Abstracts, Division of Biological Chemistry, 226th National Meeting of the American Chemical Society, September 7−11, 2003

Carol A. Fierke, Program Chair

Sunday Morning: Structure and Function of Metalloenzymes

David Christianson, Organizer

1. Crystal structure of LpxC, a zinc-dependent deacetylase required for lipid A biosynthesis. Douglas A. Whittington,[1] Kristin M. Rusche,[2] Hyunshun Shin,[1] Carol A. Fierke,[2] and David W. Christianson.[1] [1]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 (dwhit@anchor.chem.upenn.edu), and [2]Department of Chemistry, University of Michigan

Lipid A is the membrane anchor of lipopolysaccharide (or endotoxin), the principal component of the outer leaflet of the outer membrane of Gram-negative bacteria. Lipid A is essential for cell viability, and enzymes involved in its biosynthesis are antibiotic targets. The first committed step in lipid A biosynthesis is catalyzed by UDP-[3-*O*-(R-3-hydroxymyristoyl)]-*N*-acetylglucosamine deacetylase (LpxC), a zinc-dependent deacetylase that has no significant sequence identity to proteins of known structure. To define its structure and mechanism, we determined the crystal structure of LpxC from *Aquifex aeolicus* to 2.0 Å resolution. The structure reveals a unique $\alpha+\beta$ fold with a catalytic zinc ion located at the bottom of an active site cleft and adjacent to a hydrophobic tunnel that plays an important role in defining substrate specificity. We also propose a mechanism for LpxC and present thermodynamic data for compounds that bind to the enzyme with micromolar affinity.

2. Biochemical and biophysical characterization of particulate methane monooxygenase from *Methylococcus capsulatus* (Bath). Raquel L. Lieberman,[1] Deepak B. Shrestha,[1] Peter E. Doan,[1] Brian M. Hoffman,[1] Timothy L. Stemmler,[2] and **Amy C. Rosenzweig**.[1] [1]Northwestern University, Evanston, IL 60208 (amyr@northwestern.edu), and [2]Wayne State University School of Medicine

Particulate methane monooxygenase (pMMO) is a membrane-bound enzyme that catalyzes the oxidation of methane to methanol in methanotropic bacteria. Understanding how this enzyme hydroxylates methane at ambient temperature and pressure is of fundamental chemical and potential commercial importance. Difficulties in solubilizing and purifying active pMMO have led to conflicting reports regarding its biochemical and biophysical properties, however. We have purified pMMO from *Methylococcus capsulatus* (Bath) and detected activity. The purified enzyme has a molecular mass of ~200 kDa. Each 200 kDa pMMO complex contains 4.8 ± 0.8 copper ions and 1.5 ± 0.7 iron ions. Electron paramagnetic resonance spectroscopic parameters corresponding to 40−60% of the total copper are consistent with the presence of a mononuclear type 2 copper site. X-ray absorption near edge spectra indicate that purified pMMO is a mixture of Cu(I) and Cu(II) oxidation states. Finally, extended X-ray absorption fine structure data are best fit with oxygen/nitrogen ligands and a 2.57 Å Cu−Cu interaction, providing the first direct evidence of a copper-containing cluster in pMMO.

3. Anatomy of a Radical Protein. Frederick Berkovitch,[1] Yvain P. Nicolet,[1] Luke J. Higgins,[1] Michael D. Sintchak,[1] Mohammad Seyedsayamdost,[1] Jason T. Wan,[2] JoAnne Stubbe,[3] Joseph T. Jarrett,[2] and **Catherine L. Drennan**.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (cdrennan@mit.edu), [2]Department of Biochemistry and Biophysics, University of Pennsylvania, and [3]Department of Chemistry and Biology, Massachusetts Institute of Technology

Nature uses organic radical species to catalyze some of the most chemically challenging enzymatic reactions. Enzymes that use radical chemistry are involved in fundamental cellular processes such as DNA biosynthesis and repair. To generate radical species, enzymes require cofactors such as adenosylcobalamin or adenosylmethionine. Adenosylcobalamin, the largest and most complex vitamin known, generates an adenosyl radical species by homolytic cleavage of a carbon−cobalt bond. In contrast, adenosylmethionine (SAM) has been termed a "poor man's adenosylcobalamin", since it can generate an adenosyl radical using only SAM and a [4Fe-4S] cluster. Our laboratory uses X-ray crystallography to investigate the structures and mechanism of radical enzymes such as ribonucleotide reductase and biotin synthase. Here, we will compare structural information for adenosylcobalamin-dependent enzymes and radical SAM proteins to investigate the structural requirements for radical generation; can we define the anatomy of a radical protein?

4. Metal substitution in enzymes from marine microorganisms. François Morel. Department of Geosciences, Princeton University, Princeton, NJ 08544 [fax (609) 258-5242, morel@princeton.edu]

Marine microorganisms have evolved strategies for coping with an environment extraordinarily impoverished in some essential trace metals. Substitution of one metal with another in the active center of some metalloenzymes is one of these strategies. We have been studying the replacement of zinc with cobalt and cadmium in marine phytoplankton, focusing on carbonic anhydrase (CA), which is an essential part of the carbon concentrating mechanisms of these autotrophs. In marine diatoms, one form of CA can use indifferently Zn or Co as its metal center; another form of CA is a Cd enzyme.

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The periplasmic CA of diatoms uses their silica frustule as an efficient buffer for rapid proton exchange. Preliminary evidence indicates that similar metal substitution occurs in other important enzymes such as alkaline phosphatase.

Sunday Afternoon: Directed Evolution of Enzymes

Eric Toone, Organizer

5. Chemical complementation: A genetic assay for protein evolution and proteomics. Virginia W. Cornish,[1] Hening Lin,[1] Kathleen Baker,[2] Gilda J. Salazar-Jimenez,[1] Debleena Sengupta,[1] Colleen F. Bleczinski,[1] Sang-Soo Hah,[1] and Sonja Krane.[1] [1]Department of Chemistry, Columbia University, 3000 Broadway, Havemeyer Hall, MC 3111, New York, NY 10027 [fax (212) 932-1289], and [2]Department of Pharmacology, Columbia University

A high-throughput assay for enzyme activity has been developed that is reaction-independent. In this assay, a small molecule yeast three-hybrid system is used to link enzyme catalysis to transcription of a reporter gene in vivo. Here we demonstrate the feasibility of this approach using a well-studied enzyme-catalyzed reaction, cephalosporin hydrolysis by the *Enterobacter cloacae* P99 cephalosporinase. We show that the three-hybrid system can be used to read-out cephalosporinase activity in vivo as a change in the level of transcription of a lacZ reporter gene and that the wild-type cephalosporinase can be isolated from a pool of inactive mutants using a lacZ screen. The assay has been designed so that it can be applied to different chemical reactions without changing the components of the three-hybrid system. A reaction-independent, high-throughput assay for protein function should be a powerful tool for protein engineering and enzymology, drug discovery, and proteomics.

6. Directed evolution of the pyruvate aldolases. Eric J. Toone[1] and Carol A. Fierke.[2] [1]Department of Chemistry, B120 Levine Science Research Center, Duke University, Durham, NC 27708 [fax (919) 688-5483], and [2]Department of Chemistry, University of Michigan

The pyruvate aldolases are a large group of enzymes that form carbon−carbon bonds in an aldol addition reaction using pyruvate as the nucleophilic component. We have used the key enzyme of the Entner Doudoroff glycolytic pathway, 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, for the construction of carbon−carbon frameworks in several natural products. Despite this demonstrated utility, the broader use of KDPG aldolase as a synthetic catalyst is limited by a substrate specificity that restricts the nucleophilic component to pyruvate and requires polar functionality at C2 or C3 of the electrophile. To broaden or shift these specificities, we have considered aldolases from different sources and initiated directed evolution experiments on these enzymes. Here we report on the behavior of the enzyme from *Thermatoga maratima* and consider differences in the activities on the basis of the crystal structures of both enzymes. We also report on novel unnatural enzymes derived from random mutagenesis studies and identified by a selection strategy using engineered strains of *Escherichia coli* auxotrophic for pyruvate. (Supported by NIH Grant GM61596.)

7. Discovery and optimization of enzymes for chemical transformations through biodiversity access and directed evolution technologies. Mark J. Burk. Chemical and Industrial R&D, Diversa Corporation, 4955 Directors Place, San Diego, CA 92121 [fax (858) 526-5843, mburk@diversa.com]

Despite the great potential of enzymes in many industrial applications, their use on a large scale remains fairly limited. We have attempted to develop numerous technologies that address and overcome factors that historically have restricted biocatalyst usage. In general, we take a two-pronged approach based on new enzyme discovery, followed by optimization of performance through process development and the use of powerful directed evolution technologies. Our discovery efforts are based upon extraction of genomic DNA from uncultured microbial sources collected from biodiversity around the globe. Discovery from nature affords enzymes exhibiting many novel activities, yet developing a process that meets the exact specifications for a given application often requires rapid optimization. Nature provides an excellent starting point for efficient laboratory evolution of enzyme phenotypes. We will describe our methods for directed evolution of enzymes and present numerous examples of enzyme development that highlight the advantages of our approach.

8. Directed evolution of enzyme structure and function. Donald Hilvert. Laboratory of Organic Chemistry, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zurich, Switzerland (fax +41-1-632-1486, hilvert@org.chem.ethz.ch)

Natural enzymes have evolved over millions of years by the process of Darwinian evolution. Multiple cycles of mutation, selection, and amplification can also be exploited by scientists in the laboratory to create and characterize protein catalysts on a human time scale, providing access to macromolecules with tailored activities and selectivities. Recent progress in applying evolutionary approaches to the problem of catalyst design will be discussed.

Monday Morning: Mechanism of RNA Processing

Carol Fierke, Organizer

9. Metals, folding and catalysis in ribozymes. Victoria J. DeRose. Department of Chemistry, Texas A&M University, College Station, TX 77842-3012 [fax (409) 845-4719, derose@mail.chem.tamu.edu]

Cations are generally required for RNA structure and function. In particular, at moderate ionic strength, divalent ions such as Mg(II) are critical to the function of many catalytic RNAs (ribozymes). Roles of cations in stabilizing particular structures, or in acting more directly as cofactors in chemical reactions, are often proposed on the basis of biochemical studies. Spectroscopic methods allow direct observation of these important cation sites, in particular when paramagnetic Mn(II) is used as a probe ion. Magnetic resonance and other spectroscopic methods have been successful in identifying and characterizing an important "metal switch" site in the hammerhead ribozyme. These studies, as well as experiments that aid in identifying proposed metal clusters in RNA, will be presented.

10. Hepatitis delta virus ribozyme: Structural dynamics and function of a human pathogenic RNA. Nils G.

Walter, Dinari A. Harris, Sohee Jeong, Jana Sefcikova, Rebecca A. Tinsley, Miguel J. B. Pereira, and David Rueda. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 [fax (734) 647-4865, nwalter@umich.edu]

The hepatitis delta virus (HDV), an infectious human pathogen affecting millions of people worldwide, leads to intensified disease symptoms, including progression to liver cirrhosis upon coinfection with its helper virus, HBV. Both the circular RNA genome of HDV and its complementary antigenome contain a common cis-cleaving catalytic RNA motif, the HDV ribozyme, which plays a crucial role in viral replication. Previously, the crystal structure of the product form of the cis-acting genomic HDV ribozyme has been determined, and the precursor form has been suggested to be structurally similar. In contrast, we have used fluorescence resonance energy transfer (FRET) and 2-aminopurine fluorescence to show that significant global and local conformational changes accompany catalysis of a trans-acting form of the ribozyme in solution. In addition, we have employed terbium(III) footprinting to map at nucleotide resolution structural differences between the precursor and product forms that are dependent on the 5′ substrate sequence.

11. Structural studies of the specificity domain of ribonuclease P. Alfonso Mondragón,[1] Andrey S. Krasilnikov,[1] and Tao Pan.[2] [1]Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2205 Tech Drive, Evanston, IL 60208 [fax (847) 467-6489, a-mondragon@northwestern.edu], and [2]Department of Biochemistry, University of Chicago

tRNA is produced as a precursor molecule that needs to be processed at its 3′ and 5′ ends. Ribonuclease P (RNase P) is the endonuclease responsible for processing the 5′ end of tRNA. RNase P is a multiturnover ribozyme consisting of an RNA and a protein component. Eubacterial RNase P is made of two domains: a specificity domain and a catalytic domain. We have determined a 3.15 Å resolution crystal structure of the specificity domain of *Bacillus subtilis* RNase P. The structure reveals the general features of this domain, the interactions that help maintain the overall fold of the molecule, a large nonhelical module that is conserved in all RNase P's, and the regions that are involved in pre-tRNA recognition. Sequence comparisons elucidate the common structural features in the S domain of all RNase P RNA's. Additionally, the structure helps to consolidate many emerging observations about the structure of large RNA molecules.

12. Nuclear RNase P: Why has the ribozyme become so complex? David R. Engelke, Felicia H. Scott, Shaohua Xiao, Chatchawan Srisawat, and Martin Thompson. Biological Chemistry, University of Michigan, 1150 W. Medical Center Dr., 3200 MSRB III, Ann Arbor, MI 48109-0606 [fax (734) 763-7799, engelke@umich.edu]

Ribonuclease P is one of only two enzymes found in all living cells that contain catalytic RNA subunits and are thought to be remnants of a prebiotic "RNA world". The best characterized RNase P reaction is cleavage of pre-tRNAs to produce mature 5′ termini, but the enzyme also has other RNA substrates in the cell. The catalytic RNA subunit, unlike many ribozymes, recognizes substrates through tertiary contacts rather than through Watson−Crick base pairing. The bacterial enzyme usually has only one small protein subunit that is dispensable in vitro. In contrast, the eukaryotic nuclear enzyme, while having a similar, well-conserved RNA subunit, also has at least nine protein subunits. Secondary and tertiary structure models have been derived for the RNA subunit, and a preliminary map of overall holoenzyme architecture has been developed. While all of the protein subunits are essential for enzyme function in vivo, none of the protein functions have been established unambiguously, and it is thought that few are directly involved in pre-tRNA substrate recognition and cleavage. Most of the huge increase in the number of protein subunits might be required for coping with the increased complexity of the nuclear environment and for differentiating among more types of RNA and RNP substrates and nonsubstrates. To approach this question, we have examined the spatial and temporal organization of the pre-tRNA processing pathway in *Saccharomyces cerevisiae*. The majority of both RNase P and pre-tRNAs reside largely in the nucleolus, rather than the nucleoplasm, a situation that could lead to cooperative regulation of tRNA and ribosomal biogenesis. Recently, we have shown that spatial organization of tRNA biosynthesis begins even before the early cleavage of precursors by RNase P, with nucleolar positioning of the tRNA genes. This is in keeping with a previously described interaction between RNase P and one of the tRNA gene transcription factors, and suggests a model whereby both synthesis and early processing of pre-tRNAs are coordinated at the nucleolus, along with the ribosomal pathway.

Monday Afternoon: Metabolomics

Ruma Banerjee, Organizer

13. Comprehensive metabolomics using FTMS technology: Theory and applications. Dayan Goodenowe. Phenomenome Discoveries, Inc., 204−407 Downey Rd., Saskatoon, SK S7N4L8, Canada [fax (306) 244-6730, d.goodenowe@phenomenome.com]

Phenomenome Discoveries' *DISCOV*Ametrics technology separates, quantifies, and identifies all of the metabolites in a complex biological sample quickly and simultaneously. This is achieved without any *a priori* selection of the metabolites of interest and is therefore unbiased. These data are exported to a database that allows comparison of one sample to another or searching and organizing of entire populations by metabolite expression. This powerful comprehensive capability allows one to directly correlate changes in metabolism with gene expression, clinical outcomes, etc. Discussed will be the theory behind comprehensive metabolome expression analysis, including demonstrations of bioinformatic tools and application examples from collaborative research projects in agriculture, human health, and natural product studies.

14. Profiling yeast metabolism by NMR. Thomas Szyperski. Chemistry Department, State University of New York at Buffalo, 816 Natural Sciences Complex, Buffalo, NY 14260 [fax (716) 645-7338, szypersk@chem.buffalo.edu]

The routine assessment of metabolic fluxes using NMR relies on cost-effective protocols for ^{13}C labeling and

sensitive NMR detection schemes. Fractional ^{13}C labeling of proteinogenic amino acids employed in conjunction with two-dimensional $^{13}C-^{1}H$ correlation NMR spectroscopy and software for rapid data analysis allows one to efficiently determine metabolic flux ratios and the network topology of active biochemical pathways in a single experiment. The fractional ^{13}C labeling approach has been extended to eukaryotic systems, and promises to become a potent complement to proteome and transcriptome analysis for metabolic profiling. Recent applications for comparative metabolic flux profiling of *Saccharomyces cerevisiae* and *P. stipitis* are discussed.

15. Metabonomics in safety and medicine: Biochemistry lives again. John Shockcor. Department of Biological Chemistry, Imperial College, and Metabometrix Ltd., RSM, Prince Consort Road, London SW7 2BP, United Kingdom (fax +44 20 7594 6818, j.connelly@metabometrix.com)

Metabonomics is the quantitative, dynamic study of metabolite profiles in biofluids and tissues, employing NMR spectroscopy or mass spectrometry coupled with multivariate statistical spectral analysis. It is an extremely powerful and versatile technique, with uses in nonclinical and clinical drug safety and efficacy, medical diagnostics, functional genomics, characterization of genetically modified models, and environmental and forensic toxicology. Time-dependent, site- and mechanism-specific systemic changes can be readily monitored, noninvasively and with minimal sample preparation. As a measurement of the "real world" outcome of gene and protein events, metabonomics has also been used to direct genomic and proteomic sampling schedules. The basic concepts and methodologies developed at Imperial College and Metabometrix are described, along with specific applications, including prediction of longer-term toxicity from acute experiments, improved understanding of animal models of disease, and both NMR- and MS-based medical classification of stages of atherosclerosis, providing a safer equivalent to angiography.

16. Metabolic profiling used for development of new diabetes therapies. Christopher B. Newgard. Department of Pharmacology and Cancer Biology, Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, DUMC 3813, LSRC C351, Durham, NC 27710 [fax (919) 668-6044, newga002@mc.duke.edu]

Our laboratory studies metabolic regulatory mechanisms as they pertain to the development of new therapies for diabetes and obesity. We use an interdisciplinary approach, involving development of novel cellular models, gene discovery and genetic engineering, and metabolic profiling by NMR [in collaboration with A. Dean Sherry and associates (Dallas, TX)] and mass spectroscopy [in collaboration with David Millington and associates (Department of Pediatrics, Duke University Medical Center)]. We have carried out several studies that suggest mechanisms for overcoming syndromes of tissue dysfunction (β-cell failure, insulin resistance, and failure to regulate hepatic glucose production and disposal) that constitute type 2 diabetes. (Supported by grants from the National Institutes of Health and a sponsored research agreement from Takeda Chemicals Inc.)

Monday Evening: Sci-Mix (posters)

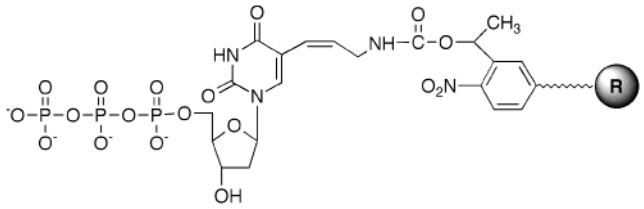
17. A combined in vitro/in vivo selection scheme for RNA polymerases with novel promoter and nucleotide specificities. Jijumon P. Chelliserrykattil and Andrew D. Ellington. Department of Chemistry and Biochemistry, University of Texas at Austin, 1 University Station, A 5300, Austin, TX 78712 [fax (512) 471-7014]

The DNA-dependent RNA polymerase from T7 bacteriophage (T7 RNA polymerase) is highly specific for its promoter and also exhibits stringent substrate specificity. A combined in vitro/in vivo selection method has been developed for the isolation of T7 RNA polymerases with altered promoter specificities and novel substrate specificities. In short, a library of T7 RNA polymerase variants was cloned downstream of a T7 or other promoter, generating a so-called autogene library. Following transformation, those polymerase variants that best recognized their adjacent promoter self-amplified both their mRNAs and themselves in vivo. The variant mRNAs were extracted from the population as a whole, and were roughly represented according to the activities of their corresponding variant polymerases. Following reverse transcription and PCR amplification in vitro, the most abundant polymerase genes were carried into subsequent rounds of selection. This method allows large (10^3-10^6) polymerase libraries to be efficiently searched for their promoter recognition ability and fidelity. We have used this method to select variants of T7 RNA polymerase that can utilize a T3 RNA polymerase promoter, and also to select novel polymerase variants that can utilize novel promoters in vivo. Autogene libraries are currently being used to screen polymerase variants that can synthesize modified RNA for a variety of applications in biotechnology.

18. Photocleavable nucleotide analogues for DNA analysis. Xiaopeng Bai,[1] Zengmin Li,[2] Sobin Kim,[2] Hameer D. Ruparel,[2] Nicholas J. Turro,[1] and Jingyue Ju.[2] [1]Department of Chemistry, Columbia University, Havemeyer Hall, MC 3156, New York, NY 10027 [fax (212) 851-5215, xb8@columbia.edu], and [2]Department of Chemical Engineering and Columbia Genome Center, Columbia University

We report the synthesis of photocleavable nucleotide analogues and their applications in DNA analysis. The nucleotide analogues have a functional moiety attached to the 5 position of 2′-deoxyuridine triphosphate via a photocleavable 2-nitrobenzyl linker. These nucleotide analogues were shown to be faithfully incorporated by a DNA polymerase into a growing DNA strand in a DNA polymerase reaction. A photocleavable fluorescent nucleotide analogue was successfully applied to a DNA sequencing by synthesis system, and the fluorescent dye was shown to be removed completely with UV irradiation ($\lambda = 340$ nm) after detection. Another nucleotide analogue containing a photocleavable biotin was used in a multiplex genotyping system. After solid phase capture of the extended DNA fragments carrying the nucleotide analogue with streptavidin-coated magnetic beads, UV irradiation ($\lambda = 340$ nm) was used to release the DNA samples into the solution, facilitating the simultaneous

detection of the DNA fragments by MALDI-TOF mass spectrometry.



19. First PCR amplification of DNA containing a nonstandard base pair A. Michael Sismour,[1] Stefan Lutz,[2] Jeong-Ho Park,[3] and Steven A. Benner.[1] [1]Chemistry, Chemistry Research Building, University of Florida, P.O. Box 117200, Gainesville, FL 32611 [fax (352) 392-7918, msismour@chem.ufl.edu], [2]Emory University, and [3]Chemical Technology, Hanbat National University

For the first time, an oligonucleotide containing a nucleoside with a nonstandard hydrogen bonding pattern (NSB) has been successfully amplified over multiple rounds by the polymerase chain reaction utilizing a single enzyme. We have shown, via a novel "sequencing" technique exploiting the pausing propensity of Taq polymerase when challenged with a NSB, that the Y188L/E478Q mutant of HIV I RT was capable of amplifying an expanded genetic alphabet containing xanthosine and its complement pyDAD nucleoside over five rounds of PCR. The enzyme exploited for this novel function resulted from the combined application of site-directed mutagenesis and screening of inhibitor resistant enzyme variants generated in clinico, representing a new strategy for the generation of altered biocatalysts. Such technology represents the first step in the generation of a functional artificial expanded genetic information system (AEGIS) capable of revolutionizing many facets of biotechnology.

20. Effects of nucleotide modification on the stability and structure of helix 69 from 23S and 28S rRNAs. Minako Sumita,[1] Helen M.-P. Chui,[2] and Christine S. Chow.[2] [1]Department of Chemistry, Wayne State Universtiy, 5101 Cass Ave., Detroit, MI 48202 [fax (313) 577-8822, msumita@chem.wayne.edu], and [2]Department of Chemistry, Wayne State University

Helix 69 is located at the interface between the large and small subunits of the ribosome. The sequence of helix 69 is highly conserved throughout phylogeny and has similar modifications, namely, pseudouridines, in *Escherichia coli* and *Homo sapiens*, at three loop positions, 1911, 1915, and 1917 (*E. coli* numbering). The sequences differ at one critical position in helix 69, A1918 in bacteria and G3734 in human, as well as in the number of pseudouridine modifications in the stem. A series of 19-nucleotide RNAs corresponding to helix 69 of *E. coli* 23S and *H. sapiens* 28S rRNA have been synthesized and studied using biophysical techniques such as circular dichroism (CD) spectroscopy, thermal melting, and one-dimensional NMR spectroscopy. The nucleotide modifications and sequences both affect the rRNA hairpin structure and stability; significant differences in melting temperatures and CD spectra are observed between the *E. coli* and *H. sapiens* helix 69 rRNAs.

21. Human Arginase II: Crystal structure and physiological role in male and female sexual arousal. Evis Cama,[1] Diana M. Colleluori,[2] Frances A. Emig,[3] Hyunshun Shin,[1] Soo Woong Kim,[4] Noel N. Kim,[4] Abdulmaged M. Traish,[4] David E. Ash,[3] and David W. Christianson.[1] [1]Department of Chemistry, University of Pennsylvania, S. 34th St., Philadelphia, PA 19104 (evis@anchor.chem.upenn.edu), [2]Temple Univeristy, [3]Department of Biochemistry, Temple University School of Medicine, and [4]Departments of Urology and Biochemistry, Boston University School of Medicine

Arginase II, a binuclear manganese metalloenzyme, is extrahepatic (e.g., kidneys, small intestine, lactating mammary gland, and penile corpus cavernosum) and is localized subcellularly in the mitochondrial matrix of kidney cells. The X-ray crystal structure of a fully active, truncated form [ΔM1-V23/ΔH331-I354 (114 kDa trimer)] of human arginase II complexed with a boronic acid transition state analogue inhibitor has been determined at 2.7 Å resolution. This structure is consistent with the hydrolysis of L-arginine through a metal-activated hydroxide mechanism. Human arginase II is present in human clitoral corpus cavernosum and vagina tissues and functions to regulate L-arginine bioavailability to NO synthase in these tissues. Accordingly, hemodynamic studies conducted with a boronic acid arginase inhibitor in vivo suggest that the extrahepatic arginase plays a role in both male and female sexual arousal. Therefore, arginase II is a potential target for the treatment of male and female sexual arousal disorders.

22. Crystal structure at 1.3 Å resolution of GDP-mannose mannosyl hydrolase (GDPMH), an unusual Nudix enzyme. Sandra B. Gabelli,[1] Patricia M. Legler,[2] Mario A. Bianchet,[1] Albert S. Mildvan,[2] and L. Mario Amzel.[1] [1]Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 [fax (410) 955-0637, sandra@groucho.med.jhmi.edu], and [2]Department of Biological Chemistry, Johns Hopkins School of Medicine

GDPMH, a homodimer, catalyzes the hydrolysis of GDP-mannose and GDP-glucose to yield GDP and sugar, by substitution with inversion at C1′ of the sugar. One divalent cation is required for catalysis which bridges the enzyme to both the α- and β-phosphates of GDP. NMR and mutational studies suggest that Glu-70 functions as a metal ligand, His-124 functions as the general base, Arg-52 and Arg-65 function as electrostatic catalysts, and His-88 functions in an unknown way [Legler, P. M., et al. (2002) *Biochemistry 41*, 10834]. The 1.3 Å X-ray structure of the GDPMH−Na^+−GDP complex (R = 19%) reveals a dimer, and shows the altered Nudix motif forms a typical loop−helix−loop structure. Metal coordination by both phosphates of GDP, and by Glu-70, is confirmed, together with Gly-50, Gln-123, and a water to form an octahedral complex. His-124, in the second sphere of the enzyme-bound metal, approaches both an inner sphere and second sphere water. Guanine is recognized by Arg-52 which donates hydrogen bonds to the C6═O group and N7, and by the C′═O group of Leu-4. Arg-65 is 5.6 Å from the α-phosphate, with an intervening water. His-88 approaches the sugar binding site which, in this structure, is occupied by a Tris molecule. Two additional

active site residues are found, Arg-37 and Tyr-103, which donate hydrogen bonds to the β-phosphate. Acid catalysis by the metal ion, Arg-37, Tyr-103, and Arg-65 and base catalysis by His-124 may contribute to the 10^{12}-fold rate acceleration produced by GDPMH.

23. Analysis of activity, substrate specificity, and kinetic mechanism of the *Staphylococcus aureus* sortase transpeptidase SrtA. Ryan G. Kruger,[1] Balint Otvos,[1] Patrick J. Dostal,[1] **Brenda A. Frankel**,[1] Dana Robinson,[2] Neil L. Kelleher,[2] and Dewey G. McCafferty.[1] [1]Department of Biochemistry and Biophysics and Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059 (kruger@mail.med.upenn.edu, bfrankel@mail.med.upenn.edu), and [2]University of Illinois at Urbana-Champaign

Sortase enzymes play a key role in the virulence of several Gram-positive bacteria by catalyzing the covalent anchoring of surface protein virulence factors to the bacterial cell wall peptidoglycan. We have determined the kinetic mechanism and substrate specificity of the *Staphylococcus aureus* sortase SrtA. Using a novel high-throughput HPLC assay we devised, revised steady state kinetic parameters have been determined for SrtA. To probe the substrate specificity of SrtA, we synthesized and evaluated the activity of a >90-member peptide substrate library. Although peptides containing LPXTG were preferred, positional tolerances within the LPXTG recognition sequence generally track with sequence variations of known sortase substrates. Furthermore, we have employed bisubstrate kinetics, inhibitor studies, and rapid kinetic analyses to identify the kinetic mechanism of SrtA as a ping-pong bi-bi mechanism with a hydrolytic shunt. We also provide here the first direct evidence for an SrtA enzyme−acyl intermediate using high-resolution Fourier transform ESI-MS methods.

24. Opening a novel window on the mechanism of β-lactamases. A. Monica Totir,[1] S. Marion Helfand,[2] D. Michael Altose,[1] M. Andrea Hujer,[2] P. Marianne Carey,[1] A. Robert Bonomo,[2] and R. Paul Carey.[1] [1]Biochemistry, Case Western Reserve University, 2109 Adelbert Rd., Cleveland, OH 44106 (mat12@cwru.edu), and [2]Research Division, Veterans Administration Medical Center

The reaction between a β-lactamase and its substrates in single β-lactamase crystals has been followed using a Raman microscope. The Raman difference data identify and quantify intermediates on the reaction pathway. Moreover, changes in band intensities with time provide kinetic data. Using the deacylation deficient mutant of SHV-1 β-lactamase (E166A), we are able to observe characteristic Raman bands for Michaelis-type complexes and for acyl−enzyme species. Moreover, we are able to follow the different rates of acylation for tazobactam, sulbactam, and clavulanic acid by the characteristic β-aminoacrylate bands from the acyl−enzyme species that appear at 1590 cm^{-1} for tazobactam or sulbactam and at 1600 cm^{-1} for clavulanic acid. The method has considerable potential for mechanistic studies involving drug-resistant mutants.

25. Trafficking of Hg^{2+} ions from the N-terminal domain to the catalytic core of Tn501 mercuric ion reductase: A thermodynamic and kinetic characterization. Richard Ledwidge and Susan M. Miller. Department of Pharmaceutical Chemistry, University of California at San Francisco, 600 16th St., San Francisco, CA 94143 [fax (415) 514-3221, ledwidg@itsa.ucsf.edu]

The controlled mobility and trafficking of metal ions in the cell are critically important in preventing unwanted ligand binding and catalytic chemistry. Bacterial mercuric ion reductases (MerA) catalyze the reduction of Hg^{2+} to Hg^0 as a means of detoxification. MerA is composed of two structural components. (1) The catalytic core (amino acids 96−562), homologous to other members of the FAD-containing disulfide oxidoreductase family, contains the active site where Hg^{2+} reduction takes place, and (2) the N-terminal domain (NmerA, amino acids 1−69) has unknown function. NmerA contains a MTCXXC metal ion binding motif common among proteins that control the mobility of Cu^+ and Zn^{2+} metal ions. To simplify analysis of NmerA, we cloned and expressed NmerA and the catalytic core as separate proteins. We have previously demonstrated that NmerA binds Hg^{2+} with a 1:1 stoichiometry via its two cysteine thiols and delivers Hg^{2+} to the catalytic core at kinetically competent rates. Our model (based on solution studies) describing the mobility of Hg^{2+} from NmerA onto the catalytic core occurs via a series of Hg−thiol ligand exchanges. We use a combination of site-directed mutagenesis, absorption and NMR spectroscopy, and rapid reaction stopped-flow spectroscopy to characterize the binding and exchange of Hg^{2+} from NmerA onto the core.

