronic strain as playing a fundamental role in enhancing the catalytic efficiency of these enzymes. An agreement of

quantum mechanical modeling of the ligand at the active site with the spectroscopic observations permits a quantitation of the electronic rearrangement and consequently a direct assessment of the role that ground-state electronic strain plays in enhancing the enzymatic reaction.

113. Substrate oxidation at the urate oxidase active site. Peter A. Tipton. Department of Biochemistry, University of Missouri, 117 Schweitzer Hall, Columbia, MO 65211 (fax: 573-884-4812, tiptonp@missouri.edu)

Urate oxidase catalyzes the O_2-dependent oxidation of urate to form 5-hydroxyisourate and H_2O_2. The catalytic reaction is unusual in that the enzyme does not utilize any transition metal or organic cofactor. Stopped-flow spectroscopic studies have provided evidence that a urate hydroperoxide species forms as a key intermediate during the catalytic reaction. The urate hydroperoxide intermediate has been trapped at the active site by dithiothreitol and tris(carboxyethyl)phosphine. Ab initio calculations suggest that the urate dianion should be very reactive toward O_2, which implies that a general base must exist at the active site to deprotonate the substrate. Site-directed mutagenesis studies suggest that Thr69 and Lys9 could potentially form a catalytic diad to abstract the proton from the substrate. Data from titration microcalorimetry, computational studies, and characterization of other site-directed mutants are being used to explore the role played by the protein in modulating the reactivity of urate toward O_2.

Wednesday Evening: Poster Sessiom II

John Blanchard, Organizer

114. Biochemical and physical properties of lipoic acid synthase. Robert M. Cicchillo, Natasha M. Nesbitt, and Squire J. Booker. Department of Biochemistry, Microbiology, and Molecular Biology, Penn State University, 101 Althouse Laboratory, University Park, PA 16802 (fax: 814-863-7024)

Lipoic acid (6,8-thioctic acid) is an essential cofactor in many multienzyme complexes that are involved in energy metabolism, such as the pyruvate dehydrogenase complex, the 2-oxoketoglutarate dehydrogenase complex, the branched-chain oxo-acid dehydrogenase complex, and the glycine cleavage system. The biosynthesis of this cofactor involves the insertion of two sulfur atoms into two completely unactivated carbon atoms of octanoic acid or a derivative thereof. The inertness of the substrate limits the possible mechanisms for sulfur incorporation, and should rule out polar processes. The reaction is believed to be catalyzed primarily by a 38 kDa metalloprotein, which has been designated lipoic acid synthase. The amino acid sequence of the protein indicates that it might belong to an emerging class of enzymes that use *S*-adenosyl-L-methionine and iron−sulfur clusters to catalyze reactions by high-energy radical mechanisms. We have subcloned and isolated the enzyme from *Escherichia coli*, and show that it contains Fe_4S_4 clusters that can easily form Fe_3S_4 clusters. In addition, we show that the enzyme contains ∼1 equiv of zinc per polypeptide, and can bind up to 1 equiv of the cofactor pyridoxal 5′-phosphate. We provide evidence for the involvement of the acyl-carrier protein in the reaction. Last, we present results from the characterization of the enzyme using a number of biophysical studies, including electron paramagnetic resonance spectroscopy, EXAFS, and Mössbauer spectroscopy.

115. Isoelectric charge patterning in the design of tetrameric coiled-coils. Dennis T. Bong and and M. Reza Ghadiri. Department of Chemistry and Molecular Biology, The Scripts Research Institute, 10550 N. Torrey Pines Rd., LaJolla, CA 92037 (fax: 212-854-2755, dbong@chem.columbia.edu)

Ionic interactions in water are of fundamental and practical importance to the chemistry of biomolecules. We present herein the first systematic study of the influence of electrostatic patterning along protein interfaces on the folding behavior of peptides predisposed to form parallel coiled-coil tetramers by their hydrophobic core. Certain charge patterns are completely disastrous for peptide folding, while other patterns appear to direct folding along alternate pathways and oligomerization states. Complementarily patterned peptides could be induced to form heteromeric assemblies. Charge interactions can therefore have large effects on assembly state, helix orientation, and selectivity of assembly; denaturation experiments revealed that these interactions have an energetic influence on the order of 3−5 kcal/mol, roughly 20−30% of the total free energy of folding. This supports the notion that the details of coiled-coil folding (beyond actual assembly) are controlled by small energetic increments and that natural coiled-coil configurations may represent relatively shallow energetic minima

116. Methyl transfer between amines: a new biomimetic reaction. B. Patrick Callahan and Richard Wolfenden. Department of Biochemistry and Biophysics, University of North Carolina, CB #7260, Chapel Hill, NC 27599 (bcallaha@email.unc.edu)

During the spontaneous decarboxylation of amino acids in water, we observed the accumulation not only of the expected methylamine but also of dimethylamine and trimethylamine. Incubating any one of these amines alone under the same conditions, we observed its disproportionation, at a velocity proportional to the square of the amine concentration. Reaction velocities reached a maximum at the amine's pK_a value, as expected for methyl transfer from the protonated to the unprotonated species. When the donor was the (nondissociable) tetramethylammonium ion, the reaction velocity approached a constant value at and above the acceptor's pK_a value. Enthalpies of activation for methyl transfer to a common acceptor, from trimethylsulfonium and from tetramethylammonium, differ by 7 kcal/mol. That difference presumably explains Nature's preference for sulfur as a methyl donor.

117. Binding properties of deglycosylated human orosomucoid gene products I and II. Andris Kronbergs and H. Brian Halsall. Department of Chemistry, University of Cincinnati, P.O. Box 21072, Cincinnati, OH 45221-0172 (kronbea@email.uc.edu)

Human α1-acid glycoprotein (also known as orosomucoid, OMD) is a plasma glycoprotein of 36.4 kDa in molecular weight, approximately 45% of which consists of carbohy-

drate, attached in the form of 5 N-linked glycans. It belongs to the group of the positive acute phase proteins, and although it has been extensively studied over many years, its exact physiological function is still unknown. Drug−protein interaction is one of the most important aspects of pharmacology. OMD is one of the major drug binding proteins in plasma, with a preference for basic and neutral drugs, although acidic drugs with a carboxyl group are also bound. OMD is also able to bind steroid hormones and vanilloids. Difference in binding properties between two gene products of a native human orosomucoid has been studied. Most studies showed that drug−OMD binding was not affected by the removal of sugar residues such as sialic acid; however, this could not exclude the possibility of inducing different effects in case of complete deglycosylation of OMD (dg OMD). The main scope of this project is to conduct calorimetric studies of drug−dg OMD to examine the thermodynamics of the interaction and describe the effect of the absence of sugar residues on the interaction as well as to investigate the differential binding properties of the native and deglycosylated OMD gene products.

118. Kinetic studies on mutants of picromycin thioesterase domain. Hongxiang Lu and David E. Cane. Department of Chemistry, Brown University, 324 Brook St., Providence, RI 02912 (Hongxiang_Lu@Brown.edu)

We have cloned and overexpressed the thioesterase (TE) domain of picromycin synthase (PICS) in *E. coli*. Kinetic data were obtained on the protein with different *N*-acetyl cysteamine thioester substrates. The results indicate that the TE domain prefers the substrate that mimics the important features of its natural substrate. At the same time, the cystral structure of TE was solved. By docking its natural substrate into the substrate pocket computationally and comparing the structure with the TE structure of 6-deoxyerythronolide B synthase (DEBS), we speculate that several amino acids in the substrate pocket of PICS TE are important for the substrate specificity. A series of site-directed mutations were made on these amino acids, and their kinetic parameters were determined.

119. Theoretical study on spectral tuning in bacteriorhodopsin and sensory rhodopsin II. Lei Ren,[1] Charles H. Martin,[1] Kevin Wise,[2] Nathan Gillespie,[1] Hartmut Luecke,[3] Janos K. Lanyi,[4] John L. Spudich,[5] and Robert R. Birge.[6] [1]Department of Chemistry, Syracuse University, 1-014 CST, Syracuse, NY 13244 (fax: 860-486-2981, lren@syr.edu), [2]Department of Chemistry and of Molecular and Cell Biology, University of Connecticut, [3]Department of Molecular Biology and Biochemistry, University of California, [4]Department of Physiology and Biophysics, University of California, [5]Department of Microbiology and Molecular Genetics and Structure Biology Center, University of Texas Medical School, and [6]Department of Chemistry, University of Connecticut and Syracuse University

Quantum mechanical and molecular mechanical calculations of bacteriorhodopsin (bR) and sensory rhodopsin II (SRII) have been carried out to determine the difference of opsin shifts between these two proteins. SRII is unique among the archaeal rhodopsins in having an absorption maximum near 500 nm, roughly 70 nm blue-shifted from the other pigments, such as bR. The molecular origins responsible for both photophysical properties are examined here with reference to the 2.4 Å crystal structure of sensory rhodopsin II (NpSRII) from *Natronobacterium pharaonis*. We use semiempirical molecular orbital theory (MOZYME) to optimize the chromophore within the chromophore binding site, and MNDO−PSDCI molecular orbital theory to calculate the spectroscopic properties. Through a comparison of corresponding calculations on the 1.55 Å crystal structure of bacteriorhodopsin (bR), we identify the principal molecular mechanisms, and residues, responsible for the spectral blue shift in NpSRII. We conclude that the major source of the blue shift is associated with significantly different positions of Arg-72 (Arg-82 in BR) in the two proteins.

120. $^{13}C/^{15}N$ Labeled 2-amino-2-deoxy-aldohexoses and their derivatives: synthesis, *J*-coupling, and DFT studies. Yuping Zhu,[1] **Qingfeng Pan**,[1] Ian Carmichael,[2] and Anthony S. Serianni.[1] [1]Department of Chemistry and Biochemistry and [2]Notre Dame Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556-5670 (fax: 219-631-6924, yuping_zhu@merck.com, qpan@nd.edu)

Biologically relevant glycosidic linkages involving 2-amino-2-deoxy-D-aldohexoses and their *N*-acetylated derivatives yield *trans*-*O*-glycoside $^3J_{COCC}$ that cannot be interpreted using a Karplus relationship recently developed for *O*-substituted coupling pathways. Therefore, a series of 2-amino-2-deoxy-D-[1-^{13}C]-aldohexose glycosides was prepared using a new chemical route. D-Aldopentoglycosylamines were prepared, and subsequent cyanohydrin reduction using K^{13}CN gave 2-aminosugar epimers which were separated on Dowex 50 (H^+) columns. *N*-Acetylation and methyl glycosidation, followed by purification, gave ^{13}C-labeled *N*-acetylated pyranosides. J_{CH} and J_{CC} were obtained by NMR and compared to those observed in aldohexopyranosides [(1995) *J. Am. Chem. Soc. 117*, 8635]. Model compounds of *N*-acetylated saccharides were devised to calculate *J*-couplings using DFT [(1999) *J. Am. Chem. Soc. 121*, 9843]. This work provides a test of predictions made in a recent theoretical study [(2000) *J. Am. Chem. Soc. 122*, 6435] in which effects of *N*-substitution on J_{CH} and J_{CC} were evaluated. This synthetic method was also modified for ^{15}N-labeling.

121. An essential but paradoxical glutamyl-tRNA synthetase defines the inception of glutaminyl-tRNA synthetase. Tamara L. Hendrickson,[1] Stéphane Skouloubris,[1] Hilde de Reuse,[2] and Agnes Labigne.[2] [1]Department of Chemistry, Johns Hopkins University, Remsen Hall, 3400 N. Charles St., Baltimore, MD 21218 (fax: 410-516-8420, Tamara.Hendrickson@jhu.edu), and [2]Pasteur Institute-IPBM

All life uses essentially the same basic machinery for protein translation. Consequently, many of the components of the protein translation apparatus were fixed in evolution prior to divergence of the tree of life. Thus, efforts that seek to delineate the evolution of components of this apparatus are often limited to phylogenetic and experimental analyses of existing genomes, without the ability to directly observe the origin of a given enzyme function. Glutaminyl-tRNA synthetase (GlnRS) is a unique exception. GlnRS is missing in most bacteria and seemingly all archaea and apparently

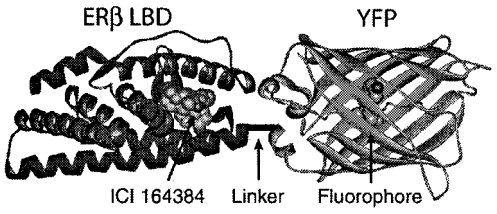
evolved after divergence of eukarya and bacteria. [Instead, in these organisms, Gln-tRNA(Gln) is biosynthesized indirectly.] Thus, the evolution of GlnRS activity offers a unique opportunity to explore one aspect of the emergence of specificity in protein translation. Phylogenetic comparisons suggest that GlnRS evolved from glutamyl-tRNA synthetase (GluRS), via a recent gene duplication event, followed by evolution of specificity for tRNA(Gln) and glutamine. Here we report the identification of an essential but paradoxical wild-type GluRS (GluRS2), which coexists with a canonical GluRS (GluRS1), in the bacterium *H. pylori*. These two paralogous GluRSs have opposing and complementary tRNA specificities. GluRS2 preferentially generates Glu-tRNA(Gln) rather than Glu-tRNA(Glu). But, it has not yet evolved the ability to generate Gln-tRNA(Gln). Thus, GluRS2 is an ancestor of the modern GlnRS, but it has been retained in an extant organism.