26. Characterization of the metal binding properties of Zn(II)CopY by site-directed mutagenesis. Kristina O. Pazehoski and Charles T. Dameron. Department of Chemistry and Biochemistry, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282

Copper is a trace element that is essential to all living organisms because of its ability to serve as a cofactor for several enzymes. Copper also has the potential to be toxic to cells; therefore, its cellular levels must be carefully controlled. Zn(II)CopY is a Cu(I) responsive repressor protein that regulates the expression of the copper homeostasis genes in *Enterococcus hirae*. The protein binds metals in a Cys-x-Cys-xxxx-Cys-x-Cys motif. Site-directed mutagenesis experiments were performed on each cysteine of the metal binding motif to define how Zn(II) and Cu(I) bind to the protein. Future site-directed mutagenesis experiments will investigate the binding interaction between Zn(II)CopY and a copper chaperone protein during Cu(I) transfer.

27. Investigation of the mechanism of LpxC, a zinc-dependent deacetylase. Carol A. Fierke, **Marcy Hernick**, and Kristin M. Rusche. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 [fax (734) 647-4865, fierke@umich.edu, hernickm@umich.edu]

LpxC catalyzes the deacetylation of UDP-3-*O*-(R-3-hydroxymyristoyl)GlcNAc, the committed step in the biosynthesis of Lipid A. A complete understanding of this enzyme and its catalytic mechanism will aid in the development of inhibitors that may be used as novel antibiotics. The

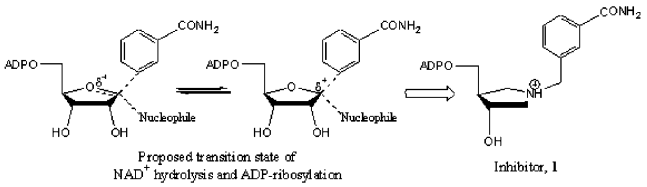
pH dependence of steady-state turnover catalyzed by *Escherichia coli* and *Aquiflex aeolicus* LpxC indicates that multiple ionizations are important for maximal activity. Assignment of the observed pK_a's to ionization of specific amino acid side chains has been examined by characterization of the pH dependence of single-amino acid mutants of LpxC. Kinetic solvent isotope experiments have been used to examine the importance of general acid−base catalysis in this catalytic mechanism. These data, in the context of the recently determined X-ray crystal structure of the *A. aeolicus* LpxC, suggest a mechanism similar to that of zinc metalloproteinases. (Supported by NIH Grant GM 40602.)

28. Exploring the role of the ribonuclease inhibitor protein in angiogenesis. Kimberly A. Dickson,[1] Peter A. Leland,[1] and Ronald T. Raines.[2] [1]Department of Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706-1544 (kdickson@biochem.wisc.edu), and [2]Department of Biochemistry and Department of Chemistry, University of Wisconsin−Madison

Human angiogenin (ANG) is a potent inducer of blood vessel growth and can stimulate the proliferation of human umbilical venous endothelial (HUVE) cells *in vitro*. ANG, a homologue of bovine pancreatic ribonuclease (RNase A), is the only angiogenic protein that possesses ribonucleolytic activity. In the cytoplasm, ANG forms a complex with the ribonuclease inhibitor protein (RI) that is amongs the tightest protein·protein interactions identified ($K_d = 10^{-16}$ M). We hypothesize that the RI·ANG complex protects the cell from the deleterious effects of free cyotplasmic ANG. To test this hypothesis, we have constructed a variant of ANG that maintains the ribonucleolytic and DNA binding activities required for angiogenesis but exhibits a 10^7-fold weaker affinity for RI ($K_d = 10^{-9}$ M). The ability of the variant ANG to stimulate proliferation of HUVE cells will be presented.

29. Transition state inhibitors for ADP-ribosylating bacterial toxins. Guo-Chun Zhou,[1] Sapan L. Parikh,[1] Peter C. Tyler,[2] Richard H. Furneau,[2] and Vern L. Schramm.[1] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 (gzhou@aecom.yu.edu), and [2]Carbohydrate Chemistry Team, Industrial Research Ltd.

Exotoxins of cholera toxin (CT), pertussis toxin (PT), and diphtheria toxin (DT) are the causative agents of severe diarrhea and dehydration (cholera), whooping cough (pertussis), and mucous membrane infection (diphtheria). These toxins have a common toxic mechanism to mono-ADP-ribosylate to specific amino acids in $G_{s\alpha}$, $G_{i\alpha}$, and eEF-2, respectively. In the absence of acceptor proteins, these toxins can act as NAD^+ glycohydrolases. The transition state structures for NAD^+ hydrolysis and ADP ribosylation reactions have oxocarbenium ion character in the ribose. On the basis of the transition state structure, we designed and synthesized transition state analogues of NAD^+ for these toxins. Inhibitor **1** has good affinity for cholera toxin {[$K_{m(NAD)}$ of 5 mM]/(K_i of 11 μM) = 450}. **1** also has inhibitory activity on pertussis toxin ($K_i = 28.6$ μM) and diphtheria toxin ($K_i = 27.5$ μM).



30. Evolution of KGPDC to HPS: Investigations of functional plasticity within the OMPDC suprafamily. Julie Ann Bauer,[1] Wen Shan Yew,[1] Eric Wise,[2] Ivan Rayment,[2] and John A. Gerlt.[1] [1]Department of Biochemistry, University of Illinois, Urbana, IL 61801 [fax (217) 244-7421, jbauer1@uiuc.edu], and [2]Department of Biochemistry, University of Wisconsin

The orotidine 5′-monophosphate decarboxylase (OMPDC) suprafamily is the first unequivocal example of a group of structurally homologous enzymes that are mechanistically distinct. Two members of the OMPDC suprafamily are 3-keto-L-gulonate-6-phosphate decarboxylase (KGPDC), which catalyzes the conversion of 3-keto-L-gulonate-6-phosphate to L-xylulose-6-phosphate in L-ascorbate metabolism and hexulose phosphate synthase (HPS), found in methylotrophs that fix formaldehyde and ribulose-5-phosphate to form D-arabino-3-hexulose-6-phosphate. To probe the functional plasticity of the OMPDC suprafamily, HPS activity was enhanced in the KGPDC scaffold using rational design. Upon inspection of the sequence alignment of the two homologues and examination of the KGPDC structure, several residues in KGPDC (E112D, T169A, R192A, and R139V) were targeted for mutation to the corresponding residue in HPS. The triple mutant E112D/T169A/R139V had an enhancement in k_{cat}/K_M of 260-fold compared ot that of wild-type KGPDC. The structure of the triple mutant has been determined and strengthens our understanding of the mechanistic diversity between KGPDC and HPS.

31. Synthesis and evaluation of aryloxymethyl and aryloxyethyl phosphonates as inhibitors of phosphatases. Subashree S. Iyer, Jared M. Younker, Przemyslaw G. Czyryca, and Alvan C. Hengge. Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300 [fax (435) 797-3390, subas@cc.usu.edu]

Protein phosphatases in conjunction with protein kinases control a diverse array of cellular processes. Linear free energy relationships, kinetic isotope effects, and quantum mechanical calculations suggest a loose transition state for the reaction catalyzed by phosphatases. On the basis of this, a number of aryloxymethyl and aryloxyethyl phosphonates were synthesized. These were evaluated as inhibitors against a set of phosphatases, including the *Escherichia coli* and human placental alkaline phosphatases, the PTPase from *Yersinia*, and the Ser/Thr phosphatases Lambda and PP2C. These compounds exhibited competitive inhibition toward the phosphatases using *p*-nitrophenyl phosphate as the substrate. In general, inhibition was weakest toward the PTPases, with inhibition constants in the millimolar range. Inhibition was most effective against Ser/Thr phosphatases

PP2C and Lambda, with inhibition constants in the micromolar range.

32. Chemoenzymatic synthesis of chiral aza-arene ligands. **Deirdre M. Murphy**,[1] Derek R Boyd,[1] Narain D Sharma,[1] and A. John Blacker[2]. [1]Department of Chemistry, Queen's University Belfast, Belfast BT9 5AG, United Kingdom (D.Murphy@qub.ac.uk), and [2]PTD, Avecia

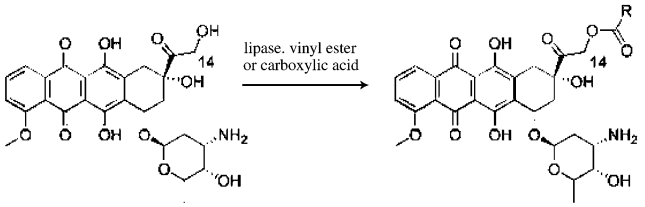
Biotransformations with high regio- and enantioselectivity have been found to provide a valuable synthetic route to new chiral amino alcohol and bipyridine ligands. Enantiopure *cis*-dihydrodiol metabolites, resulting from the synthesis and biotransformation of bi- and tricyclic aza-arene substrates, were produced. Bioproducts from dihydroxylation of the 5,6 and 7,8 bonds of 2-chloroquinoline, catalyzed by toluene dioxygenase, were isolated from the intact cells of *Pseudomonas putida* UV4. Biphenyl dioxygenase-catalyzed dihydroxylation of 2-chlorobenzo[*h*]quinoline, using whole cell cultures of *Sphingomonas yanoikuyae* B8/36, was found to yield a 9,10-diol bioproduct. The bioproducts were utilized in the synthesis of a series of chiral bipyridine-type ligands which under the Kharasch−Sosnovsky reaction conditions were found to catalyze allylic oxidation of cycloalkenes with good enantioselectivity. Amino alcohol derivatives of the *cis*-dihydrodiols were also synthesized and evaluated as catalysts for asymmetric hydrogenation.

33. Biocatalysis as a tool to generate 14-*O*-acyl doxorubicin derivatives with improved therapeutic activity. **Ian C. Cotterill**, Peter C. Michels, Harold Meckler, and Joseph O. Rich. Biosciences, Albany Molecular Research, Inc., 601 E. Kensington Rd., Mount Prospect, IL 60056 [fax (847) 506-4261, ian.cotterill@albmolecular.com]

Doxorubicin is a natural product of *Streptomyces peucetius* that exhibits the broadest antitumor activity of current chemotherapeutics. Treatment with doxorubicin has the disadvantage of dose-limiting cardiotoxicity and the susceptibility of tumors developing resistance to the drug. The sensitivity of doxorubicin to pH, heat, metal ions, and light combined with its polyfunctional structure exacerbates conventional efforts to effect controlled derivatization.

Albany Molecular Research has successfully applied its biocatalytic technology to achieve regioselective acylation of doxorubicin under mild conditions. A rapid screening program identified the most suitable lipase and solvent system for acylating doxorubicin at the C-14 position.

Derivatives with an improved therapeutic index and novel activity have been identified *via* screening against breast cancer cell lines. Multigram quantities of the desired derivatives can be easily accessed by this biocatalytic route.



34. Phosphospecific proteolysis: A chemoenzymatic approach for mapping sites of protein phosphorylation. **Zachary A. Knight** and Kevan M. Shokat. Department of Cellullar and Molecular Pharmacology, University of California, 600 16th St., Box 2280, Genentech Hall, Room N514, San Francisco, CA 94143-2280 [fax (415) 514-0822, knight@itsa.ucsf.edu]

Protein phosphorylation is a dominant mechanism of information transfer in cells, and a major goal of current proteomic efforts is to generate a system-level map describing all the sites of protein phosphorylation. Recent efforts have focused on developing technologies for enriching and quantitating phosphopeptides. In contrast, identification of the sites of phosphorylation typically relies on the use of tandem mass spectrometry to sequence individual peptides. Herein is described a novel approach for phosphopeptide mapping that makes it possible to interrogate a protein sequence directly with a protease that recognizes sites of phosphorylation. The key to this approach is the selective chemical transformation of phosphoserine residues into lysine isosteres (aminoethylcysteine). Aminoethylcysteine-modified peptides are then selectively cleaved with a lysine-specific protease to map sites of phosphorylation. A blocking step enables single-site cleavage when desired, and adaptation of this reaction to the solid phase facilitates phosphopeptide enrichment and modification in one step.

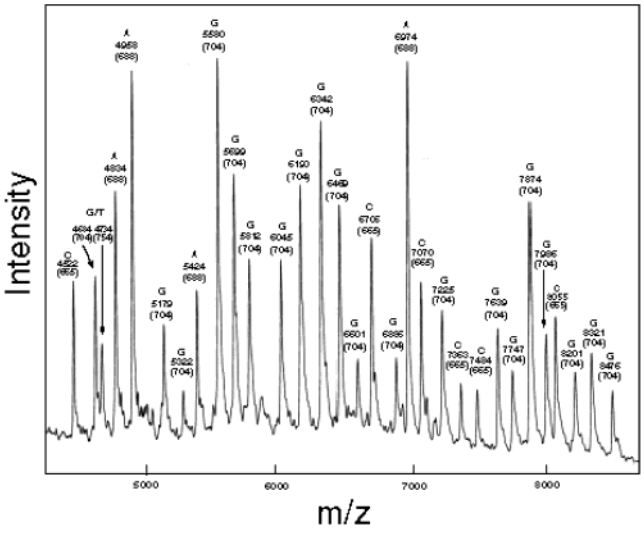
35. Isolation of protein-binding molecules from large combinatorial peptoid libraries. **Muralidhar Reddy Moola**, Prasanna G. Alluri, Kiran Bachhawat-sikder, Hernando J. Olivos, and Thomas Kodadek. Center For Biomedical Inventions, Departments of Internal Medicine and Molecular Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9185 [fax (214) 648-4156, reddy.moola@utsouthwestern.edu]

Most of the biological functions are mediated by proteins. Therefore, it is important to develop technologies capable of analyzing proteins directly. An attractive solution to this problem is the development of protein-detecting microarrays consisting of arrayed capture molecules that would allow the simultaneous analysis of many proteins in blood, cell extracts, etc. We have recently developed a general protocol for the synthesis, screening, and characterization of large combinatorial peptoid libraries. This strategy will allow the isolation of high-affinity and -specificity protein-binding molecules against a large number of proteins in a high-throughput fashion. Our efforts in this direction with its application in proteomics will be presented.

36. Multiplex genotyping using molecular affinity and mass spectrometry. **Sobin Kim**, **Michael E. Ulz**, John Edwards, and Jingyue Ju. Department of Chemical Engineering and Columbia Genome Center, Columbia University, 1150 St. Nicholas Ave. #403, New York, NY 10032 [fax (212) 851-5215, sk474@columbia.edu, meu2001@columbia.edu]

We report the simultaneous genotyping of 30 nucleotide variations in exons 5, 7, and 8 of the human p53 gene in one tube using molecular affinity and MALDI-TOF mass spectrometry. Thirty primers specific for each mutation site were designed to yield single-base extension (SBE) products with sufficient mass differences. This was achieved by tuning the masses of some primers using methyl dCTP and dUTP

to replace dCTP and dTTP, respectively. These 30 primers were mixed with multiplexed PCR products and biotinylated dideoxynucleotides for the SBE reaction. The 3′-biotinylated extension DNA products were then captured by streptavidin-coated magnetic beads and analyzed with mass spectrometry. Using this method, we simultaneously genotyped 30 nucleotide variations in the p53 gene from genomic DNA of 35 patients with either Wilms' tumor or head and neck tumors. This is the highest level of multiplexing ever reported thus far using mass spectrometry for SNP analysis.



37. Study of β-amyloid aggregate at the air−water interface. Changqing Li, Jhony Orbulescu, and Roger M. Leblanc. Department of Chemistry, University of Miami, 1301 Memorial Dr., Coral Gables, FL 33124 [fax (305) 284-1880, cli@umsis.miami.edu]

β-Amyloid peptide plays a central role in the neuropathogenesis of Alzheimer's disease (AD). This study investigates the driving forces that cause fragmental β-amyloid peptides to aggregate and form amyloid fibril by employing a two-dimensional approach. Both peptidolipids (C_{18}-GSNKGAIIGLM) that include the peptide known to form amyloid aggregate in vitro and the aggregation inhibitors (C_{18}-GVVIA and C_{18}-RVVIA) were designed by using the C-terminal sequence of the β-amyloid peptide as a template. The aggregation and inhibition processes were followed by several techniques: surface pressure−area isotherms to simulate the behavior of the biomembrane and epifluorescence microscopy to monitor the aggregate formation/inhibition process at the air−water interface. IR reflection absorption spectroscopy and ATR-FTIR were used to determine secondary structual motifs of the aggregate.

38. Synthetic protein folding and self-assembly: Computational library design and yeast expression screening. Sheldon J. Park,[1] Magdalena Jonikas,[2] **Eric T. Boder**,[1] and Jeffery G. Saven.[1] [1]Department of Chemistry, University of Pennsylvania, 231 S. 34th St., Philadelphia, PA 19104 (spark@saven5.chem.upenn.edu, boder@seas.upenn.edu), and [2]Department of Bioengineering, University of Pennsylvania

Folded proteins have the potential to serve as well-structured building blocks of ordered biomaterials having a variety of functionalities. We aim to surmount the difficulties of designing folded, self-associating proteins by generating partially random but focused libraries of sequences and assaying for desired properties. Design of these libraries is guided by theory, and the stringent post-translational quality control machinery of yeast is used to screen for folded domains. The initial target is a three-helix bundle (alpha3D), which is stable but has a fluctuating interior structure. A single-helix library has been designed and screened by yeast surface display to isolate individual clones demonstrating varying levels of protein expression and/or stability. Results of this proof-of-concept study and plans to extend this approach to design self-associating three-helix bundles will be discussed.

39. Using multivalent ligands to study B cell activation. Jessica J. Hollenbeck,[1] Erik B. Puffer,[1] Jason K. Pontrello,[2] and Laura L. Kiessling.[3] [1]Department of Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (jhollenbeck@biochem.wisc.edu), [2]Department of Chemistry, University of Wisconsin−Madison, and [3]Departments of Chemistry and Biochemistry, University of Wisconsin−Madison

The B cell antigen receptor (BCR) is a multiprotein complex on the surface of B cells that consists of a membrane-bound immunoglobulin (mIg) and a noncovalently associated heterodimer, Igα/Igβ. Each mature B cell expresses multiple copies of a BCR specific for a unique antigen. Cross-linking of the BCR by multivalent antigens initiates a signaling cascade that leads ultimately to cellular proliferation and differentiation into antibody-secreting plasma cells and memory B cells. The B cell coreceptor complex of CD19 and CD21 functions synergistically with the BCR to reduce the threshold for B cell activation. Using multivalent scaffolds that display multiple antigenic epitopes for the BCR and peptides that bind to CD21, we are elucidating the structural requirements for CD19/CD21 signal amplification.

40. Hydroxyl radical-induced H/D exchange mapping the structure of hirudin. Benlian Wang and Vernon E. Anderson. Department of Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4935 (bxw37@po.cwru.edu)

Hydroxyl radical-induced H/D exchange reaction is used to map the solvent accessible surface and structure of hirudin, a small protein. The mixture of structured and unstructured hirudin was exposed to γ-irradiation in N_2O-saturated D_2O containing dithiothreitol (DTT, D-atom donor). Trypsin-digested hirudin modified with acrylamide on cysteine residues served as unstructured hirudin. Further tryptic digestion on the H/D-exchanged mixture was performed, and the cysteine residues were alkylated with iodoacetamide. The digested peptides from structured and unstructured hirudin therefore can be identified on the same mass spectra measured by matrix-assisted laser desorption ionization time of flight mass spectrometry. The exposure of structured and unstructured hirudin to γ-irradiation in the presence of a 2H donor results in the incorporation of D into C−H bonds and an increase in the isotope ratio. C-Terminal peptides from structured and unstructured hirudin always exhibit a high

isotope ratio, while the N-terminal peptide has a high isotope ratio for only unstructured hirudin but not for structured hirudin. Analysis of the isotope ratio indicated that hirudin has a structure with an extended C-terminus but a compacted N-terminus.

41. Investigating nucleotide interaction in the ATP-dependent protease Lon. Jennifer Thomas-Wohlever,[1] Ramona Behshad,[1] Morris Burke,[2] and Irene Lee.[1] [1]Department of Chemistry, Case Western Reserve University, 2074 Adelbert Rd., Cleveland, OH 44106 (j_thomas_wohlever@hotmail.com), and [2]Department of Biology, Case Western Reserve University

Lon is an ATP-activated serine protease. Although ATP is the optimal activator, certain nonhydrolyzable ATP analogues as well as other purine or pyrimidine nucleoside triphosphates also activate the enzyme's peptidase activity. In this study, we compared the steady-state kinetic parameters of peptide cleavage and nucleotide hydrolysis by Lon in the presence of ATP, GTP, UTP, and CTP. Our data indicated that while the K_m of the peptide substrate was independent of the nucleotide structure, the k_{cat} and $K_{act,nucleotide}$ values were affected. The k_{cat} and $K_{m,nucleotide}$ values for nucleotide hydrolysis were affected by both the structure of the base and the presence of peptide substrate. We performed limited tryptic digestion of Lon to probe the physical changes in enzyme upon binding to different nucleotides. Lon's susceptibility to tryptic digestion revealed that the nucleotide-binding site of Lon is highly specific for adenine. In assessing the peptidase data, we propose that Lon undergoes a conformational change upon binding to the adenine nucleotide base. This complex mechanism for activating peptide hydrolysis is consistent with the regulatory function of Lon.

Tuesday Morning: Lilly Award Symposium

Andrea Matouschek, Organizer

42. DNA packaging by the bacteriophage ϕ29 portal motor. Carlos Bustamante. Howard Hughes Medical Institute, University of California, Berkeley, CA 94720-3206 [fax (510) 642-5943]

As part of its infection cycle, bacteriophage ϕ29 packages its 6.6 mm long double-stranded DNA into a 42 nm wide and 54 nm high capsid via a portal complex that hydrolyzes ATP. We have used optical tweezers to demonstrate that the portal complex is a force-generating motor. The motor can work against loads of up to ~57 pN on average, making it one of the strongest molecular motors ever reported. Movements of more than 5 mm are observed, indicating high processivity. The rate-limiting step of the motor's cycle is force-dependent even at low loads. Interestingly, the packaging rate decreases as the prohead is filled, indicating that an internal pressure builds up due to DNA compression. We estimate that at the end of the packaging the capsid pressure is ~15 MPa, corresponding to an internal force of ~50 pN acting on the motor.

43. Chaperonin-mediated protein folding. Arthur Horwich,[1] Eric Bertelsen,[1] Wayne Fenton,[1] Krystyna Furtak,[1] Jocelyne Fiaux,[2] Charu Chaudhry,[3] Paul Adams,[4] Neil Ranson,[5] Helen Saibil,[5] Tapan Chaudhuri,[1] and Kurt Wüthrich.[2] [1]HHMI and Department of Genetics, Yale School of Medicine, New Haven, CT 06510, [2]Institute for Molecular Biology and Biophysics, ETH, [3]Department of Molecular Biophysics and Biochemistry, Yale University, [4]Lawrence Berkeley Laboratory, and [5]Department of Crystallography, Birkbeck College

Chaperonins are large ring assemblies that provide essential ATP-dependent assistance to protein folding in a variety of cellular compartments. The bacterial chaperonin, GroEL, assists folding by alternately binding non-native proteins in an open ring through multivalent hydrophobic contacts and releasing them. Release occurs either into an encapsulated hydrophilic cavity formed upon binding ATP and the single-ring cochaperonin, GroES, to the same ring as polypeptide (*cis* folding), or into the bulk solution after binding ATP/GroES to the opposite ring (*trans*), the latter a process required for assistance in the folding of proteins too large to be encapsulated. Recent experiments have focused on examining GroEL-bound proteins by NMR, on crystallographic, cryoEM, and fluorescence dynamic studies of ATP and ADP *cis* complexes, and on better resolving the *trans* mechanism using *trans*-only complexes studied both *in vitro* and *in vivo*.

44. Signal-dependent proteolysis by proteasomes. Cecile M. Pickart. Biochemistry and Molecular Biology, Johns Hopkins University, 615 North Wolfe St., Baltimore, MD 21205 [fax (410) 955-2926, cpickart@jhmi.edu]

The 26S proteasome is unique because of the breadth of its cellular functions and because substrate unfolding and proteolysis are coupled to the recognition of a covalent signal, a specific polyubiquitin chain, that is linked to the substrate by upstream conjugating enzymes. Our long-term goal is to define the molecular interactions and mechanisms through which recognition of the polyubiquitin signal is transduced into proteolysis. At the level of the signal, we have defined tetraubiquitin as the minimum signal for efficient targeting. At the level of the enzyme, we have shown that one subunit of the proteasome's regulatory (19S) complex contacts the bound chain in an ATP-modulated manner, and is likely to play an important role in chain recognition. We have also studied the potential trans-targeting function of polyubiquitin-binding proteins of the UbL-Uba family, such as Rad23. Recent progress in these and other areas will be discussed.

45. Protein unfolding in the cell. Andreas Matouschek, Sumit Prakash, and Lin Tian. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Hogan 2-100, 2205 Tech Dr., Evanston, IL 60208-3500 [fax (847) 467-6489]

Protein unfolding is a key step in several cellular processes, including protein translocation across some membranes and protein degradation by ATP-dependent proteases. The proteasome, the major eukaryotic ATP-dependent protease, catalyzes unfolding by processively unraveling its substrates from their degradation signal. To fully engage the unfolding activity, substrate proteins have to contain an extended unstructured region, which will serve as the start point of degradation. Degradation can then occur in either direction along the polypeptide chain (N to C and C to N). In multidomain proteins, a degradation-resistant domain protects

the following part of the substrate from proteolysis. The unfolding activity of the proteasome can be attenuated by the substrate's amino acid sequence immediately preceding a folded domain. We show that, together, these results can explain the limited degradation by the proteasome that occurs in the processing of the precursor of transcription factor NF-κB.

Tuesday Afternoon: Protein Design

E. Neil Marsh, Organizer

46. Design and selection of antiparallel coiled coils. Martha G. Oakley. Department of Chemistry, Indiana University, 800 East Kirkwood Ave., Bloomington, IN 47405 [fax (812) 855-8300, oakley@indiana.edu]

Coiled coils consist of two or more α-helices that associate with a parallel or an antiparallel relative alignment of helices. Although antiparallel coiled coils were once thought to be rare in nature, recent structural studies have highlighted the importance of this class of coiled coils. Studies using both rational design and selection methods for understanding the features that lead to helix orientation preference in coiled coils will be described.

47. Designing self-assembling materials and regular protein materials. Todd O. Yeates, Jennifer Padilla, and Stephanie Yoshido. Department of Chemistry and Biochemistry, University of California at Los Angeles, UCLA-DOE Center for Genomics and Proteomics, Los Angeles, CA 90095-1570 [fax (310) 206-3914, yeates@chem.ucla.edu]

Natural proteins offer a rich source of starting materials for designing novel structures and assemblies on the mid-nanometer scale. Many proteins have evolved naturally to form noncovalent but tight and specific homo-oligomeric complexes, with dimers, trimers, and tetramers being especially common. Two or more proteins of this type can be joined together by genetic engineering to create novel fusion proteins that self-assemble to form structures and materials that are much more elaborate than the simple assemblies formed by their separate parts. Progress, obstacles, and ideas for creating materials by this approach will be discussed.

48. Lanthanide binding tags: New tools for chemical biology. Barbara Imperiali. Department of Chemistry and Department of Biology, Massachusetts Institute of Technology, 77 Mass Ave., Cambridge, MA 02139 [fax (617) 452-2419, imper@mit.edu]

This presentation will discuss a new class of protein coexpression tag that is based on minimal polypeptide motifs that bind lanthanide ions. Because the tags comprise only the encoded amino acids, they can be introduced using standard molecular biology techniques, thereby creating fusion proteins that contain a built-in, site-specific, and multitasking probe of protein structure and function. The lanthanide binding tags (LBTs) were developed using a combination of design, parallel synthesis, and high-throughput screening. Peptide sequences with Tb^{3+} binding and luminescence properties that are significantly improved relative to those of native calcium-binding loops from which they were derived have been identified. Postexpression addition of a particular Ln(III) ion provides a variety of biophysical properties that can be broadly exploited in the field of chemical biology. The multiple potential applications of LBTs include acceleration of protein structure determination by NMR and X-ray methods, as well as development of functional assays that utilize the lanthanide luminescence.

49. De novo design of peptides, proteins, and peptide mimetics. William DeGrado. Department of Biochemisty and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6059 (wdegrado@mail.med.upenn.edu)

Our group has developed computational and synthetic methods for understanding the structural bases of protein function. To test some of the rules and concepts that are important for protein folding and function, we have designed several simple proteins that fold into predetermined three-dimensional structures. Further, the sequences of the designed helical bundles have been elaborated to introduce binding sites for small ligands and metal ions, including a diiron cluster similar to that observed in enzymes such as the R2 subunit of ribonucleotide reductase R2 from *Escherichia coli* and methane monooxygenase. We have also investigated the features required for folding and assembly of membrane proteins. In a similar vein, we have designed nonpeptidic oligomers that mimic the structures and biological properties of helical peptides that interact with phospholipid bilayers (e.g., antimicrobial peptides such as magainin), as well as helices that engage in protein−protein interactions.

Wednesday Morning: Enzyme Action: Structure, Dynamics, and Function (Pfizer Symposium)

Dorothee Kern, Organizer

50. Analysis of all covalent intermediates in thiamin diphosphate enzymes during catalysis by NMR. Gerhard Huebner. Biochemistry and Biotechnology, Halle University−Wittenberg, Halle, Germany (fax 345-552-7011, huebner@biochemtech.uni-halle.de)

Thiamin diphosphate (ThDP), the biologically active form of vitamin B_1, is an essential cofactor in biocatalysis, being involved in numerous metabolic pathways. Studies on the mechanism of activation of enzyme-bound ThDP have revealed that the 4′-amino group of ThDP and a highly conserved interaction between a glutamate and N1′ of the cofactor are integral components of a proton relay catalyzing fast deprotonation of C2 in ThDP [Kern, D., Kern, G., Neef, H., Tittmann, K., Killenberg-Jabs, M., Wikner, C., Schneider, G., and Hübner, G. (1997) How thiamine diphosphate is activated in enzymes, *Science 275*, 67−70]. In addition, proton−nitrogen correlated NMR measurements performed on the ThDP-dependent tetrameric 240 kDa pyruvate decarboxylase reconstituted with ^{15}N-labeled ThDP showed an increase in the basicity of the 4′-amino group. The subsequent steps to C2−H dissociation in catalysis, which proceeds via covalent adducts at C2 of ThDP, have been observed by the quantitative analysis of all intermediates during catalysis using ^1H NMR spectroscopy. As demonstrated for pyruvate decarboxylase, this method allows the determination of the microscopic rate constants of individual catalytic steps and, in combination with site-directed mutagenesis, the assignment of individual side chains to single steps in catalysis. Comparative experiments on other ThDP enzymes, such as

transketolase, pyruvate oxidase, and the E1 component of the pyruvate dehydrogenase complex, shed light on the molecular details within the active site that are responsible for catalytic diversity.

51. Weak orientation allows a very precise NMR view of biomolecular structure. Tobias S. Ulmer[1] and **Ad Bax**.[2] [1]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, and [2]Laboratory of Chemical Physics, National Institutes of Health, Building 5, Bethesda, MD 20892 [fax (301) 402-0907, bax@nih.gov]

Biomolecular structure determination by NMR to date has relied almost exclusively on local parameters, such as NOEs and *J* couplings. In contrast, dipolar couplings measured in weakly aligned macromolecules provide information about the orientation of individual internuclear vectors relative to the molecular alignment tensor. Next to restraining local geometry, they therefore also have an intrinsic global character and can constrain the relative orientation of parts of a structure that are not connected by NOEs. This is particularly useful in the study of nonglobular and multimeric structures. Dipolar couplings can be measured at very high relative accuracy, and thereby provide precise monitors of local structure. In general, the higher the resolution of the reference structure, the better the agreement between measured dipolar couplings and protein coordinates. For example, excellent agreement is observed between $^{13}C-^{1}H$ dipolar couplings measured for the third Igg-binding domain of protein G and its 1 Å X-ray structure. This agreement can be further improved if the X-ray structure is refined with one-bond dipolar couplings, measured for backbone $^{13}C'-^{13}Ca$ and $^{13}C'-N$ dipolar couplings. This refined structure also provides precise information about the position of the amide protons, and insight into the question on how far amide protons can fall outside the C′−N−Ca plane.

52. Mechanisms in prokaryotic chloride ion channels. Christopher Miller. Department of Biochemistry, Brandeis University, Waltham, MA 02454 [fax (781) 736-2365, cmiller@brandeis.edu]

CLC-type Cl^- channels constitute a molecular family of membrane proteins found ubiquitously throughout the biological world. They act in many different physiological contexts that require gated membrane permeability to Cl^-, such as electrical excitation in skeletal muscle and HCl pumping in endosomes. A prokaryotic CLC homologue of known structure, clcA of *Escherichia coli*, is now generally used as a model for eukaryotic CLC channels, but the biological function of clcA is unlike that of the eukaryotic channels studied electrophysiologically. We have therefore developed radioactive flux and electrophysiological approaches to study the Cl transport function of clcA itself. The channel is directly activated by low pH (<4), and appears to lack the voltage and Cl sensitivity characteristic of its eukaryotic homologues.

53. Enzymes in action in the NMR Tube: Protein dynamics during catalysis and signaling. Dorothee Kern. Department of Biochemistry, Brandeis University, 415 South St., Waltham, MA 02453 (dkern@brandeis.edu)

A fundamental challenge for understanding protein function is the characterization of proteins as dynamic objects. In this talk, I will present new experimental approaches for going beyond static structures with the ultimate goal being to "see" macromolecules reacting at atomic resolution. Nuclear magnetic resonance spectroscopy is used not only to determine structures of proteins but also to look to the "dynamic hot spots" in these structures. Modern spin relaxation experiments on many atoms within the protein allow a quantitative analysis of the movements. We have developed methods that allow measurements of dynamics in enzymes in action. With this technique, dynamics can be linked to function as demonstrated in two examples: activation of a signaling protein [Volkman, B., Lipson, D., Wemmer, D., and Kern, D. (2001) Two-state allosteric behavior in a single domain signaling protein, *Science 291*, 2429−2433] and dynamic behavior of an enzyme during turnover [Eisenmesser, E. Z., Bosco, D. A., Akke, M., and Kern, D. (2002) Enzyme Dynamics During Catalysis, *Science 295*, 1520−1523]. The experimental results clearly reveal collective motions that are correlated with the chemical steps of substrate turnover. Finally, NMR relaxation experiments are applied to exploit the mechanism of HIV virulence promotion by the human enzyme CypA [Bosco, D. A., Eisenmesser, E. Z., Pochapsky, S., Sundquist, W. I., and Kern, D. (2002) Catalysis of cis/trans isomerization in native HIV-1 capsid by human cyclophilin A, *Proc. Natl. Acad. Sci. U.S.A. 99* (8), 5247−5252]. The presented experiments demonstrate how physical techniques applied to biological systems advance our fundamental understanding of complex biological molecules.

Wednesday Afternoon: Repligen Award: New Insights into Enzyme Action

John Gerlt, Organizer

54. Ribonucleotide reductase: Unnatural amino acids for probing proton-coupled electron transfer. JoAnne Stubbe, Cyril S. Yee, Michelle C. Y. Chang, Jie Ge, and Daniel G. Nocera. Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139 (stubbe@mit.edu)

Escherichia coli ribonucleotide reductase (RNR) catalyzes the conversion of NDPs to dNDPs, an essential step in DNA replication and repair. The enzyme is composed of two subunits: R1 and R2. R1 contains the active site for nucleotide reduction and the allosteric effector sites that regulate specificity and turnover rate. R2 contains the diferric tyrosyl (Y•) radical cofactor that initiates nucleotide reduction by a proposed long-range proton-coupled electron transfer pathway (35 Å). Initiation is thought to involve specific amino acid radical intermediates (Y122, W48, and Y356 within R2 to Y731, Y730, and C439 within R1). To study radical initiation, R2 (375 amino acids) has been synthesized semisynthetically and Y356 has been replaced with 3-nitroY and 2,3-difluoroY. Results from experiments with mutant R2 subunits provide the first demonstration of a kinetically competent conformational change triggered by nucleotides in the R1−R2 complex. They also suggest that the mechanism for radical inititation involving hydrogen atom transfer beween W48 and Y731 is not viable.

55. Mechanistic and genomic imprints of the evolution of antibiotic resistance in pathogenic microorganisms.

Richard N. Armstrong. Department of Biochemistry, Vanderbilt University, 842 Robinson Research Building, Nashville, TN 37232-0146 [fax (615) 343-2921, r.armstrong@vanderbilt.edu]

An analysis of the genomes of numerous microorganisms reveals the existence of at least three mechanisms of resistance catalyzed by metalloenzymes in the vicinal oxygen chelate (or VOC) superfamily. The selective pressure of fosfomycin resistance has led to a divergence in the catalytic mechanism of fosfomycin resistance proteins to match the biochemical imperative of the microorganisms. The data indicate that several pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus anthracis*, *Brucella melitensis*, *Listeria monocytogenes*, and *Clostridium botulinum*, may harbor an intrinsic resistance to fosfomycin. Structural and mechanistic analysis suggests that the resistance has evolved in a relatively narrow region of mechanism space perhaps involving a catalytically promiscuous progenitor. (Supported by NIH Grant AI42756.)

56. Enzyme chemistry and proteomics: together again for the first time. J. W. Kozarich. ActivX Biosciences, Inc., 11025 North Torrey Pines Rd., La Jolla, CA 92037 [fax (858) 558-4878, johnk@activx.com]

More than half a century of bioorganic chemistry has established the framework for our understanding of the mechanism and function of proteins from enzymes to receptors. Recently, genomics has spawned proteomics which is largely an intellectual and analytical restructuring of protein space into cell-specific, networked ensembles called proteomes. The proteomic scientist is faced with the daunting challenge of intelligently interrogating proteomes for high-impact biological and pharmaceutical discovery. The protein chemistry lessons of the past are poised to be reharvested to solve this new problem. This presentation will describe the development and application of chemical tools designed to capture and analyze large families of functionally related proteins in proteomes. Specific examples will focus on serine hydrolases and kinases with applications to drug discovery and development.

57. Discovering, evolving, and designing new enzymes. John A. Gerlt. Department of Biochemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave., Urbana, IL 61801 [fax (217) 244-6538, j-gerlt@uiuc.edu]

For many years, enzymologists studied a single enzyme to elucidate structure−function relationships. With the availability of complete genomes for many organisms, an alternate strategy can be used that involves parallel structure−function studies of enzymes derived from a single progenitor, thereby providing an understanding of how sequence and structure together deliver enzymatic function. The $(\beta/\alpha)_8$-barrel fold is perhaps the most common fold found in enzymes. This fold provides the active site scaffold for both the enolase superfamily, homologous enzymes that share Mg^{2+}-assisted enolization of a carboxylate substrate, and the OMP decarboxylase suprafamily, homologous enzymes that do not share any mechanistic features. This lecture will summarize recent studies of members of both homologous groups and highlight the importance of functional promiscuity in both natural and unnatural evolution of new functions.

Wednesday Evening: General Poster Session

Carol Fierke, Organizer

58. Novel binding interactions of the DNA fragments d(GpG) and d(ApA) bound to the antitumor active compound bis(*μ*-*N*,*N*′-di-*p*-tolylformamidinato)dirhodium-(II). Helen Chifotides and Kim R. Dunbar. Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77842-3012 [fax (979) 845-7177, chifotides@mail.chem.tamu.edu]

Recently, there has been a resurgence of interest in the dirhodium tetracarboxylate $Rh_2(O_2CR)_4L_2$ (R = Me, Et, or Pr, L = solvent) and the mixed formamidinate/carboxylate $Rh_2(DTolF)_2(O_2CCF_3)_2(H_2O)_2$ (DTolF = *N*,*N*′-di-*p*-tolylformamidinate) compounds due to their carcinostatic activity against various types of cell lines. Recent studies in our laboratories have unequivocally established that the interactions of guanine, adenine derivatives, and dipurine-containing DNA fragments (GG and AA) with the dirhodium core involve *unprecedented* equatorial bridging interactions. We are currently studying the dirhodium adducts $Rh_2(DTolF)$-{d(GpG)} and $Rh_2(DTolF)${d(ApA)} (DTolF = *N*,*N*′-*p*-ditolylformamidinate) by one- and two-dimensional NMR spectroscopic techniques. The data suggest that the d(GpG) and d(ApA) dinucleotides bind to the dirhodium unit via N7−O6 and N6−N7 bridges that span the Rh−Rh bond. The guanine and adenine rings are found in the enolate and imino forms, respectively, facts which may have interesting biological implications due to disruption of the usual Watson−Crick hydrogen bonding in DNA.

59. Packaging and targeting of mixed backbone antisense oligonucleotides to bcl-2 mRNA in non-Hodgkin's lymphoma cells. Jean-Paul Desaulniers,[1] Nayera El-Shakankiry Hamdy,[2] Anton Scott Goustin,[3] Azmath Mohammed,[1] Ayad Al-Katib,[2] and Christine S. Chow.[1] [1]Deparment of Chemistry, Wayne State University, Detroit, MI 48202 [fax (313) 577-8822, jdesauln@chem.wayne.edu], [2]Division of Hematology and Oncology, Wayne State University, and [3]Center for Molecular Medicine and Genetics, Wayne State University

Non-Hodgkin's lymphoma (NHL) is a cancer of the lymphatic system and is the fifth most common cancer in the United States. NHL is often characterized by overexpression of the gene product, Bcl-2, due to the translocation of the gene to the *IgH* gene locus. The focus of this project is to identify, package, and target an antisense (AS) oligonucleotide to the mRNA region encoding the Bcl-2 protein, *in vivo*. We have identified a short mixed backbone (MBO) oligonucleotide that causes growth inhibition in follicular small cleaved cell lymphoma (FSCCL) cells at low concentrations, targeting the ribosome-binding site in the *bcl-2* mRNA. An immunoliposome (IL) was generated by the noncovalent attachment of a genetically engineered antibody comprised of a GPI anchored to single-chain variable fragments of anti-CD20 to MBO-containing liposomes. Our efforts are currently focused on determining the effect that AS MBOs, MBO-containing liposomes, and ILs have on severe combined immunodeficient (SCID) mice bearing an NHL tumor.

60. Protein−protein and protein−DNA interactions of the b/HLH/z transcription factors Myc/Max/Mad. Jianzhong

Hu, Anamika Banerjee, and Dixie J. Goss. Department of Chemistry, Hunter College and Graduate Center, City University of New York, 695, Park Avenue, New York, NY 10021 [fax (212) 772-5332]

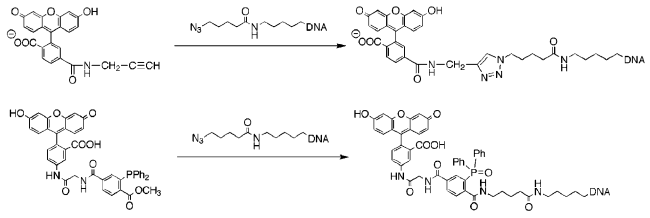
Max and Myc form a heterodimer that has strong oncogenic potential, while Mad and Max form a heterodimer that acts as a tumor suppressor. These proteins bind to a DNA E-box (CACGTG). We have used fluorescence anistropy to measure protein−protein and protein−DNA affinity. The enthalpy and entropy of these interactions were determined from the temperature dependence. The overall $\Delta G°$ for formation of the protein dimer−DNA complex was almost 4 kJ less favorable for Mad−Max−DNA formation at 20 °C compared to the formations of Max_2−DNA or Myc−Max−DNA complexes which were essentially the same. However, at 37 °C, Mad−Max−DNA and Myc−Max−DNA complexes have essentially the same $\Delta G°$, while the Max_2−DNA complex was ∼7 kJ less stable. The enthalpy values for Max_2−DNA formation were significantly higher than for either of the heterodimer−DNA interactions. The largest contribution was assigned to the protein−DNA interaction rather than the protein−protein interaction. These results suggest that the formation of heterodimers between Max and other proteins is a more important function of Max than the selection of DNA sequences. Kinetic analysis of these pathways is currently underway.

61. Regulation of gene expression with the small molecule biotin: Covalent biotinylation of an artificial transcription factor. Sonalee Athavankar[1] and Blake R. Peterson.[2] [1]Chemistry, The Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802 [fax (814) 863-8403, sonalee@chem.psu.edu], and [2]Department of Chemistry, The Pennsylvania State University

Small molecules that dimerize proteins in living cells can reconstitute bipartite transcription factors to regulate the expression of specific genes. These types of compounds provide powerful probes of biological systems and may have clinical applications in gene therapy. We report a yeast genetic system that places gene expression under the control of the nontoxic and cell permeable small molecule biotin as a mediator of protein dimerization. We fused the 15-amino acid biotin acceptor peptide termed Avitag to the B42 transcriptional activation domain and expressed this protein in yeast. This protein was coexpressed with both the BirA biotin protein ligase from *Escherichia coli* and streptavidin fused to the LexA DNA binding domain. Addition of biotin to recombinant yeast was found to reconstitute a functional transcription factor and activate expression of a lacZ reporter gene. In appropriately engineered cells, biotin can be used as a small molecule-based transcriptional regulator.

62. Site-specific fluorescent labeling of DNA using the 1,3-dipolar cycloaddition reaction and Staudinger ligation. Tae Seok Seo,[1] Charles C.-Y. Wang,[2] Zengmin Li,[1] Hameer D. Ruparel,[1] and Jingyue Ju.[1] [1]Department of Chemical Engineering and Columbia Genome Center, Columbia University, 1150 Nicholas Ave., Room 403, New York, NY 10032 [fax (212) 851-5215, ts503@columbia.edu], and [2]Department of Chemistry, University of Hong Kong

We report here the site-specific fluorescent labeling of DNA using the 1,3-dipolar cycloaddition reaction and Staudinger ligation. Both chemoselective reactions produced fluorescent single-stranded DNAs in near-quantitative yield under aqueous conditions. The resulting oligonucleotides were characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. In the 1,3-dipolar cycloaddition, the electron-deficient alkyne was conjugated with an azido-DNA by a simple synthetic protocol without catalysts at room temperature using water as a solvent. In the Staudinger ligation, the azido-DNA was selectively reacted with 5-{[*N*-(3′-diphenylphosphinyl-4′-methoxycarbonyl)phenylcarbonyl]aminoacetamido}fluorescein (FAM) under aqueous conditions to produce a Fam-labeled oligonucleotide. The fluorescent oligonucleotides were successfully used as primers in the Sanger dideoxy sequencing reaction to produce fluorescent DNA extension fragments, which were detected with single-base resolution in a capillary electrophoresis DNA sequencer by laser-induced fluorescence detection.



63. Structural consequences of the incorporation of conformationally restricted nucleosides into DNA. Melissa Maderia,[1] Shilpa Shenoy,[2] Barry O'Keefe,[2] Victor E. Marquez,[1] and Joseph J. Barchi, Jr.[1] [1]Laboratory of Medicinal Chemistry, National Institutes of Health, National Cancer Institute, CCR, NCI-Frederick, Frederick, MD 21702 [fax (301) 846-6033, mmaderia@helix.nih.gov], and [2]Laboratory of Drug Discovery Research, National Institutes of Health, National Cancer Institute, CCR

DNA−protein binding often results in a change in conformation of one or both of the interacting macromolecules. For example, protein binding to oligonucleotides may result in bending or kinking of the DNA. These structural adjustments are often associated with rearrangement of the furanose sugar pucker within the base pairs that comprise the bend. Standard B-form double-stranded DNA contains nucleotides puckered in the 2′-endo ("South") conformation. We have incorporated nucleotide building blocks that are conformationally "locked" in a 2′-exo ("North") conformation (a disposition associated with nucleotides within a bend) into B-form DNA to study any resulting structural changes. In the studies presented here, North thymidine nucleoside analogues, with a [3.1.0] bicyclic template in place of the furanose ring, have been incorporated into the Dickerson−Drew dodecamer (5′-CGCGAATTCGCG-3′). Studies to date that included calorimetry, circular dichroism, and NMR measurements have indicated that DNA species with a single modification retain B-form characteristics with localized perturbations in the structure, while modification of both thymidine residues results in a structure quite different from that of the native DNA.

64. Photogeneration of nitro derivatives of BeP and BaP for evaluating their mutagenic activity. Silvina E. Fior-

essi[1] and Rafael Arce.[2] [1]Department of Science and Technology, Universidad del Turabo, P.O. Box 3030, Gurabo, PR 00778 (sfioressi@yahoo.com), and [2]Department of Chemistry, University of Puerto Rico, Río Piedras Campus

Polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs are ubiquitous atmospheric pollutants associated with airborne particulate matter. Many of them present mutagenic activity, and their presence in the atmosphere has been related with an increase in cancer incidence, particularly lung cancer. During their residence in the atmosphere, PAHs can react with nitrogen oxides to form mono- and dinitro derivatives. These reactions are of particular interest because both types of contaminants are emitted simultaneously from combustion sources and the nitro-PAHs are often much more potent carcinogens than the parent PAHs. We are studying the photochemistry and mutagenic properties of nitro polycyclic aromatic hydrocarbons adsorbed on model surfaces of the atmospheric particulate matter and in solution with the purpose of providing information about its ultimate environmental and biomedical fate. Benzo[*e*]pyrene (BeP) and benzo[*a*]pyrene (BaP) were selected as representative PAHs. The photoreactivity of BeP and BaP toward nitrogen oxides is investigated. The nitro derivatives produced by irradiation of BeP and BaP under simulated atmospheric conditions will be isolated and characterized. The mutagenic properties of the isolated photoproducts will be evaluated by the Ames *Salmonella* assay. This will help in assessing their possible harmful effects on human health and in establishing if nitration increases the toxicity of these pollutants.

65. Synthesis of the thymidine−thymidine (6−4) photolesion and its incorporation into oligonucleotides for DNA repair mechanism studies. Julianne M. Caton-Williams[1] and Zhen Huang.[2] [1]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, 1248 Ryder St., Brooklyn, NY 11234 [fax (718) 951-4607, jcwill_tt@yahoo.com], and [2]Department of Chemistry, Brooklyn College, and Programs of Biochemistry and Chemistry, The City University of New York Graduate School and University Center

Ultraviolet (UV) light is known to induce mutations in base moieties of nucleic acids. We have strategically planned routes to synthesize a DNA containing the T(6−4)T photolesion. Two methods have been reported for the preparation of photolesions at adjacent pyrimidine sites. The methods are the direct UV irradiation of DNAs and the direct solid-phase oligonucleotide synthesis using a phosphoramidite building block of the photolesion. These two methods cannot lead to satisfactory preparation of large-scale photolesion DNAs for further X-ray crystal structure studies. We are in the process of developing a biochemical approach. The photolesion that was obtained was first synthesized, then purified, and characterized. The yield of the photolesion was 80%. This purified lesion was incorporated into oligonucleotides via enzymatic ligation procedures. Ultimately, the DNA lesion will be crystallized with DNA repair enzymes in an effort to further understand the DNA repair mechanism. Results of chemical synthesis and enzymatic preparation will be presented.

66. Synthesis past a cis−syn thymine dimer by yeast DNA Pol eta is much less efficient than past the undimerized thymines. Hanshin Hwang and John-Stephen A. 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]

DNA polymerase eta is a member of the Y family of DNA polymerases that have been implicated in the bypass of DNA damage. Pol eta has been reported to efficiently bypass the cis−syn thymine dimer, the major photoproduct of DNA, in a relatively error-free manner under steady state conditions by an induced-fit mechanism of nucleotide incorporation. Herein, we report the single-turnover, pre-steady state, and steady state kinetics of nucleotide insertion opposite the cis−syn thymine dimer and the corresponding undimerized site by the catalytic core of yeast pol eta. In contrast to what was expected from previous reports, we find that the pre-steady state and steady state insertion efficiencies opposite the 3′-T of the dimer are 30- and 20-fold less efficient, respectively, than opposite the corresponding undimerized T. We attribute these altered kinetics to the distorted nature of the dimer and an inability of the dimer to fit properly within the active site of pol eta.

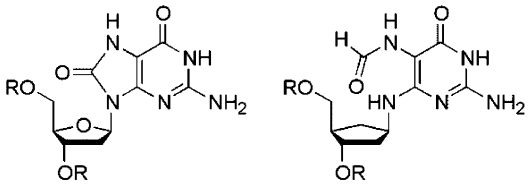
67. Thermodynamics of DNA minor groove interactions: Sequence dependence of the binding enthalpy and entropy. Binh Nguyen, Farial Tanious, Donald Hamelberg, and **W. David Wilson**. Department of Chemistry, Georgia State University, 50 Decatur St., Atlanta, GA 30303

The effect of DNA sequence on the thermodynamics of DNA interactions has been studied with a series of short DNA sequences and some DNA minor-groove binders. The results from a wide range of biophysical techniques, including spectroscopy, biosensor-surface plasmon resonance (SPR), calorimetry, and molecular dynamics (MD) simulations, have shown that DNA sequence can significantly influence the thermodynamics of binding. The enthalpic and entropic contributions to the total free energies of binding vary significantly with the DNA sequences. Analysis of temperature effects on the thermodynamic parameters shows that the heat capacities vary significantly among different DNA sequences. Molecular dynamics calculation results suggest that these differences may arise from different hydration patterns and minor groove widths associated with different DNA sequences.

68. Two main oxidative DNA lesions 8-oxodG and FapydG feature strongly different pairing properties. Matthias Ober and Thomas Carell. Department of Chemistry, Philipps-Universitaet Marburg, Hans-Meerwein-Strasse, Marburg 35032, Germany (fax +49-6421-28-22189)

The formamidopyrimidine of 2′-deoxyguanosine (β-FapydG) and 7,8-dihydro-8-oxo-2′-deoxyguanosine (β-8oxodG) are two significant DNA lesions induced by oxidative stress. As a monomer, β-FapydG readily anomerizes in solution, impeding its stereospecific incorporation into oligonucleotides during automated DNA synthesis. A phosphoramidite building block of β-cFapydG, a stable analogue of β-FapydG in which the ribose moiety was replaced with a carbocyclic ribose mimic, was synthesized from (1*R*)-(−)-2-azabicyclo[2.2.1]hept-5-ene-3-one. This new building block and β-8oxodG were both incorporated into oligonucleotides with like sequences. The first direct comparison of

the pairing properties of the oxidative lesions β-8oxodG and β-cFapydG revealed unexpected large base pairing differences. Whereas β-8oxodG exhibited a preference for both dC and dA as a counterbase, double strands with β-cFapydG opposing dC exhibit a melting behavior, which is similar to a mismatch. It is found that β-cFapydG double strands with the counter base dT possess the highest melting point.



69. Isothermal titration calorimetric studies of drug−DNA interactions. Challa V. Kumar and **Jyotsna Thota**. Department of Chemistry, University of Connecticut, Unit 3060, 55 North Eagleville Rd., Storrs, CT 06269 [fax (860) 486-2981, cvkumar@nucleus.chem.uconn.edu, reachthota@yahoo.com]

Understanding the binding of small molecules to DNA is important in studying drug−DNA interactions. We chose anthracene derivatives as model drugs to study drug−DNA interactions. To quantitate thermodynamics of DNA−anthracene systems, we employed isothermal titration calorimetry (ITC). Here, we describe the binding of four anthracene derivatives, MEA, BMEA, DEA, and BDEA (Figures 1−4), to calf thymus DNA (CTDNA). It was observed that binding of MEA and BMEA to CTDNA was enthalpic and binding of DEA and BDEA to CTDNA was entropic. This follows the conclusions of spectroscopic studies that the order of degree of intercalation is as follows: MEA > BMEA ∼ DEA > BDEA. To understand the effect of the presence of additional charges and the effect of the NH group as the hydrogen bonding group rather than the OH group as in ethanolammines, binding of AODA (Figure 5) was studied.

Fig.1 MEA

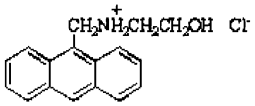


Fig.2 BMEA

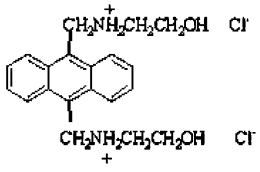


Fig.3 DEA

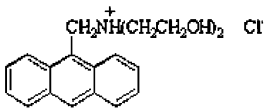


Fig.4 BDEA

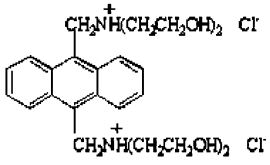
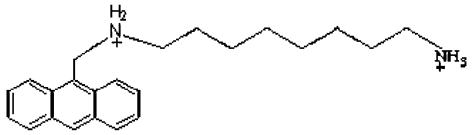


Fig.5 AODA



According to ITC studies, AODA binds to CTDNA with a binding constant of 7×10^5 M^{-1}, which is in agreement with spectroscopic studies.

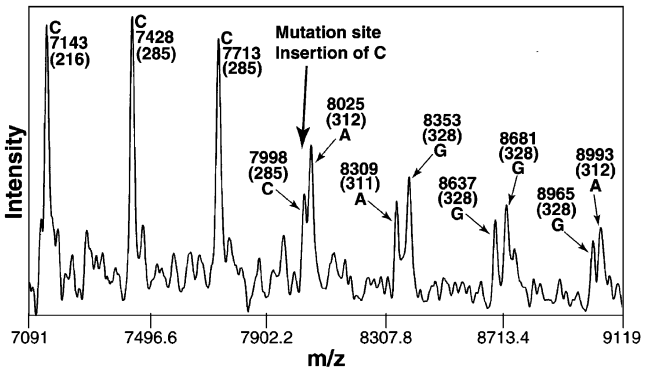
70. Nucleosomes inhibit nucleotide excision repair of site-specific platinum−DNA adducts. Dong Wang,[1] Ryujiro Hara,[2] Gitanjali Singh,[1] Aziz Sancar,[2] and Stephen J. Lippard.[3] [1]Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139, [2]Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina at Chapel Hill, and [3]Department of Chemistry, Massachusetts Institute of Technology

DNA is a major biological target for cisplatin. Understanding the cellular responses to platinum−DNA adduct formation is crucial to elucidating the cytotoxic mechanism of cisplatin. Eukaryotic DNA is packaged into chromatin, a complex composed of fundamental units called nucleosomes. The local environment in nucleosomal and free DNA differs dramatically. Detailed knowledge about the repair of platinum−DNA adducts in nucleosomes has thus far been lacking. In this study, mononucleosomes with site-specific platinum−DNA intrastrand GG−Pt and GTG−Pt cross-links were prepared as probes for nucleotide excision repair (NER). In vitro repair experiments reveal that nucleosomes significantly inhibit the excision of platinum−DNA adducts. The effect of post-translational histone modification on NER was also investigated by comparing native and recombinant nucleosomes. Excision from the native nucleosome is ∼2-fold higher than in recombinant material, indicating that post-translational histone modifications can modulate excision repair of damaged nucleosomes. (Supported by the National Cancer Institute.)

71. Digitally detecting frameshift mutations by DNA sequencing using solid phase capturable dideoxynucleotides and mass spectrometry. Hameer D. Ruparel, Michael E. Ulz, Sobin Kim, and Jingyue Ju. Department of Chemical Engineering and Columbia Genome Center, Columbia University, 1150 St. Nicholas Ave. #403, New York, NY 10032 [fax (212) 851-5215, hdr25@columbia.edu]

Deletion or insertion mutations may lead to a frameshift that causes misalignment between wild-type and mutated alleles, making it difficult to identify such mutations using electrophoresis-based sequencing. We have developed a DNA sequencing method using solid phase capturable dideoxynucleotides and MALDI-TOF mass spectrometry. Here, we report the use of this approach to identify the 185delAG and 5382insC frameshift mutations in the BRCA1 gene. In this method, Sanger DNA sequencing fragments are generated in one tube using biotinylated dideoxynucleotides. The sequencing fragments carrying a biotin at the 3′ end are captured on a streptavidin-coated solid phase to eliminate excess primer, primer dimers, and false stops. Only correctly terminated DNA fragments are captured, subsequently released, and analyzed by mass spectrometry to obtain digital DNA sequencing data. This method produces distinct doublet mass peaks at each point in the mass

spectrum beyond the mutation site, facilitating the accurate characterization of the mutation.



72. Effect of different cations on the conformation of DNA adsorbed on hydrophilic and hydrophobic surfaces. Emilios K. Dimitriadis,[1] James Pascual,[1] and **Ferenc Horkay**.[2] [1]Division of Bioengineering and Physical Science, OD, National Institutes of Health, 13 South Dr., MSC 5766, Building 13, Room 3N17 (dimitria@helix.nih.gov), and [2]Laboratory of Integrative and Medical Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, 13 South Dr., Bethesda, MD 20892 [fax (301) 435-5035, horkay@helix.nih.gov]

Despite intense efforts in studying DNA conformation in solutions, little work has been done to understand the effect of surface properties on the morphology. We studied by AFM the conformation of DNA strands adsorbed on different surfaces (hydrophobic, moderately hydrophilic, and hydrophilic) from solutions containing monovalent (Na^+), divalent (Ca^{2+}), and trivalent (spermidine) cations. It has been demonstrated that substrate surface energy mediates DNA−DNA interactions, leading to the formation of a variety of structures: DNA adsorbed from NaCl solution onto hydrophilic surfaces remains linear. Increasing ion valence causes DNA aggregation and formation of complex structures (bundles and loops with Ca^{2+} and toroids and "flowers" with spermidine). Increasing surface hydrophobicity favors the formation of compact structures. A synergetic effect is found between surface hydrophobicity and cation valence. The morphological features observed in the presence of cations are qualitatively consistent with the predictions of molecular dynamics simulations of DNA condensation.

73. Incorporation of the cationic 5-(3-aminopropyl)-2′-deoxyuridine (Z3dU) into the Dickerson dodecamer: evidence for site-specific DNA bending. Feng Wang,[1] Zhijun Li,[1] Tandace A. Scholdberg,[1] Jian-Sen Li,[2] Barry Gold,[2] and **Michael P. Stone**.[1] [1]Department of Chemistry, Vanderbilt University, Box 1822, Station B, Nashville, TN 37235 (feng.wang.1@vanderbilt.edu), and [2]Eppley Institute for Research in Cancer and Allied Diseases and Department of Pharmaceutical Sciences, University of Nebraska Medical Center

The dodecamers d(CGCGAATXCGCG)$_2$ (**I**), d(CGCGAAXTCGCG)$_2$ (**II**), and d(CGCGAAXXCGCG)$_2$ (**III**), in which X is 5-(3-aminopropyl)-2′-deoxyuridine (Z3dU), were compared with the Dickerson dodecamer d(CGCGAATTCGCG)$_2$. At neutral pH, Z3dU tethered a positive charge into the major groove. The presence of Z3dU perturbed the dodecamers in a sequence-dependent manner. The melting temperatures of **I** and **III** decreased compared to that of the Dickerson dodecamer, whereas the melting temperature of **II** increased slightly. NMR revealed that in all three duplexes, Watson−Crick base pairing was intact. Duplexes **I** and **III** showed an approximate 0.25 ppm upfield shift of only the ^{31}P resonance, corresponding to the linkage between C^9 and G^{10}, the next-nearest-neighbor phosphodiester in the 3′ direction from X^8. This suggested that placement of Z3dU at position X^8 did not result in formation of a salt bridge to the X^8 phosphate, but instead positioned the tethered cationic amine on the floor of the major groove and oriented in the 3′ direction. Molecular dynamics calculations on **I** and **III**, restrained by ^{1}H NOEs and ^3J couplings, and in which the tethered amine of X^8 was restrained proximate to G^{10} O^6, yielded ensembles of bent structures. NOE intensities calculated using complete relaxation matrix theory were in agreement with experimental NOE intensities. The lack of any ^{31}P chemical shift perturbation in **II** suggested that placement of Z3dU at position X^7 did not restrain the tethered cationic amine. Molecular dynamics calculations in which the cationic Z3dU at X^7 was not restrained did not yield bent structures and were consistent with experimentally determined NOE intensities for **II**. (Supported by NIH Grant CA-76049.)

74. Detection of a C·C mismatched base pair by SPR assay using sensors immobilized with a cytosine-specific ligand. Akio Kobori,[1] Kazuhiko Nakatani,[2] and Isao Saito.[2] [1]Department of Synthetic Chemistry and Biological Chemistry, PRESTO, Kyoto 606-8501, Japan, and [2]Department of Synthetic Chemistry and Biological Chemistry, Kyoto University

We have designed and synthesized ligands that specifically bind to cytosine·cytosine (C·C) and cytosine·thymine (C·T) mismatched base pairs. The binding of ligands was verified by observing an increase in the melting temperature specific for 11mer duplexes containing the mismatches. T_m measurements also showed that the sequence flanking the mismatch has little effect on the binding. The cytosine-specific ligand was immobilized on the surface of sensors for the surface plasmon resonance (SPR) assay. A distinct SPR signal was obtained for the duplexes containing the C·C mismatch, and a weaker signal was detected for the C·T mismatch, whereas other mismatches did not produce any significant signals.

75. Delivery of antisense phosphorodiamidate morpholino oligomers by arginine-rich peptides. Hong M. Moulton, Michelle C. Hase, and Patrick L. Iversen. Biology, AVI BioPharma Inc., 4575 SW Research Way, Suite 200, Corvallis, OR 97333 [fax (541) 754-3545, moulton@avibio.com]

Phosphorodiamidate morpholino oligomers (PMOs) are antisense molecules that inhibit gene expression by preventing translation or interfering with pre-mRNA splicing. These molecules, however, have limited permeability through the plasma membrane of cultured cells. Using an antisense functional assay, fluorescent microscopy, and flow cytometry, we investigated factors influencing the ability of a "cell

permeable" arginine-rich peptide, RRRRRRRRRFFC (R9F2C), to deliver PMOs into cultured cells. These factors included the type of cross-linker that connected PMO and peptide, the size of the peptide's cargo, and the sites within the peptide where the PMO was conjugated. We found that conjugates with long and flexible cross-linkers were more effective than short and bulky ones, and that a linker, which can be cleaved intracellularly, was more effective than a noncleavable linker. Increasing cargo size by increasing the length of PMO did not decrease the peptide's delivery efficacy. Variation of the site of PMO attachment within the peptide did not affect the antisense activity of the PMO. We conclude that cellular delivery of various length PMOs to their target mRNA can be significantly enhanced by conjugating PMO to the R9F2C peptide through a long, flexible, and cleavable linker.