122. An SDS−PAGE assay to detect kinetically stable proteins. Marta Manning and Wilfredo Colón. Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180 (kepinm@rpi.edu)

Kinetically stable proteins are resistant to unfolding even under thermodynamically unfavorable conditions. Their kinetic stability is made possible by the presence of a high activation energy barrier, which prevents unfolding from taking place without the use of extreme solvent conditions, such as high temperature or high concentrations of chemical denaturants. The purpose of our research is to develop an assay of kinetic stability in proteins using sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). The behavior of several kinetically stable proteins treated with SDS was examined using gel electrophoresis before and after the protein samples were boiled. We observed a difference in migration on the gel between the boiled and unboiled protein samples studied, attesting to their resistance to SDS-induced denaturation. The relationship between kinetic stability and resistance to SDS seems to be accompanied by a correlation between resistance to SDS denaturation and the presence of β-sheet protein structure.

123. Analysis of small molecule−protein interactions in recombinant yeast: coupling folding of yellow fluorescent protein to the stability of estrogen receptor ligand binding domains. Smita S. Muddana and Blake R. Peterson. Department of Chemistry, The Pennsylvania State University, 152 Davey Lab, University Park, PA 16802 (fax: 814-863-8403)

Small molecule−protein interactions underlie many fundamental processes in biology and form the basis for pharmacological intervention in human disease. The binding of ligands to proteins often stabilizes protein folding, and this enhanced stability can be coupled to the folding of an appropriately fused reporter domain. We engineered yeast to express truncated estrogen receptor (ER) ligand binding domains (LBD) fused to yellow fluorescent protein (YFP) to couple ligand binding-induced stabilization of the ER to folding of YFP. Analysis by flow cytometry revealed that the fluorescence of YFP was enhanced by as much as 10-fold in yeast treated with the ER ligands estradiol or ICI 182780. Comparisons of dose-dependent fluorescence enhancements with analogous yeast two-hybrid transcriptional assays showed strong correlations between these complementary methods of analysis of small molecule−protein interactions. This method has significant potential as an inexpensive assay for high-throughput screening of compounds against nuclear hormone receptor drug targets.



124. Antibacterial protein-based surfaces. JaimeLee I. Cohen,[1] **Karin Melkonian**,[2] Maya Filshtinskaya,[1] Tanya Abel,[1] Jasmine Escalera,[1] Alice Melkonian,[2] Russell Fincher,[2] and **Robert Engel**.[3] [1]Department of Chemistry and Physical Sciences, Pace University, 1 Pace Plaza, New York, NY 10038 (fax: 212-346-1256, jcohen@pace.edu), [2]Department of Biology, Long Island University, C.W. Post Campus, Northern Blvd., Greenville, NY 11548 (kmelkoni@liu.edu), and [3]Department of Chemistry, Queens College and GSUC CUNY, Queens College of the City University of New York and the Graduate Center of CUNY, 65-30 Kissena Blvd., Flushing, NY 11367 (fax: 718-997-5198, robert_engel@qc.edu)

Surfaces bearing protein units (wool, silk) have been modified in a two-step process to incorporate units that bear antibacterial activity. Specifically, surface serine units have been activated at the free hydroxyl groups by tosylation followed by displacement of the tosylate functionality with a tertiary amine bearing a variety of alkyl functionalities. The effectiveness of these modified surfaces for antibacterial action against a series of seven Gram-positive and Gram-negative bacteria is reported. Structural factors maximizing the antibacterial activity have been studied, and surfaces have been prepared that exhibit bactericidal activity against all bacteria tested. Procedures for the generation of such surface materials are discussed along with the methods of determination of antibacterial activity and the mode of activity.

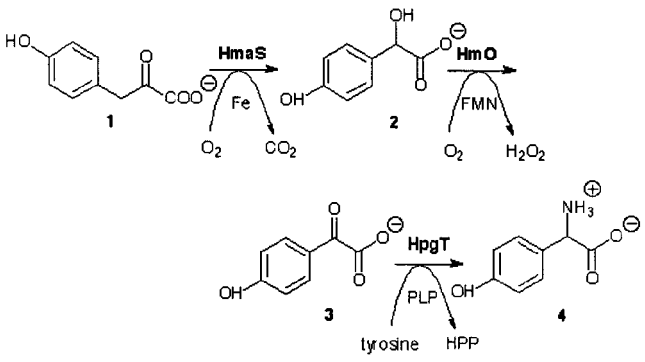
125. Biochemical and spectroscopic characterization of human heart L-3-hydroxyacyl-CoA dehydrogenase. Pravin A. Nair,[1] Jiaquan Wu,[1] Alasdair F. Bell,[1] Barycki J. Joseph,[2] Leonard J. Banaszak,[2] and Peter Tonge.[1] [1]Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400 (fax: 631-632-5797, pnair@ic.sunysb.edu), and [2]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN

Human heart L-3-hydroxyacyl-CoA dehydrogenase (HAD) is the penultimate enzyme in the oxidation pathway and catalyzes the NAD-dependent oxidation of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. We are using site-directed mutagenesis (SDM), kinetics, and Raman spectroscopy to elucidate the mechanism of the enzyme. X-ray crystallography studies suggested key residues that hydrogen bond to the substrate and cofactor, namely, histidine 158, glutamate 170, serine 137, and asparagine 208. Histidine 158 has been

proposed to be the key active site residue acting as a catalytic base, and when abstracted from the 3-OH group of the substrate, the glutamate residue is proposed to stabilize the positive charge that results from the proton abstraction. Raman spectroscopy has been used to characterize the structure of the complex of wild-type HAD+NAD^++AcAc-CoA which was discovered to form a charge-transfer complex. Labeled compounds will be used to assign the Raman bands from the 3-ketoacyl-CoA and find structure−reactivity correlation.

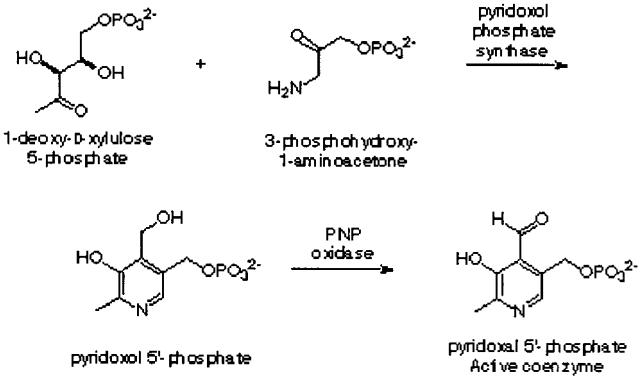
126. Biosynthesis of the vancomycin group of antibiotics: three steps lead to the formation of (*S*)-4-hydroxyphenylglycine. Jonathan B. Spencer, **Oliver W. Choroba**, Tsung-Lin Li, Simon W. O'Brien, and Dudley H. Williams. Cambridge Centre for Molecular Recognition, Department of Chemistry, Cambridge University, Lensfield Rd., Cambridge CB2 1EW, United Kingdom (fax: 0044-1223-336362, jbs20@cam.ac.uk, owc20@cam.ac.uk)

The vancomycin family of glycopeptide antibiotics are important as drugs of last resort against Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). Bacterial strains resistant to vancomycin have been isolated in ever increasing numbers, raising the possibility that a MRSA strain may soon acquire resistance to vancomycin. An attractive approach to the synthesis of new glycopeptide antibiotics, with potentially greater potency than vancomycin, is to manipulate the biosynthetic pathway of the producing organism. We have set out to elucidate the biosynthetic pathway of chloroeremomycin, a close homologue of vancomycin. 72 kb of genomic DNA from *Amycolatopsis orientalis* has been sequenced, and we cloned and studied the enzymes coded for by *orf* 21, 22, and 17 and showed their involvement in the biosynthesis of (*S*)-4-hydroxyphenylglycine **4**. 4-Hydroxymandelate synthase (HmaS, Orf 21) was characterized as an unusual iron(II)-containing dioxygenase which converts 4-hydroxyphenylpyruvate **1** into 4-hydroxymandelate **2**. Oxidation by hydroxymandelate oxidase (HmO, Orf 22) delivers the keto acid 4-hydroxyphenylglyoxylate **3**. HmO is FMN-dependent and utilizes molecular oxygen as electron acceptor, which is reminiscent of oxidases rather than dehydrogenases which use ubiquinone or cytochrome b_5 instead. A PLP-dependent transaminase (HpgT, Orf 17) finally yields 4-hydroxyphenylglycine **4** with tyrosine as amino donor.



127. Biosynthesis of vitamin B_6: an investigation of the pyridoxol phosphate synthase mechanism. Valerie A. Frydrychowski and David E. Cane. Department of Chemistry, Brown University, 324 Brook St., Providence, RI 02912 (fax: 401-863-2594, Valerie_Frydrychowski@brown.edu)

Pyridoxol phosphate synthase is the penultimate enzyme involved in the biosynthesis of pyridoxal 5′-phosphate, the active B_6 vitamen, in *Escherichia coli*. This enzyme catalyzes the formation of pyridoxol 5′-phosphate, the precursor to pyridoxal 5′-phosphate, from 1-deoxy-D-xylulose 5-phosphate and, presumably, 3-phosphohydroxy-1-aminoacetone. We are interested in the mechanism of the pyridoxol phosphate synthase reaction. An investigation of the stereochemical aspects of this reaction will be described. In addition, ongoing efforts to identify reaction intermediates by rapid mix−rapid quench experiments will be discussed.



128. Characterization of a complex between biotin synthase and flavodoxin. Jason T. Wan and Joseph T. Jarrett. Department of Biochemistry and Biophysics, University of Pennsylvania, 904 Stellar-Chance Labs, 422 Curie Blvd., Philadelphia, PA 19104 (fax: 215-573-8052)

E. coli flavodoxin has been shown to be necessary for the catalytic activity of many enzymes. Upon reduction to the semiquinone or fully reduced hydroquinone form, flavodoxin (Fld1) can provide electrons to enzymes that require low-potential electrons for activation or catalysis, including cobalamin-dependent methionine synthase and several AdoMet-dependent radical enzymes. AdoMet-dependent radical enzymes utilize electrons from flavodoxin to catalyze reductive cleavage of AdoMet and generation of the strongly oxidizing 5′-deoxyadenosyl radical. In the last step of biotin biosynthesis, biotin synthase is a 80 kDa dimeric iron−sulfur protein that requires Fld1 for generation of 5′-deoxyadenosyl radicals necessary for oxidation of and insertion of sulfur into dethiobiotin. Using chemical cross-linking, ultracentrifugation, and spectrophotometric methods, we have identified a tight stoichiometric complex between BioB and Fld1. Data will be presented describing factors affecting the affinity of this complex and implicating specific protein residues essential for the protein−protein interaction.

129. Characterization of the branched-photocycle intermediates P and Q of bacteriorhodopsin. Nathan B. Gillespie,[1] Kevin J. Wise,[2] Jeffrey A. Stuart,[1] Duane L. Marcy,[1] Qun Li,[1] Lavoisier Ramos,[3] Kevin Jordan,[3] Lei Ren,[1] and Robert R. Birge.[3] [1]Department of Chemistry, Syracuse University, 1-014 CST, Syracuse, NY 13244 (nbgilles@syr.edu), and [2]Departments of Biology and [3]Chemistry, University of Connecticut

The bacteriorhodopsin branched-photocycle intermediates P and Q are studied with respect to photochemical origin and photoreversibility, and compared to spectrally similar thermal denaturation products of purple membrane. Suspensions of the purple membrane form of bacteriorhodopsin containing low concentrations of water result in increased accumulation of P, which can be attributed to the role that water plays in the hydrolysis reaction that converts the P-state to Q. Relatively large photostationary state populations of these intermediates can be generated in both high glycerol content suspensions and acrylamide gels, and then fully regenerated to bR with UV/violet light. Chromophore extraction and HPLC analysis reveal that P, Q, and spectrally similar thermal products contain a 9-*cis*-retinal chromophore. The thermal products are also found to contain the 7-cis (1−3%) isomer of retinal. Time-resolved spectra of bR reveal that P is comprised of two components. The extinction coefficients of the two components of P ($\lambda_{max} = 445$ and 525 nm, respectively) and pure Q in a glycerol/water suspension are calculated from steady-state spectra. As applied to temporal kinetic studies, they provide further evidence that the P-state is predominantly formed from photocycle intermediates rather than via direct excitation of light-adapted bR.