76. (1*S*)- and (1*R*)-*trans*-*anti*-benzo[*c*]phenanthrene alter the structure of the *Salmonella typhimurium* hisD3052 gene iterated repeated sequence containing the $(CpG)_3$ frameshift hotspot. Yazhen Wang,[1] Nathalie Schnetz-Boutaud,[1] Heiko Kroth,[2] Haruhiko Yagi,[2] Jane M. Sayer,[2] Subodh Kumar,[3] Donald M. Jerina,[2] and Michael P. Stone.[1] [1]Chemistry Department, Center in Molecular Toxicology, and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN 37235, [2]Laboratory of Comparative Carcinogenesis, National Cancer Institute-Frederick, and [3]Great Lakes Laboratory, State University College at Buffalo

The oligodeoxynucleotide 5′-d(ATCGCGCGGCATG)-3′•5′-d(CATGCCGCGCGAT)-3′, derived from the *Salmonella typhimuium* hisD3052 gene, is a hotspot for frameshift mutagenesis. Frameshift mutations at this locus probably result from transient strand slippage during error-prone lesion bypass. The (1*S*)- and (1*R*)-*trans*-*anti*-benzo[*c*]phenanthrene adducts were site-specifically introduced into 5′-d(ATCGCX-CGGCATG)-3′•5′-d(CATGCCGCGCGAT)-3′, where X is *trans*-*anti*-[BcPh]-N2-deoxyguanosine. As anticipated, both the 1*S* and 1*R* forms intercalated from the minor groove with the orientation dependent on stereochemistry. Intercalation occurred to the 5′-side of the modified strand for the 1*S* adduct and to the 3′-side for the 1*R* adduct [Lin, C. H., Huang, X., Kolbanovskii, A., Hingerty, B. E., Amin, S., Broyde, S., Geacintov, N. E., and Patel, D. J. (2001) *J. Mol. Biol. 306*, 1059−1080]. Structures for the two intercalated adducts were refined using molecular dynamics calculations restrained by ^{1}H NOEs and ^3J coupling constants. Subsequently, the 13−11mer 5′-d(ATCGCXCGGCATG)-3′•5′-d(CATGCCGCGAT)-3′, containing the (1*R*)-*trans*-*anti*-benzo[*c*]phenanthrene adduct opposite a CpG deletion in the complementary strand, was constructed. The structure of the 1*R* adduct in the bulged sequence was compared with corresponding structures for the nonbulged sequence, the unmodified bulged sequence, and the corresponding bulged sequence containing the malondialdehyde exocyclic 1,N2-dG M1G adduct. Whereas the bulge within the M1G-modified 13−11mer was localized at the damage site, the intercalated (1*R*)-*trans*-*anti*-benzo[*c*]phenanthrene adduct facilitated bulge migration on the NMR time scale. [Supported by NIH Grant CA-55678 (M.P.S.).]

77. Zinc-dependent cleavage in the hammerhead ribozyme: Characterization and use in studying the mechanisms of inhibition by antibiotics. Emily J. Borda and Snorri Th. Sigurdsson. Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195 [fax (206) 616-4209, eborda@u.washington.edu]

We have characterized a cleavage site between nucleotides C3 and U4 in the catalytic core of the hammerhead ribozyme, which is only observed in the presence of Zn^{2+}. This site has an unusual pH dependence: U4 cleavage products are only observed above pH 7.9 and reach a maximum yield at pH ~8.5. These data, together with the fact that no metal ion binding site is observed near the U4 cleavage site in the crystal structure, suggest a pH-dependent conformational change in the hammerhead ribozyme. We have previously described Zn^{2+}-dependent cleavage between G8 and A9 in the hammerhead ribozyme and have discovered that U4 cleavage occurs only after A9 cleavage. To our knowledge, this is the first example of sequential cleavage as a possible regulatory mechanism in ribozymes. We have used both cleavage sites to explore the mechanism of action of antibiotics neomycin B and chlortetracycline on the hammerhead ribozyme.

78. Local conformational changes in the catalytic core of the trans-acting hepatitis delta virus ribozyme accompany catalysis. Dinari A. Harris, David Rueda, and Nils G. Walter. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 (dinarih@umich.edu)

The hepatitis delta virus ribozyme is a self-cleaving catalytic RNA motif that processes multimeric intermediates during replication of its genome. Kinetic analyses suggest that a particular cytosine residue (C75) acts as a general acid or base in cleavage chemistry. The crystal structure of the product form of a cis-acting HDV ribozyme shows this residue positioned close to the 5′-OH leaving group of the reaction by a trefoil turn in the RNA backbone. By modifying G76 of the trefoil turn of a synthetic trans-cleaving HDV ribozyme to the fluorescent 2-aminopurine (AP), we can monitor local conformational changes in the catalytic core. In the precursor, AP fluorescence is strongly quenched, while the product shows a significant increase in fluorescence, consistent with AP76 becoming unstacked and solvent-exposed as evidenced in the trefoil turn. Our data show that C75 becomes positioned for reaction chemistry only along the trajectory from precursor to product.

79. Interaction of magnesium ion with tRNA-Phe. Martin J Serra,[1] Benjamin M. Whittam,[1] and E. Westhof.[2] [1]Department of Chemistry, Allegheny College, 520 N. Main St., Meadville, PA 16335 [fax (814) 332-2789, mserra@allegheny.edu], and [2]UPR9002, IBMC-CNRS

Optical melts were performed on tRNA-Phe in varying concentrations of magnesium ion (from 0 to 50 mM) at two different NaCl concentrations (0.1 and 0.01 M). The melt curve analysis yielded thermodynamic data (T_M and $\Delta H°_{VH}$) used to examine the interaction of RNA and Mg^{2+}. Results indicated a stabilization of RNA by Mg^{2+}, shown by T_M increases (from 48 to 79 °C) and $\Delta H°_{VH}$ increases (from 102.4 to 149.0 kcal/mol) in 100 mM NaCl buffer, and increases in T_M (from 35 to 79 °C) and $\Delta H°_{VH}$ (from 77.1 to 171.6 kcal/mol) in 10 mM NaCl buffer. The stabilization of tRNA-Phe by magnesium ion can be modeled by

nonspecific interactions of the metal ions with the RNA. Further examination of the data noted an increased stabilization of the RNA at lower concentrations of NaCl and a constant concentration of magnesium ion. This suggested the existence of monovalent−divalent cation competition for RNA binding.

80. Kinetic solvent isotope effect studies on the folding mechanism of the hepatitis delta virus ribozyme. Rebecca A. Tinsley, Dinari A. Harris, and Nils G. Walter. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 (rtinsley@umich.edu)

The exchange of deuterium for hydrogen in water often produces solvent kinetic isotope effects (KSIEs) on the rate constants associated with enzyme reactions, including those catalyzed by RNA. Recently, KSIEs were used to show that proton transfer occurs in the rate-limiting step of cleavage by the hepatitis delta virus (HDV) and other ribozymes. To test the underlying assumption that KSIEs are related to the chemistry step of ribozyme-mediated cleavage reactions, we developed fluorescence resonance energy transfer assays to measure KSIEs on the rate constants of conformational changes associated with substrate binding and dissociation by a trans-acting HDV ribozyme. We observe comparable KSIEs (2−3-fold) on rate constants of conformational change and cleavage, with proton inventory experiments supporting a two-proton transfer mechanism during conformational change. Together, these results challenge traditional views that the observation of a KSIE implies that chemistry is the rate-limiting step in an RNA-catalyzed reaction.

81. Binding of the bovine immunodeficiency virus Tat peptide with modified BIV TAR RNA. Jeffrey B. H. Tok, Lanrong Bi, and Shuyin Huang. Chemistry, York College, and Graduate Center of The City University of New York, The City University of New York, 94-20 Guy R. Brewer Blvd., Jamaica, NY 11451 [fax (718) 262-2652, tok@york.cuny.edu]

Besides generation of novel binding peptides or small molecules to their RNA target, successful design of chemically modified RNA constructs capable of tighter binding with their binding peptides is also of significant interest. Herein, the synthesis and binding studies of both wild-type and mutant BIV TAR RNA constructs against its Tat peptide are reported. Understanding the requirements that enable RNA construct binding properties, especially at the hairpin loop or internal bulge, would enable a potential therapeutic approach to control the BIV life cycle.

82. Structures and stability of unilamellar phospholipid vesicles. Baohua Yue,[1] Chien-Yueh Huang,[2] Otto H. York,[2] Muping Nieh,[3] John Katsaras,[4] and Charles J Glinka.[5] Department of Chemistry and Environmental Science, New Jersey Institute of Technology, 161 Warren St., Newark, NJ 07102 (by2@njit.edu), [2]Department of Chemical Engineering, New Jersey Institute of Technology, [3]Steacie Institute for Molecular Science, National Research Concil, [4]Neutron Program for Materials Research, National Research Council, and [5]Center for Neutron Research, National Institute of Standards and Technology

Unilamellar vesicles (ULVs) are vesicles consisting of single-bilayer shells. These vesicles have potential applications for drug delivery and gene therapy vessels, and microreactors. In this work, we describe biomimetic ULVs that are formed spontaneously with a narrow size distribution. The vesicles are composed of phospholipids dimyristoyl- and dihexanoylphosphorylcholine (DMPC and DHPC, respectively) doped with negatively charged lipid dimyristoylphosphorylglycerol (DMPG) and cations such as Ca^{2+}, Na^{+}, and K^{+}. Our experimental data from dynamic light scattering show that the ULV structures are stable over a wide range of lipid concentrations and temperatures (between 10 and 40 °C) and are independent of the concentrations of univalent ions but are sensitive to divalent ions possibly due to the surface couplings between calcium ion and the lipid membranes. The structures and the stability of the ULVs are also supported by data from our recent small angle neutron scattering (SANS) experiment.

83. Atomic force microscopic study on supported bilayers composed of lipopolysaccharides and bacteria phospholipids. Jihong Tong and Thomas J. McIntosh. Department of Cell Biology, Duke University Medical Center, Box 3011, Durham, NC 27710 [fax (919) 681-9929, j.tong@cellbio.duke.edu]

Atomic force microscopy (AFM) was used to study the structure of supported bilayers composed of lipopolysaccharide (LPS), the primary lipids in the outer membrane of Gram-negative bacteria, and bacteria phospholipids (BPL). A vesicle fusion technique was used to deposit negatively charged LPS, BPL, and LPS/BPL mixtures onto a polyethylenimine (PEI)-covered mica substrate. The BPL and LPS bilayers on the PEI-coated mica were quite smooth, and the thickness data of the supported bilayers measured with contact mode AFM (5.0 ± 0.3 nm for BPL, 6.9 ± 0.4 nm for Rd LPS, and 8.8 ± 0.6 nm for Ra LPS) were consistent with previous X-ray diffraction measurements. It was found that the coverage of the bilayers decreased when the bilayers were dried, indicating a reorganization of the supported bilayer structure. The possible domain formation in BPL/LPS mixtures with different ratio was probed by tapping mode AFM in solution.

84. Protein interactions and signaling activity in a self-assembled receptor system. Abdalin E Asinas and Robert M. Weis. Department of Chemistry, University of Massachusetts, 701 N. Pleasant St., 701 LGRT, Amherst, MA 01003 [fax (413) 545-4490, aasinas@chem.umass.edu]

In the bacterial cell, a ternary complex of receptors, an adaptor protein CheW, and a kinase CheA is essential for chemotactic signaling. Previously, we used lipid vesicles to direct the assembly of active complexes composed of receptor cytoplasmic fragments (CFs), CheW and CheA. Here we report the effect of introducing point mutations into the CFs on assembly and signaling activity. The dominance of these mutations, which are known to influence interactions between receptor subunits, correlates with the extent to which activity can be rescued in mixtures with wild-type CF. The effects of the mutations on CheA and CheW binding explain this activity−rescue correlation; superdominant mutants bind CheA and CheW more tightly than wild-type CF, while rescuable mutants bind CheA and CheW more weakly.

85. Transmethylation of receptors in the bacterial chemotaxis signaling pathway. Frances M. Antommattei and

Robert M. Weis. Chemistry Department, University of Massachusetts, LGRT 701, 710 Pleasant St., Amherst, MA 01003 [fax (413) 545-4490, fantomma@chemistry.umass.edu]

Adaptation to sensory stimuli in bacterial chemotaxis is mediated by the reversible methylation of certain glutamate residues in the signaling domains of receptors. Methylation, and perhaps demethylation, occur by a mechanism involving interactions between receptors of different ligand specificity, yet adaptation exhibited by the cell is ligand specific. The receptors are found in large clusters, which are undoubtedly heterogeneous with respect to both the methylation level and ligand specificity. Our goal is to understand how ligand-specific adaptation is maintained within this heterogeneous environment. The approach involves generating membrane samples in which the aspartate and serine receptors are coexpressed, and are engineered so that methylation of one receptor is dependent on the other. *In vivo* and *in vitro* experiments demonstrate that transmethylation is obligatory with these engineered receptors. *In vitro* methylation reactions are being used to determine the extent of ligand-specific activation.

86. Biosynthesis of transmembrane domains of the *Saccharomyces cerevisiae* α-factor receptor. Racha Estephan,[1] Enrique Arevalo,[1] Jacqueline Englander,[1] Jeffrey M. Becker,[2] and Fred Naider.[2] [1]Department of Chemistry, The College of Staten Island/Cicty University of New York, 2800 Victory Blvd., Staten Island, NY 10314 [fax (718) 982-3910, racha5@hotmail.com], and [2]Department of Microbiology, University of Tennessee

The yeast *Saccharomyces cerevisiae* α-factor pheromone receptor (Ste2p) that is required for mating belongs to the GPCR family. The P258L mutation in transmembrane domain 6 (TM6) results in a constitutively active receptor. Wild-type TM6, a 33-residue peptide, was biosynthesized and labeled with ^{15}N in minimal medium as a 17.3 kDa fusion protein using histidine-tagged TrpΔLE as the N-terminus. Biosynthesis, isolation, and purification of the ^{15}N-labeled TM6 fusion protein (M6FP) gave 10 mg of pure fusion protein/L of bacterial culture. The TrpΔLE was released from M6FP by CNBr cleavage to yield 1 mg of pure ^{15}N-labeled TM6 peptide/8 mg of cleaved fusion protein. Its ^{15}N-edited HSQC spectrum indicates a uniform incorporation of the label throughout the peptide. Biosyntheses of the ^{15}N-labeled constitutive mutant TM6 peptide (P258L) and ^{15}N-labeled TM7 (Ste2p 267−339), hypothesized to interact with TM6, are being optimized.

87. Characterization of hydraphile ion channel activity using an ISE method. Michelle E. Weber,[1] Natasha K. Djedovic,[1] Paul H. Schlesinger,[2] and George W. Gokel.[1] [1]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8103, St. Louis, MO 63110, and [2]Department of Cell Biology and Physiology, Washington University School of Medicine

Nature has developed complex methods for the transport of biologically relevant ions across cell membranes. To better understand these complicated systems, our group has reported several molecules capable of ion transport across membranes. This work focuses on hydraphile channels, a series of compounds designed for sodium transport. Past work has used ^{23}Na NMR to assess ion transport across liposome membranes. We will show that ion-sensitive electrodes (ISE) have recently proved to be an appreciably more sensitive tool. Using an ISE-based system, we have determined that our channels release sodium from liposomes in a concentration-dependent manner. Additionally, the ISE method has enabled us to better understand the transport activity of the compounds in the membrane. We will discuss the results of a structure−activity study using the ISE system as a measurement of the extent of sodium release. Additionally, we will present a model of how these compounds might be situated in the membrane to facilitate ion release.

88. Effects of receptor oligomerization on *N*-formyl peptide receptor: A model of G-protein-coupled receptor signaling. Allison C. Lamanna,[1] Steven D. Brown,[2] and Laura L. Kiessling.[3] Department of Biochemistry, University of Wisconsin−Madison, Madison, WI 53706 (alamanna@biochem.wisc.edu), [2]Department of Chemistry, University of Wisconsin−Madison, and [3]Departments of Chemistry and Biochemistry, University of Wisconsin−Madison

N-Formyl peptide receptor (FPR) is a heptahelical transmembrane G-protein-coupled receptor (GPCR) that is found on the surface of neutrophils, where it is active in chemotaxis and phagocytosis. Recent studies suggest that GPCRs can homo- or hetero-oligomerize. Here, we demonstrate that ring opening metathesis polymerization (ROMP)-derived ligands for FPR can alter the signals and biological responses produced upon FPR activation. Multivalent ligands enhance signal induction through FPR, at both the intracellular signaling level and the behavioral level. Polymeric ligands of varying lengths differentially affect the downregulation of FPR signaling. These data suggest that oligomerization of this GPCR by multivalent ligands can change signal output. We anticipate that multivalent ligands will be useful for both studying the importance of oligomerization and modulating responses mediated by GPCRs.

89. Role of Tyr179 in the catalytic activity and stability of phenylalanine hydroxylase from *Chromobacterium violaceum*. Jerome Zoidakis, Mui Sam, and Mahdi Abu-Omar. Department of Chemistry and Biochemistry, University of California, 607 Charles E. Young Dr. E., Los Angeles, CA 90095 [fax (310) 206-4038, zoidakis@chem.ucla.edu]

The non-heme iron enzyme phenylalanine hydroxylase catalyzes the conversion of phenylalanine to tyrosine by using molecular oxygen and biopterin. The crystal structure of the protein shows that tyrosine 179 is in the proximity of the active site iron and biopterin. The point mutants Y179F and Y179A were purified, and their kinetic parameters and stabilities were determined. Y179F has a lower k_{cat} than the wild-type (WT) enzyme and binds phenylalanine and biopterin with higher affinity. Y179A has a lower k_{cat} than the WT enzyme and a lower affinity for phenylalanine. The stability of Y179F is comparable to that of WT, whereas Y179A is less stable. In conclusion, Tyr179 is involved in substrate binding and catalysis.

90. Observation of the tetrameric form of pig heart lipoamide dehydrogenase in reverse micelles. Irina G.

Gazaryan,[1] Valentina Schedrina,[2] Natalia Klyachko,[2] Alexander Efimov,[3] 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, and [3]Institute of Protein Research of the Russian Academy of Sciences

Pig heart lipoamide dehydrogenase (EC 1.6.4.3, LADH) is believed to exist as a homodimeric molecule in aqueous solution. Structural studies of the multienzyme complexes that contain LADH as a component are consistent with a dimeric form. However, modeling studies of the complex can accommodate the compact LADH tetramer. We have investigated the state of oligomerization of pig heart LADH using a reversed micelle system, where activity peaks when the micelle size [which is determined by the ratio of surfactant to water (W_o)] closely matches the protein volume. At pH 7.5, the dependence of K_m and V_m on W_o for the LADH-catalyzed diaphorase reaction exhibits two clear peaks at W_o values of 19 and 35, which correspond to the dimeric and tetrameric forms of the enzyme, respectively. Micelle volumes combined with sedimentation analysis will allow prediction of the shape of the dimer and tetramer. The pig heart solution tetramer was modeled on the basis of the tetrameric form of pea lipoamide dehydrogenase within the crystal cell [Faure et al. (2000) *Eur. J. Biochem. 267*, 2890−2898]. The modeling suggests a weak tetramer, which is held together by a small number of electrostatic interactions. These weak contacts are consistent with the low-stability tetramer that we have observed in solution. (Supported by Grant NS38741 and NATO Linkage Grant LST.CLG.978592 to A.M.B.)

91. Function of Arg70 in *Saccharomyces cerevisiae* phosphoenolpyruvate carboxykinase. Emilio Cardemil, M. Cristina Ravanal, Michel Flores, Francisco Aroca, and Liliana Llanos. Departamento de Ciencias Químicas, Universidad de Santiago de Chile, Casilla 40, Santiago 33, Chile [fax +56(2)681-2108, ecardemi@lauca.usach.cl]

Saccharomyces cerevisiae phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the decarboxylation and phosphorylation of oxaloacetate in the presence of ATP and Mn^{2+} to yield phosphoenolpyruvate (PEP), ADP, and CO_2. Homology modeling based on the crystal structure of the *Escherichia coli* PEPCK−ATP−pyruvate−Mg^{2+}−Mn^{2+} complex indicates that Arg70 is located close to the carboxyl group of enzyme-bound PEP [Krautwurst et al. (2002) *Biochemistry 41*, 12763−12770]. The function of Arg70 in catalysis was investigated employing Arg70Lys, Arg70Gln, and Arg70Met mutant PEPCKs. The relative V_{max} values for the wild type and Lys, Gln, and Met mutants were 1, 0.9, 0.0002, and 0.0002, respectively, with only small alterations in K_m. The binding affinity of PEP was lowered in the Arg70Gln and Arg70Met mutant PEPCKs. These results indicate that Arg70 is a critical catalytic residue in *S. cerevisiae* PEPCK, and suggest that it helps orienting and stabilizing PEP binding through electrostatic interactions. (Supported by FONDECYT 1000756-1030760.)

92. Gln^{212}, Asn^{270}, and Arg^{301} are critical for catalysis by adenylosuccinate lyase. Mark L. Segall and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 [fax (302) 831-6335, markasl@chem.udel.edu]

Adenylosuccinate lyase (ASL) of *Bacillus subtilis* is a tetrameric enzyme that catalyzes the cleavage of adenylosuccinate (SAMP) to produce AMP and fumarate. We mutated three conserved amino acid residues found in the region of the succinyl moiety of adenylosuccinate when docked into crystalline ASL. Q212 was replaced with Glu or Met, N270 with Asp or Leu, and R301 with Lys or Gln. The mutants were expressed in *Escherichia coli* and purified to homogeneity. With the exception of Q212E, the mutant enzymes displayed specific activities equal to or less than 0.0003 μmol min^{-1} mg^{-1}, as compared to a value of 1.56 μmol min^{-1} mg^{-1} for wild-type adenylosuccinate lyase. Nonetheless, SAMP binding was observed for Q212M, N270D, N270L, R301K, and R301Q, with K_D's of 30 ± 6, 121 ± 8, 77 ± 21, 41 ± 8, and 113 ± 25 μM, respectively. Laser light scattering measurements at a protein concentration of ∼0.2 mg/mL revealed similarity in the oligomeric state among the wild-type enzyme and the Q212E, N270D, N270L, and R301K mutants. Wild-type secondary structure was also retained in these mutants, as shown by CD measurements. Q212E ASL, displaying a specific activity of 0.0025 μmol min^{-1} mg^{-1}, exhibited a K_m of 14.6 μM, only modestly increased as compared to the value of 3.5 μM for the wild-type enzyme, indicating that the mutation primarily affects V_{max}. ASL mutants at all three positions were partially reactivated through intersubunit complementation by mixing with mutants at residues contributed to the active site by other subunits. Hence, mixing Q212M ASL with an equal amount of N270L, K268Q, or H141Q ASL led to specific activity enhancements of at least 250-fold. Similarly, N270L in the presence of H68Q resulted in a 260-fold increase in specific activity, and the combinations of R301K ASL with the H141Q and K268Q mutants showed similar activation. H68Q ASL, however, does not complement R301K ASL since both of these residues are contributed to the active site by the same subunit. The results of this study suggest that Q212, N270, and R301 perform critical roles as participants in the active site of adenylosuccinate lyase. (Supported by NIH Grant R01-DK60504.)

93. HMG-CoA synthase: Mutagenesis and ^{13}C NMR studies on the influence of conserved aromatic amino acids. Chang-zeng Wang, Ila Misra, and Henry M. Miziorko. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226

To evaluate the potential contribution of conserved aromatic residues to the hydrophobic active site of 3-hydroxy-3-methylglutaryl-CoA synthase, site-directed mutagenesis was employed to produce Y130L, Y163L, F204L, Y225L, Y346L, and Y376L proteins. Each mutant protein was expressed and isolated in highly purified form. Kinetic characterization indicated that F204L exhibits a substantial (>300-fold) decrease in catalytic rate (k_{cat}). Upon modification with the mechanism-based inhibitor, 3-chloropropionyl-CoA, or in formation of a stable binary complex with acetoacetyl-CoA, F204L exhibits binding stoichiometries comparable to that of the wild-type enzyme, suggesting substantial retention of active site integrity. Y130L and

Y376L exhibit inflated values (80- and 40-fold, respectively) for the K_m for acetyl-CoA in the acetyl-CoA hydrolysis partial reaction; these mutants also exhibit a 1 order of magnitude decrease in k_{cat}. Formation of the acetyl−S− enzyme reaction intermediate by Y130L, F204L, and Y376L proceeds slowly in comparison with that of the wild-type enzyme. However, $H_2{}^{18}O$ solvent exchange into the thioester carbonyl oxygen of these acetyl−S−enzyme intermediates is not slow in comparison with previous observations for D159A and D203A mutants, which also exhibit slow formation of the acetyl−S−enzyme intermediate. The magnitude of the differential isotope shift (measured by ^{13}C NMR at 151 MHz) upon exchange of $H_2{}^{18}O$ into the [^{13}C]acetyl−S−enzyme intermediate suggests a polarization of the thioester carbonyl and a reduction in bond order. Such an effect may substantially contribute to the upfield ^{13}C NMR shift observed for the [^{13}C]acetyl−S−enzyme intermediate. The influence on acetyl−S−enzyme formation, as well as observed k_{cat} (F204L) and K_m (Y130L and Y376L) effects, implicates these invariant residues as part of the catalytic site. Substitution of phenylalanine (Y130F and Y376F) instead of leucine at residues 130 and 376 weakens the effects on the catalytic rate and substrate affinity observed for Y130L and Y376L, underscoring the influence of aromatic side chains near the active site. (Supported by NIH Grant DK21491.)

94. Asn460 of *β*-galactosidase (*Escherichia coli*) plays a role in the binding and transition state stabilization of galactosides. John C. Kappelhoff, Jen Hahn, and Reuben E. Huber. Department of Biological Sciences, University of Calgary, 2500 University Dr. N.W., Calgary, AB T2N 1N4, Canada [fax (403) 289-9311, jckappel@ucalgary.ca]

Asn460 of *β*-galactosidase may provide a substantial amount of transition state stabilization energy in the "deep" binding mode. The nitrogen of Asn460 is thought to bind the 2-hydroxyl of the transition state, while the carbonyl group hydrogen bonds to the guanidium group of Arg388. Asn460 was substituted with Ser, Thr, Gly, Ala, and Asp. The k_{cat} values decreased 26 times for N460D-*β*-galactosidase and 82−230 times for the other mutants with *o*-nitrophenyl *β*-D-galactopyranoside and 77−1200 times with *p*-nitrophenyl *β*-D-galactopyranoside. K_m values were quite similar to those of the wild type. Only N460S-*β*-galactosidase inhibition was as expected. It bound galactose poorly, but D-2-deoxygalactose and D-talose (with an axial configuration at the C2 position) were bound normally. N460D-*β*-galactosidase was inhibited to a greater extent by all of the inhibitors than were the rest of the substituted enzymes. Glu537 of *β*-galactosidase also hydrogen bonds with the C2 hydroxyl of the galactose moiety. Since aspartic acid is a carboxylate like Glu537, it may help bind the inhibitors more tightly. 2-Aminogalactose was an especially good inhibitor of N460D-*β*-galactosidase. This is probably because the negative charge of aspartic acid binds the positive charge of the 2-aminogalactose. All of the substituted enzymes were inhibited as well or somewhat better by inhibitors that bind the shallow mode. This shows that Asn460 is not involved in shallow mode binding. Each substituted enzyme except N460D-*β*-galactosidase was inhibited more poorly than the wild type by D-galactonolactone, showing that Asn460 is important for deep mode binding of the transition state. The extent of inhibition of the substituted enzymes by pentoses was decreased. Of the pentoses that were tested, the level of L-ribose inhibition was decreased the most. L-Ribose binds in the pyranose form in the wild type in a position similar to that of galactose, but slightly shifted toward the sodium ion and rotated 60° with respect to the ring oxygen. Thus, the poor binding of pentoses could be due to effects of Asn460 on their unique positioning.

95. Molecular mechanism of *Escherichia coli* aspartate decarboxylase activation is revealed by site-directed mutagenesis and crystallography. Michael E Webb[1] and Chris Abell.[2] [1]University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge, United Kingdom (fax +44 1223 330190, mew23@cam.ac.uk), and [2]Department of Chemistry, University of Cambridge

Aspartate decarboxylase is an essential component of the pathway to pantothenate (vitamin B_5) in bacteria. This pathway is a viable target for novel antibacterial and herbicidal therapeutics. Aspartate decarboxylase is a pyruvoyl-dependent decarboxylase in which the prosthetic pyruvoyl group is formed via an autocatalytic rearrangement from a precursor serine. The mechanism of catalysis of this activation and the mechanism of the subsequent decarboxylation have been studied by combined crystallographic and mutagenic studies. These studies have identified functional roles for the majority of conserved residues.

96. Human versus *Plasmodium falciparum* purine nucleoside phosphorylase: kinetic isotope effects and crystallographic structures. Andrzej Lewandowicz,[1] Wuxian Shi,[1] Li-Min Ting,[2] Gary B. Evans,[3] Richard H. Furneaux,[3] Peter C. Tyler,[3] Kami Kim,[2] and Vern L. Schramm.[1] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 (alewand@medusa.bioc.aecom.yu.edu), [2]Departments of Medicine and Microbiology and Immunology, Albert Einstein College of Medicine, and [3]Industrial Research Ltd.

Inhibition of purine nucleoside phosphorylase (PNP) by Immucillin H revealed the purine-deficient death of *Plasmodium falciparum*. This logically designed transition state analogue inhibitor may provide an alternative target for multidrug resistant malaria treatment. It is not known if inhibition of only the *P. falciparum* enzyme or both human and parasite PNPs is required for parasite death. Transition state analogue inhibitors with specificity for *P. falciparum* PNP were designed from crystallographic analysis of active sites. Kinetic isotope effects were used to compare the transition states for host and parasite PNPs. For the arsenolysis reaction of inosine, the observed 1′-^{14}C KIE near unity points to an S_N1 reaction mechanism with a crucial role for the 5′-hydrogen (5′-^{3}H KIE of 1.06) in stabilizing the oxacarbenium-like transition state for both enzymes. Examination of the active cavity of *P. falciparum* PNP revealed cocrystallized isopropanol. Hydrogen bond networks provided an opportunity to discriminate between enzymes by adding steric hindrance at the 5′-carbon or modifying hydrogen bonds.

97. Structure of a thermophilic dihydrofolate reductase. Steven M. Damo,[1] Hui Sun Kim,[2] Seok-Yong Lee,[3] Olay-

inka Oyeyemi,[2] Judith P. Klinman,[4] and David E. Wemmer.[5] [1]Department of Chemistry, University of California, 1 Cyclotron Rd 3-350D, Calvin Lab, Berkeley, CA 94720 [fax (510) 486-6059, smdamo@lbl.gov], [2]Department of Chemistry, University of California, [3]Howard Hughes Medical Institute, Rockefeller University, [4]Departments of Chemistry and Molecular and Cell Biology, University of California, and [5]Department of Chemistry, and Lawrence Berkeley National Laboratory, University of California

Dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolate to tetrahydrofolate, a precursor essential for the synthesis of methionine, serine, and purines in both eukaryotes and prokaryotes. Here, we present the 2.0 Å crystal structure of DHFR from the moderate thermophile *Bacillus stearothermophilus* (optimum growth temperature of 65 °C). The crystals belong to space group $P42_12$ with the following unit cell dimensions: $a = 104.65$ Å, $b = 104.65$ Å, and $c = 116.37$ Å. This is the first monomeric DHFR characterized from a thermophilic source. The structure is compared with DHFRs from other organisms.

98. NMR structure of the FAD and NADH binding domains of the reductase component of soluble methane monooxygenase from *Methylococcus capsulatus* (Bath). Lisa L. Chatwood,[1] Jens Müller,[1] John D. Gross,[2] Gerhard Wagner,[2] and Stephen Lippard.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139 (llchat@mit.edu), and [2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Soluble methane monooxygenase (sMMO) catalyzes the hydroxylation of methane by dioxygen to methanol, the first step in carbon assimilation by methanotrophs. This multicomponent system transfers electrons from NADH through a reductase component to the non-heme diiron center in the hydroxylase, where O_2 is activated. The reductase component consists of three distinct domains, a [2Fe-2S] ferredoxin domain along with FAD and NADH binding domains. We report the solution structure of the reduced 27.6 kDa FAD/NADH domain (MMOR-FAD) of the reductase from *Methylococcus capsulatus* (Bath) determined by NMR spectroscopy. The FAD binding domain consists of a seven-stranded antiparallel β-barrel and one α-helix, with the first 10 N-terminal residues unstructured. In the interface between the two domains, the FAD cofactor is tightly bound. The NADH binding domain consists of a five-stranded parallel β-sheet surrounded by four α-helices. MMOR-FAD is structurally homologous to other FAD-containing oxidoreductases.

99. NMR and mutagenesis studies of the structure of the MutT−Mg^{2+}−8-oxo-dGMP complex. Vibhor Saraswat, Michael A. Massiah, Hugo F. Azurmendi, and Albert S. Mildvan. Department of Biological Chemistry, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 [fax (410) 955-5759, saraswat@bs.jhmi.edu]

The MutT pyrophosphohydrolase from *Escherichia coli* (129 residues) catalyzes the hydrolysis of nucleoside triphosphates (NTPs), including 8-oxo-dGTP, by substitution at P-β, to yield NMP and pyrophosphate. The product, 8-oxo-dGMP, is a tight binding, slowly exchanging, mixed inhibitor with a K_I(slope) of 49 nM, $10^{4.6}$-fold tighter than the K_I(slope) of dGMP [Saraswat, V., et al. (2002) *Biochemistry 41*, 15566]. The solution structures of free MutT [Abeygunawardana, C., et al. (1995) *Biochemistry 34*, 14997] and of the E−Mg^{2+}−AMPCPP− complex [Lin, J., et al. (1997) *Biochemistry 36*, 1199] were similar, showing a hydrophobic nucleotide-binding cleft with Asn-119 and Arg-78 among the few polar residues. The N119D and N119A mutations increased K_I(slope) of 8-oxo-dGMP 36- and 1650-fold, respectively, but did not greatly affect K_I(slope) of dGMP or dAMP, k_{cat}, or K_m(dGTP). The R78A mutation increased the K_I(slope) of 8-oxo-dGMP 6.6-fold, with smaller effects on the K_I(slope) values of dGMP and dAMP. Formation of the ternary MutT−Mg^{2+}−8-oxo-dGMP complex altered the backbone ^{15}N and NH chemical shifts of 62 residues throughout the protein, slowed the NH exchange of 45 residues, and induced the disappearance of the Asn-119−Nδ H2 resonances and the appearance of two Arg−Nϵ H signals. The structure of the MutT−Mg^{2+}−8-oxo-dGMP complex, based on 1746 NOEs (13.5 NOEs/residue), 186 ϕ and ψ values, and 53 N−H residual dipolar couplings, showed a narrowing of the nucleotide-binding cleft, likely driven by the interaction of the 8-oxoguanine ring with Asn-119 and Arg-78.

100. Proposed mechanism for ketopantoate hydroxymethyltransferase. Louise M. Birch and Chris Abell. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom (fax 01223 330190, lmb32@cam.ac.uk)

Ketopantoate hydroxymethyltransferase (KPHMT) is the first enzyme on the bacterial pathway used to form pantothenic acid (vitamin B_5). The first step in this biosynthesis is the one-carbon transfer of a methylene group from the cofactor (6*S*)-N^5,N^{10}-5,6,7,8-tetrahydrofolate (CH_2THF) to α-ketoisovalerate by KPHMT, to give ketopantoate. A high-resolution crystal structure of KPHMT with ketopantoate bound had been determined, and through the use of computer modeling and ^{18}O labeling, a mechanism for this reaction is proposed.

101. An undergraduate teaching laboratory involving the isolation and characterization of lactate dehydrogenase. Resa M. Kelly and Richard M. Hyslop. Department of Chemistry and Biochemistry, University of Northern Colorado, 1380 Ross Hall, Campus box 98, Greeley, CO 80639 [fax (970) 351-1269, R6kelly@aol.com]

Providing students with laboratory experiences that reinforce biochemical concepts taught via lectures is important in the development of students' understanding. Protein purification and enzyme characterization are fundamental laboratory concepts that are typically introduced in biochemistry lectures pertaining to proteins and enzymes. These concepts may have more meaning if they are associated with laboratory experiences. The focus of this research was to develop robust isolation and characterization techniques of the enzyme lactate dehydrogenase for the eventual implementation into a general undergraduate biochemistry laboratory setting. The characterization of the enzyme involved studying effects of temperature and pH on enzyme activity. In addition, three chemicals, α-ketobutyric acid, 2-chloro-

propionic acid, and 3-chloropropionic acid, with structures similar to the substrate pyruvate were studied to learn how they affect the lactate dehydrogenase-catalyzed reaction between pyruvate and the reduced form of nicotinic adenine dinucleotide. Currently, methods for isolating lactate dehydrogenase from bovine heart are being developed.

102. Structure and mechanism of enzymes mediating oxalate metabolism. Stefan Jonsson,[1] Drazenka Svedruzic,[1] Ewa Wroclawska,[1] Christopher H. Chang,[1] **Nigel G. J. Richards**,[1] Ylva Lindqvist,[2] and Stefano Ricagno.[2] [1]Department of Chemistry, University of Florida, Box 117200, Gainesville, FL 32611-7200 [fax (352) 392-7918, sjonsson@chem.ufl.edu, richards@qtp.ufl.edu], and [2]Molecular Structural Biology, Karolinska Institute

Almost all of the enzymes that are involved in the synthesis and degradation of oxalate are poorly understood. This poster will describe recent results from kinetic and site-directed mutagenesis experiments, and theoretical calculations that set the scene for a detailed mechanistic understanding of *Oxalobacter formigenes* formyl-CoA transferase and *Bacillus subtilis* oxalate decarboxylase. The CoA transferase is a member of a new class of coenzyme A transferases, while the decarboxylase is an Mn-dependent enzyme the catalytic mechanism of which remains to be fully elucidated.

103. Mechanistic insights revealed through characterization of a novel chromophore in selenophosphate synthetase from *Escherichia coli*. Matt D. Wolfe. Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, 50 South Dr., Bethesda, MD 20892-8012 (wolfem@nhlbi.nih.gov)

Selenium commonly performs its biological role as either selenocysteine or a base modification in tRNA. The specific incorporation of selenium into both molecules requires selenophosphate ($SePO_3$), the formation of which is catalyzed in an Mg/ATP-dependent reaction by selenophosphate synthetase (SPS). Here, the characterization of a previously unrecognized chromophore covalently attached to SPS is presented. The UV−vis spectrum of SPS has a feature centered at 315 nm ($\epsilon_{315} \sim$ 2-4 mM^{-1}) that ionizes with a pK_a of ~4. Zn^{2+}, an inhibitor of SPS, reversibly quenches the 315 nm absorption, and mutagenesis studies indicate two N-terminal cysteine residues are involved in chromophore formation. Upon incubation with both Mg^{2+} and ATP, the chromophore shifts to 340 nm, and the shift requires a nucleotide having a hydrolyzable γ-phosphate. These data indicate that the chromophore either is directly involved in phosphoryl transfer or reflects a phosphorylation-dependent conformational change in SPS.

104. Ricin A chain substrate specificity and role of binding interactions in catalysis. Timothy K. Amukele and Vern L. Schramm. Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., F304, Bronx, NY 10461 [fax (718) 430-8565, amukele@aecom.yu.edu]

Ricin A chain is the catalytic monomer of the heterodimeric cytotoxin, Ricin, which depurinates the ribosome at A^{4324}, resulting in ablation of protein translation. The structure of the ribosome in this region is a stem with a GAGA tetraloop. Short stem−loop motifss with GAGA tetraloops are also substrates for RTA. Earlier characterization of RTA activity on short stem−loop motifs showed two clear trends. The first is that RNA 10-mers (3 bp stem) are 10-fold more active as substrates than DNA 10-mers. The second is that RNA stem−loop motifs show an up to 500-fold change in k_{cat} as the number of base pairs in the stem goes from 2 to 5; however, this characterization of activity as a function of stem length had not been carried out on DNA substrates. To deduce the structural correlates of these phenomena, we kinetically characterized RNA−DNA hybrid stem−loop motifs and DNA stem−loop motifs of varying stem lengths as substrates for RTA.

105. Mechanistic investigation of MEP synthase: Syntheses and enzymic evaluation of fluorinated DXP analogues. David T. Fox and C. Dale Poulter. Department of Chemistry, University of Utah, 315 S. 1400 E, Salt Lake City, UT 84105 [fax (801) 581-4391, davidfox@chem.utah.edu]

Methylerythritol phosphate synthase (MEP synthase) catalyzes the first committed step in the recently discovered methylerythritol phosphate pathway. This route for isoprenoid biosynthesis, found in eubacteria, green algae, and plant chloroplasts, presents a new target for antibacterial drugs and herbicides. MEP synthase catalyzes a rearrangement of the carbon skeleton and NADPH-dependent reduction of 1-deoxy-D-xylulose 5-phosphate (DXP) to give 2C-methylerythritol 4-phosphate (MEP). The stereochemical course for the transformation is well-documented; however, the detailed reaction mechanism remains elusive. The chemical syntheses of fluorinated DXP analogues are presented as putative substrates/inhibitors of MEP synthase. Steady-state kinetic studies with the fluorinated analogues provide evidence the MEP synthase active site has strict size and functionality requirements. Additional kinetic studies providing insight into the reaction mechanism will be discussed.

106. Studies on the mechanism of estrone sulfatase. Cheri L. Stowell and Theodore S. Widlanski. Department of Chemistry, Indiana University, Bloomington, IN 47405 [fax (812) 855-8300, cstowell@indiana.edu]

Estrone sulfatase is a membrane-bound enzyme that is selectively overexpressed in breast cancer cells. Recent discoveries involving the active site of other sulfatases suggest that estrone sulfatase undergoes a rare posttranslational modification that results in an active site formyl glycine. The proposed mechanism is similar to the cleavage of phosphate esters by alkaline phosphatase. While much is known about the mechanism of alkaline phosphatase, relatively little is known about the mechanism of estrone sulfatase. To further study the mechanism of estrone sulfatase, we determined a β_{lg} of −0.7 for sulfate esters. We have also developed a method for looking at the ^{34}S isotope effects on the cleavage of sulfate esters by estrone sulfatase.

107. Mechanistic studies of reactions catalyzed by 2,4-dienoyl-CoA reductase. Ding Li, Gong Chen, Xiusheng

Chu, and Wenhua Yu. Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, Hong Kong (fax 852 2788 7406, bhdingli@cityu.edu.hk)

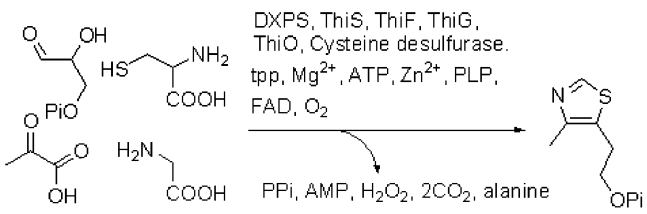
Numerous diseases have been reported in relation to fatty acids, such as cardiovascular disease, cancer, and diabetes. The regulation of fatty acid oxidation has been reported as a potential method of treating non-insulin-dependent diabetes mellitus (NIDDM), and inhibitors of enzymes involved in the metabolism of fatty acids have been studied as potential medicines. Mitochondrial 2,4-dienoyl-CoA reductase is a key enzyme for the β-oxidation of unsaturated fatty acids. The deficiency of 2,4-dienoyl-CoA reductase is one of seven common fatty acid oxidation disorders. We have overexpressed active human mitochondrial 2,4-dienoyl-CoA reductase in a bacterial system and purified the enzyme in one step with a metal affinity column. A variety of substrate analogues have been synthesized and incubated with 2,4-dienoyl-CoA reductase for the mechanistic study of enzyme-catalyzed reactions. Site-directed mutagenesis of some highly conserved residues has also been carried out.

108. Studies on the mechanism of histone deactylase enzymes. Jennifer L. Gronlund, Michael K. Yu, Stephen Stayrook, A. Melissa Concepcion, Shu He, and Dewey G. McCafferty. Department of Biochemistry and Biophysics and Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 (gronlund@mail.med.upenn.edu)

Histone deacetylases regulate chromatin remodeling and transcriptional silencing by removing acetyl groups from conserved lysine residues located on the N-terminal tails of histones. A novel continuous assay was developed to examine the activity of histone deacetylases. Four representative HDACs (Hos3, HDLP, HDAC2, and HDAC8) were employed to verify the broad utility of this assay. Additionally, mechanistic inhibition studies were performed using the Hos3 enzyme with several known HDAC inhibitors, including trichostatin A. A stimulatory effect on enzyme activity of Mn(II) was observed for Hos3 and HDLP. Crystals of HDLP soaked in buffer containing Mn(II) (5 mM) and Zn(II) (100 μM) diffracted to 2.1 Å. Anomalous diffraction experiments revealed that Mn(II) is localized to the previously reported Zn(II) binding site, possibly suggesting that high levels of Mn(II) may stimulate activity by replacing the catalytic Zn(II) ion. Potential implications for histone deacetylase function are discussed.

109. The mechanism to the thiazole−phosphate moiety of thiamine in *Bacillus subtilis*. Pieter C. Dorrestein, Huili Zhai, Ethan Settembre, Joo-heon Park, Fred W. McLafferty, Steven Ealick, and Tadhg Begley. Department of Chemistry and Chemical Biology, Cornell University, G75 Chemistry Research Building, Ithaca, NY 14850 (pcd3@cornell.edu)

This poster describes the elucidation of the function and mechanism of all the enzymes involved in the formation of the thiazole moiety of vitamin B_1. Thiazole phosphate biosynthesis in *Bacillus subtilis* requires four substrates, six enzymes, two metals, oxygen, and four additional cofactors. We describe the mechanism and roles of each of the enzymes involved in the biosynthesis.



110. Kinetic characterization of carbonyl reductase activities from rabbit heart cytosol. Henry A. Charlier, Jr., Mark B. Cheney, and C. Mark Maupin. Department of Chemistry, Boise State University, 1910 University Dr., Boise, ID 83725-1520 [fax (208) 426-3027, hcharlier@chem10.boisestate.edu]

Anthracyclines are highly effective antitumor drugs, but are known to induce a potentially life-threatening cardiotoxicity, which severely limits their effectiveness. Alcohol metabolites of anthracyclines have been linked to the development of cardiotoxicity. Conversion of anthracyclines to these metabolites is catalyzed by carbonyl reductase (CR). Ion exchange chromatography was used to isolate CR activities present in rabbit heart cytosol, and these activities were kinetically analyzed using several CR substrates. Several chromatographically distinct enzymatic activities were found in rabbit hearts that have unique specificities for the anthracyclines, daunorubicin and doxorubicin, as well as the non-anthracycline substrates, menadione and 4-benzoylpyridine. From kinetic studies, estimates of V_{max}, K_m, and V_{max}/K_m were determined for each of the activities for the CR substrates. In some instances, partial substrate inhibition was observed for menadione. Results from kinetic analyses will be used to design anthracyclines that are poor substrates for CR, which may lower the risk of cardiotoxicity.

111. Monobromobimane occupies a distinct xenobiotic substrate site in glutathione *S*-transferase π. Luis A. Ralat and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 [fax (302) 831-6335, ralat@udel.edu]

Monobromobimane (mBBr) functions as a substrate of porcine glutathione *S*-transferase π (GST π): the enzyme catalyzes the reaction of mBBr with glutathione. In the absence of glutathione, monobromobimane inactivates GST π at pH 7.0 and 25 °C as assayed using mBBr as a substrate, with a weaker effect on the enzyme's use of 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. These results suggest that the sites occupied by CDNB and mBBr are not identical. The extent of inactivation is proportional to incorporation of 2 mol of bimane/mol of subunit. Modification of GST π with mBBr does not interfere with its binding of 8-anilino-1-naphthalene sulfonate, indicating that the hydrophobic liganding site is not the target of monobromobimane. *S*-Methylglutathione and a nonreactive analogue of monobromobimane each yield partial protection against inactivation and decrease the level of reagent incorporation, while glutathionyl-bimane protects completely against inactivation. Peptide analysis after trypsin digestion indicates that mBBr modifies Cys^{45} and Cys^{99} equally. The level of modification

of Cys^{45} is reduced in the presence of *S*-methylglutathione, indicating that this residue is at or near the glutathione binding region. In contrast, the level of modification of Cys^{99} is reduced in the presence of *S*-(hydroxyethyl)bimane, suggesting that this residue is at or near the mBBr xenobiotic substrate binding site. Modification of Cys^{99} can best be understood by reaction with monobromobimane while it is bound to its xenobiotic substrate site in an alternate orientation. These results support the concept that glutathione *S*-transferase accomplishes its ability to react with a diversity of substrates in part by harboring distinct xenobiotic substrate sites. (Supported by NIH Grant R01 CA66561.)

112. N-Terminal region of the *Escherichia coli* pyruvate dehydrogenase complex E1 subunit interacts with the E2 subunit. Yun-Hee Park, Wen Wei, Leon Z. Zhou, Natalia Nemeria, and Frank Jordan. Chemistry, Rutgers University, 73 Warren St., Newark, NJ 07102 [fax (973) 353-1264, yhpark@pegasus.rutgers.edu]

The recently determined structure of the *Escherichia coli* pyruvate dehydrogenase complex E1 subunit (PDHc-E1) did not display electron density for N-terminal residues 1−55. FT-ICR MS experiments suggested that this region likely interacts with the E2 subunit. To test this finding, the following PDHc-E1 deletion variants were constructed: Δ6−15, Δ16−25, Δ26−35, Δ36−45, and Δ46−55. The overall PDHc activity was severely reduced for the first four constructs, but not for the Δ46−55 variant, while the E1-specific activity assay exhibited only small impairment. A functional assay (MALDI-TOF MS to ascertain reductive acetylation of E2 by E1 and pyruvate), along with light scattering and size-exclusion HPLC, showed that residues 6−45 are important in the interaction between E1 and E2. Significant differences were revealed in the functional assay relying on the isolated lipoyl domain rather than with the entire E2, pointing out the importance of using the intact subunits in such studies. (Supported by NIH Grant GM 62330.)

113. New role for the amino-terminal proline in the tautomerase superfamily: *trans*-3-Chloroacrylic acid dehalogenase. Hugo F. Azurmendi,[1] Susan C. Wang[2], Michael A. Massiah,[1] Christian P. Whitman,[2] and Albert S. Mildvan.[1] [1]Department of Biological Chemistry, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 [fax (410) 955-5759, azur@jhu.edu], and [2]Division of Medicinal Chemistry, University of Texas at Austin

CAAD converts 3-CAA to malonate semialdehyde and HCl. Sequence homology between CAAD, an $(\alpha/\beta)_3$ hexamer, and 4-oxalocrotonate tautomerase (4-OT) distinguishes CAAD from other dehalogenases, which form alkyl−enzyme intermediates. Mutagenesis of CAAD implicated Pro-1′(β) and Arg-11(α) as being mechanistically important. In the 4-OT-catalyzed conversion of β,γ-unsaturated keto acids to their α,β isomers, Pro-1 ($pK_a = 6.4$) functions as a base by abstracting the 3-proton while Arg-11 participates in both substrate binding and catalysis [Harris, T. K., et al. (1999) *Biochemistry 38*, 12343]. With CAAD, the effect of pH on k_{cat}/K_m indicates a catalytic base ($pK_a = 8.0$) and an acid ($pK_a = 9.1$). NMR titration of ^{15}N-labeled CAAD yields a pK_a of 9.3 for Pro-1, implicating Pro-1 as the acid catalyst. $^1H-^{15}N$ HSQC spectra of CAAD (3.6 mM sites, 5°, pH 7.6) showed nine of the ten Arg NϵH resonances (R_A to R_I), one of which (R_D) disappeared in the R11A mutant. At pH 8.9, all Arg−NϵH resonances disappeared due to fast exchange. Saturating with the substrate analogue, 3-chlorobutenoic acid (3-CBA) (16 mM), induced the reappearance of two Arg−NϵH signals, those of Arg-11 and of R_G which are protected against exchange by 3-CBA.

114. Understanding HTLV-I protease. Bryan E. Herger, Victoria L. Mariani, Kelly J. Dennison, and Suzanne B. Shuker. School of Chemistry and Biochemistry, Georgia Institute of Technology, 315 Ferst Dr., Atlanta, GA 30332-0363 [fax (404) 894-2295, herger@chemistry.gatech.edu]

HTLV-I protease is essential in the life cycle of HTLV-I. It is important to understand the properties of the protease in developing therapeutic agents which target this essential enzyme. In this work, we present several new observations. We have determined that the C-terminal region of the protease is not necessary for activy. We have also studied the effect of several mutations on proteolytic activity. Finally, we identified a new protease cleavage site in the pol polyprotein.

115. Investigation of substrate modulation in wild-type and disease-causing mutations of human short-chain acyl-CoA dehydrogenase. Amy K. Saenger,[1] Jerry Vockley,[2] and Marian T. Stankovich.[1] [1]Department of Chemistry, University of Minnesota, 207 Pleasant St. SE, Minneapolis, MN 55455 [fax (612) 626-7541, saenger@chem.umn.edu], and [2]Department of Medical Genetics, Mayo Clinic and Mayo Foundation

Human short-chain acyl-CoA dehydrogenase (hSCAD) is a flavoprotein that catalyzes the first step in the β-oxidation cycle, which produces up to 40% of the total human energy requirement. Previous work with acyl-CoA dehydrogenases (ACDs) has shown that these enzymes are specifically modulated upon binding of the substrate/product couple, allowing the reaction to proceed in a thermodynamically favorable manner. Deficiencies in hSCAD can lead to serious metabolic disorders, due to inhibition of energy production. Two nucleotide variations in the hSCAD gene, G625A and C511T, have been identified and associated with SCAD deficiency. These mutants have been purified and their redox properties characterized. Although substrate binding appears to be uncompromised by these mutations, spectroelectrochemical investigation reveals a lack of electron transfer from the substrate to the FAD cofactor, thus rendering the reaction unlikely. Electrochemical and binding studies with wild-type and mutant hSCAD will be presented and compared to studies with other ACD systems.

116. Establishing paradigms in the orotidine 5′-monophosphate decarboxylase suprafamily from studies of the reaction catalyzed by 3-keto-L-gulonate 6-phosphate decarboxylase. Wen Shan Yew,[1] Eric Wise,[2] Ivan Rayment,[2] and John A. Gerlt.[1] [1]Department of Biochemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave., Urbana, IL 61801 (wenyew@uiuc.edu), and [2]Department of Biochemistry, University of Wisconsin

The orotidine 5′-monophosphate decarboxylase (OMPDC) suprafamily bears testament to the opportunistic nature of

enzyme evolution. Although OMPDC and 3-keto-L-gulonate 6-phosphate decarboxylase (KGPDC) are two homologous members of this suprafamily that share both the $(\beta/\alpha)_8$-barrel fold and a conserved constellation of active site residues, they catalyze mechanistically distinct reactions. OMPDC catalyzes a metal ion-independent reaction that must avoid a vinyl anion intermediate, and KGPDC catalyzes an Mg^{2+}-dependent decarboxylation reaction found in the catabolic pathway of L-ascorbate utilization by *Escherichia coli* K-12. We present stereochemical and structural insights for the reaction catalyzed by KGPDC that are consistent with a mechanism involving an Mg^{2+}-stabilized enediolate anion intermediate. The results of these studies emphasize the opportunistic nature of divergent enzyme evolution, and form the basis for rational design and directed evolution efforts in the OMPDC suprafamily.

117. Contrasts between farnesyl pyrophosphate feedback inhibition of prokaryotic and eukaryotic mevalonate kinase. Henry M. Miziorko[1] and Natalya Voynova.[2] [1]Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226, and [2]Biochemistry Department, St. Petersburg State University

Feedback inhibition of mevalonate kinase (MK, EC 2.7.1.36) by farnesyl pyrophosphate (FPP), which is a competitive inhibitor with respect to ATP, has been reported for the enzyme from several sources. However, literature K_i values range from 10^{-8} to 10^{-5} M; this wide range may reflect differences in either the assay method or the enzyme source. To determine whether intrinsic differences in FPP inhibition distinguish prokaryotic and eukaryotic MKs, we isolated recombinant forms of *Staphylococcus aureus*, rat, and human MKs and, under consistent assay conditions, tested the inhibition of these enzymes by both FPP and (*S*)-farnesyl thiodiphosphate (FSPP). Competitive inhibition with respect to ATP is observed for both compounds. The thio analogue FSPP exhibits inhibitory effects that are comparable to those of FPP. However, both eukaryotic proteins (human and rat MK) are more sensitive to these inhibitors than is the *S. aureus* MK. For FPP, human MK $K_i = 35$ nM, rat MK $K_i = 348$ nM, and *S. aureus* MK $K_i = 46$ μM. For FSPP, human MK $K_i = 29$ nM, rat MK $K_i = 473$ nM, and *S. aureus* MK $K_i = 45$ μM. Thus, the heterologous nature of eukaryotic and prokaroytic MK proteins may correlate with differences in sensitivity to feedback inhibition. (Supported by NIH Grant DK53766.)

118. Kinetic and chemical mechanism of 1-deoxy-D-xylulose-5-phosphate isomeroreductase from *Mycobacterium tuberculosis*. Argyrides Argyrou and John S. Blanchard. Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 [fax (718) 430-8565]

1-Deoxy-D-xylulose-5-phosphate (DXP) isomeroreductase catalyzes the first committed step of the 2-*C*-methylerythritol-4-phosphate pathway of isoprenoid biosynthesis. We have cloned, expressed, and purified the *Mycobacterium tuberculosis* DXP isomeroreductase. DXP isomeroreductase is a metal-activated enzyme displaying superior specificity for Co^{2+}, good specificity for Mn^{2+}, and poor specificity for Mg^{2+}. Although NADPH is preferred over NADH by ~100-fold as evaluated by the relative k_{cat}/K_m values, the maximum turnover numbers are similar, suggesting that the 2′-phosphate of NADPH contributes mostly to binding and not to transition-state stabilization. The results of product inhibition studies and isotope effects are most consistent with a random mechanism. Significant isotope effects were observed with [4*S*-^{2}H]NAD(P)H, establishing that the enzyme promotes transfer of the C_4-*proS* hydride of the reduced pyridine nucleotide. The magnitude of these isotope effects varied with metal and pyridine nucleotide identities. The results are discussed in terms of significant differences in the commitment factors with the various metals and pyridine nucleotides.

119. Chemical probes of the catalytic mechanism of UDP-galactopyranose mutase. Erin E. Carlson,[1] Michelle L. Soltero-Higgin,[2] and Laura L. Kiessling.[3] [1]Department of Chemistry, University of Wisconsin—Madison, 1101 University Ave., Madison, WI 53706 [fax (608) 262-0381, carlson@chem.wisc.edu], [2]Department of Biochemistry, University of Wisconsin—Madison, and [3]Departments of Chemistry and Biochemistry, University of Wisconsin—Madison

According to the World Health Organization, tuberculosis (TB) has recently made a resurgence, infecting one-third of the world's population. The increased death toll is partially a result of antibiotic resistant strains. Consequently, development of new drugs is important. UDP-galactopyranose mutase (UGM) is the enzyme responsible for the isomerization of UDP-galactopyranose (UDP-galp) into UDP-galactofuranose (UDP-galf) during cell wall construction of a number of bacteria, protozoa, and fungi and is necessary for cell viability. Although the mechanism of this transformation is controversial, enzymatic activity is dependent on a fully reduced flavin adenine dinucleotide cofactor (FAD). We propose that FAD is directly involved in the transformation, acting as a nucleophile. Computational studies were carried out to examine the nucleophilicity of FAD. We plan to monitor enzymatic activity in the presence of several FAD analogues; new synthetic routes to both 1-deaza- and 5-deazariboflavin were completed. Design and application of a high-throughput screen for UGM are currently under investigation.

120. Circular dichroism signatures for the amino and imino tautomers of thiamin diphosphate on enzymes. Ahmet T. Baykal, **Ebenezer Joseph**, Natalia Nemeria, and Frank Jordan. Chemistry, Rutgers University, 73 Warren St., Newark, NJ 07102 [fax (973) 353-1264, atbaykal@hotmail.com, edj@pegasus.rutgers.edu]

We recently reported that there are two circular dichroism (CD) signatures evident in the spectra of the E477Q variant of yeast pyruvate decarboxylase (YPDC), a positive one centered near 305 nm and a negative one centered at 320 nm. We have assigned the band at 305 nm to the 1′,4′-imino tautomer of thiamin diphosphate (ThDP) both on YPDC and on the E1 subunit of the *Escherichia coli* pyruvate dehydrogenase complex (PDHc-E1). There is evidence for the 320 nm band both on YPDC and on PDHc-E1 in the presence of substrate or substrate analogues. On the basis of models for the aromatic rings of ThDP, the CD band at 320 nm is now associated with a charge transfer transition

within the coenzyme itself, ThDP being constrained in the V conformation, and the 4′-aminopyrimidine ring being in its neutral ionization state in the amino tautomeric form. (Supported by NIH Grant GM-62330.)

121. Direct binding of the N-terminus of HTLV-1 Tax oncoprotein to cyclin-dependent kinase 4 is a dominant path for stimulating the kinase activity. Junan Li,[1] Ming-Daw Tsai,[2] and Hongyuan Li.[2] [1]Department of Chemistry, The Ohio State University, Johnston Lab, Room 316, 176 W. 19th Ave., Columbus, OH 43202 [fax (614) 292-1532, li.225@osu.edu], and [2]Department of Chemistry, The Ohio State University

The involvement of Tax oncoprotein in the INK4-CDK4/6-Rb pathway has been regarded as a key factor for immortalization and transformation of human T-cell leukemia virus 1 (HTLV-1)-infected cells. In both p16 −/− and +/+ cells, expression of Tax has been correlated with an increase in CDK4 activity, which subsequently increases the level of phosphorylation of Rb and drives the infected cells into cell cycle progression. In relation to these effects, Tax has been shown to interact with two components of the INK4-CDK4/6-Rb pathway, p16 and cyclin D(s). While Tax competes with CDK4 for p16 binding, thus suppressing p16 inhibition of CDK4, Tax also binds to cyclin D(s) with concomitant increases in both CDK4 activity and the level of phosphorylation of cyclin D(s). Here we show that both Tax and residues 1−40 of the N-terminus of Tax, Tax40N, bind to and activate CDK4 in vitro. In the presence of INK4 proteins, binding of Tax and Tax40N to CDK4 counteracts the inhibition of p16 and p18, and acts as the major path for regulating Tax-mediated activation of CDK4. We also report that Tax40N retains the transactivation ability. These results of in vitro studies demonstrate a potentially novel, p16-independent route for regulating CDK4 activity by the Tax oncoprotein in HTLV-1-infected cells.

122. Loop movements and catalysis in creatine kinase. George L. Kenyon,[1] Pan-Fen Wang,[1] Allen J. Flynn,[1] Michael J. McLeish,[1] Sushmita D. Lahiri,[2] and Karen N. Allen.[2] [1]College of Pharmacy, University of Michigan, 428 Church St., Ann Arbor, MI 48109 (gkenyon@umich.edu), and [2]Department of Physiology and Biophysics, Boston University School of Medicine

Recently, the X-ray structure of creatine kinase from *Torpedo californica* (TcCK) was determined to 2.1 Å resolution with a substrate, MgADP, and a transition state analogue complex (TSAC) composed of MgADP, nitrate, and creatine, bound in the active site. The latter structure differs significantly from that of the MgADP-bound structure, with the most striking difference being the movement of two loops (comprising residues 60−70 and residues 323−332) into the active site in the transition state structure. This movement effectively occludes the active site from solvent, and the loops appear to be locked into place by a salt bridge formed between His66 and Asp326. Subsequently, we have obtained structures of TcCK bound to several substrates and/or products. These have confirmed that the major loop movement is confined to the enzyme bound to the TSAC. Further, we have used site-directed mutagenesis to demonstrate that both His66 and Asp326 play significant roles in creatine kinase catalysis.

123. Mechanistic studies of FtsZ, the tubulin homologue in *Mycobacterium tuberculosis*. Pravin A. Nair,[1] Loertha Carlisle-Moore,[1] Caroline Kisker,[2] Richard Slayden,[3] Teruo Kirikae,[4] Iwao Ojima,[1] and Peter J. 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], [2]Department of Pharmacological Sciences, State University of New York at Stony Brook, [3]Department of Microbiology, Immunology and Pathology, Colorado State University, and [4]Department of Infectious Diseases and Tropical Medicine, International Medical Center of Japan

FtsZ is the prokaryotic homologue of tubulin. In a process strongly reminiscent of microtubule formation by tubulin, FtsZ polymerizes into filaments, which assemble into a highly dynamic structure known as the Z-ring on the inner membrane during cell division. Inactivation of FtsZ results in the complete loss of septum formation, making this protein essential for cell division. Compounds originally designed to interfere with the (de)polymerization of tubulin have antibacterial activity against *Mycobacterium tuberculosis*, the organism that causes tuberculosis, and we are currently testing the hypothesis that these compounds exert their anti-mycobacterial activity by inhibiting FtsZ (de)polymerization. We are initially focusing on the tubulin polymerization inhibitors albendazole and thiabendazole. These compounds have MICs toward H37Rv of 61 and 80 mM, respectively, and inhibit cell division, resulting in the formation of abnormally elongated cells. In our lab, we are investigating the effect of these compounds on the polymerization and GTPase activity of FtsZ. The tools being used to elucidate the effect of these inhibitors are light scattering assays, GTPase assays, X-ray crystallography, and analytical ultracentrifugation.