130. Chitin-induced biosynthesis of phytoalexin 4′-deoxyaurones in cell suspension cultures of *Cephalocereus senilis*. Isagani D. Padolina and Tom J. Mabry. Section of Molecular Cell and Developmental Biology, University of Texas at Austin, BIO 311 Biological Laboratories, School of Biological Sciences, Austin, TX 78712 (fax: 512-232-3402, gdpadolina@mail.utexas.edu)

Chitin-elicited cell suspension cultures of *Cephalocereus senilis* produce several unusual flavonoids lacking the normal 4′-hydroxyl substitution in the B-ring, including the red pigment cephalocerone, a 4′-deoxyaurone. Previous enzymological studies showed that the elicited cell suspension cultures have the necessary enzymatic activities required for the conversion of L-phenylalanine to the 4′-deoxyflavonoids. Explanations for the 4′-deoxyflavonoid biosynthesis include: (1) a bypass of cinnamic acid 4-hydroxylase, therefore leading to an accumulation of 4′-deoxy precursors available for the 4′-deoxyflavonoid production: and (2) a dehydroxylation step within the flavonoid biosynthesis pathway. The current biochemical investigations into the biosynthesis of the phytoalexin cephalocerone using HPLC metabolic profiling of isotope dilution experiments established the existence of a novel 4′-dehydroxylation step instead of a 4′-hydroxy "bypass". Our results strongly suggest that the metabolic flux involved in the selective production of 4′-deoxyflavonoids may be controlled by a specific enzyme activity that dehydroxylates the 4′-position of the tetrahydroxychalcone B-ring.

131. Cloning and expression of thioredoxin and thioredoxin reductase from *Thermoplasma acidophilum*. Hector H. Hernandez,[1] Bernard A. Brown II,[2] and Catherine L. Drennan.[1] [1]Departments of Chemistry and [2]Biology, Massachusetts Institute of Technology, 56-546, 77 Massachusetts Ave., Cambridge, MA 02139 (hectorh@mit.edu)

Thioredoxin (TRX) and thioredoxin reductases (TRR) are responsible for the rereduction of ribonucleotide reductases (RNRs). RNRs are found in all organisms and catalyze the conversion of nucleotides to deoxynucleotides. This reaction is an essential step in DNA biosynthesis. To completely characterize RNR from the thermophile *Thermoplasma acidophilum*, TRX and TRR are required. Thus, we have identified the *T. acidophilum* genes encoding proteins TRX (ta0866) and TRR (ta0984) by sequence homology, and these genes have been amplified and cloned. Expression of recombinant $(His)_6$-tagged TRX and TRR proteins was confirmed by SDS−PAGE. Progress is being made in the biochemical characterization of these enzymes in relation to the ribonucleotide reductase system.

132. Creation of enantioselective hydrolases by engineered substrate assisted catalysis. Anders Magnusson and Karl Hult. Department of Biotechnology/Biocatalysis Group, Royal Institute of Technology/KTH, Roslagstullsbacken 21, 106 91 Stockholm, Sweden (fax: +46-8-55378468, andersm@biochem.kth.se)

We have investigated a strategy to change the enantioselectivity of hydrolases toward substrates carrying a hydrogen-bond donor. A rationally designed mutation in *Candida antarctica* lipase B gave remarkable results. The oxyanion hole, a spatial arrangement of hydrogen-bond donors, plays a major role in the stabilization of the transition state conformation. By changing threonine 40, we have removed one of the hydrogen-bond donors capable of stabilizing the transition state. With a hydroxyl group as hydrogen-bond donor, we have earlier showed a 14-fold increase for the enantioselectivity. Here we have used the corresponding substrate but with an amino group. The total effect on the enantioselectivity is very similar, and for both cases, the R-enantiomer is favored by the mutation. With the amino group, we have also reversed the selectivity. Thus, we can use different hydrogen-bond donors, and this strategy should also be useful for other enzymes with similar active site conformation.

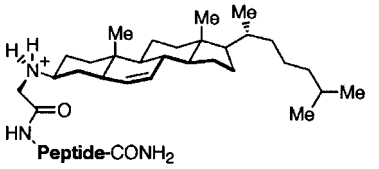
133. Crystal structure of B_{12}-dependent ribonucleotide reductase: a paradigm for allosteric regulation in a monomeric enzyme. Michael D. Sintchak, Gitrada Arjara, Brenda A. Kellogg, JoAnne Stubbe, and Catherine L. Drennan. Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (fax: 617-258-7847, oxalis@mit.edu)

Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to deoxyribonucleotides, an essential step in DNA biosynthesis and repair. Here we present the crystal structure of class II (coenzyme B_{12}-dependent) ribonucleoside triphosphate reductase from *Lactobacillus leichmannii* in the apo enzyme form and in complex with the B_{12} analogue adeninylpentylcobalamin at 1.75 and 2.0 Å resolution, respectively. This monomeric, allosterically regulated, class II RNR retains all the key structural features associated with the catalytic and regulatory machinery of oligomeric RNRs. The structure described here completes the trilogy of structures defining the catalytic subunits of the three well-characterized classes of RNR, and provides the first opportunity to observe the juxtaposition of active site, cofactor site, and allosteric specificity site in a ribonucleotide reductase. Surprisingly, the dimer interface responsible for effector

binding in class I RNR is preserved through a single 130 residue insertion in the class II structure. The *L. leichmannii* class II RNR structure provides insight into chemistry-driven divergent evolution and allosteric regulation of specificity.

134. Delivery of peptide-binding proteins into mammalian cells with synthetic cholesterylamine-terminated peptides. Scott E. Martin and Blake R. Peterson. Department of Chemistry, Pennsylvania State University, 152 Davey Lab, University Park, PA 16802 (fax: 814-863-8403, sem225@psu.edu)

The efficacy of macromolecular therapeutics and cellular probes is often limited by poor uptake of macromolecules by mammalian cells. We report here the synthesis and biological evaluation of novel cholesterylamine-derived lipopeptides that promote endocytosis of peptide-binding proteins and protein complexes by enabling strong noncovalent interactions at cellular plasma membranes. The plasma membranes of Jurkat lymphocytes were decorated with antigenic peptides and related protein ligands by treatment of cells with short cholesterylamine-terminated peptides. These peptides comprised HA-Tag, Flag-Tag, and Strep-Tag II peptide sequences. Subsequent addition of cognate peptide-binding antibodies or streptavidin to cells renders these proteins intracellular within 12 h by accessing endogenous mechanisms controlling clathrin-mediated endocytosis. The synthesis of these agents, mechanistic studies of cellular uptake, and potential applications in the areas of DNA delivery, tumor therapy, and stimulation of immune responses will be presented.



Peptide = HA-Tag (GYPYDVPDYA)
Peptide = Flag-Tag (GDYKDDDDK)
Peptide = Strep-Tag II (GSNWSHPQFEK)

135. Detection of an interchain cross-link formed in a CpG sequence by the acrolein DNA adduct γ-OH-1,N^2-propano-2′-deoxyguanosine. Hye-Young H. Kim, Markus Voehler, Thomas M. Harris, and Michael P. Stone. Department of Chemistry, Vanderbilt University, 1822-B, Nashville, TN 37235 (fax: 615-343-0384, hye-young.h.kim@vanderbilt.edu)

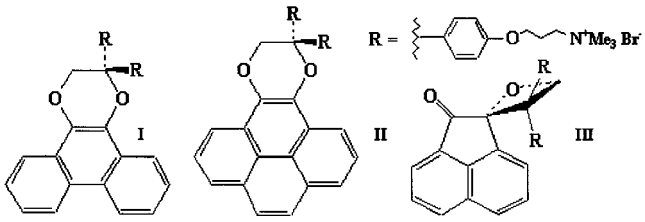
The primary adduct formed by acrolein in duplex DNA is the 1,N^2-exocyclic propano lesion on deoxyguanosine, γ-OH-pdG. In duplex DNA, this exocyclic lesion can open to the N^2-(3-oxopropyl)-deoxyguanosine derivative. Evidence for formation of interchain cross-linking in the CpG sequence was reported. However, it was not possible to determine the chemical identity of the DNA cross-link with certainty. Chemical synthesis of γ-OH-pdG allowed us to exploit the chemical species that were present in the DNA duplex. Herein, NMR spectroscopy revealed reaction between the ring-open N^2-(3-oxopropyl)-deoxyguanosine derivative and the exocyclic amino group of the deoxyguanosine base of the 5′-neighboring base pair. The cross-link was identified by placing deoxyguanosine, isotopically labeled with ^{15}N at the N^2 position, into the complementary strand of the acrolein-modified duplex. A series of ^{15}N-edited NMR spectra revealed cross-peaks from the amino proton of the ^{15}N-labeled deoxyguanosine in the complementary strand, to the propyl protons of the acrolein-modified deoxyguanosine nucleotide.

136. Development of a molecular sensor for detection of thymine glycol. Jay R. Carreon and Shana O. Kelley. Department of Chemistry, Boston College, 2609 Beacon St., Merkert Chemistry Center, Chestnut Hill, MA 02467 (carreon@bc.edu)

Thymine glycol (5,6-dihydro-5,6-dihydroxythymine) is a major product of oxidative DNA base damage caused by cellular radicals. Thymine glycol interferes with DNA replication, and its introduction into genomic DNA is therefore proposed to have a deleterious effect on cellular function. To facilitate the study of the causes and effects of DNA damage manifested as thymine glycol, we have developed a detection method that exploits the chemical reactivity of the unique 5,6-diol of this base lesion. We have generated a fluorescent molecular sensor reporting the presence of thymine glycol that will be useful for the detection of this damaged base within genomic DNA.

137. Dihydrodioxins and oxetanes: masked *o*-quinones as DNA cleaving agents. Dagne Birzniece, Eric T. Mack, and R. Marshall Wilson. Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221-0172 (fax: 513-556-9239)

The water-soluble masked quinones I−III have been synthesized and their binding to DNA studied by a variety of methods. The relative efficiency of each of these masked quinones as DNA photocleaving agents has been evaluated using ϕX-174. Correlations between binding constant/modes and cleaving efficiencies will be discussed.



138. DNA strand scission and mutagenesis at DNA deoxyribonolactone lesions. Terry L. Sheppard, Yingli Wang, and **Yan Zheng**. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113 (fax: 847-491-7713, sheppard@chem.northwestern.edu)

Reactive oxygen species lead to oxidative damage of the nucleobase and sugar components of nucleotides in DNA, which may lead to DNA strand scission and misincorporation of nucleotides during cellular nucleic acid synthesis. The deoxyribonolactone lesion results from the oxidation of the C-1′ position of nucleotides in DNA. Our laboratory previously reported a photochemical method for the site-specific introduction of deoxyribonolactone lesions in DNA oligonucleotides. These modified oligonucleotides were used to assess the biochemical properties of the lesion. The half-

life for DNA strand scission at the lesion site was measured under simulated physiological conditions. The DNA cleavage products were characterized by chemical fingerprinting and MALDI-TOF mass spectrometry. Direct evidence for the beta-elimination product was obtained. DNA templates containing lactone lesions were used as substrates in polymerase extension studies. DNA polymerases insert dA opposite the deoxyribonolactone lesion, following the "A-rule" of abasic site mutagenesis.