124. Biochemical characterization of 4-hydroxymandelate synthase (HmaS): A non-heme iron-dependent dioxygenase. Oliver W Choroba[1] and Jonathan B. Spencer.[2] [1]Cambridge Centre for Molecular Recognition, Department of Chemistry, Cambridge University, Lensfield Road, Cambridge CB2 1EW, United Kingdom (fax 0044-1223-336362, owc20@cam.ac.uk), and [2]Cambridge Center for Molecular Recognition, Department of Chemistry, Cambridge University

Non-heme iron-dependent dioxygenases have increasingly become the focus of many studies within the large field of metalloproteins. We recently described a non-heme iron-dependent dioxygenase from the biosynthetic gene cluster of glycopeptide antibiotics which is most homologous to 4-hydroxyphenylpyruvate dioxygenase. The enzyme was shown to catalyze the conversion of 4-hydroxyphenylpyruvate to 4-hydroxymandelate, the first committed precursor toward the rare amino acid constituent 4-hydroxyphenylglycine, and subsequently named 4-hydroxymandelate synthase (HmaS). The reaction requires molecular oxygen which is fully incorporated into the product molecule. Here we present a comprehensive study of the stereochemical course of the reaction together with a kinetic and mechanistic characterization.

125. Spectral and kinetic properties of a novel periplasmic catalase-peroxidase. Cornelius L. Varnado, Kristen Hertwig,

Robert Thomas, J. Kenneth Roberts, and Douglas C. Goodwin. Department of Chemistry, Auburn University, 179 Chemistry Blvd., Auburn, AL 36849 [fax (334) 844-6959, varnaco@auburn.edu]

Enterohemorrhagic *Escherichia coli* O157:H7 is a highly virulent and deadly food-borne pathogen. Enterohemorrhagic *E. coli* O157:H7 carries a unique plasmid-encoded periplasm-targeted catalase-peroxidase (KatP) that is absent from nonpathogenic *E. coli*. Thus, KatP may be a 0157:H7 virulence factor. Electrophoretic analyses of expressed and partially purified recombinant KatP revealed the presence of two polypeptides. Further purification by FPLC showed that heme and enzyme activity were associated with only one of these. The absence of the targeting signal peptide from the active polypeptide was demonstrated by N-terminal sequencing. Visible absorption spectra recorded for KatP were consistent with similar spectra recorded for other catalase-peroxidases. Likewise, the k_{cat} for the catalase activity of KatP (7500 s^{-1}) was nearly identical to that observed for other similar enzymes. However, the K_M of KatP for H_2O_2 was 5-fold greater than that of other catalase-peroxidases. Potential mechanisms by which KatP may act as a virulence factor will be discussed.

126. Catalytic contribution of a conserved histidine in a mobile loop of phosphoribulokinase. Jennifer A. Runquist and Henry M. Miziorko. Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226

The structure of *Rhodobacter sphaeroides* phosphoribulokinase (PRK) indicates there are several mobile regions of the protein that are not well-defined in the published model, which contains no bound substrates. Included among these regions is the loop containing invariant residues Y98 and H100. To evaluate the potential contribution of these invariant residues to substrate binding and/or catalytic efficiency, Y98L, H100A, H100N, and H100Q mutants have been constructed, expressed, and isolated. The structural integrity of the mutants has been tested by measuring the binding stoichiometry of the fluorescent alternative substrate, trinitrophenyl-ATP; binding stoichiometries of the mutant PRKs are comparable to that of wild-type PRK, suggesting that the substrate binding site is substantially intact. Similarly, ATP saturation curves for mutant PRKs are comparable to that of wild-type PRK. Y98L exhibits a 40-fold increase in K_m for ribulose 5-phosphate, whereas H100 mutants exhibit only 8-14-fold effects. The V_m of Y98L is 27-fold smaller than the V_m for wild-type PRK. In contrast, H100A, H100N, and H100Q exhibit significant decreases in V_m of 2600-, 2300-, and 735-fold, respectively. The results suggest that, during turnover, the mobile region containing Y98 and H100 must be positioned as part of the PRK active site. Moreover, the basic side chain of H100 significantly contributes to the catalytic efficiency. (Supported by Department of Energy Grant DE-FG02-00ER15100.)

127. Distributive substrate processing mechanism for the *Saccharomyces cerevisiae* histone deacetylase Hos3. Shu He,[1] Jennifer L. Gronlund,[1] Fanyu Meng,[2] Neil L. Kelleher,[2] and Dewey G. McCafferty.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 (shuhe@mail.med.upenn.edu), and [2]University of Illinois at Urbana-Champaign

Histone deacetylases catalyze the removal of acetyl groups from acetylated lysine residues on the highly conserved histone N-terminal tails. Limited information exists on both the substrate specificity and enzymatic mechanism of these enzymes. We have overexpressed and purified Hos3, a histone deacetylase from *Saccharomyces cerevisiae*, and synthesized hyperacetylated peptide substrates corresponding to the N-termini of histones H2A, H2B, H3, and H4 using SPPS. The deacetylation process and substrate specificity of Hos3 were investigated using MALDI-TOF and high-resolution FT-ESI mass spectrometry. Results of the enzyme assay indicated that Hos3 deacetylates nearly *every* physiologically relevant Ac-Lys residue on these peptides. Furthermore, time course analysis confirmed a distributive deacetylation mechanism whereby each binding event is accompanied by a single Ac-Lys hydrolysis. These data suggest that the exquisite positional specificity exhibited by histone deacetylases *in vivo* is likely directed by the protein microsequence context, fold, posttranslational modification states, or interactions with partner proteins or DNA.

128. Cloning and optimizing recombinant expression of human histone deacetylase 8. Stephanie L. Gantt and Carol A. Fierke. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 [fax (734) 647-4865, gantts@umich.edu]

Histone deacetylase 8 (HDAC8) is a member of the class I family of histone deacetylases. These metalloenzymes play an important role in the regulation of gene expression by removing acetyl groups from Ac-Lys residues on the N-terminal histone tails. HDAC8 was cloned from a human cDNA library into a bacterial expression vector with a C-terminal His tag. The initial HDAC8 protein expression level in *Escherichia coli* was lower than desired; therefore, the effects of improving codon usage and varying growth conditions (growth media, inducer concentration, temperature, time of induction, and presence of protease inhibitors) on HDAC8 expression were studied. Future work will look at the chemical mechanism and metal dependence of HDAC8 using a novel radioisotope assay that is under development. [Supported by NIH Grant RO1 GM40602 (to C.A.F.) and an NSF Predoctoral Fellowship (to S.L.G.).]

129. Deuterium isotope effects on ^{13}C chemical shifts and one-bond coupling behavior in hydrogen-bonded alcohol−amine complexes. Nakul C. Maiti,[1] Anthony S. Serianni,[2] and Vernon E. Anderson.[1] [1]Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106 (nxm50@po.cwru.edu), and [2]Department of Chemistry and Biochemistry, University of Notre Dame

NMR investigations have been carried out on hydrogen-bonded complexes between hexafluoroisopropanol (HFIP) and different amines with varying pK_a values. Deuterium isotope (H/D) effects on the chemical shift of the secondary carbon, $^1\Delta C$(C−D), were measured for HFIP and its H-bonded complex with trimethylamine. $^1\Delta C$(C−D) was greater in the amine complex than in the monomer in chloroform. $^1\Delta C$(C−D) was calculated for the gas phase

monomer and trimethylamine−HFIP complex. The geometries of the free and complexed HFIP were optimized with density functional theory [B3LYP/6-31G (d,p)]. Following Wolinski et al. [(1990) *J. Am. Chem. Soc. 112*, 8251] and Abildgaard et al. [(1998) *J. Am. Chem. Soc. 120*, 9063−9069], the nuclear shielding and the first derivative of the ^{13}C nuclear shielding with respect to C−H bond stretching ($d\sigma/dR_{CH}$) have been calculated with the same basis set using the gauge-independent atomic orbital (GIAO) method. The change in the R_{CH} distance upon deuteration [$\Delta R_{CH(D)}$] was obtained from a potential scan of C−H bond stretching and by analyzing the data with a fitted Morse function. $^1\Delta C$(C−D) was calculated as the product of $d\sigma/dR_{CH}$ and $\Delta R_{CH(D)}$. A one-bond coupling constant ($^1J_{CH}$) was also measured as a potential spectroscopic indicator of H-bond strength. As the strength of an H-bond increases, $^1J_{CH}$ decreases. The maximum $^1J_{CH}$ of HFIP was 149 Hz in $CHCl_3$ and decreased to 142 Hz in the triethylamine (complex) that formed a strong H-bond with the OH group of HFIP. Calculation of $^1J_{CH}$ in amine complexes with MeOH also shows decreases with H-bond formation. These NMR results are consistent with the observation that H-bond donation by alcohols results in a decrease in the bond order of αC−H groups arising from enhanced negative hyperconjugation. The decrease in bond order (increase in bond length) causes the decrease of the $^1J_{CH}$ coupling. The larger $^1\Delta C$(C−D) in the amine complex results from an increase in the anharmonicity of the weakened bond. Both NMR parameters could be used as gauges for H-bond strength in macromolecular complexes.

130. Expression and refolding of recombinant tobacco peroxidase from *Escherichia coli* inclusion bodies. Irina G. Gazaryan,[1] Pavel A. Savitski,[2] Alexandra M. Rojkova,[3] Dmitry M. Hushpulian,[3] Tatyana A. Chubar,[3] Victoria A. Fechina,[2] Ivan Yu. Sakharov,[3] Vladimir I. Tishkov,[3] and L. Mark Lagrimini.[4] [1]Burke Medical Research Institute, 785 Mamaroneck Ave., White Plains, NY 10605 [fax (914) 597-2757, igazarya@burke.org], [2]A. N. Bach Institute of Biochemistry of the Russian Academy of Sciences, [3]Department of Chemical Enzymology, School of Chemistry, Moscow State University, and [4]Syngenta Biotechnology

The properties of native tobacco anionic peroxidase superproduced by tobacco and tomato plants were studied in detail in this laboratory. The enzyme exhibited a number of unusual catalytic characteristics, such as high activity in the luminescence reaction with luminol and stability in extremely acidic media in the presence of calcium ions. To evaluate the contribution of Glu-141 at the entrance to the heme-binding pocket to the unique properties of the enzyme, the recombinant tobacco peroxidase have been produced and compared to its Glu141Phe mutant form mimicking the active site of horseradish peroxidase. Coding DNA of the tobacco anionic peroxidase gene was cloned into the pET40b vector. The problem of 11 "rare" arginines encoded in the tobacco peroxidase gene was solved by using the *Escherichia coli* BL21(DE3) CdPlus strain. The expression level of the tobacco peroxidase apoprotein in the above strain was ca. 40% of that of the total protein. The optimization of tobacco peroxidase refolding was performed on the basis of the protocol developed earlier for recombinant horseradish peroxidase. The refolding was followed by ultrafiltration and gel filtration to yield active enzyme (RZ > 3.0) with a specific activity of 900 units/mg with ABTS as a substrate. The Glu141Phe mutant was constructed with the QuikChange kit from Stratagene. Wild-type and mutant forms of the recombinant tobacco enzyme (MW = 33 kDa, pI 3.5) were compared in their activity toward ABTS, phenol, luminol, and veratryl alcohol. (Supported by INCO-Copernicus Grant ICA2-CT-2000-10050.)

131. Reduction of oxyferrous cytochrome P450 2B4 by 5′-deazaFAD T491V cytochrome P450 reductase. Lucy Waskell,[1] Haoming Zhang,[1] Larry Gruenke,[1] Dave Arscott,[1] Anna Shen,[2] and Charles Kasper.[2] [1]Department of Anesthesiology, University of Michigan, 2215 Fuller Rd., Ann Arbor, MI 48105 [fax (734) 213-6985, waskell@umich.edu], and [2]Department of Oncology, University of Wisconsin

The rate of reduction of microsomal oxyferrous cyt P450 2B4 by 5′-deazaFAD T491V cyt P450 reductase has been measured using a reductase capable of donating a single electron. A mutant reductase in which FAD has been replaced with a redox inactive 5′-deazaFAD cofactor has been prepared. Using 5′-deazaFAD T491V reductase, it has been possible to demonstrate with stopped-flow spectrophotometry that a step following reduction of oxyferrous cyt P450 is rate-limiting. This step may be protonation of the peroxo or hydroperoxo cyt P450 intermediate. Under these experimental conditions, 33% of the cyt P450 produces the product, norbenzphetamine, which was quantitated by LC−MS/MS. The rate constant for oxyferrous cyt P450 oxidation (k = 0.09 s^{-1}) accounts for the rate of product formation at 15 °C under steady state conditions. In a similar experiment, cyt b_5 reduces oxyferrous cyt P450 which immediately oxidizes without formation of an intermediate and produces the product with an efficiency of 52%.

132. Camphor analogues as probes for the investigation of the involvement of second oxidants in the mechanism of cytochrome P450-CAM. Shengxi Jin, Thomas A. Bryson, and John H. Dawson. Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter St., Columbia, SC 29208

Cytochrome P450 is a versatile heme-containing oxygenase that transfers oxygen atoms from dioxygen to a wide range of organic substrates. Hydroxylation involves substrate binding to the ferric enzyme followed by reduction and oxygen binding to give the oxyferrous state. Addition of a second electron yields a peroxoferric intermediate, protonation of which generates a hydroperoxoferric state; loss of water then forms an oxoferryl porphyrin radical (Compound I). Although the latter is thought to be the ultimate oxidant, the peroxo- and hydroperoxoferric species have been proposed as secondary oxidants. The T252A mutant of P450-CAM does not hydroxylate camphor; therefore, we believe it must not form P450 Compound I. However, it still accepts electrons from NADH to give hydrogen peroxide, presumably via the peroxo- and hydroperoxoferric intermediates. Thus, T252A P450-CAM is the ideal mutant for testing whether one and/or the other of these two species is capable of O-atom transfer.

We have prepared a series of camphor analogues that contain reactive functional groups that are the site of O-atom transfer to investigate the mechanism of olefin epoxidation,

ether O-dealkylation, amine N-dealkylation, thioether sulfoxidation, and *N*-hydroxyimine denitrosation. With 5-methylenylcamphor, for example, we find that the T252A mutant is enzymatically active in olefin epoxidation, producing epoxide at 20% of the wild-type rate. This clearly demonstrates that a second active oxidant is formed in the P450 reaction cycle. Results of our studies of oxygen activation by P450 with these model substrates, with emphasis on the involvement of a second oxidant in O-atom transfer, will be presented.

133. Mechanism of mammalian protein geranylgeranyltransferase type I: Role of Mg(II). Heather L. Hartman, Katherine E. Bowers, and Carol A. Fierke. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055 (hhartma@umich.edu)

Protein geranylgeranyltransferase type I (GGTase-I) and protein farnesyltransferase (FTase) are collectively known as CaaX prenyltransferases, catalyzing thioether bond formation between a cysteine sulfur near the C-terminus of the protein substrate and C1 of the isoprenoid substrate. FTase and GGTase-I both contain a catalytically essential zinc ion. Additionally, Mg(II) accelerates the catalytic activity of FTase by coordinating and activating the pyrophosphate leaving group of the isoprenoid substrate. In contrast, Zhang and Casey in 1996 demonstrated that addition of either Mg(II) or metal chelating reagents has little effect on the steady-state turnover rate of GGTase-I, suggesting Mg(II) is not required for maximal activity. To further investigate the catalytic mechanism of GGTase-I, we have used single-turnover experiments to measure the rate constant of the prenyl transfer step catalyzed by GGTase-I in the presence of various Mg(II) and metal chelator concentrations. (Supported by NIH Grant GM40602 and GAANN Grant 037733.)

134. Metal binding Asp120 in metallo-β-lacatamse L1 from *Stenotrophomonas maltophilia* plays a crucial role in catalysis. James D. Garrity[1] and Michael W. Crowder.[2] [1]Department of Chemistry and Biochemistry, Miami University, 112 Hughes Hall, Oxford, OH 45056 (garritjd@muohio.edu), and [2]Department of Chemistry, Miami University

Metallo-β-lactamase L1 from *Stenotrophomonas maltophilia* is a dinuclear Zn(II) enzyme that contains a metal binding aspartic acid (common to most dinuclear, metal-containing, hydrolytic enzymes, particularly those with a β-lactamase fold) in a position to potentially play an important role in catalysis. Three site-directed mutants (D120C, D120N, and D120S) were prepared and characterized using metal analyses, CD spectroscopy, and pre-steady state and steady state kinetics. The mutants exhibited 10−1000-fold drops in k_{cat} values as compared to wild-type L1, and the following general trend of activity was observed for all substrates that were tested: wild type > D120N > D120C and D120S. We conclude that Asp120 plays a significant role in catalysis by properly orienting the bridging hydroxide, via an electrostatic interaction, for nucleophilic attack on the substrate. Data collected also demonstrate that Asp120 is crucial for binding of L1 to its full complement of Zn(II) and subsequently for proper substrate binding to the enzyme.

135. Determinants of metal ion specificity in carbonic anhydrase II. Tamiika K. Hurst, Andrea Stoddard, and Carol A. Fierke. Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109 (tkhurst@umich.edu)

Carbonic anhydrase II (CAII) is a zinc metalloenyzme that catalyzes the reversible hydration of CO_2. The zinc cofactor of CAII lies at the bottom of the active site cleft where it is coordinated to H94, H96, H119, and a solvent molecule in a tetrahedral geometry. Additionally, each of the direct ligands forms hydrogen bonds with a second shell of residues, Q92, E177, and T199. Wild-type CAII binds Cu(II) and Zn(II) with affinities of 17 fM and 1 pM, respectively. The zinc and/or copper affinity can be altered by substitutions in the direct ligands or the surrounding hydrophobic amino acids. Here we investigate the function of the second-shell hydrogen bond interactions in determining metal ion specificity. Knowledge of the structural features leading to high metal specificity and affinity in CAII will aid in the design and optimization of CAII-based metal ion biosensors.

136. Determinants of the peptide specificity of protein farnesyltransferase. Katherine A. Hicks,[1] Pam T. Wong,[2] Katherine E. Bowers,[2] and Carol A. Fierke.[2] [1]Department of Biological Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109-1055, and [2]Department of Chemistry, University of Michigan

Protein farnesyltransferase (FTase) catalyzes the addition of a 15-carbon lipid group to the C-terminus of a number of proteins. These proteins include important signaling proteins, such as Ras, that are involved in cancer progression. Thus, FTase inhibitors have been developed that target both Ras and additional, unidentified proteins. Previous work has indicated that FTase recognizes a C-terminal CAAX box sequence in its target proteins, in which C is the conserved cysteine, the A residues are aliphatic, and X is thought to determine specificity (Met, Ser, or Gln). To further characterize the peptide specificity of FTase, binding and kinetic studies have been carried out which indicate that the identity of the X group mainly influences binding affinity while an upstream polybasic sequence affects both binding affinity and catalytic efficiency. These studies thus highlight the thermodynamic and kinetic parameters underlying FTase specificity.

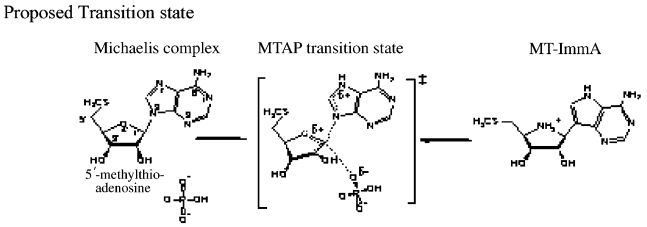
137. Structural investigations of the metal binding site in CopY. Melinda A. Harrison,[1] Kristina E. Odonish,[1] Paul A. Cobine,[2] and Charles T. Dameron.[1] [1]Department of Chemistry and Biochemistry, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282 [fax (412) 396-5683, harriso760@duq.edu], and [2]Department of Medicine and Biochemistry, University of Utah

Copper homeostasis in the Gram-positive bacterium *Enterococcus hirae* is controlled by the cop operon which encodes four genes: copY, copZ, copA, and copB. The zinc/copper binding site of the CopY repressor in *E. hirae* is the key to metal regulation of a copper uptake and resistance pathway. CopY has a unique metal binding site that requires Zn(II) for DNA binding activity. As part of the regulatory process, the Zn(II) is displaced by Cu(I). Although the structure of the protein has not been elucidated, previous research has shown that the copper:protein stoichiometry is two Cu(I) ions per monomer and copper is necessary for

dimer formation. A 19-amino acid fragment containing the Cu(I)CopY binding site was selected and studied through spectral methods. The peptide was studied to confirm that its metal binding properties are similar to those of CopY.

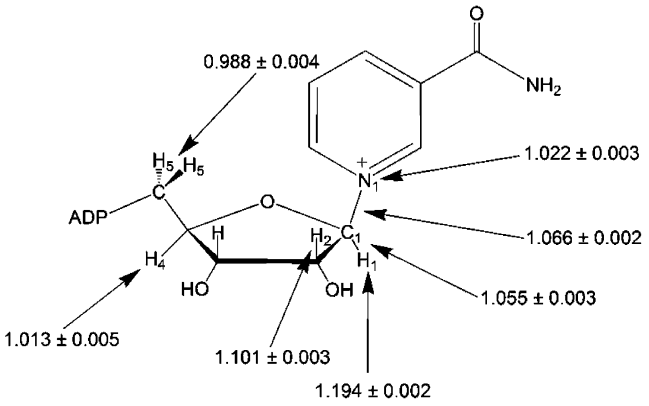
138. Targeting polyamine biosynthesis: Transition state of 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAP) and transition state analogue inhibitors. Vipender Singh,[1] Gary B. Evans,[2] Richard H. Furneaux,[2] Peter C. Tyler,[2] and Vern L. Schramm.[1] [1]Biochemistry, Albert Einstien College of Medicine, 1925 Eastchester Rd., 11E, New York, NY 10461 (vsingh@aecom.yu.edu), and [2]Industrial Research Ltd.

Polyamine synthesis is critical for DNA packaging in actively dividing cells. Inhibitors of polyamine biosynthesis are known anticancer agents. The MTAP reaction is unique to this pathway. Kinetic isotope effects (KIEs) have been used systematically to study the transition of enzymatic reactions. KIEs for the arsenolysis reaction of methylthioadenosine were measured with specifically labeled methylthioadenosine with the following labels: 5′-^{14}C, 1′-^{3}H, 1′-^{14}C, 2′-^{3}H, 3′-^{3}H, 4′-^{3}H, and 5′-^{3}H. Preliminary data suggest that the reaction proceeds through a transition state with ribooxacarbenium character. Phosphorolysis of purine *N*-ribosides by the closely related purine nucleoside phosphorylase is known to involve a similar transition state. On the basis of this precedent, we have synthesized a series of 5′-alkylthio derivatives of Immucillin-A, which we call alkylthio-Immucillin-A. Several inhibitors with picomolar affinity are described.



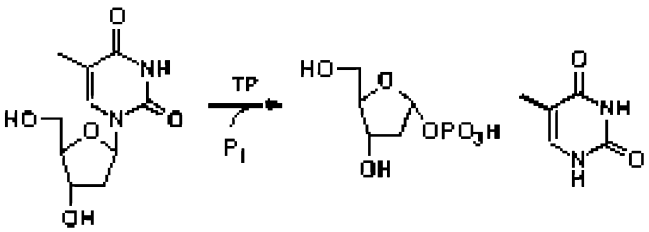
139. Transition state analysis for ADP ribosylation of eEF-2 by diphtheria toxin. Sapan L. Parikh[1] and Vern L. Schramm.[2] [1]Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Forch 304, Bronx, NY 10461 [fax (718) 430-8565, sparikh@aecom.yu.edu], and [2]Department of Biochemistry, Albert Einstein College of Medicine

Diphtheria toxin ADP ribosylates diphthamide, a post-translationally modified histidine of eukaryotic elongation factor 2 (eEF-2). This covalent modification inactivates eEF-2, thereby inhibiting protein biosynthesis, and ultimately killing the cell. Detailed mechanistic information of ADP-ribosyltransferase activity was determined by steady state kinetics, substrate commitment factors, and competitive kinetic isotope effects (KIEs). The transition state structure was determined with molecular modeling. A relatively large 1′-^{14}C kinetic isotope effect of 1.055 ± 0.003 for the ADP ribosylation is characteristic of significant nucleophilic participation in the transition state. The α-secondary 1′-^{3}H KIE was 1.194 ± 0.002, and the β-secondary 2′-^{3}H KIE was 1.101 ± 0.003. Both values are indicative of an oxacarbenium ion-like ribose ring at the transition state. These results are characteristic of a dissociative transition state. A transition state consistent with these KIEs will be presented.



140. Transition state of thymidine phosphorylase. Matthew R. Birck[1] and Vern L. Schramm.[2] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 [fax (718) 430-8565, birckmr@medusa.bioc.aecom.yu.edu], and [2]Department of Biochemistry, Albert Einstein College of Medicine

Thymidine phosphorylase (TP) has been identified as a possible antiangiogenesis target in a wide variety of human solid tumors. Our strategy for designing a potent inhibitor for TP begins with determining the transition state of the reaction catalyzed by TP. Using specifically labeled thymidine, competitive kinetic isotope effects were measured at several positions. Unlike most known ribosyl transferases, the KIEs for TP appear to indicate an S_N2 mechanism with 1′-^{14}C and 1′-^{3}H KIEs of 1.117 ± 0.005 and 0.989 ± 0.002, respectively. In addition, the 2′-^{3}H KIEs, often as large as the 1′-^{3}H KIE in S_N1 systems, are 1.036 ± 0.002 and 0.974 ± 0.001 for the *pro-S* and *pro-R* protons, respectively. These data are indicative of a highly symmetric, associative transition state. This is the first example of a symmetric, nucleophilic transition state for any *N*-ribosyltransferase.



141. Computational investigation of the role of tryptophan 463 in modulating the electron transfer in nitric oxide synthase. Joseph A. Pichler,[1] Michael J. Tallhamer,[2] and Valentin Gogonea.[1] [1]Department of Chemistry, Cleveland State University, 2121 Euclid Ave., SI 413, Cleveland, OH 44115 [fax (216) 687-9298, j.a.pichler@csuohio.edu], and [2]Department of Chemistry, Cleveland State University

Mutation of a conserved tryptophan (Trp463) residue in inducible nitric oxide synthase (iNOS) leads to the increased mobility of the (6*R*)-tetrahydrobiopterin cofactor (BH4) and the decreased stability of the tryptophan mutant dimer. To investigate the role of Trp in arginine oxidation, molecular

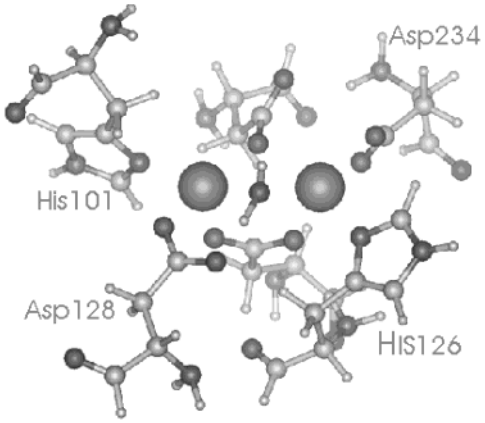
dynamic (MD) simulations on the solvated NOS monomer and quantum mechanical (QM) calculations on BH4 and Trp in the protein environment were performed. The computational analysis gives insight into the role that the conserved Trp and BH4 might play in nitric oxide synthesis. The ionization potential and electron affinity of Trp463 and BH4 were determined from analysis of the MD simulation to determine if these residues are involved in the electron transfer to protoporphyrin IX (heme) or if Trp463 plays a mechanical role in modulating the conformation of the BH4 cofactor. The analysis aims to determine the importance of Trp463 in maintaining the structural integrity of the BH4 binding site.

142. A theoretical study of the enantioselectivity of subtilisin Carlsberg during catalysis. Marc D. Legault,[1] Daniel Bacelo,[2] and Gabriel Barletta.[3] [1]Department of Physics, University of Puerto Rico at Bayamon, #170, Carr. 174 Parque Industrial Minillas, Bayamon, PR 00959 (md_legault@hotmail.com), [2]Department of Sciences and Technology, Universidad Metropolitana, and [3]Chemistry, University of Puerto Rico

The objective of this research is to study the interactions between different substrates and the active site of subtilisin Carlsberg and to relate the above interactions to the activity and enantioselectivity. The configuration energy of the enzyme−substrate system was determined using combined quantum/molecular mechanics methods such as the ONIOM methodology. The system was partitioned into two layers. The first layer, consisting of the most significant residues of the active site of the enzyme and substrate, was treated using quantum mechanics. The energy of this layer was computed at the DFT level using the three-parameter Becke hybrid with the Lee, Yang, and Parr electron correlation functional (B3-LYP) and using the 6-311G* basis set. The second layer, consisting of all other residues of the active site and the rest of system, was treated using the universal force field molecular mechanical approximation. The ONIOM energy of three sterically different chiral tetrahedral intermediates and enzyme was used to calculate $\Delta\Delta G^*$ to determine the enantioselectivities $\{E = \exp[-\Delta\Delta G^*/(RT)]\}$ of these different enzyme−intermediate systems. These calculations were repeated with the self-consistent reaction field method with different dielectric constants corresponding to different solvents.

143. Dynamical flexibility and proton transfer in the arginase active site probed by ab initio molecular dynamics methods. Ivaylo Ivanov and Michael L. Klein. Department of Chemistry, University of Pennsylvania, 231 S. 34th St., Philadelphia, PA 19104 (iivanov@cmm.chem.upenn.edu)

Ab initio molecular dynamics has provided in recent years unprecedented insight into condensed phase processes. However, applications to transition metal systems and, in particular, enzymes still present major challenges. We have used the Car−Parrinello methodology to investigate the dynamical flexibility of the bridged binuclear motif in the active site of arginase. Dynamical transformations play a crucial role in catalysis. We have provided direct insight into the motions of all first-shell ligands with emphasis on the chelating and bridging carboxylates. In the case of the terminal Asp234, we observe changes in the binding mode (carboxylate shifts), which correlate with the motions of other ligands. Also of note is the repeated proton transfer between the bridging water molecule and the catalytically essential Asp128 residue. A number of such events were observed over the duration of our trajectories, which allowed us to map the free energy surface for the process in terms of simple coordinates such as the asymmetric stretch and O−O distance.



144. Computational studies of ammonia channeling in amidotransferases. Xiang Wang,[1] Nigel Richards,[2] and Adrian Roitberg.[3] Department of Chemistry, University of Florida, Box 117200, Gainesville, FL 32611 [fax (352) 392-7918, simwang@chem.ufl.edu], [2]Department of Chemistry, University of Florida, and Quantum Theory Project, University of Florida

We are using theoretical and experimental methods to gain insight into the molecular mechanisms by which independent catalytic modules are coupled, as seen in glutamine-dependent amidotransferases. X-ray crystal structures of asparagine synthetase (AS) and glutamine 5′-phosphoribosylpyrophosphate amidotransferase (GPATase) have shown that both enzymes contain a common N-terminal, glutaminase domain fused to different C-terminal domains, which are themselves members of extant enzyme superfamilies. Both AS and GPATase therefore have two independent active sites; hence, ammonia generated in a glutaminase domain is shuttled along an interior channel to the other catalytic region of the enzyme. This poster will report molecular dynamics and free energy calculations that reveal the role of specific residues in GPATase that mediate intramolecular ammonia transfer.

145. Arg199 is a key player in the electron transfer in nitric oxide synthase. Michael J. Tallhamer and Valentin Gogonea. Department of Chemistry, Cleveland State University, 2121 Euclid Ave., SI 413, Cleveland, OH 44115 [fax (216) 687-9298, m.tallhamer@csuohio.edu]

Molecular dynamics (MD) simulations and quantum mechanical (QM) calculations on nitric oxide synthase (NOS) shed light on the role of the tetrahydrobiopterin (H4B) cofactor in the electron transfer (ET) in NOS enzymes. The analysis shows that the ionization potential of H4B fluctuates dramatically as a function of enzyme dynamics and comes close to zero for portions of the trajectory. The simulation shows an arginine residue (Arg199) making an extensive

network of hydrogen bonds with heme propionates, the H4B cofactor, and surrounding water molecules. The H4B cofactor turned 180° from its crystal structure, making hydrogen bonds with the heme propionate via a transient water molecule and one of its hydroxy groups. This is in agreement with the experimental findings which show that substitution at the sixth position in H4B inhibits the synthesis of nitric oxide. Homology studies indicate that Arg199 is conserved among different species containing inducible NOS.