139. Effects of substrate structure on the reaction pathway in chloroperoxidase catalyzed peroxidation of phenols and anilines. R. Daniel Libby, Susan Schiavo, Stephanie Horne, Leah Williams, and Eman Jarrah. Chemistry Department, Moravian College, 1200 Main St., Bethlehem, PA 18018 (fax: 610-625-7918, rdlibby@cs.moravian.edu)

A kinetic study of the chloroperoxidase catalyzed peroxidation of a series of 4-substituted phenols and anilines showed that the *V/K*'s for the reactions were dependent upon the extent of branching in the substituent. For both substituted anilines and phenols, although effects on V_{max} and K_m were mixed, *V/K* decreased in the order: butyl > *s*-butyl > *tert*-butyl. *V/K* values for similarly substituted anilines and phenols are equal within experimental error. Comparison of product distributions of CPO catalyzed reactions with HRP catalyzed reactions of the same substrates showed identical products for corresponding phenol substrates but differences for corresponding aniline substrates. Horseradish peroxidase catalyzed peroxidatic reactions are known to occur entirely through a radical pathway. The product distribution studies suggest that phenol reactions occur by a radical pathway while aniline may not. The results will be discussed in terms of a proposed reaction mechanism.

140. Electrostatic stabilization of an oxacarbenium ion by uracil DNA glycosylase. Yu Lin Jiang,[1] Fenhong Song,[2] and James T. Stivers.[1] [1]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 (fax: 410-955-3023), and [2]Center for Advanced Research in Biotechnology, National Institute of Standards and Technology, and University of Maryland Biotechnology Institute

Hydrolytic cleavage of the glycosidic bond of unwanted deoxyuridine residues in DNA is catalyzed by the powerful DNA repair enzyme uracil DNA glycosylase (UDG). We have previously established that the reaction proceeds through a stepwise mechanism involving an unprecedented positively charged oxacarbenium ion sugar and uracil anion leaving group. Here we evaluate the catalytic contribution of electrostatic interactions between four phosphodiester groups of the substrate and the cationic intermediate by systematic deletion of the phosphates, or by substituting each group with an uncharged *R*- or *S*-methylphosphonate linkage (MeP). Deoxyuridine, which has no anionic groups at all, has a k_{cat} for hydrolysis that is 1 million-fold less than the optimal substrate A+2pA+1pU-1pA-2pA, indicating that the combined catalytic effect of all anionic charges on the substrate cannot exceed 9.2 kcal/mol. Further investigation at the individual positions revealed that (i) the +2 phosphodiester makes no contribution to catalysis, (ii) the +1 position contributes 4−7 kcal/mol to catalysis, (iii) the C1 group contributes 2.5 kcal/mol, and (iv) the C2 position makes a large ~ 8 kcal/mol catalytic contribution. These results, which reveal the net energetic effect of each phosphodiester substituent, provide upper limit estimates of electrostatic effects in catalysis. These estimates are significantly less than the net 22 kcal/mol contribution calculated in a recent QM/MM computational study.

141. Energetic contribution of stacking interaction of U1A−U1snRNA complex. Anne M. Baranger and **Yulianingsih Benitex**. Department of Chemistry, Wesleyan University, Middletown, CT 06459 (abaranger@wesleyan.edu, ybenitex@wesleyan.edu)

The crystallographic and NMR studies of the U1A−U1snRNA complex show specific hydrogen bonding and stacking interactions between highly conserved residues of the protein and the bases of the RNA. We are interested in determining the contribution of the stacking interaction between Tyr13 of the U1A protein and C5 of U1snRNA stem loop 2 to the stability of the complex. The energetic contribution of the stacking interaction between Tyr13 and C5 was probed by substituting C5 with a series of aromatic base analogues that have no capability of hydrogen bonding with the protein. The ability of these modified nucleosides to participate in stacking interactions was also varied. Specifically, a series of fluoro-substituted phenyl ribonucleosides were synthesized and incorporated into oligonucleotides. The affinities of these modified oligonucleotides for the U1A protein were measured.

142. Enzyme inhibitory effects and base pairing properties of 8-chloroadenosine derivatives. Terry L. Sheppard and **Lisa S. Chen**. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113 (fax: 847-491-7713, sheppard@chem.northwestern.edu, lisachen@chem.nwu.edu)

8-Modified nucleoside analogues have displayed significant potential for cancer chemotherapy via apoptosis induction. To elucidate their effects on nucleic acid biochemistry, these analogues were incorporated into both DNA and RNA, and their corresponding triphosphate derivatives were synthesized. 8-Chlorodeoxyadenosine (8-Cl-dAdo) and 8-chloroadenosine (8-Cl-Ado) have been incorporated into synthetic oligonucleotides to determine the base pairing properties of 8-chloroadenine (8-Cl-A). 8-Cl-Ado destabilizes RNA duplexes by approximately 5 kcal/mol per modification, as demonstrated by UV thermal denaturation experiments. The coding potential of 8-Cl-A during enzymatic nucleic acid synthesis was assessed. DNA templates containing 8-Cl-dAdo were synthesized and used to measure the incorporation efficiencies of natural nucleotides by polymerases. 8-Cl-Ado derivatives were shown to be inhibitors of polyadenylation by yeast poly(A) polymerase. 8-Cl-ATP and synthetic RNA oligonucleotides containing terminal 8-Cl-Ado residues were not substrates for yeast poly(A) polymerase. Results examining 8-Cl-ATP with human polyadenylation extracts and a dual expression phagemid also will be reported.

143. Evaluation of the role of tyrosines in the enzyme coproporphyrinogen oxidase. Nancy E. Thomas, Jon A.

Friesen, and Marjorie A. Jones. Department of Chemistry, Illinois State University, Campus Box 4160, Normal, IL 61790-4160 (fax: 309-438-5538, nethoma@ilstu.edu)

Coproporphyrinogen oxidase (CO), the sixth enzyme in the heme biosynthetic pathway, catalyzes the conversion of coproporphyrinogen III to protoporphyrinogen IX. Deficiency of this enzyme in humans leads to the disease hereditary coproporphyria. Three highly conserved tyrosines (187, 255, and 292) were investigated to determine their role in catalysis. PCR-mediated site-directed mutagenesis was used to generate mutant forms of CO where each tyrosine was replaced by leucine and/or phenylalanine. Wild-type and mutant proteins were expressed in *E. coli* and purified using Ni^{2+} affinity chromatography. The purity of the enzyme was evaluated using SDS electrophoresis. Specific activities for Y187 and Y265 mutant enzymes were found to be similar to wild-type CO. A substantially lower specific activity found for Y292 mutant enzymes suggests that this amino acid may be critical for catalysis. Future kinetic studies will help to provide insight into the specific roles of tyrosine in the catalytic site.

144. Exploring the structural selectivity of enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis.* Richa Rawat and Peter J. Tonge. Department of Chemistry, SUNY−Stony Brook, Stony Brook, NY 11794-3400 (rrawat@ic.sunysb.edu)

There has been a renewed interest in elucidating the mode of action of the well-known antitubercular drugs, such as isoniazid, in the wake of reemergence of tuberculosis during the past decades. The major problem associated with this disease is drug resistance. It is known that isoniazid specifically targets enoyl-ACP reductase from *M. tuberculosis*, encoded by the inhA gene. Isoniazid forms a covalent adduct with NADH, and this adduct is a tight binding inhibitor of InhA. It has been proposed that some of the drug-resistant mutants must interfere with the binding of the isoniazid metabolite and/or the formation of isoniazid-NAD adduct directly. To understand the drug resistance, we are trying to probe the interactions between InhA and the inhibitor by exploring the structural selectivity of the isoniazid-NAD adduct and another analogue, the benzoic hydrazide-NAD adduct, toward InhA and drug-resistant mutants by kinetic and radioactive experiments.

145. Fragmentation studies for peptide using FTICR mass spectrometry and LC/MS/MS with IRMPD: experiment and simulation. Kazuhiko Fukui, Katsutoshi Takahashi, and Yutaka Akiyama. Computational Biology Research Center (CBRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-41-6 Aomi, Koto-ku, Tokyo 135-0064, Japan (fax: 81-3-3599-8081, k-fukui@aist.go.jp)

In recent years there has been significant interest in fragmentation analysis of polypeptide and protein using mass spectrometry (MS) following infrared multiphoton dissociation (IRMPD) and collision induced dissociation (CID) techniques. We investigate the cleavage of polypeptide (Angiotensin II) induced at the backbone amine bonds by using mass spectrometry combined with the laser peptide cleavage IRMPD technique as a "photo-protease" method. MS experiments were performed in electrospray ionization (ESI) Fourier Transform Ion Cyclotron Resonance (FTICR) MS and LC/MS/MS. In addition, we theoretically seek to model the fragmentation of the peptide under collision-free, vacuum conditions using molecular dynamics. The dissociation energy and proton affinity for the peptide bonds (C′−N bond) of Angiotensin II are also obtained using ab initio calculations. Taking advantage of the sensitivity and adaptability of MS, the fragmented peptides of Angiotensin II were screened in the mass range from 200 to 2000 Da. The peaks for the photoproducts were observed as y7 [Arg-Val-Tyr-Ile-His-Pro-Phe+H]$^+$ at m/z = 931.507, y2 [Pro-Phe+H]$^+$ at m/z = 263.139, and b6 [Asp-Arg-Val-Tyr-Ile-His+H]$^+$ at m/z = 784.432 type ions, corresponding to the cleavage of Asp-Arg and His-Pro in the parent amino acid sequence. The tendency in the cleavage of His-Pro was also observed in the spectrum for the LC/MS/MS experiment. The computational simulations which predicted the probable products (dissociation of Asp-Arg and His-Pro bonds) were in very good agreement with the experimental data. The calculated results of ab initio (B3LYP/6-31+G*) and classical molecular dynamics for the photodissociation process will be presented.

146. How do ADP-ribosylating toxins discriminate for their target substrates? Rakhi Patel, Heidi Peltier, Jeremy Read, and **Steven R. Blanke**. Department of Biology and Biochemistry, University of Houston, 369 Science and Research Building II, 3201 Cullen Blvd., Houston, TX 77204-5001 (fax: 713-743-8351, rpatel@uh.edu, sblanke@uh.edu)

ADP-ribosylating toxins are major virulence factors in many important infectious diseases. All ADP-ribosyltransferases catalyze the transfer of the ADP-ribose from nicotinamide adenine dinucleotide (NAD) to an acceptor nucleophile. However, the mechanism by which ADP-ribosylating toxins allow their cognate ADP-ribose acceptors to access the N-glycosidic bond of bound NAD, while excluding all other potential acceptors, is not understood. To elucidate the mechanism of ADP-ribose acceptor discrimination, we are investigating acceptor recognition demonstrated by two distinct toxins: diphtheria toxin, which modifies the imidazole ring of an unusual posttranslationally modified histidine residue called diphthamide; and, cholera toxin, which modifies the guanidino side chain of arginine. Using entirely synthetic genes, we are attempting to interconvert the ADP-ribose acceptor specificities of diphtheria toxin and cholera toxin. Data suggest that a conserved structural element at the active-site face of these enzymes may function as molecular gates to dictate ADP-ribose acceptor discrimination.

147. Hydrogen isotope tracing in the reaction of OMP decarboxylase. Beth A. Simpson, **Brian DelFraino**, and Jeffrey A. Smiley. Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH 44555 (fax: 330-742-1579)

Orotidine-5′-phosphate decarboxylase (ODCase) catalyzes the decarboxylation of OMP to UMP with a rate enhancement of approximately 10^{17}; its incompletely characterized mechanism has led to keen interest among enzymologists.

Some of the proposed mechanisms for the ODCase reaction suggest the possibility of hydrogen exchange at position 5 of the pyrimidine, a normally nonexchangeable position. We have addressed the possibility of this mechanistic feature by (1) NMR observation of the extent of hydrogen exchange from H5 of the product UMP in the presence of ODCase in D_2O and (2) synthesis of [^{2}H5]-OMP and quantification of the amount of deuterium remaining in the enzymatically decarboxylated product. The absence of measurable isotope exchange in both experiments limits the possibilities of reaction mechanisms for ODCase.

148. In vivo and ex vivo NMR in the study of the pathogenesis of AIDS dementia. Jane Brock Greco,[1] Ken E. Sakaie,[1] Susan Westmoreland,[2] Julian He,[1] Sahar Aminipour,[1] Prabhat Seghal,[3] L. Ling Cheng,[1] P. Lani Lee,[1] Andrew Lackner,[2] and R. Gilberto Gonzalez.[4] [1]Martinos Center for Biomedical Imaging, Massachusetts General Hospital, 149 13th St., Charlestown, MA 02129 (fax: 617-726-7422, greco@nmr.mgh.harvard.edu), [2]Division of Pathology and [3]Primate Medicine, New England Primate Research Center, and [4]Department of Neuroradiology, Massachusetts General Hospital and Harvard Medical School

In vivo magnetic resonance spectroscopy assesses neurochemistry noninvasively. We have applied this technique in the SIV-infected macaque, the premiere model for AIDS. Viremia peaks at 11 days post infection (dpi), with a 30% increase in the inflammatory markers choline/creatine and myo-inositol/creatine. At 13 dpi, these resolve, and *N*-acetylaspartate/creatine, a marker of neuronal integrity is decreased. The choline/creatine continues to decrease below preinfection values, possibly representing a cellular repair mechanism, a novel observation. The decrease in Cho/Cr between 11 and 25 dpi, at the time of systemic control of virus, is similar to that observed in patients whose viral load is controlled by therapy. The SIV macaque model permits direct correlation of pathological and high-resolution NMR data with the in vivo data. The high-resolution NMR data resolve the overlapping resonances observed in vivo, allowing the precise identification of the metabolic changes that accompany neuroAIDS pathology.

149. Induced cell cycle arrest of CAPE on C6 glioma cells. Tsui-Hwa Tseng,[1] Wan-Chyi Lin,[1] and **Yean-Jang Lee**.[2] [1]Institute of Biochemistry, Chung Shan Medical & Dental University, Taichung, Taiwan, 110, Section 1, Chien Kuo N. Rd., Taichung 400, Taiwan (tht@mercury.csmc.edu.tw), and [2]Department of Chemistry, Changhua University of Education, 1 Chieng-Derg Rd., Changhua 50058, Taiwan (fax: 886-4-7211190, leeyj@cc.ncue.edu.tw)

As in our previous studies, we found caffeic acid phenethyl ester (CAPE) and ethyl caffeicate (EC) exhibited significant cytotoxicity on oral cancer cells. Herein we further investigated caffeic acid; EC and CAPE were tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay for the growth of H3B hepatocytes and C6 glioma cells. CAPE showed significant cytotoxicity on C6 glioma cells, and then analysis cell cycles. Flow cytometric analysis observed that in the hypodiploid phase (apoptosis peak, 37%), DNA content analysis exhibited 55% of cells in the G0/G1 phase, 8% in the S phase, and 0% in the G2/M phase after 36 h treatment with CAPE. Further investigation showed that C6 cells underwent internucleosomal DNA fragmentation and morphological changes characteristic of apoptosis after 36 h treatment with CAPE (50 μM). Finally, C6 cells treated with CAPE for 6 h could increase tumor suppressor-p53, p53 target protein-p21, and cyclin dependent kinase inhibitor (CDKI/CKI)-p27 expression. These results suggest that CAPE induce cell cycle arrest and apoptosis on C6 glima cells via the p53 pathway.

150. Interfacial orientation of *Thermomyces lanuginosa* lipase on phospholipid vesicles investigated by ESR relaxation spectroscopy. Eva M. K. Hedin,[1] Pernille Hoyrup,[2] Shamkant A. Patkar,[3] Jesper Vind,[3] Allan Svendsen,[3] and Karl Hult.[1] [1]Department of Biotechnology, Royal Institute of Technology, Stockholm Center for Physics, Astronomy, and Biotechnology, SE-106 91 Stockholm, Sweden (fax: +46-8-5537 8468, eva@biochem.kth.se), [2]Department of Chemistry, Technical University of Denmark, and [3]Novozymes A/S

The 269 amino acid fungal triacyl glycerol lipase (EC 3.1.1.3) *Thermomyces lanuginosa* lipase (TLL) displays classical interfacial activation. The orientation of TLL on phospholipid vesicle membranes was investigated employing site-directed spin labeling and electron spin resonance (ESR) relaxation spectroscopy. Eleven TLL single-cysteine mutants, each with the mutation positioned at the surface of the enzyme, were selectively spin-labeled with a nitroxide spin probe and studied together with vesicles of varying size and surface charge. The orientation of the enzyme with respect to the vesicle membrane was probed using the water-soluble spin relaxant chromium(III)oxalate. In addition, the interaction between the spin labels and the lipid phase was monitored as the fluorescence quenching of dansyl-labeled vesicles. On the basis of the obtained results, the binding orientations of TLL on the different phospholipid vesicles are presented. The importance of vesicle size and surface charge for productive binding is discussed.

151. Investigation of early glycosyltransferases on the dolichol pathway. M. K. O'Reilly,[1] Ziye Liu,[2] Peng George Wang,[2] and Barbara Imperiali.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (fax: 617-452-2799), and [2]Department of Chemistry, Wayne State University

The biosynthesis of asparagine-linked glycoproteins begins with the assembly of a dolichol-linked tetradecasaccharide core, Dol-PP-$GlcNAc_2Man_9Glc_3$, by a series of glycosyltransferases on the dolichol pathway. Alg1p is a β-1,4-mannosyltransferase that catalyzes the mannosylation of Dol-PP-$GlcNAc_2$. We have cloned and expressed this enzyme in *E. coli*, purified it to homogeneity, and confirmed its activity. Alg1p was then used to prepare the next intermediate in the pathway. A yeast knock-out that accumulates Dol-PP-$GlcNAc_2Man$ and Dol-PP-$GlcNAc_2Man_2$ has a mutated *ALG2* gene, suggesting that Alg2p catalyzes the addition of the second and third mannoses of the tetradecasaccharide. In contrast, an *ALG2* mutant of the *Rhizomucor pusillus* accumulates only Dol-PP-$GlcNAc_2Man$. *ALG2* has been cloned, expressed, and purified in *E. coli*. Work is in progress to determine whether Alg2p completes the second, third, or

both mannosylations. Alg1p and Alg2p may facilitate chemoenzymatic synthesis of glycolipids and glycoproteins. Abbreviations: Dol-PP, dolichyl pyrophosphate; GlcNAc, *N*-acetylglucosamine; Man, mannose; Glc, glucose.

152. Investigation of proteins of cytosol in sheep liver for copper deficiency. Mars G. Safin,[1] **Nurali Q. Mukhamadiev**,[1] Shuhrat M. Sayitkulov,[2] and Ismoil M. Ergashev.[2] [1]Biological Chemistry Department and [2]Physical Chemistry Department, Samarkand State University, University Blvd., 15, Samarkand 703004, Uzbekistan (fax: 998-662-333487, m_nurali@hotmail.com)

It is known that liver cells and its cytosol take an active part in the metabolism of copper in sheep. Elucidation of the biochemical mechanisms of the origin, growth, and increasing seriousness of diseases connected with copper deficiencies in sheep requires thorough study of the proteins of cytosol in liver. In connection with this, the purpose of this work is to study proteins of cytosol in sheep liver for copper deficiency by the methods of gel-filtration and electrophoresis. Gel-filtration of cytosol in the liver has been carried out on Sephadex S-100 balanced in a 0.25 M solution of sodium chloride. This solution was used for elution of protein fractions also. Cytosol of and its protein fractions, obtained after chromatography, was subjected to disk electrophoresis in a polyacrylamide gel. Electrophoretic investigation of proteins of cytosol in each case was used to identify the appropriate protein fractions, to determine the quota taking part in copper exchange in the future. Based on investigations we carried out, we have established that in cytosol five protein fractions (peaks I−V) were discovered, all of which contain zinc and copper. Of these, the second (ceruloplasim), fourth (superoxide dismutase), and fifth (metallothionine) fractions are copper-containing (microelements of Zn and Cu were determined by the atom-adsorption method). They have been evaluated for their fermentative activity. In this way, using gel-filtration and electrophoresis, determination of the activity of copper-containing ferments and the content of copper and zinc in cytosol and its fractions allow a greater understanding of the etiological factor of copper deficiency in sheep.

153. Isolation and characterization of water-soluble fluorescent species from human serum. Ashraf H. Elamin and David W. Seybert. Department of Chemistry & Biochemistry, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15205 (fax: 412-396-5683, elamin115@duq.edu)

Lipid peroxidation has been linked to the pathogenesis of numerous diseases including atherosclerosis, cancer, and Parkinson's disease as well as being associated with drug-associated toxicity and aging. Aldehydes such as malondialdehyde (MDA) and 4 hydroxy-2-nonenal (HNE) are end products of this process, and they exhibit facile reactivity with various biomolecules, such as proteins and phospholipids, generating stable products. Previous in vitro studies have shown that modification of proteins by lipid peroxidation derived products produces fluorescence with excitation maxima at 350 nm and emission maxima at 460 nm. We have begun characterization of the water-soluble fluorescent (WSF) species present in human serum. Most of this fluorescence appears to be covalently associated with various serum proteins, and we have determined serum albumin is one of these modified proteins.

154. Mechanism and kinetics of the reactions of hydroxyurea derivatives and hemoglobin. Jinming Huang,[1] Zhou Zou,[1] Daniel Kim-Shapiro,[2] and S. Bruce King.[1] [1]Departments of Chemistry and [2]Physics, Wake Forest University, Salem Hall, Winston Salem, NC 27109 (fax: 336-758-4656, huangj@wfu.edu)

Hydroxyurea represents a new treatment for sickle cell disease. Recent evidence indicates that a portion of hydroxyurea's beneficial actions may be mediated by nitric oxide (NO). We have already shown that hydroxyurea reacts with hemoglobin to produce nitric oxide. Here, we report results regarding the mechanism of nitric oxide formation and also reaction kinetics using various synthetic hydroxyurea derivatives with different groups substituted on either the acylhydroxylamine group (−NHOH) or the amine group. Electron paramagnetic resonance experiments indicate that NO formation and binding to the heme iron require an unsubstituted −NHOH group and an amine group with at least one hydrogen atom. All hydroxyurea derivatives reacted faster with hemoglobin than hydroxyurea did. Rate constants have no relationship with the pK_a of hydroxyurea derivatives, but correlated well with the chemical potential of these hydroxyurea derivatives. This indicates that hydroxyurea nitroxide radical represents the key step in the nitric oxide producing reactions.

155. Molecular interactions that govern the cellular response to DNA damage in *E. coli.* **Penny Beuning**,[1] Laurie Waters,[1] Mark D. Sutton,[2] and Graham Walker.[1] [1]Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Ave., 68-653, Cambridge, MA 02139 (beuning@mit.edu), and [2]SUNY Buffalo

The umuDC gene products are part of the SOS system in *E. coli* and are expressed in response to DNA damage. Moreover, overexpression of the umuDC gene products results in a cold-sensitive phenotype. Expressing, in addition, the beta (processivity) subunit of the replicative polymerase polIII results in exacerbation of the cold sensitivity, while overexpression of the epsilon (proofreading) subunit results in suppression of the phenotype. We have exploited this observation to select for mutations in the umuDC gene products that fail to exacerbate this cold-sensitive phenotype when expressed with the beta subunit. Mapping of these mutations in a homology model shows that all appear to be within one region of the UmuC protein. We are pursuing a similar strategy to determine the interactions of the umuDC gene products with the epsilon subunit. The results of these experiments will help to illuminate the molecular interactions involved in replication fork management following DNA damage.

156. Molecular level interactions between guanidinium salts and polypeptides. Hasnain Gulam Jaffer, Gilles Peslherbe, and Ann English. Department of Chemistry & Biochemistry and Centre for Research in Molecular Modeling, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, QC H3G 1M8, Canada (fax: 514-848-2868, jafferhasnain@hotmail.com)

The protein folding problem has generated a lot of interest in recent times. To investigate the types of interaction that hold a protein in its native structure, its unfolding mechanism is often investigated. Chemical denaturants such as guanidine hydrochloride (GdnHCl) or guanidine thiocyanate (GdnHSCN) are routinely used to denature proteins. However, not much is known about their interaction with the proteins especially at the molecular level. Here, we attempt to explain the types of interaction involved between these denaturants and homopolypeptides such as poly-L-lysine (PLL) and poly-L-aspartic acid (PLA). A combination of FTIR spectroscopy and molecular modeling is used to gain insight into the nature of the interactions involved. Interestingly, we find evidence for denaturant−side chain interactions that vary in strength with the homopolypeptide investigated.