146. A theoretical study of the active site imidazole rotation in subtilisin Carlsberg during catalysis. Marc D. Legault,[1] Gabriel Barletta,[2] **Myritza Castillo Irrizary**,[1] and **Brenda De Leon Rivera**.[2] [1]Department of Physics, University of Puerto Rico at Bayamon, #170, Carr. 174 Parque Industrial Minillas, Bayamon, PR 00959 (md_legault@hotmail.com, myritza@yahoo.com), and [2]Department of Chemistry, University of Puerto Rico at Humacao, Station CUH, Humacao, PR 00791 (deb_ren@yahoo.com)

The objective of this research is to investigate the proton transfer that occurs between the active site residues of the serine protease subtilisin Carlsberg during catalysis, according to the imidazole ring-flip mechanism. Accurate quantum mechanical calculations were performed on a very restricted part of the enzyme, the active site. The ring-flip mechanism was studied by performing various partial geometrical optimizations as a function of the rotation of the imidazoles ring (of His64) with and without the presence of a tetrahedral intermediate. These calculations were also compared to similar molecular mechanics calculations performed on a much greater portion of the enzyme, using the software Insight and Discover. For this last study, the energy of the imidazole ring was calculated at every 2° of rotation around its axis, with and without a substrate and/or a tetrahedral intermediate structure. This combination of techniques facilitated the study of the ring-flip mechanism in greater detail and ascertains its feasibility.

147. Theoretical study of the peroxidative reactions. **Daniel Bacelo**,[1] Carmen L. Cadilla,[2] Juan Lopez-Garriga,[3] and Marc D. Legault.[4] Department of Sciences and Technology, Universidad Metropolitana, P.O. Box 21150, San Juan, PR 00928-1150 [fax (787) 751-5386, dbacelo@netscape.net], [2]Biochemistry Department, University of Puerto Rico at Medical Sciences Campus, [3]Department of Chemistry, University of Puerto Rico at Mayaguez Campus, and [4]Department of Physics, University of Puerto Rico at Bayamon

One of the most active topics in biomedical research is the understanding of the reactions between Hb, Mb, and other heme proteins with H_2O_2. These tissue oxidants lead, for example, to pathological conditions in mechanisms of ischemia and reperfusion injury. This research involves the study of the structure, energy, and interactions of the native and wild-type hemoglobins of the bivalve mollusk *Luciana pectinata*. Particular attention is given to the investigation of how the mutations changed the binding of hydrogen peroxide (H_2O_2) and other ligands of interest (O_2 and SH_2) with the heme group. We are reporting the optimized structures of the porphyrin ring with different ligands (H_2O_2, O_2, and SH_2) using the density functional theory approximation based on the three-parameter functional proposed by Becke for the exchange electronic energy plus the functional proposed by Lee, Yang, and Parr for the correlation energy (B3-LYP) at the 6-311G* level. The iron atom was treated using the pseudopotentials proposed by Hay and Wadt. Eighteen electrons were considered internal and simulated by the pseudopotentials, while the rest of the electrons were treated with the (5s5p4d)/[4s3p3d] basis set. After we had successfully completed these calculations, we increased the system by the addition of histidine 96. The optimization of these new systems, at the same level as that described above, presented structures with shorter distances between the ligands and the iron atom. The detailed study of the electronic density of the results demonstrated the strong effect that the presence of His96 has on the ligand binding. There is an effective electron transfer from the iron atom to His96. This created a positive density around the iron that generates stronger interactions with the ligands. We can conclude that the interaction between the iron of the heme protein and the ligand is predominantly electrostatic.

148. Calculation of the electron transfer reorganization energy from molecular dynamics simulations. **David Justice**, Sean Laing, and Valentin Gogonea. Department of Chemistry, Cleveland State University, 2121 Euclid Ave., SI 413, Cleveland, OH 44115 [fax (216) 687-9298, DaJustice1@aol.com]

The estimation of the Franck−Condon (FC) factor for nonadiabatic electron transfer reactions in proteins requires the determination from experiment or by calculation of the reorganization energy and the free energy for the electron transfer process. Emphasis in the study is placed on the control of the reaction by the FC factor that determines the thermally averaged density of states in which the donor and acceptor electronic states are resonant and therefore electron tunneling between them is possible. The product of the electronic coupling and the FC factor is then used to obtain the reaction rate constant by means of Fermi's golden rule. Calculations of the reorganization energy, free energy, and rate constant are being carried out on a modified myoglobin and cytochrome *c*, which have a $Ru(NH_3)_5$ compound attached to a histidine residue situated on the protein surface. The theoretically derived quantities are compared with those obtained experimentally.

149. Theoretical study of the short Fe−Fe distance in peroxodiferric compounds. **Daniel Bacelo**[1] and **Silvina E. Fioressi**.[2] [1]Department of Sciences and Technology, Universidad Metropolitana, P.O. Box 21150, San Juan, PR 00928-1150 [fax (787) 751-5386, dbacelo@netscape.net], and [2]Department of Science and Technology, Universidad del Turabo, San Juan, PR 00778 (sfioressi@yahoo.com)

Several biological processes occur through iron and oxygen chemistry at iron proteins. Between them, diiron proteins are involved in important functions such as respiration, oxidation and desaturation of organic substrates, and others. Ferritins are the proteins that concentrate iron in cells as a mineral. Recently, Hwang et al. reported the unusually short Fe−Fe distance of 2.53 Å in the diiron (μ-1,2-peroxodiferric) intermediate occurring in the first stages of ferritin biomineralization. The Fe−Fe distance characterized

earlier in similar complexes ranged from 3.1 to 4.0 Å. This theoretical study explored the potential energy surface of a diferric system and identified as many stable configurations as possible, including the most stable system that is consistent with the short Fe−Fe distance reported by Hwang et al. in their spectroscopic studies. The systems that have been treated are small molecule analogues with peroxodiferric centers. Calculations were carried out with the B3-LYP density functional method. The B3-LYP functional consists of Becke's three-parameter hybrid plus the Lee, Yang, and Parr correlation functional. This methodology has proved be a useful tool in the treatment of transition metal compounds. The "frozen core" geometry optimizations were performed using the GAUSSIAN 98 suite of programs. The 6-311++G-(d,p) basis sets were employed. Our results show that only one configuration presents stability with the Fe atoms being separated by no more than 3 Å. The structure has two single-oxygen atom bridges and one peroxo bridge. The predicted Fe−Fe distance is 2.499 Å, very close to the reported distance of 2.53 Å. The peroxide bond is found to be 1.436 Å in length, and the Fe−O distances in the peroxide moiety are 1.767 Å. The Fe−O−O angle equals 107.51°. The other oxygen atoms are bonded to the iron atoms with distances of 1.929 Å. All other possible structures that were treated resulted in Fe−Fe distances larger than 3 Å in the optimization process. Our calculations show a large charge transfer from the Fe atoms to the oxo bridge oxygens. Also, some small charge transfer from the Fe atoms to the peroxide bridge oxygens is found.

150. Inhibitors tethered near the acetylcholinesterase active site serve as molecular rulers of the peripheral and acylation sites. Joseph L. Johnson,[1] Bernadette Cusack,[1] Thomas F. Hughes,[1] Ellizabeth H. McCullough,[1] Abdul Fauq,[1] Peteris Romanovskis,[2] Arno F. Spatola,[2] and Terrone L. Rosenberry.[1] [1]Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Rd., Jacksonville, FL 32224 (johnson.joseph@mayo.edu), and [2]Department of Chemistry and Institute for Molecular Diversity and Drug Design, University of Louisville

Acetylcholinesterase terminates synaptic transmission by hydrolyzing the neurotransmitter acetylcholine. It is inactivated by organophosphates (developed not only as pesticides but also, deplorably, as chemical warfare agents) as they pass through a transient ligand binding site (P-site) and phosphorylate the catalytic serine. One strategy for protecting against organophosphate inactivation is to design cyclic ligands that bind specifically to the P-site and block the passage of organophosphates but not acetylcholine. To accelerate the process of identifying cyclic compounds with high affinity for the P-site, we combined mutagenesis, combinatorial, and molecular modeling methods to design, develop, and tether potential lead compounds near the P-site. The advantages of this approach were confirmed with tethered cations of varying length and composition. Results from molecular modeling calculations were verified experimentally by measuring competitive inhibition constants for the acetylcholinesterase inhibitors propidium and tacrine. Relative changes in propidium and tacrine affinities provided a sensitive molecular ruler for assigning the binding locations of the tethered cations.

151. Anthracyclines inhibit calcium release from the sarcoplasmic reticulum and bind directly to calsequestrin. Carissa Thornock,[1] Henry A. Charlier, Jr.,[1] Richard D. Olson,[2] Wendy K. Mercer,[1] T. Steven Broyles,[1] Hervé A. Gambliel,[2] Barry J. Cusack,[2] and **Susan E. Shadle**.[1] [1]Department of Chemistry, Boise State University, 1910 University Dr., Boise, ID 83725 [fax (208) 426-3027, carissathornock@mail.boisestate.edu, sshadle@chem.boisestate.edu], and [2]Research Service (151), VA Medical Center

Anthracyclines are widely used anticancer drugs which suffer from a dose-limiting cardiotoxicity. Calsequestrin (CSQ), a lumenal protein of the sarcoplasmic reticulum (SR), is involved in calcium sequestration and is a potential target for anthracyclines. In experiments with isolated SR vesicles, anthracyclines and the calsequestrin inhibitor, trifluoperazine (TFP), caused a dose-dependent inhibition of caffeine-induced SR calcium release ($IC_{50} = 45$ μM for TFP and 3 μM for daunorubicin). This effect was dependent on SR calcium loading; drug added before calcium loading inhibited release significantly more than drug added later. Quinone-containing anthracyclines have a larger effect than imino analogues. Drug binding to CSQ was assessed by protein fluorescence quenching. The drugs bound with K_d values in the micromolar range. Quinone-containing drugs were found to bind with slightly lower affinities than the imino analogues. These results support a model for anthracycline-induced cardiotoxicity, whereby the anthracycline disrupts calcium flux in the SR, presumably through interactions with CSQ.

152. Progress toward the development of allele-specific inhibitors of phosphatidylinositol 3-kinase. Peter J. Alaimo, Zachary A. Knight, and Kevan M. Shokat. Department of Cellullar and Molecular Pharmacology, University of California, 600 16th St., Box 2280, Genentech Hall, Room N514, San Francisco, CA 94143-2280 [fax (415) 514-0822, alaimo@cmp.ucsf.edu]

The lipid kinase family known as phosphatidylinositol 3-kinase (PI 3-K) is a key component in many cellular signaling pathways. Widespread study of this small class of kinases has contributed much to our understanding of the roles of this enzyme subfamily and its chemical products in cell function. Nonetheless, there is much that is still unknown about PI 3-K-mediated cellular signaling. A novel approach to studying kinase-mediated cellular signaling that combines the strengths of chemical and genetic techniques was recently developed in our laboratory. Using this approach, one can synthesize inhibitors that are specific for a single mutant allele of a kinase of interest. Accordingly, we have generated mutants of p110α, a PI 3-K, that can be selectively inhibited by small organic molecules we have synthesized. Our progress toward obtaining monospecific inhibitors of p110α will be presented.

153. Pressure-induced aggregation of interleukin-1 receptor antagonist (IL-1ra): mechanism and thermodynamics. Matthew B. Seefeldt,[1] Yong-Sung Kim,[2] Brent S. Kendrick,[3] Kevin Tolley,[1] John F. Carpenter,[2] and Theodore W. Randolph.[1] [1]Department of Chemical Engineering, University of Colorado, Campus Box 424, Boulder, CO 80309

(seefeldt@colorado.edu), [2]Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, and [3]Amgen, Inc.

Purpose. Application of high hydrostatic pressure (1−4 kbar) has previously been shown to efficiently refold aggregated proteins. Interestingly, we found that pressures of 2000 bar instigated aggregation of the protein IL-1ra. Studies were conducted to determine the mechanism and thermodynamics of pressure-induced aggregation.

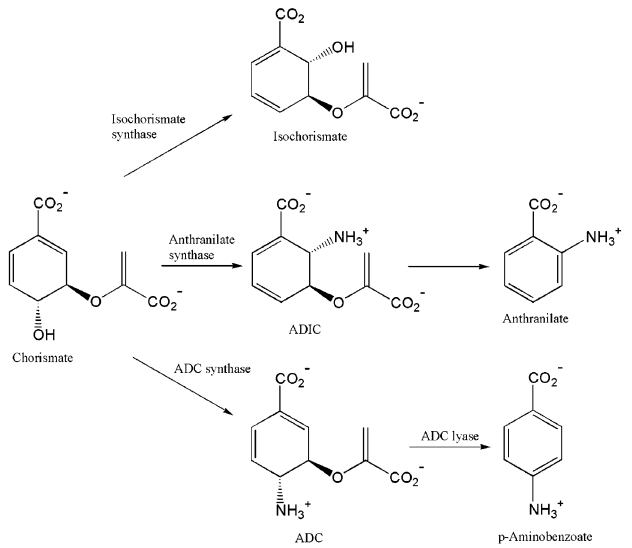
Results. Aggregates of IL-1ra formed rapidly at 2000 bar and 31 °C; however, the addition of DTT (reducing agent) inhibited aggregation at all pressures. To explore this further, the aggregation rate was monitored as a function of pressure. The activation volume and free energy were determined to be -102 ± 23 mL/mol and 33 ± 5 kJ/mol, respectively. Pressure unfolding with DTT yielded a $\Delta V_{unfolding}$ of -127 ± 60 mL/mol, while the unfolding free energy was 25 ± 6 kJ/mol.

Conclusions. The similar thermodynamic parameters of aggregation and unfolding verify that aggregation occurs upon protein denaturation. Since reducing agents inhibit aggregation, we conclude that pressure-induced aggregation is instigated by exposure of free cysteines which cross-link and aggregate.

154. Mechanistic and inhibition studies on chorismate-utilizing enzymes. Miguel D. Toscano and Chris Abell. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom (fax 0044-1223-336362, mdgpt2@cam.ac.uk)

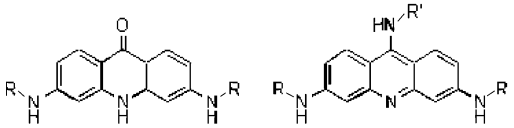
Chorismate is the key branch point metabolite in the shikimate pathway and the substrate for a number of enzymes involved in the biosynthesis of the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), the folate coenzymes, and a number of vitamins and aromatic secondary metabolites. This pathway has only been found in plants, fungi, and microorganisms, and it is therefore a target for the development of antibiotic, antiparasitic, and herbicidal compounds.

Three chorismate-utilizing enzymes [aminodeoxychorismate (ADC) synthase, anthranilate synthase (AS), and isochorismate synthase (ICS)] exhibit remarkable sequence similarity and structural homology, although they catalyze different reactions. This work involves the use of structural information and molecular modeling in elucidating the mechanisms and design of potent inhibitors for these enzymes.



155. Rational design and evaluation of substituted acridones and acridines as telomerase inhibitors. Michael J. Moore, Christoph M. Schultes, and Stephen Neidle. Cancer Research UK BioMolecular Structure Group, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, United Kingdom [fax +44 (0)20 7753 5970, michael.moore@ulsop.ac.uk]

Telomerase represents an attractive target for the rational design of new anticancer agents. The determination of quadruplex structures formed from human telomeric DNA has provided new opportunities for structure-based design of potent and selective G-quadruplex (GQ) telomerase inhibitors. Both 3,6-disubstituted acridones (**1**) and 3,6,9-trisubstituted acridines (**2**), where R is a propionic side chain bearing a tertiary amino group, have been shown recently to inhibit telomerase. For inhibitor optimization, we have investigated extension of the R substitutents based on the hypothesis that positioning of the charged tertiary amino function within the GQ grooves and loops results in a favorable electrostatic interaction and an increase in potency. Inhibitors designed selectively for GQ over duplex DNA should also exhibit low cytotoxicity. Tools utilized include (1) modeling of ligand GQ complexes (to provide binding energies) and (2) the FRET GQ stability assay (T_m values). The correlation of these techniques with telomerase IC_{50} (Tel_{50}) values has been examined for rational design purposes. The "in silico" and FRET screening of **1** and **2**, where R varies in length from propionyl 3C to hexanoyl 6C, is presented together with telomerase activity.



156. Interaction of a novel carbazole derivative with human telomere: A potent telomerase inhibitor. Cheng Chung Chang, Jin-Yi Wu, and Ta-Chau Chang. Institute of Atomic and Molecular Sciences, Academia Sinica, P.O. Box 23-166, Taipei 106, Taiwan (fax +886-2-23620200, tcchang@po.iams.sinica.edu.tw)

We have synthesized a novel molecule of 3,6-bis(1-methyl-4-vinylpyridium) carbazole (BMVC) for stabilizing the quadruplex structure of the human telomeric sequence of $d(T_2AG_3)_4$ and inhibiting telomerase activity. Mixing BMVC with the $d(T_2AG_3)_4$ can increase the melting temperature of $d(T_2AG_3)_4$ by ~13 °C in vitro, implying that BMVC is a good quadruplex stabilizer. The IC_{50} value of BMVC (0.5 μM) suggests that BMVC is a good telomerase inhibitor. On the other hand, the cell viability assay suggests that the cytotoxic EC_{50} values of BMVC concentrations against a panel of human lung adenocarcinoma CL1-1 cells and mouse embryonic fibroblast NIH3T3 cells are similar

and slightly larger than 20 μM. Absorbance titration indicates the affinity of binding of BMVC to $d(T_2AG_3)_4$ is larger than that for binding to $[d(GCGCA_2T_2GCGC)]_2$. The observations of quadruplex stability, telomerase inhibitory activity, cellular cytotoxicity, and binding selectivity suggest that BMVC might be a good candidate for the application of the clinical telomerase inhibitor.

157. Irreversible inhibition of the *Staphylococcus aureus* transpeptidase sortase (SrtA) by vinyl sulfones. Brenda A. Frankel, Balint Otvos, Shu He, Ryan Kruger, and Dewey G. McCafferty. Department of Biochemistry and Biophysics and Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059 (bfrankel@mail.med.upenn.edu)

The sortase enzyme family is made up of cell wall-associated transpeptidases that play a key role in the virulence of Gram-positive bacteria by catalyzing the covalent linkage of LPXTG-containing virulence and adhesin proteins, such as protein A or fibronectin binding protein, to the peptidoglycan layer of the bacterial cell wall. Selective inhibition of sortase constitutes a novel avenue of antivirulence chemotherapy against Gram-positive bacterial infections. Sortase contains a reactive cysteine (C184) that is purported to function as an active site nucleophile. Here we show that vinyl sulfones, a newly emerging class of electrophilic inactivators of cysteine proteinases, potently and irreversibly inactivate the SrtA sortase from *Staphylococcus aureus*. Vinyl sulfones therefore represent a novel general inhibitor class for the sortase superfamily, whose potential applications include the treatment of Gram-positive drug-resistant bacterial infections, the analysis of substrate specificity and isoform selectivity using peptidyl vinyl sulfones, and sortase activity-based protein profiling.

158. Nicotinamide inhibition of SIR2 is a consequence of chemical competition for an ADPR−peptidyl intermediate. Anthony A. Sauve[1] and Vern L. Schramm.[2] [1]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461 (sauve@medusa.bioc.aecom.yu.edu), and [2]Department of Biochemistry, Albert Einstein College of Medicine

The Sir2 enzymes are NAD^+-dependent protein deacetylases. They catalyze the deacetylation of acetyllysine residues utilizing NAD^+ as a cosubstrate to form the free amino group, nicotinamide, and the novel compounds 2′- and 3′-*O*-acetyl ADPR [Sauve, A. A., et al. (2001) *Biochemistry 40*, 15456]. Nicotinamide is a base exchange substrate and an effective inhibitor of the SIR2 reaction. Biological effects, including lifespan extension by caloric restriction, may be controlled by nicotinamide regulation of SIR2. We provide kinetic characterization of the base exchange reaction and provide k_{cat} (exchange) and K_m values for enzymes derived from *Archeaglobus fulgidus* AF2, *Saccharomyces cerevisiae* Sir2p, and mouse SIR2a. The measured K_m values are in the range of 40−150 μM. Inhibition at variable nicotinamide concentrations indicated that the K_i values are equal to the K_m values for exchange, implying that exchange and inhibition can be traced to the same binding event. Inhibition-plotted $1/v$ versus I showed that inhibition was nonlinear hyperbolic in nature with a plateau in excess of 10-fold K_i. Comparisons of k_{cat}(deacetylation) and k_{cat}(exchange) predict the inhibition results. The ratio k_{cat}(exchange)/k_{cat}(deacetylation) is tightly correlated with the extent of inhibition at high nicotinamide concentrations. These data indicate that nicotinamide exchange is responsible for inhibition. The bifurcated reactivity of a nicotinamide−ADPR−peptidyl SIR2 intermediate that can react forward to deacetylate or react backward to re-form substrates accounts for the hyperbolic inhibition. Inhibition by nicotinamide isosteres corroborates this mechanism. Exchange data provide insight into the reaction mechanism and reaction coordinate energetics of the SIR2 enzymes. This unique exchange/inhibition reaction mechanism provides a strategy for upregulation of SIR2 catalysis by inhibition of chemical exchange but not deacetylation.

159. Screening UDP-galactopyranose mutase using fluorescence polarization. Michelle Soltero-Higgin,[1] John H. Phillips,[2] Erin E. Carlson,[2] and Laura L. Kiessling.[3] [1]Department of Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (msoltero@biochem.wisc.edu), [2]Department of Chemistry, University of Wisconsin−Madison, and [3]Departments of Chemistry and Biochemistry, University of Wisconsin−Madison

UDP-galactopyranose mutase (UGM) catalyzes the interconversion of UDP-galactopyranose and UDP-galactofuranose. This flavoenzyme, which is involved in the cell wall biosynthesis of various pathogenic species, is essential for the viability of *Mycobacterium tuberculosis*. Because tuberculosis affects almost 2 million people annually and is a leading health issue worldwide, the need for new therapeutics that target *M. tuberculosis* is urgent. To discover new leads, we have developed a high-throughput assay based on the tight and specific interaction of UGM and a fluorescent derivative of UDP. Using this compound, we developed a high-throughput fluorescence polarization assay. We have screened a library of 16 000 pharmacophore-based compounds, and several leads have been identified. The activities of these compounds with respect to enzyme inhibition will be presented.

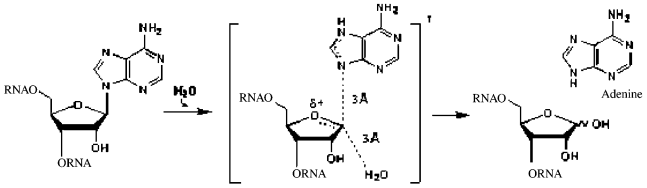
160. Elucidation of fluoroaromatic−amino acid contacts between inhibitors and viral neuraminidase. Ahamindra Jain, **Kathryn M. Cantrell**, and **Irene Wong**, Chemistry, University of California, 325 Latimer Hall, Mail Code 1460, Berkeley, CA 94720-1460 (ahamindra@cchem.berkeley.edu, cantrell@uclink.berkeley.edu, airiin@uclink.berkeley.edu)

Fluorobenzyl ether analogues of Tamiflu have been prepared, and their binding to viral neuraminidase has been quantified via a fluorescence-based assay, with the goal of identifying novel intermolecular forces between fluoroaromatic groups and amino acid side chains. Results from biochemical measurements of the affinity of these compounds, as well as from X-ray crystallography of cocrystals, will be presented.

161. Stem−loop RNA analogues as transition state inhibitors of the ricin toxin A-chain (RTA). Setu Roday,[1] Richard Furneaux,[2] Gary Evans,[2] Peter Tyler,[2] and Vern Schramm.[1] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY

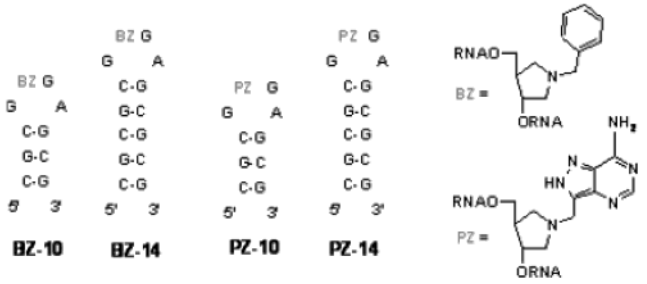
10461 (sroday@aecom.yu.edu), and [2]Carbohydrate Chemistry Team, Industrial Research Ltd.

Ricin is a cytotoxic protein isolated from castor beans. The B-chain lectin component of this heterodimeric protein directs the A-chain to the cell surface. Within the cytosol, RTA binds to a specific "GAGA" base sequence on the sarcin−ricin loop (SRL) of the 28S ribosomal RNA and specifically depurinates the first adenosine in that sequence. Kinetic isotope effects on the hydrolysis of a small 10-base stem−loop oligonucleotide substrate established the mechanism of depurination as $D_N{*}A_N$ involving an oxacarbenium ion intermediate in a highly dissociative transition state.



Reaction catalyzed by RTA

Stem−loop structures incorporating a non-natural site were synthesized and tested as inhibitors of RTA. The 2′-deoxypyrrolidine nucleoside resembles the transition state by protonation of the ring nitrogen, while the methylene bridge provides torsional flexibility in the active site. The dissociation constants for BZ-10, BZ-14, PZ-10, and PZ-14 are 77, 682, 163, and 94 nM, respectively. These inhibitors are the tightest single-molecule catalytic site inhibitors known for RTA.



162. Structure−activity relationships for synthetic peptides and peptidomimetics of thrombin. Cristina C. Clement and Manfred Philipp. Chemistry Department, Lehman College, City University of New York, 250 Bedford Park Blvd. W., Bronx, NY 10468 (cclement_us@yahoo.com)

This investigation is focused on discovery of peptides and peptidomimetics that reversibly inhibit thrombin. The experimental approach combines molecular docking using "Sculpt" software provided by "MDL" and automatic chemical synthesis of the candidate compounds using Fmoc chemistry. The peptides were docked into active site of thrombin and assigned scoring functions based on the predicted free energy of interaction with thrombin, ranging from −20 to −100 kcal/mol. Lead inhibitors were selected from two classes of sequences: 1-D-Phe-Pro-Arg-D-Pro-P2′-P3′ and 2-D-Phe-Pro-D-Arg-P1′-P2′. Both the use of D-Pro in the P1′ position and the use of D-Arg instead of Arg in the P1 position are expected to inhibit the thrombin-mediated hydrolysis of candidate peptides. We varied positions P1′, P2′, and P3′ using a selection of the naturally encoded amino acids in the L- and D-forms. Peptidomimetics were designed by keeping constant P2, P1, and P1′ in the leads but varying the P3 position with Phe analogues like *trans*-cinnamic and dihydrocinnamic acids, L/D-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) and L/D-Thi (thienylala). Peptides with the sequence space 2-D-Phe-Pro-D-Arg-P1′-P2′ were better inhibitors than the compounds from the D-Phe-Pro-Arg-D-Pro-P2′-P3′ series. Lead compounds having an experimentally determined inhibitory constant (K_i) between 16.5 and 2.5 μM are potential competitive inhibitors. The results are discussed with respect to the computationally calculated structural determinants involved in predicting the best fits between peptides or peptidomimetics and thrombin.

163. Immunoassay for drugs in a blood substitute. Rebecca Eileen Barlag, H. Brian Halsall, and W. R. Heineman. Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221-0172 [fax (513) 556-9239, reb7511@yahoo.com]

The purpose of this research is to determine an optimal method for detecting both hydrophilic and hydrophobic drugs in a blood substitute. An immunoassay has been developed to determine the concentration of drugs using spectrophotometric detection. Samples in whole blood, however, require a different method of detection. This research uses time-based single-potential hydrodynamic voltammetry, an electrochemical detection method. This research shows that this is a suitable method of detection for the immunoassay of both the hydrophilic drug theophylline and the hydrophobic drug phenytoin in the presence of a blood substitute.

164. Studies on the intracellular release of genetic drugs from pharmaceutical carriers. Bart L. Lucas, **Katrien Remaut**, Stefaan C. De Smedt, Jr., and Jo Demeester. Faculty of Pharmacy, Ghent University, Ghent, Belgium (bart.lucas@rug.ac.be, katrien.remaut@rug.ac.be)

This study focuses on the advantages of using dual-color fluorescence fluctuation spectroscopy (FFS) to study the fate of DNA−polymer complexes in living cells. Whereas confocal laser scanning microscopy (CLSM) evaluates the spatial fluorescence distribution, FFS monitors the temporal fluorescence fluctuations caused by the diffusion of fluorescently labeled molecules through the fixed excitation volume of a microscope. Dual-color FFS answers the question of whether the DNA and the cationic carrier move together (i.e., when they are associated) or separately (i.e., when they are dissociated) through the excitation volume. CLSM just answers the question of whether both fluorescent species are located in the same area (colocalization). The dissociation of oligonucleotides (ONs) from poly(L-lysine) (pLL) was considered. Both the DNA and the cationic carrier were labeled with spectrally different, fluorescent markers [rhodamine green (RhGr) and Cy5]. The DNA complexes were either applied to a monolayer of cells or microinjected into the cytoplasm. Nuclear colocalization of red and green fluorescence in the CLSM images showed that both Cy5-pLL and RhGr-ONs were present in the nucleus. However, dual-color FFS measurements revealed that no intact Cy5-pLL−RhGr-ONs complexes are found in the nucleus. Therefore, dual-color FFS proves to be a very valuable tool

for obtaining information about the cellular pathway of DNA complexes.

165. Antibacterial activity of polystyrene-*block*-poly(4-vinylpyridine) and poly(styrene-*random*-4-vinylpyridine). Eun-Soo Park,[1] Gasan-Dong,[2] Mal-Nam Kim,[3] Ji Hye Shin,[4] and Jin-San Yoon.[5] [1]Department of Product Development, Youngchang Silicone Co., Ltd., 481-7, [2]Kumchun-Gu, Seoul 153-803, South Korea (fax 82-2-803-8380, t2phage@hitel.net), [3]Department of Biology, Sangmyoung University, [4]AVI-CORE-Biotechnology Institute, and [5]Department of Polymer Science Engineering, Inha University

Styrene was polymerized in the presence of benzoyl peroxide (BPO) and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to yield polystyrene−TEMPO (PS−TEMPO) macroinitiators. The PS−TEMPO macroinitiators with different molecular weight were reacted with 4-vinylpyridine to synthesize polystyrene-*block*-poly(4-vinylpyridine) (PS-*b*-PVP), which was than quaternized with 1-iodooctane. Antibacterial activity of the quaternized copolymer was assessed against a Gram-negative bacteria (*P. aeruginosa*) and a Gram-positive one (*S. aureus*) by using the shake flask test method, and the results were compared with those of poly(styrene-*random*-4-vinylpyridine) [P(ST-*r*-VP)]. PS-*b*-PVP was more active than P(ST-*r*-VP) for both the two bacteria, which was ascribed to the fact that the content of quaternized 4-VP units on the surface of the copolymer particles of the former copolymer was higher than that corresponding to the later copolymer.

166. Surfaces antibacterial toward *Pseudomonas aeruginosa*. JaimeLee I. Cohen,[1] Jasmine Escalera,[1] Tanya Abel,[1] Tatiana Hatchett,[1] Karin Melkonian,[2] 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, and [3]Department of Chemistry and Biochemistry, Queens College and City University of New York Graduate Center, 65-30 Kissena Blvd., Flushing, NY 11367 [fax (718) 997-5198, robert_engel@qc.edu]

Recent efforts of this laboratory have been concerned with the preparation of porous surfaces that exhibit antibacterial and antifungal activity by contact with the organism. Activity has been achieved by incorporation of a detergent-like adjunct into the structure of the surface material. While activity against a wide range of microbes had been observed with such adjunct incorporation, *Pseudomonas aeruginosa* was found to be resistant to the action by agents active against other bacteria and fungi. We herein report on modifications to carbohydrate- and protein-based porous surfaces that exhibit complete antibacterial activity against *P. aeruginosa* as well as a wide range of other microbial species. This is accomplished by a modification of the nature of the lipophilic chain associated with the adjunct. A mixture of adjuncts incorporated into the surface provides activity against the full range of bacteria and fungi.