157. Multiple reaction ability (MRA) of 2-thioxopyrimidinones-4. Kasim A. Zakhidov. Organic Chemistry, Samarkand State University, University Blvd., 15, Samarkand 703004, Uzbekistan (fax: 998-662-333487, m_nurali@hotmail.com)

2-Thioxopyrimidinones-4, like 2-oxopyrimidinones-4, have five tautomeric forms and in coordination with metals might participate four heteroatoms. Comparative study UV spectra of neutral molecules and sodium salts have shown that for 6-methyl-2-thioxopyrimidinone-4 form I is thermodynamically stable (X = s), but in its anions, metal coordination proceeds with an atom of oxygen and is available in form X. Alkylation of 6-methyl-2-thioxopyrimidinone-4 by methyliodine in alcohol forms a mixture of 6-methyl-2-methylpyrimidinone-4 (XI) and 3,6-dimethyl-2-methylthiopyrimidinone-4 (XII). Methylation by methylthiazolate gives the third reaction product, 1,6-dimethyl-2-thioxopyrimidinone-4 (XIII). The N3-methylproduct (XIV) is formed when carrying out the reaction under heat. The reaction in acetonitrile gives predominantly S2-methylproduct. Increasing amounts of products methylating on the NI-center occur in dimethylformamide. With the purpose of elucidation of the influence of the substituent's nature for position 6, methylation of 6-phenyl-2-thioxopyrimidinone-4 (XV) has been carried out. Its alkylation by methyliodine proceeds selectively on the S-center with formation of compound XVI. Utilization of methylthiazolate results in the formation of N3-methylproduct XVII. In contrast to 6-methyl-2-thioxopyrimidinone-4, alkylation of 6-phenyl-2-thioxopyrimidinone-4 by methylthiazolate in dimethylformamide is accompanied by side-by-side formation of special products and products of alkylation of O4 (XVIII). In this case, the NI-methylproduct is not discovered. The direction of the reaction of 6-methyl-phenyl-2-thioxopyrimidinones-4 with the polarity of solvents influences the majority of reaction centers, which is changed by the dependence on the nature of substituent S-6.

158. Oxidative modification of Cu/Zn superoxide dismutase leads to a functional protein with altered biophysical properties. Mitchel D. de Beus and Wilfredo Colon. Department of Chemistry, Rensselaer Polytechnic Institute, Cogswell Room 125, 110 Eighth St., Troy, NY 12308 (fax: 518-276-4887, debeum@rpi.edu)

The enzyme Cu/Zn superoxide dismutaes (SOD1) protects the cell against oxygen free radical damage by converting superoxide radicals into molecular oxygen and hydrogen peroxide. In recent years, SOD1 has received increased attention because of its involvement in some cases of the motor neuron disease familial amylotrophic lateral sclerosis (FALS). The mechanism by which SOD1 mutations cause FALS involves a gain of function; however, the molecular detail of this pathological function remains unknown. During purification of SOD1 from human erythrocytes, SOD1 protein undergoes an oxidative modification that has no effect on its activity. Therefore, it was suggested that this modification is unimportant. The biophysical characterization of the oxidized SOD1 shows that the oxidative modification to SOD1 results in a more kinetically stable protein, which unfolds irreversibly at low pH. However, when SOD1 is reduced with sulfhydryl reagents, its biophysical properties are restored to those of the unmodified protein. We are interested in determining whether the intrinsic capability of SOD1 for autoxidation is enhanced by FALS-related SOD1 mutations.

159. Peptide fragments from the DNA binding protein Fis: influence of proline on secondary structure. Daniel F. Moriarty and Wilfredo Colón. Department of Chemistry, Rensselaer Polytechnic Institute, Cogswell Building, Room 125, 110 Eighth St., Troy, NY 12180 (fax: 518-276-4887, moriad@rpi.edu)

The homodimeric DNA binding protein Fis contains four helices per subunit, with a kinked central helix that has a proline at position 61. To investigate the effect of proline, several peptides were synthesized corresponding to different sections of Fis. It was found that the individual helices by themselves, as well as constructs corresponding to the N-terminal and central helices (helix A and B)and the central and C-terminal helices (helix B, C, and D), contain little to no structure. Substitution of alanine for proline at position 61 has a large effect on the structure of the isolated B-helix. The Ala61 A-B and B-C-D peptides also contain a high degree of helical structure, but the B-C-D peptide is much more prone to aggregation. These results show that while the full-length protein is able to maintain a stable helix despite the presence of the centrally located proline, an isolated peptide corresponding to this sequence cannot. However, with a single proline to alanine mutation, we have been able to stabilize portions of the protein in a nativelike conformation.

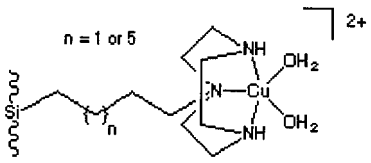
160. Physicochemical study of DNA binding properties of the naphthylimido-imidazoacridone WMC79 and related compounds. Sergey G. Tarasov,[1] Chris Nelson,[1] Wieslaw M. Cholody,[2] Humcha Hariprakasha,[2] Teresa Kosakowska-Cholody,[2] Jose R. Casas-Finet,[3] and Christopher J. Michejda,[2] [1]Structural Biophysics Laboratory and [2]Structural Biophysics Laboratory, Molecular Aspects of Drug Design Section, NCI−Frederick, P.O. Box B, Frederick, MD 21702 (tarasovs@ncifcrf.gov), and [3]Analytical Biochemistry Division, Medimmune, Inc.

Imidazoacridones are DNA-interactive agents with potent antiviral and anticancer activity. The properties of the naphthylimido-imidazoacridone WMC79 and its constituent moieties, imidazoacridone WMC77 and mitonafide(naphthylimide), were studied by steady-state and time-resolved

fluorescence spectroscopy, UV-spectroscopy, and gel-electrophoresis. The environmental sensitivity of spectral properties suggests that WMC79 adopts a ring-stacked conformation in aqueous solution, whereas in hydrophobic environments (organic media, vicinity of DNA bases) the conformation is more relaxed. WMC79, binding to poly(dA)-poly(dT) with 20 nM K_d, induced a biphasic melting curve and increased T_m less than WMC77, whereas mitonafide decreased T_m. WMC79 induced significant band shifts in a gel shift assay when added to supercoiled plasmid DNA, consistent with intercalation. Preliminary time-resolved fluorescence spectroscopy data support a model wherein WMC79 may bind to DNA as a dimer, with only one chromophore intercalated, while the others residues most probably are in the minor groove, accessible for additional interactions

161. Preparation of silica-bound copper(II) macrocycles for phosphate ester cleavage. Anthony W. Ehrbar. Brett R. Bodsgard, and Judith N. Burstyn. Department of Chemistry, University of Wisconsin−Madison, 1101 University Ave., Madison, WI 53706 (awehrbar@students.wisc.edu)

Silica-bound copper(II) triazacyclononane has been prepared in two forms, characterized, and shown to cleave an activated phosphodiester. The materials were prepared by a rhodium-catalyzed hydrosilation reaction between a silica-hydride intermediate and triazacyclononane bearing a pendant alkyl chain with a terminal alkene. Kinetic data for reactions with bis(*p*-nitrophenyl)phosphate (BNPP) show an initial slow induction period followed by a faster linear increase in product formation. At extended times, product inhibition is observed. Initial reaction rates increase to a maximum when the sample is washed and recycled with fresh substrate before the product inhibition period. The rate of reaction for the butyl-linked material is faster than that for the octyl-linked material. Another direction we are pursuing to increase the hydrolytic efficiency is to embed the active copper species into a porous silica matrix. Preliminary results for this route are also presented.



162. Preparation, characterization, and optical properties of a new chiral calizarene derivative. Limin Zheng and Han Zheng. College of Chemistry and Chemical Engineering, Dong Hua University, 1882 W. Yan An Rd., Shanghai 200051, China (acsprof@yahoo.com)

A new derivative of chiral calix[4]arene, 5-aldehydo-25,-27-diacetyl-calix[4]arene, was synthesized and characterized by ESI-MS, UV−Vis, IR, and DSC. By the aid of the UV−Vis spectrum and polarimetric studies, it is desirable that the compound is levorotatory. By means of ESI-MS, the value of *m*/*z* corresponds to the chiral calixarene derivative. And it acts as a molecular receptor to recognize and combine with amino acids to construct a supramolecular system. This kind of recognition is a chiral one. The interaction of this compound with L-threonine and D-threonine leads to non-enantiomer, which is demonstrated by the absorption spectra and polarimetric experiment results. It is noteworthy that the new chiral Schiff base calix[4]arene has shown its potential to be used in separating enantiomers of amino acids, or as a signaling label of optical isomers. At the same time, the synthesis of a new calixarene derivative with a fluorescent group has also been discussed. Most of the synthetic work will be presented.

163. Probing the protein conformation by hydroxyl radicals. Jing-Qu Guan,[1] Steve C. Almo,[2] and Mark R. Chance.[1] [1]Department of Physiology & Biophysics and [2]Department of Biochemistry, Center for Synchrotron Biosciences, Albert Einstein College of Medicine, 1300 Morris Park Ave., Ullmann 315, Bronx, NY 10461 (jguan@aecom.yu.edu)

Monomeric actin (G-actin) is thought to be in the Mg^{2+}-bound state in vivo, taking into consideration the higher concentration of free Mg^{2+} (∼0.5 mM) in the cytosol of most cells than that of Ca^{2+} (∼0.2 mM). It has been shown that Mg^{2+}-ATP-actin has a different conformation from that of Ca^{2+}-ATP-actin, which may account for the greater tendency of Mg^{2+}-actin to polymerize to form filaments compared to Ca^{2+}-ATP-actin. We present here data showing specific structure differences between Ca^{2+}-ATP-G-actin and Mg^{2+}-ATP-G-actin probed by hydroxyl radicals and mass spectrometry. While peptides 85−95 and 118−125 in subdomain 1 and peptide 316−326 in subdomain 4 did not show any change in modification by hydroxyl radicals, when comparing the two actin forms, most peptides in subdomain 2 were shown to be less accessible to solvent for the Mg^{2+}-G-actin form, and a slight protection of peptides 196−207 and 242−253 in subdomain 4 was also observed. These results suggest a smaller cleft between subdomains 2 and 4 in Mg^{2+}-ATP-actin than in Ca^{2+}-ATP-actin. The lower rate of modification of peptide 360−372 in Mg^{2+}-ATP-actin suggested this peptide is closer to the core of subdomain 1. These conformational changes in Mg^{2+}-ATP-actin made peptides 337−359 and 157−178 more exposed to the solvent, which showed higher modification rates. These results show Ca^{2+}-ATP-G-actin and Mg^{2+}-ATP-G-actin have different conformations, which are likely to be related to the variation in their biochemical function.

164. Rapid cleavage of SNAP-25 by BoNT/E and BoNT/A. Richard B. Lomneth and Melissa Mahlen. Department of Chemistry, University of Nebraska at Omaha, 6001 Dodge St., Omaha, NE 68182-0109 (rlomneth@mail.unomaha.edu)

Botulinum neurotoxins (BoNTs) are proteases which inhibit exocytosis. BoNT/A and BoNT/E both cleave synaptosomal associated protein of 25 kDa (SNAP-25). BoNT/E is more efficacious in vitro, in part because it removes more of the C-terminus of SNAP-25. The work presented here demonstrates there is also a difference in the rate at which BoNT/A and BoNT/E cleave SNAP-25 in permeabilized PC12 cells. SNAP-25 cleavage by BoNT/E begins immediately, while there is a brief lag before cleavage by BoNT/A. Maximum cleavage (∼50%) of the SNAP-25 was achieved by both toxins within 5 min, but they differed greatly in their ability to inhibit Ca^{2+}-stimulated norepinephrine release. These results are consistent with significant

amounts of SNAP-25 unavailable for cleavage during the relatively brief duration of these experiments, and that BoNT/A cleaves SNAP-25 after exocytosis occurs or SNAP-25 cleaved by BoNT/A retains some functionality.

165. Resonance Raman investigation of structural properties of DNA photolyase. Olga Sokolova,[1] Anand Gopal,[1] Stacey Wagner,[2] Meghan Ramsey,[2] Yvonne Gindt,[2] and Johannes Schelvis.[1] [1]Department of Chemistry, New York University, 100 Washington Square E., New York, NY 10003, and [2]Department of Chemistry, Lafayette College

DNA photolyase is a photoactive enzyme that repairs lesions formed in the DNA by UV-light. Although the crystal structure is known for this enzyme, our interest is in the elucidation of the structural basis of the enzymatic processes. Accordingly, we have been looking at flavin radical stabilization, structural function of the MTHF cofactor, and the enzyme−DNA interactions. DNA photolyase has been characterized using resonance Raman spectroscopy at 350.7, 530.9, and 568 nm wavelengths of excitation. The enzyme was studied with and without the MTHF cofactor present, and the question of stability has been addressed. H−D exchange was conducted to help assign the flavin vibrations. Interactions of the DNA photolyase with the cyclobutane pyrimidine dimer of DNA substrate and its effect on the flavin radical vibrations have been investigated as well.

166. Saccharide display on microtiter plates. Marian C. Bryan, Oliver Plettenburg, Pamela Sears, and Chi-Huey Wong. Department of Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037 (fax: 858-784-2409, mcbryan@scripps.edu)

New insight into the importance of oligosaccharides and glycoconjugates in biological systems underscores the need for rapid synthetic and screening procedures for carbohydrates. A facile method for attaching carbohydrates to the surface of microtiter plates was therefore undertaken to facilitate research in glycobiology. Galactosyllipids containing small, hydrophobic groups at the anomeric position were screened for noncovalent binding to microtiter plates. When the lipid component was a saturated hydrocarbon between 13 and 15 carbons in length, the monosaccharide showed complete retention after several aqueous washes and could successfully be utilized in biological assays. This alkyl chain was then successfully employed with oligosaccharides in biological assays. Based on these findings, we feel this method for attachment of carbohydrates to microtiter plates should be highly efficacious to a variety of biological assays.

167. Sequence dependent mechanisms of DNA recognition by the Tc3 transposase DNA-binding domain. Martin Thompson,[1] Douglas C. Daniel,[2] and Neal W Woodbury.[2] [1]University of Michigan Medical Center, 1150 W. Medical Center Dr., MSRB 2, Ann Arbor, MI 48109 (proteios@umich.edu), and [2]Department of Chemistry and Biochemistry, Arizona State University

Mechanisms of DNA recognition by the helix-turn-helix (HTH) motif from the Tc3 transposase were studied by thermodynamic and spectroscopic methods. Circular dichroism and thermodynamic analysis of the 51 amino acid Tc3-HTH indicate the domain is disordered in solution and only adopts its native helical structure in the presence of DNA containing its cognate site. Further analyses using single-photon counting and steady-state fluorescence resonance energy transfer indicate that in addition to the base substitutions within the cognate site, flanking sequences affect the binding event. This makes an argument that the induced fit surface between the protein and the DNA has effects beyond the interaction surface itself. It is becoming generally accepted that protein−DNA complex formation involves more subtle and seemingly indirect elements relating dynamic properties of the double helix that are functions of its base sequence with the binding induced structural changes of the protein.

168. Simple mathematical test for identification of enzyme active sites from structure using THEMATICS. Huyuan Yang and Mary Jo Ondrechen. Department of Chemistry, Northeastern University, Boston, MA 02115 (fax: 617-373-8795)

THEMATICS, Theoretical Microscopic Titration Curves, is a successful method for the identification of enzyme active sites from the structure alone. A small fraction of the ionizable residues in a protein deviate from Henderson−Hasselbalch behavior; these perturbed residues are functionally significant. Predicted titration curves for all of the ionizable residues in a protein molecule are presented as C(pH), the calculated mean net charge on each residue as a function of pH. Typical residues obey the Henderson−Hasselbalch equation and have sigmoid shape, with a sharp falloff near the pK_a. We fit the calculated *C*(pH) curves to a two-parameter sigmoid function using a Levenberg−Marquardt procedure. A residue with a poor quality fit is deemed positive; positive residues tend to form a cluster at the active site, such that a cluster of two or more positive residues reliably predicts the active site. Implications for enzyme reaction mechanisms and for functional genomics are discussed.

169. Specific substrates of bacterial DD-peptidases. Rajesh Nagarajan and R. F. Pratt. Department of Chemistry, Wesleyan University, Lawn Ave., Middletown, CT 06459 (fax: 860 685 2211, rnagarajan@mail.wesleyan.edu)

D-Alanyl-D-alanine peptidases/transpeptidases catalyze the final (cross-linking) step in the biosynthesis of bacterial cell walls. The natural substrates of these enzymes should be segments of peptidoglycan structures, but enzyme specificity toward these structures has not yet been convincingly demonstrated. Previous research in our laboratory established that glycyl-L-α-amino-ϵ-pimelyl-D-alanyl-D-alanine is a very specific substrate for the *Streptomyces* R61 DD-peptidase (k_{cat} = 69 s^{-1}, K_m = 7.9 mM, k_{cat}/K_m = 8.7 × 10^{-6} M^{-1} s^{-1}).We have now employed a more versatile approach toward the synthesis of peptide substrates for DD-peptidases by introducing a sulfur in the pimelyl side chain. We are currently investigating the role of charges and side-chain length in substrate binding to these enzymes. We are also investigating methodologies to link these side chains to penicillins and cephalosporins. Such products might have improved affinity toward antibacterial targets.

170. Spectrophotometerical control of composition of products of oxidation of adrenalin in the presence of

cobalttyurfyrin, Rahima V. Tashmatova and Robiya A. Nasimova. Analytical Chemistry, Samarkand State University, University Blvd., 15, Samarkand 703004, Uzbekistan (fax: 998-662-333487, chem@samarkand.uz)

In view of the possible practical use of porphyrins as biologically active substances, an important study of their catalytic influence at near-physiological pH is presented. Oxidation of adrenalin in basic medium at pH 8.5 occurs concomitant with generation of superoxide anion radical (O_2^-). By reducing the pH, the speed of oxidation of adrenalin decreases, and at pH <8.5 practically no adrenalin is oxidized. If a small quantity of Co-porphyrin was injected into the system, then oxidation of adrenalin occurs at pH 8.0. In this case, CoP plays a catalytic role. The absence of Co-porphyrin oxidation products of adrenalin was observed at any rate for several hours. When Co-porphyrin was injected at concentrations of $10^{-5}-10^{-6}$ M, oxidation occurs with noticeable speed. The formation process of products of adrenalin oxidation was observed in absorption spectra in the visible region. Side-by-side formation of oxidation products occurs at the expense of Co-porphyrin, apparently on increasing the intensity of Sor-Co-porphyrin ($\lambda = 429$ nm). Analysis of absorption spectra of oxidation products has shown that under these conditions only adrenochrome (Ach) and the product of its oxidation, P1, are formed. Product P2, which was discovered in the oxidation of adrenalin in basic medium (pH 10), in the current conditions (pH 8.0) is barely formed. For more detailed investigation, the following experiments of the catalyst's transformation have been carried out. Solutions of adrenalin and Co-porphyrin were poured into a vacuum and kept in these conditions for 24 h, so that practically all of the Co-porphyrin turned into the oxygen-less form. Then air was turned on and the changes in absorption spectra were followed. In this case, the concentration of adrenalin [$(0.2-5) \times 10^{-5}$ M] was small enough so that absorption of products of its oxidation did not interfere with the transformation of Co-porphyrin. Analysis of spectral changes has shown that in air occur two processes: formation of the oxygen complex, which is accompanied by displacement of the maximum of absorption bands of Sor from 417 to 429 nm; and destruction of the porphyrin ligand, causing a common drop in absorption of the short-wave zone of the spectrum.

171. Spectroscopic properties of simple linear polyenes as a function of conjugation length. Jesusa S. Josue,[1] Harry A. Frank,[1] Elizabeth A. Barney,[2] Richard D. Broene,[2] and Ronald L. Christensen.[2] [1]Department of Chemistry, University of Connecticut, 55 N. Eagleville Rd., Storrs, CT 06269 (fax: 860-486-2981, jesusa.josue@uconn.edu), and [2]Department of Chemistry, Bowdoin College

Simple, α,ω-methyl-substituted polyenes containing different conjugation lengths were synthesized using living polymerization techniques, and the all-trans isomers were separated by high-performance liquid chromatography. Absorption, fluorescence, and fluorescence excitation spectra of the molecules were obtained in *n*-hexane at 293 K. The absorption and fluorescence spectra shift to lower energy with increasing conjugation. The emission spectra display well-resolved vibronic structure from which the energies of the S_1 (2^1A_g) $\rightarrow$ S_0 (1^1A_g) (0−0) transitions may be accurately determined for this series of molecules. These data are important for understanding the controlling features of the spectroscopic and photochemical properties of linearly-conjugated p-electron molecules that play important roles in numerous biological systems.

172. Structural models and modifications of HTLV-I protease. Bryan E. Herger, Kelly J. Dennison, Victoria L. Mariani, and Suzanne B. Shuker. School of Chemistry and Biochemistry, Georgia Institute of Technology, 315 Ferst Dr., Atlanta, GA 30332-0363 (herger@chemistry.gatech.edu)

Two approaches were employed to prepare structural models for HTLV-I protease: sequence homology modeling and "in silico" mutation of known structures. For homology modeling, MODELLER was used to generate structures based on homology to 1D4L, an HIV-1 protease mutant, and 1BAI, Rous sarcoma virus protease. Mutation "in silico" involved iterative modification and energy minimization using MacroModel. These models suggest that the active site contains a narrow, hydrophobic channel and that Leu30 and Gly34 play an important role in the selectivity for P1 residues. In addition, the models suggest that there are hydrophobic residues that may be on the surface and thereby contribute to aggregation through intermolecular hydrophobic interaction. Mutation of these residues is currently underway.

173. Structural perturbations in a human mitochondrial tRNA caused by pathogenic mutations. Melissa A. Lapierre, **Marc D. Roy**, and Shana O. Kelley. Department of Chemistry, Boston College, Merkert Chemistry Center, 2609 Beacon St., Chestnut Hill, MA 02467 (melissa.lapierre.1@bc.edu, mark.roy.1@bc.edu)

Mutations in human mitochondrial transfer RNAs (hs mt tRNAs) are correlated with a variety of neuromuscular and metabolic diseases. A hotspot for pathogenic point mutations is the gene encoding hs mt $tRNA^{Leu}$. Presently, 13 disease-related mutations have been identified within this region of the hs mt genome. To elucidate the effects of these mutations at the molecular level, we are investigating the structures of the wild-type tRNA and pathogenic mutants. Using tRNAs generated via bacterial overexpression, we have explored the role of modified nucleotides in stabilizing the folded structure. In addition, we have implemented enzymatic and chemical probing methods to assemble a solution structure of the native tRNA and to visualize disruptions in folding caused by disease-related mutations. These studies indicate that both local and global structural disruptions may potentially contribute to the loss of molecular and cellular function of mutated hs mt tRNAs.

174. Synthesis of bio-available inhibitors of oligosaccharyl transferase. Maria L. Ufret and Barbara Imperiali. Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (marial@mit.edu)

Protein glycosylation is an important process because of the great diversity of glycoproteins that can be produced by the introduction of different oligosaccharide sequences. Our group has made significant progress in the study of asparagine-linked glycosylation. This process is catalyzed by oligosaccharyl transferase (OT), which is a membrane-

associated enzyme found in the lumen of the endoplasmic reticulum (ER). A variety of inhibitors that bind tightly to OT in vitro, with K_is as low as 10 nM, have been synthesized. The development of peptides capable of inhibiting OT in vivo would be desirable, since there is no bio-available inhibitor that targets N-linked glycosylation directly. Both active and passive strategies for delivering inhibitors to the site of OT will be discussed.

175. Synthesis of ethyl-3-bromooxindoleacetate as a potential mechanism-based inhibitor of cysteine proteases. Edward J. Brush, Asako Enomoto, and Takafumi Iba. Department of Chemical Sciences, Bridgewater State College, Bridgewater, MA 02325 (ebrush@bridgew.edu)

The mammalian cysteine proteases play key roles in a variety of pathological processes, and are attractive targets for the design of specific therapeutic agents. Medically interesting proteases in the papain family include cathepsins B and L (cancer growth and metastasis) and cathepsin K (bone degradation and osteoporosis). We have synthesized ethyl-3-bromooxindoleacetate (BOAA-EE) as a potential mechanism-based inhibitor of the cysteine protease papain. BOAA-EE was prepared by bromination of indole-3-acetic acid in *tert*-butyl alcohol with NBS, producing 3-bromooxindole acetic acid (BOAA), and subsequent DCC-mediated esterification with ethyl alcohol. We propose that enzymatic hydrolysis of BOAA-EE will produce BOAA, which under physiological conditions undergoes spontaneous decarboxylation with elimination of bromide forming 3-methyleneoxindole (MOI). MOI may then alkylate the catalytic site of the enzyme through Michael addition with nucleophilic amino acid residues. (This research was supported in part by grants from the Bridgewater Foundation and Research Corporation.)

176. The effect of side chains on the conformation and stability of helical peptides as studied by IR spectroscopy. R. A. Gangani Silva, Julie Nguyen, and Sean M. Decatur. Department of Chemistry, Mount Holyoke College, Carr Laboratory, South Hadley, MA 01075 (fax: 413-538-2327, gsilva@mtholyoke.edu)

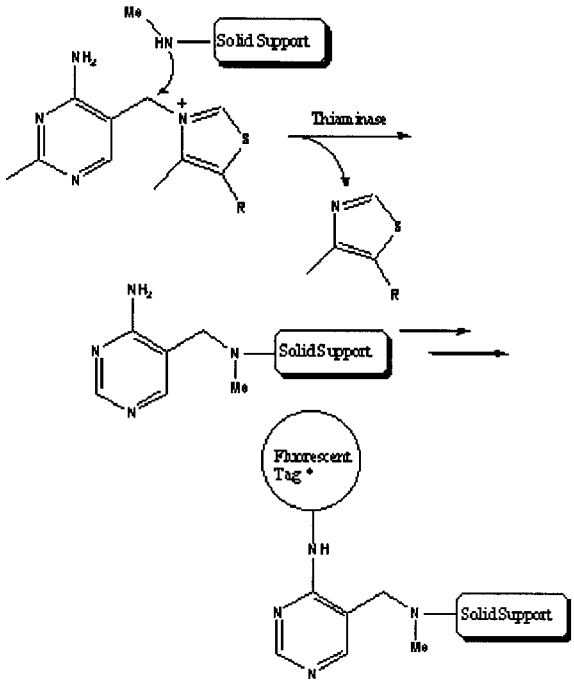
Infrared spectroscopy, particularly of the amide I bands, has been widely applied to probe the conformation and dynamics of peptides and proteins. Introduction of ^{13}C-labeled carbons into selected backbone carbonyls provides residue-specific infrared probes of protein structure; the amide I bands of these "heavy" carbonyl vibrations appear at $\sim 30-40$ cm^{-1} lower frequency from major (^{12}C) amide I and can be used to gain conformational and environmental evidence of the residue(s) of interest. In previous studies, we have applied this technique of isotope-edited infrared spectroscopy to the study of helix stability in model peptides and the effect of capping groups of local conformation. In this study, we report the study of a 25 residue alanine-rich peptide, Ac-$(AAAAK)_4$-$AAAAY$-NH_2, and its leucine variants, Ac-$LLLLK$-$(AAAAK)_3AAAAY$-NH_2 and Ac-$(AAAAK)_3$-$LLLLY$-NH_2. The amide I bands of these peptides have very similar shapes and intensities at a given temperature but have clearly different melting temperatures. Introduction of ^{13}C labels onto the consecutive backbone carbonyls in each peptide brings out significant differences in both the ^{12}C and ^{13}C amide I band shapes and intensities. Upon analysis of spectra as a function of temperature and label position, differences in site-specific helix stability can be extracted. We will discuss the effect in terms of differences in structure and helix propensity of leucine compared to alanine.

177: The elusive C-terminus of ribonucleotide reductase. Luke James Higgins, Herman Lelie, and Catherine L. Drennan. Department of Chemistry, Massachusetts Institute of Technology, 56-546 77 Massachusette Ave., Cambridge, MA 02139 (higginsl@mit.edu)

Ribonucleotide reductase catalyzes the conversion of ribonucleotides to deoxynucleotides. The reductive equivalents for this reaction are provided by the thioredoxin/thioredoxin reductase (Tr/TrR) redox system. The redox cascade of Tr/TrR is coupled to active site reduction via a C-terminal disulfide of RNR. In class II ribonucleotide triphosphate reductase (RTPR) from *Lactobacillus leichmannii*, this rereduction reaction is the rate-limiting step. The structure of RTPR has been solved in our laboratory, yet the C-terminal portion of the structure remains disordered. To investigate the properties of the C-terminus, 15 residue peptides have been synthesized on solid support. A truncated mutant of RTPR has been cloned and purified. We are currently using ITC to characterize the binding specificity of a C-terminal peptide mimetic to the truncated mutant. To obtain structural information, cross-linking experiments are being employed to generate a stable covalent complex between the C-terminal peptide mimetic and the mutant enzyme.

178. Thiaminase: progress toward a novel fluorescent microplate assay. R. Marshall Werner, Michael O'Toole, and Richard Federley. Department of Chemistry, Lake Superior State University, 650 W. Easterday Ave., Sault Ste. Marie, MI 49783 (fax: 906-635-2266)

Progress toward a newly developed assay for thiaminase (type I) involving use of a covalently attached secondary



amine will be discussed. This assay utilizes the covalently transferred pyrimidine moiety from thiamine, and functionalizes it with a fluoresecent tag. This assay will be compared with the traditional thiochrome assay used for thiaminase analysis.

179. To elucidate the mechanism of chromophore formation in red fluorescent protein (RFP) for *Discosoma* species (Ds). Pravin A. Nair, Yuguo Feng, Alasdair F. Bell, and Peter Tonge. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400 (fax: 631-632-5797, pnair@ic.sunysb.edu)

RFP from Ds have been a keenly researched field for some time now. RFP has potential uses as a reporter protein on being tagged with the protein of interest. Our primary interest is to study the stepwise chromophore formation and to throw light on how the protein environment affects the chromophore formation. Toward this end, we are cloning the RFP gene into pTWIN vector from Clontech and expressing the protein in fragments. The fragments would be the initial 1−61 amino acids and the 69−225 section of the protein. The chromophore consists of 66−68 amino acids. The in-between fragment 62−68 would be synthesized using peptide synthesizer with the incorporation of unnatural amino acids. This intein−extein ligation strategy we believe would help us shed light on our aforementioned objectives. The ligated fragments would also be subjected to UV−Vis and fluorescence spectroscopic inspection to learn of its chromophoric properties and maturation time.

180. Uncatalyzed peptide bond formation and transition state stabilization by the ribosome. Annette Sievers and Richard Wolfenden. Department of Biochemistry and Biophysics, University of North Carolina, CB #7260, Chapel Hill, NC 27599 (sievers@email.unc.edu)

The crystal structure of the ribosome reveals that it is a ribozyme. A catalytic mechanism involving the conserved adenine residue A2451 in the *E. coli* 23S RNA has been proposed, but recently it was shown that this adenine residue can withstand mutations without causing loss of activity in vitro. To determine whether the ribosome acts as a chemical catalyst or by juxtaposition of the reactants, the nonenzymatic rate of peptide bond formation was examined at a series of different pHs and temperatures, yielding liner Arrhenius plots. Comparison with the thermodynamic parameters for peptide bond formation by the *E. coli* ribosome reveals that the rate enhancement is achieved by lowering the entropy of activation. This is is accord with the hypothesis that the ribosome acts as an entropy trap and promotes catalysis by bringing the two reactants into proximity.

181. Vibrational coupling between residues in specifically labeled helices: dependence of the amide I bands on the number and position of labeled residues. Wendy Barber-Armstrong, **Teraya M. Donaldson**, R. A. Gangani Silva, and Sean M. Decatur. Department of Chemistry, Mount Holyoke College, Carr Laboratory, South Hadley, MA 01075 (fax: 413-538-2327, wbarber@mtholyoke.edu)

Infrared spectroscopy of peptides incorporating site-specific ^{13}C labels is becoming a standard tool for probing secondary structure at the residue level; the frequency and intensity of the ^{13}C amide I band can be observed and analyzed independently of the ^{12}C amide I band arising from the unlabeled portions of the peptide. Despite the growing popularity of this technique, there are still fundamental questions about the nature of coupling between ^{13}C-labeled residues which have not been explored in detail. In this study, we have synthesized a series of alanine-rich peptides based on the sequence repeat $(AAKAA)_n$ incorporating varying numbers and arrangements of ^{13}C labels. All of these peptides form stable alpha helices in aqueous solution. Labeled residues adjacent in sequence couple more strongly then separated labels, resulting in a 6 cm^{-1} shift in the amide I band. Frequency shifts are also observed when the number of labeled residues gradually increases from 1 to 4. Disruption of the coupling between ^{12}C residues by introducing ^{13}C residues in the center of the helix results in a significant decrease in the intensity of the ^{12}C amide I band. These results provide important insight on how the frequency of bands arising from labeled residues can vary as a function of label position as opposed to backbone conformation.

Thursday Morning: Role of Mechanistic Studies in Enzyme Inhibitor Design

Ross Stein, Organizer

182. Transition state analogues: pushing the limit? Richard V. Wolfenden. Department of Biochemistry & Biophysics, University of North Carolina−Chapel Hill, Chapel Hill, NC 17599 (water@med.unc.edu)

An enzyme's affinity for the altered substrate in the transition state matches the value of k_{cat}/K_m divided by the rate constant for the uncatalyzed reaction in water. The validity of this relationship is not affected by the detailed mechanism by which any particular enzyme may act, or by whether enzyme conformation changes accompany catalysis. It subsumes potential effects of substrate desolvation, H-bonding and other polar attractions, and the approximation of several substrates in a configuration appropriate for reaction. Recent observations of the rate enhancements that are actually produced by enzymes indicate that the potential binding affinities of ideal transition state analogue inhibitors are very large indeed. In several cases, transition state binding discimination appears to be critically dependent on connectivity effects, and on the displacement of solvent water from the binding cavity.

183. Transition state analogue inhibitor design for *N*-ribosyltransferases. Vern L. Schramm. Albert Einstein College of Medicine, Bronx, NY 10461 (fax: 718-430-8565, verb@aecom.yu.edu)

Transition state structures for *N*-ribosyltransferases have been established by the combination of kinetic isotope effects and computational chemistry. Purine nucleoside phosphorylase catalyzes the formation of a ribooxacarbenium ion with residual bond order to hypoxanthine, but only van der Waals interaction with phosphate. Immucillin-H, a mimic of the transition state, is a 23 pM inhibitor. Purine and pyrimidine phosphoribosyltransferases have also been identified as forming ribooxacarbenium-like transition states with similar properties. The 5′-phosphorylated Immucillins are nanomolar inhibitors for several purine phosphoribosyltransferases,

including that from malaria. Nucleoside *N*-ribosyl hydrolases provide purines for salvage by protozoan parasites, and constitute a group of isozymes with varying specificity for the purine or pyrimidine leaving group. Transition state structure and leaving-group activation have permitted synthesis of nanomolar transition state analogues with strong isozyme specificity. Ricin A-chain differs from the other *N*-ribosyltransferases by fully dissociated ribooxacarbenium transition states. Therefore, transition state analogues with dissociated leaving groups are superior to those with covalent leaving groups. NAD^+-ADP-ribosyl transferases have transition states with residual bond order to nicotinamide but have resisted inhibitor design based on ribooxacarbenium ion mimics. (Supported by research grants from the NIH.)

184. Inhibition of IMP dehydrogenase and mechanisms of drug selectivity. Lizbeth Hedstrom. Department of Biochemistry, Brandeis University, 415 South St., MS 009, Waltham, MA 02454-9110 (fax: 781-736-2349, hedstrom@brandeis.edu)

Drug resistance often results from mutations that are located far from the drug binding site. The inhibition of IMPDH by mycophenolic acid (MPA) is an example of this phenomenon. MPA is a potent inhibitor of mammalian IMPDHs but a poor inhibitor of the microbial enzymes. MPA traps the covalent intermediate E−XMP*, and binds in the nicotinamide half of the dinucleotide site. Approximately half of the difference in sensitivity derives from residues in the MPA binding site. The remainder can be attributed to the adenosine subsite of the NAD^+ site. The nicotinamide and adenosine sites are tightly coupled in *Tritrichomonas foetus* IMPDH but independent in the human type 2 isozyme. This difference in coupling can account for the remaining difference in MPA affinity. Coupling derives from the competition between MPA and the active site flap for the drug binding site. This conformational change is required for completion of the catalytic cycle.

185. Integration of genomic, structural and thermodynamic information in drug design. Ernesto Freire. Department of Biology, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218 (fax: 410-516-6469, ef@jhu.edu)

Enzymatic targets for drug design, especially those of viral or bacterial origin, display different levels of heterogeneity due to: (1) genetic diversity; (2) drug resistant mutations; (3) binding site dynamics. Structure-based drug design against heterogeneous targets requires a departure from the classic "lock and key" paradigm that leads to the development of conformationally constrained molecules unable to adapt to target variations. Heterogeneous targets need adaptive drug molecules, characterized by the presence of flexible elements at specific locations that sustain a viable binding affinity against existing or expected polymorphisms. Adaptive ligands have characteristic thermodynamic signatures that distinguish them from their rigid counterparts. This realization has led to new design guidelines involving: (1) a new representation of binding sites that include a structural mapping of genetic polymorphisms and conformational dynamics; and (2) a close monitoring of the enthalpic and entropic components of the binding affinity during lead identification and optimization. The application of these guidelines to the design of HIV-1 protease and plasmepsin inhibitors will be discussed. (Supported by Grant GM57144 from the NIH and Grant MCB9816661 from the NSF.)

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