167. Colocalization of ramoplanin and vancomycin peptide antibiotics to *Bacillus subtilis* divisional septa and MreB/Mbl-like helical cables. Wenkai Li,[1] Xiangwei He,[2] Predrag Cudic,[1] William Margolin,[3] and Dewey G. McCafferty.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 422 Curie Blvd., Philadelphia, PA 19104 [fax (215) 573-8052, wenkaili@mail.med.upenn.edu], [2]Department of Biochemistry and Molecular Biology, University of Texas Medical School, and [3]Department of Microbiology and Molecular Biology, University of Texas Medical School

The peptide antibiotics ramoplanin and vancomycin act by binding to peptidoglycan biosynthesis lipid intermediates, precluding their proper incorporation into the cell wall. To determine the sites of localization of ramoplanin and vancomycin, we conducted confocal fluorescence microscopy analysis of fluorescent derivatives of these two antibiotics in viable *Bacillus subtilis*, a rod-shaped Gram-positive bacteria. Ramoplanin and vancomycin were both found to be concentrated in divisional septa, a site of intense peptidoglycan biosynthesis. In addition, with increased incubation times, both antibiotics were found to form surface-localized helical cables. Nearly identical patterns have been observed for MreB and Mbl, two actin-like cytoskeletal proteins involved in establishing the *B. subtilis* rod shape. These proteins are also believed to recruit peptidoglycan biosynthesis machinery for directed deposition of new peptidoglycan. These results suggest that ramoplanin and vancomycin likely bind to discrete yet functionally related targets in peptidoglycan biosynthesis.

168. Antimicrobial activity of synthetic hydraphile channels. W. Matthew Leevy,[1] Paul H. Schlesinger,[2] and George W. Gokel.[1] [1]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8103, St. Louis, MO 63110 (wmleevy@artsci.wustl.edu), and [2]Department of Cell Biology and Physiology, Washington University School of Medicine

Living organisms employ a delicate architecture of macromolecular interactions in their mission for viability and survival. A critical component in this framework of life is the operation of the membrane bilayer and its many proteins, such as ion channels, which regulate the transport of salts and metabolites. We have designed, synthesized, and studied a number of abiotic compounds that emulate ion channel behavior. The goal of such studies is to better understand the kinetic and structural details of ion transport. We have called one family of channel formers "hydraphiles". Channel or pore formation may cause the depletion of cellular ion gradients, resulting in cell death for bacteria. Our data show (1) localization of hydraphiles to the bacterial plasma membrane, (2) evidence of a channel mechanism for bacterial toxicity, and (3) structure−toxicity relationships that are in accordance with previous experiments in synthetic systems.

169. Two-dimensional model for peptide−protein binding. Catherine M. Cresson-Tarsa, Frank H. Stillinger, Salvatore Torquato, and George L. McLendon. Frick Laboratories, Department of Chemistry, Princeton University, Princeton, NJ 08544 [fax (609) 258-6746, ccresson@princeton.edu]

We present a reduced dimensional approach to studying peptide−protein binding. The peptides are composed of

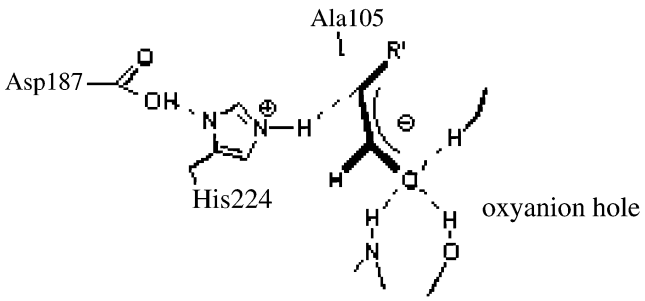
pseudoatoms, and their unbound structures are determined through a series of Monte Carlo simulated annealing (MCSA) minimizations. The intramolecular peptide potential contains three terms: bonds, angles, and van der Waals. Through our novel approach, the protein binding site is represented by a set of Gaussian functions that account for atomic shape as well as chemical binding features. This computational model has been successfully tested on Smac binding to XIAP; these two proteins are important control factors in apoptotic cell death. This two-dimensional model demonstrates that experimental binding trends can be reproduced with a minimalist model and a computationally cheap approach.

170. Transcriptional profiling of the peptide antibiotic ramoplanin in *Bacillus subtilis*. Jason E. Drury,[1] Don A. Baldwin,[2] and Dewey G. McCafferty.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 422 Curie Blvd., Philadelphia, PA 19104 [fax (215) 573-8052, jdrury@mail.med.upenn.edu], and [2]Microarray Core Facility, University of Pennsylvania School of Medicine

Ramoplanin is a cyclic glycolipodepsipeptide antibiotic involved in the inhibition of cell wall biosynthesis in Gram-positive bacteria. The primary goal of this research was to further the understanding of the mechanism of action of ramoplanin. Gene expression profiling was conducted on *Bacillus subtilis* treated with ramoplanin using GeneChip antisense genome arrays (Affymetrix). During vegetative growth, more than 90% of the *B. subtilis* genome was found to be transcriptionally active. Addition of ramoplanin at 0.5 and 4.0 times the MIC values resulted in the selective upregulation of many genes involved in cell wall metabolism and stress response. Many genes involved in protein synthesis and secondary metabolite biosynthesis were downregulated. Although no clinical or laboratory-generated resistance to ramoplanin has been reported, at 60 min, select group transferases and porins were highly upregulated. This may indicate an attempt to circumvent the action of the antibiotic by covalent modification combined with efflux mechanisms.

171. Rational redesign of a lipase to an aldolase. Cecilia Branneby,[1] Peter Carlqvist,[2] Karl Hult,[1] Tore Brinck,[2] and Per Berglund.[1] [1]Department of Biotechnology, KTH, SE-106 91 Stockholm, Sweden (fax +46-8-55378468, cecilia@biochem.kth.se), and [2]Department of Chemistry, Division of Physical Chemistry, KTH

We have rationally redesigned a lipase into an enzyme with aldolase activity. *Candida antarctica* lipase B belongs to the group of enzymes with a catalytic triad (Asp, His, and Ser) and an oxyanion hole for stabilizing the intermediate in the active site. This enzyme is stable under many conditions and active even in organic solvents. The feasibility of the aldol reaction was first investigated by quantum chemical calculations and could then be supported by experiments performed with various aldehydes and ketones. The catalytic nucleophile (Ser105) was mutated to an alanine to remove the nucleophilic ability; the histidine was kept to increase the α-proton acidity, and the oxyanion hole was left intact to stabilize the enolate intermediate. Both quantum chemical calculations and experimental data on the mutant agree that the reaction proceeds through the suggested enolate intermediate.



Ser105Ala mutant

172. Redesign of D-glucarate dehydratase as galactarate dehydratase. Cheri L. Millikin[1] and John Gerlt.[2] [1]Department of Biochemistry, University of Illinois at Urbana-Champaign, 399 Roger Adams Laboratory, 600 S. Mathews Ave., Urbana, IL 61801 [fax (217) 244-7426, millikin@uiuc.edu], and [2]Department of Biochemistry, University of Illinois at Urbana/Champaign

Members of the enolase superfamily utilize the TIM barrel fold and catalyze more than 13 diverse reactions. One member of the enolase superfamily, D-glucarate dehydratase (GlucD), catalyzes the dehydration of D-glucarate via *syn* elimination. Another member, D-galactonate dehydratase (GalD), catalyzes the dehydration of D-galactonate via *anti* elimination. Comparing the active site architectures of GlucD and GalD shows that both enzymes contain a histidine at the end of the seventh β-strand as well as metal binding ligands at the ends of the third, fourth, and fifth β-strands; however, GalD also contains a histidine at the end of the third β-strand. Since the active site residues in TIM barrels function and evolve independently, the addition of a histidine at the end of the third β-strand in GlucD allows the enzyme to catalyze the *anti* elimination of galactarate. Redesigning the active site of GlucD to catalyze the dehydration of galactarate introduces a novel reaction to the enolase superfamily.

173. A census of the "*i* − 4, *i*, *i* + 4" reductive aromatic zipper motif in the protein sequences of oxidoreductases. Chi Ming Yang. Neurochemistry and Chemical Biology and Institute for Life Science and Health, San Diego, CA, and Nankai University, Tian Jin 300071, China (fax 011 86 22 23503863)

Biochemists are familiar with nonpolar leucine zippers and polar zippers in proteins. But chemical zipper motifs in proteins that may function through sequence-specific chemical processes are rarely reported. Here, a census of reductive aromatic zipper motif in the form of "RaaXXXRaaXXXRaa", named the "$i - 4$, i, $i + 4$" reductive aromatic zipper motif, in the sequences of oxidoreductases is described. Raa's are reductive aromatic amino-acid residues (Trp, His, and Tyr), side chains of which are capable of binding to metal ions and undergoing an electron transfer-mediated redox reaction. A statistical analysis of the Protein Data Bank revealed that the total number of protein sequences carrying this putative "$i - 4$, i, $i + 4$" zipper motif in the form of "RaaXXXRaaXXXRaa" is 4040 of the 47 263 protein

sequences (up to April 16, 2003), among which there are 29 protein sequences having a zipper sequence displayed as "W_{i-4}, Hi, W_{i+4}", 96 protein sequences consisting of a zipper as "H_{i-4}, W_i, H_{i+4}", and 228 protein sequences retaining a zipper as "H_{i-4}, Y_i, H_{i+4}". The identification of this zipper motif may facilitate a delineation of the site and mode of cooperative binding of metal ions to proteins. A recognition of this reductive zipper may also provide new clues about predicting function from protein sequence.

174. Exploring the mechanistic paradigm of the crotonase superfamily: Insights from 3-hydroxyisobutyryl-CoA hydrolase. Brian J. Wong[1] and John A. Gerlt.[2] [1]Department of Biochemistry and Chemistry, University of Illinois, Urbana, IL 61801 (bjwong@scs.uiuc.edu), and [2]Department of Biochemistry, University of Illinois

3-Hydroxyisobutyryl-CoA hydrolase (HICH), a member of the crotonase superfamily, is a key enzyme in valine catabolism that catalyzes the hydrolysis of a thioester bond. Reactions catalyzed by enzymes in this superfamily proceed through a mechanism in which abstraction of a proton α to the carbonyl of a thioester leads to the formation of an enolate anion intermediate. However, we have shown through kinetic isotope and ^{18}O labeling experiments that HICH does not proceed by this general mechanism but instead utilizes an active site glutamate to form an acyl−enzyme intermediate. We also hypothesize that the enzyme uses the oxyanion hole found in the structures of all superfamily members to stabilize the formation of a tetrahedral intermediate. Although this mechanism does not support the mechanistic hypothesis proposed for this superfamily, we suspect that it will provide a crucial link to understanding the divergent evolution of these enzymes.

175. Stereochemistry of tartrate dehydrogenase and its application to the synthesis of stereospecifically monodeuterated L-serine. Mark W. Ruszczycky and Vernon E. Anderson. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106 (mwr8@cwru.edu)

Tartrate dehydrogenase catalyzes the formation of different products depending on the stereochemistry of its substrate. The effect of catalysis on *meso*-tartaric acid results in the formation of D-glyceric acid through a process of oxidation, decarboxylation, and reduction. The final reductive step in this mechanism is believed to involve the addition of hydride from NADH and a proton from solvent across the double bond of hydroxyenolpyruvate. The stereochemistry of this addition has not been determined. To investigate this process, (2*R*,3*R*)-[3-^{2}H]glyceric acid was synthesized from D-fructose 6-phosphate using the known stereospecificity of phosphoglucose isomerase to replace the *pro-R* proton at C1 with deuterium. The ^{1}H NMR of this product permitted the assignment of both chemical shifts and coupling constants for each proton in D-glyceric acid. On the basis of this information, the tartrate dehydrogenase-catalyzed reaction with *meso*-tartrate in D_2O was found to generate (2*R*,3*R*)-[3-^{2}H]glyceric acid. This indicates that tartrate dehydrogenase catalyzes decarboxylation and protonation at C3 of the respective intermediates with retention of stereochemistry such that the solvent-derived proton adds to the same face as that from which CO_2 leaves. Having determined this stereochemistry, we were then able to generate (2*S*,3*R*)-[3-^{2}H]serine in a "single-pot" reaction from *meso*-tartaric acid. The product was isolated in nearly 40% yield with greater than 90% deuteration and no evidence of racemization.

176. Synthesis and biological activity of analogues of the peptide antibiotic ramoplanin. Salim Barkallah, Predrag Cudic, Jason E. Drury, and Dewey G. McCafferty. Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 422 Curie Blvd., Philadelphia, PA 19104 [fax (215) 898-8052, barkalla@sas.upenn.edu]

Ramoplanin is a 17-residue cyclic depsipeptide antibiotic that has emerged as a promising clinical candidate for treatment of vancomycin-resistant Gram-positive bacterial infections. Previously, we and others have determined that a fundamental component of the mechanism of action of ramoplanin involves capture of peptidoglycan biosynthesis lipid intermediates. NMR studies and semisynthetic modification studies revealed a possible role in the Hpg3−Orn10 residues in ligand recognition. Here we report the results of semisynthetic modification of ramoplanin residues within this recognition sequence as well as the synthesis of cyclic ramoplanin analogues using solid-phase methods. Their syntheses and biological activities will be discussed.

177. Synthesis and biological tests of solution phase S-glycosylated porphyrin combinatorial libraries. Xin Chen, David Foster, and Charles Michael Drain. Hunter College and Graduate Center, City University of New York, 695 Park Ave., New York, NY 10021 [fax (212) 772-5332, chenxin@hunter.cuny.edu]

Photodynamic therapy (PDT) is a medical treatment which employs the combination of light and a drug to bring about a cytotoxic or modifying effect to cancerous or otherwise unwanted tissue. Because of potential selective binding to various cell types, porphyrins appended with a variety of saccharides have been examined as possible agents for PDT. Our focus has been on the preparation of S-glycosylated porphyrins, in which the hydrolytically labile acetal moiety of the sugar is substituted with a functionality more stable toward acids, bases, and enzymes. This property may increase the selectivity and reduce the dosages needed for PDT. Synthesis begins with 5,10,15,20-tetrakis(pentafluorophenyl)-porphyrin (TPFPP), because of the high reactivity of the para fluoro group in nucleophilic substitution reactions. The acetal-protected tetrathioglucosyl, tetrathioxylosyl, tetrathioglucosamino, tetrathiopyridinyl, tetrathiodimethylaminoethane, tetra-*p*-thiotoluene, and tetra-1-thiopentane derivatives are formed in >90% yield. Subsequently, 55- and 406-member solution-phase combinatorial libraries of different sugar functionalities are made successfully, which can be demonstrated by the observation of the desired porphyrin peaks via electrospray mass spectroscopy (EMS). Additions to these libraries of different "biological moieties" are also made as esters, amides, and thio esters. The selection method is being studied, using human breast cancer MDA231 cells as the biological model. Some members of the libraries have shown photodamaging effects as promising PDT agents.

178. Synthesis of new *β*-cyclodextrin derivatives. Marie Thomas[1] and Robert Engel.[2] [1]Department of Chemistry and

Biochemistry, Queens College and City University of New York Graduate Center, 65-30 Kissena Blvd., Flushing, NY 11367 [fax (718) 997-5198, malie333@hotmail.com], and [2]Department of Chemistry, Queens College and City University of New York Graduate Center

In the past, this laboratory has synthesized a series of α- and *β*-cyclodextrins substituted at each of the 6 positions with ligands bearing quatenary ammonium and lipophilic units. In the continuation of these studies, a new series of similarly substituted *β*-cyclodextrins are being synthesized bearing substituents containing quaternary phosphonium sites with lipophilic units. The approach to synthesis of such materials with capacity for selective binding and investigation of their binding characteristics are discussed.

179. Synthesis of novel isoxazolones and pyrazolones. Saba Jahangiri[1] and Rolf H. Prager.[2] [1]Department of Chemistry, The Flinders University of South Australia, GPO Box 2100, Adelaide 5001, Australia (fax 61-8-8201 2905, saba.jahangiri@flinders.edu.au), and [2]Department of Chemistry, The Flinders University of South Australia

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). The receptors to which it binds are involved in the processing of sensory information, memory, and learning (*1*). Changes in glutamate transmission have been associated with a number of CNS disorders, including Parkinson's disease and schizophrenia (*2*).

The high affinity of TAN-950A3 (*1*), an antifungal antibiotic for glutamate receptors, resulted in the synthesis and evaluation of a large number of C3-substituted alkyl and cyclic analogues (*1*). In line with these results, we have synthesised some further C3-substituted analogues (*2, 3*). The analogues have been designed to investigate the roles played by the heteroatom (O vs N) of the heterocycle and the possibility of favorable hydrogen bonding interactions between the analogues and the receptor site. This poster will outline the methodologies employed toward the synthesis of some novel isoxazolone- and pyrazolone-containing amino acids. The affinity of these compounds for the glutamate receptor sites will also be discussed.

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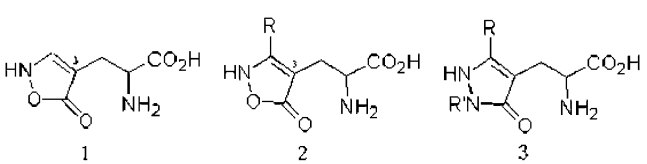


180. Three-step in vitro syntheses of C30 carotenoids on microfluidic biochips. Jae-Cheol Jeong,[1] Bo Sung Ku,[1] Aravind Srinivasan,[1] Benjamin N. Mijts,[2] Jonathan S. Dordick,[1] and Claudia Schmidt-Darnnert.[2] [1]Department of Chemical Engineering, Rensselaer Polytechnic Institute, 101 Rickettes, 110 8th St., Troy, NY 12180-3590 (jeongj@rpi.edu), and [2]Department of Biochemistry, University of Minnesota

We have expressed His-tagged farnesyl pyrophosphate synthase (ispA), dehydrosqualene synthase (crtM), and dehydrosqualene desaturase (crtN) in the active form, purified the enzyme, and performed solution-phase reactions. Isopentenyl pyrophosphate and dimethylallyl pyrophosphate, or farnesyl pyrophosphate, have been used for kinetic study of the production of C30 carotenoids, including diapophytoene, diapophytofluene, diapo-*ζ*-carotene, and diaponeurosporene. Once the optimal three-step conditions were identified, we proceeded to incorporate the enzymes into a continuous flow microfluidic biochip to generate C30 carotenoids and analogues.

181. Kinetic resolution of racemic *N*-acetyl-3-trimethylsilylalanine catalyzed by an *Aspergillus oryzae* cell. Hua Song Peng,[1] **Min-hua Zong**,[1] Ru Jin Zhou,[2] Ruo Xu,[2] and Zhi Feng Chen.[2] [1]Department of Biotechnology, South China University of Technology, Wushan, Guangzhou, China (fax 86-20-87112979, penghuasong@yahoo.com, btmhzong@scut.edu.cn), and [2]Department of Biotechnology, South China University of Technology

Optically active silicon-containing amino acids are important building blocks for asymmetric synthesis of many bioactive compounds. L-Trimethylsilylalanine preparation with asymmetric hydrolysis of racemic *N*-acetyl-3-trimethylsilylalanine catalyzed by an *Aspergillus oryzae* cell was explored for the first time. The effects of cell age, pH, buffer concentration, and temperature on the activity and enantioselectivity of aminoacylase were investigated. An *A. oryzae* cell showed the highest activity when grown in the liquid medium for 48 h. The optimum pH, phosphate buffer concentration, and temperature for the hydrolysis of racemic *N*-acyl-L-3-trimethylsilylalanine were 7.0, 0.1 mM, and 50 °C, respectively, under which a level of substrate conversion of 49.5% could be achieved upon incubation for 79 h with a product enantiomeric excess as high as 92.7%.

182. Kinetic study on (*R*)-oxynitrilase-catalyzed asymmetric transcyanation of acetyltrimethylsilane with acetone cyanohydrin. Ning Li,[1] Shun-rong Huang,[1] **Min-hua Zong**,[1] Jun He,[2] and Di-Heng Luo.[2] [1]Department of Biotechnology, South China University of Technology, Wushan, Guangzhou, China (newslx@yahoo.com.cn, btmhzong@scut.edu.cn), and [2]Department of Biotechnology, South China University of Technology

(*R*)-Oxynitrilase-catalyzed asymmetric transcyanation of silicon-containing ketones with acetone cyanohydrin was a newly discovered reaction and an efficient and facile way to prepare chiral silicon-containing cyanohydrins. To better understand the reaction, the kinetics was explored for the first time. When the enzyme concentration in the reaction system was less than 96 units/mL, there is a linear correlation between enzyme concentration and initial reaction rate, suggesting the determination of the reaction rate by enzymatic reaction at the active site of the enzyme over the range examined. The reaction was initiated by adding 96 units of (*R*)-oxynitrilase/mL from apple seed meal in a biphasic system of 23% (v/v) citrate buffer (0.1 M, pH 5.0) and

diisopropyl ether containing a predetermined amount of acetyltrimethylsilane (ranging from 5 to 16.7 mM) and acetone cyanohydrin (in great excess) at 30 °C and 150 rpm. The reaction was in accordance with the Michaelis−Menten equation as indicated by the linear plot of $1/V_0$ versus $1/C$. The parameters K_m and V_{max} were 13.2 mM and 9.6 mM/h, respectively. The activation energy (E_a) for this reaction was found to be 36.8 kJ/mol.

183. Kinetic study on lipase-catalyzed ammonolysis of trimethylsilylmethyl acetate in organic solvent. Hua Song Peng, **Min-hua Zong**, and Yi Qun Xu. Department of Biotechnology, South China University of Technology, Wushan, Guangzhou, China (fax 86-20-87112979, penghuasong@yahoo.com, btmhzong@scut.edu.cn)

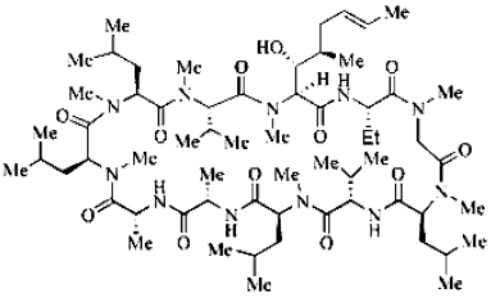
Recently, many studies have focused on the ammonolysis of carboxylic esters for its potential in the preparation of chiral amides and esters. To better understand this reaction, the kinetics of enzymatic ammonolysis of silicon-containing esters was explored for the first time. The reaction was initiated by adding 13.5 units of immobilized lipase from *Candida antarctica* per milliliter in cylohexane containing a predetermined amount of trimethylsilylmethylsilyl acetate (ranging from 5.86 to 58.6 mmol/L) and ammonium carbamate (in great excess) at 30 °C, 160 rpm, and an initial water activity of 0.55. Within the range that was studied, there was no substrate inhibition observed. When the enzyme concentration in the reaction system was less than 9 units/mL, there is a linear correlation between the enzyme concentration and the initial reaction rate, suggesting the elimination of mass transfer limitation and the determination of the reaction rate by enzymatic reaction at the active site of the enzyme over the range that was examined. The reaction was in good accordance with Michaelis−Menten kinetic equation as indicated by the linear plot of $1/V_0$ versus 1/[S]. The apparent Michaelis constant (K_m) and the maximum rate of the reaction (V_m) for trimethylsilylmethyl acetate were 44.6 mM and 3.0 mM/h, respectively. The activation energy (E_a) for this reaction was found to be 36.8 kJ/mol.

184. Facile method for the total synthesis of site-specifically modified histone proteins. Shu He,[1] David Bauman,[1] Jamaine S. Davis,[1] Jennifer L. Gronlund,[1] Fanyu Meng,[2] A. Loyola,[3] Danny Reinberg,[3] Neil L. Kelleher,[2] and Dewey G. McCafferty.[1] [1]Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 (shuhe@mail.med.upenn.edu), [2]University of Illinois at Urbana-Champaign, and [3]University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

The functional capacity of histone proteins can be powerfully expanded by posttranslational modifications. These modifications influence local and global protein architecture and promote interactions with chromatin remodeling complexes and transcriptional factors. To assist in decoding the molecular logic of histone posttranslational modification, we have employed native chemical ligation (NCL) techniques to prepare full-length, site-specifically acetylated, and methylated *Xenopus laevis* histones H3 and H4. In particular, peptides corresponding to the amino acids 15−25 of the N-termini of histones were prepared as peptide thioesters via solid phase peptide synthesis on mercaptopropionyl-MBHA resin using Boc chemistry, and then ligated with recombinantly produced histone C-terminal globular domains containing an engineered N-terminal cysteine residue. Following ligation, desulfurization of the ligated product generated a native histone protein sequence. The synthetic histones were successfully folded into H3−H4 tetramers, assembled into chromatin with recombinant histone H2A/2B using the RSF chromatin assembly complex, and served as functional substrates for histone-modifying enzymes.

185. Chemoenzymatic modification of immunosuppressant cyclosporin A. Michael H. Hemenway, **Qibo Zhang**, Peter C. Michels, Joseph O. Rich, Yuri L. Khmelnitsky, Simon N. Haydar, and Bruce F. Molino. Albany Molecular Research, Inc., Mount Prospect, IL 60056

Cyclosporin A is the most widely prescribed drug for rejection suppression after organ transplantation. However, chronic use of this agent can lead to loss of the transplanted organ due to its nephrotoxicity and neurotoxicity. Decades of investigation have led to the syntheses of more than 3000 cyclosporin analogues, but thus far, no improved cyclosporin analogues have made it to the market. Here we report a chemoenzymatic approach for efficient generation of novel cyclosporin A derivatives.



186. Chemoenzymatic synthesis of pseudosugars from iodobenzene. Nuria Martinez-Llamas, Derek R. Boyd, Narain D. Sharma, John F. Malone, Colin R. O'Dowd, and Christopher C. R. Allen. Department of Chemistry, Queen's University of Belfast, Belfast BT9 5AG, United Kingdom

The versatile (1*S*,2*S*)-3-iodo-3,5-cyclohexadiene-1,2-diol metabolite, formed by bacterial dioxygenase-catalyzed oxidation of iodobenzene, using a mutant strain of *Pseudomonas putida* UV4, has been used as an enantiopure precursor of the carbasugars pseudo-β-D-altrose, pseudo-α-L-galactose, pseudo-β-L-glucose, and pseudo-α-L-glucose. Regioselective inversion of one and two chiral centers, substitution of the iodine atom with a carbomethoxy group, and stereoselective catalytic hydrogenation of the α,β-unsaturated ester are the key steps in the synthesis of these pseudosugars. The relative and absolute configurations of the pseudosugars were established by a combination of X-ray crystallography and NMR spectroscopy.

187. Asymmetric reduction of acetyltrimethylsilane with a new isolate *Rhodotorula* sp. AS2.2241. Di-Heng Luo,[1] **Min-hua Zong**,[2] and Jian-He Xu.[3] [1]Department of Biotechnology, South China University of Technology, 1 Wushan St., Guangzhou 510640, China (fax 86-20-87112979,

luodiheng@163.com), [2]Department of Biotechnology, South China University of Technology, 1 Wushan St., Guangzhou 510640, China (fax 86-20-87112979, btmhzong@scut.edu.cn), and [3]Laboratory of Applied Biocatalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology

A new isolate, *Rhodotorula* sp. AS2.2241, capable of reducing acetophenone and α-bromoacetophenone with high stereoselectivity, was further studied in an effort to exploit its potential in asymmetric reduction of silicon-containing ketones to silyl alcohols. After encapsulation, the cells were used in an aqueous or an aqueous and organic solvent biphasic system for asymmetric reduction of acetyltrimethylsilane (ATMS) to (*S*)-1-trimethylsilylethanol (TMSE). It has been found that higher product yield and product enantiomeric excess (ee) could be achieved with immobilized cells in an aqueous and organic solvent biphasic system. Isooctane was found to be the most suitable organic phase for the reaction. The optimum volume ratio of aqueous phase to organic phase, reaction temperature, buffer pH, and shake speed were 1/1, 40 °C, 6.5, and 150 rpm, respectively, under which the product yield and the product ee were as high as 99 and 90%, respectively, which are much better than the results reported by another group.

188. Biosynthesis of quinolinate from tryptophan in bacteria. Keri L. Colabroy,[1] Vasiliy Goral,[1] Oleg Kurnasov,[2] Andrei Osterman,[2] and Tadhg Begley.[1] [1]Department of Chemistry and Chemical Biology, Cornell University, 119 Baker Laboratory, Ithaca, NY 14850 [fax (607) 255-4137, klc38@cornell.edu], and [2]Integrated Genomics Inc.

Quinolinate is a precursor to the ubiquitous redox cofactor nicotinamide adenine dinucleotide (NAD). Most prokaryotic organisms biosynthesize quinolinate from dihydroxyacetone phosphate and aspartate using only two enzymes, NadA and NadB. The alternative pathway used by eukaryotic organisms catabolizes tryptophan over five enzymatic steps to yield quinolinate. This tryptophan to quinolinate pathway has generated interest not only because of its role in NAD biosynthesis but also because of the neurotoxic effects exerted by quinolinate. The final enzymatic step controlling the formation of quinolinate is catalyzed by the non-heme Fe^{2+}-dependent 3-hydroxyanthranilate-3,4-dioxygenase (HAD). Study of HAD has been limited due to the availability of only unstable eukaryotic protein from weak expression systems. This poster describes the first identification and full characterization of the five enzymes of the tryptophan to quinolinate pathway from a bacterial species, as well as mechanistic work on HAD made possible by the overexpression of the bacterial enzyme.

189. Biosynthesis of squalamine and related antimicrobial aminosterols in cartilaginous fish liver. Ann E. Shinnar,[1] Eleanor Allen,[1] Christine Musich,[1] Joan Shu,[1] Rayna Goldstein,[1] Hege Myhre Willemsen,[2] and Yasuhiro Itagaki.[3] [1]Department of Chemistry, Barnard College, 3009 Broadway, New York, NY 10027 [fax (212) 854-2310, ashinnar@barnard.edu], [2]More Research, and [3]Chemistry Department, Columbia University

Squalamine is a sulfated aminosterol exhibiting potent antimicrobial and antiangiogenesis activity. Since this therapeutically important water-soluble steroid is produced abundantly in spiny dogfish (*Squalus acanthias*), we are investigating its biosynthetic pathway in shark liver. After devising a miniscale extraction scheme using organic solvents and C18 SPE, we found that this zwitterionic aminosterol is localized predominantly in the gray fat and total membrane (TM) fractions of spiny dogfish liver. Our subsequent survey of 20 species of cartilaginous fish representing different orders showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* in liver extracts from the gray fat and TM of six other squaliform sharks, but not in carcharhiniform and lamniform sharks or rajiform rays. Biologically active fractions analyzed by ESI-MS revealed mass spectral patterns characteristic of squalamine and related aminosterols. Our initial survey suggests that aminosterol biosynthesis is not universal among cartilaginous fish, but varies among orders within Chondrichthyes.

190. Sensing system for arsenite detection based on a regulatory protein. Jessika Feliciano, Sapna K. Deo, and Sylvia Daunert. Department of Chemistry, University of Kentucky, 125 Chemistry-Physics Building, Lexington, KY 40506-0055 [fax (859) 323-1069, jsfeli0@uky.edu]

A fluorescent sensing system for arsenite has been developed on the basis of the regulatory protein ArsR. ArsR is part of the *Escherichia coli* chromosomal ars operon which gives resistance to arsenite and antimonite. ArsR binds arsenite and antimonite in a highly specific manner. This binding induces a hinge motion in ArsR, and the resultant protein conformational change constitutes the basis of the sensing system. The gene encoding ArsR was isolated from *E. coli* using the polymerase chain reaction (PCR) and was cloned into an expression vector. An environment sensitive fluorescent probe, *N*-[2-(1-maleimidyl)ethyl]-7-(diethylamino)coumarin-3-carboxamide (MDCC), was attached to the lysine residues of the ArsR. Upon addition of arsenite, the conformational change in the protein leads to a change in the environment around the fluorophore, causing a change in the fluorescence intensity of the fluorophore. Consequently, this change in the fluorescence intensity can be directly correlated with the amount of arsenite in the sample.

191. Sensitive one-step HPLC method for determination and quantification of *N*-methyl-D-aspartate. Mara Tsesarskaia,[1] **George Fisher**,[1] Erika Galindo,[1] and Gyula Szokan.[2] [1]Department of Chemistry, Barry University, 11300 NE 2 Ave., Miami Shores, FL 33161 [fax (305) 899-3479, mtsesarskaja@mail.barry.edu, gfisher@mail.barry.edu], and [2]Institute for Organic Chemistry, Eotvos University

N-Methyl-D-aspartate (NMDA) is a widely known neuroexcitatory compound previously considered only a synthetic compound. However, since 1987 NMDA has been found in many living organisms. NMDA is predicted to play a significant role in neuroendocrinology, but further advances in the study of this compound have been hindered by the lack of a reliable and sensitive method for its determination and quantification. There are three published methods for fluorometric determination of NMDA by HPLC. All three methods require a preliminary step of removal of primary amino acids by treatment with *o*-phthaldialdehyde (OPA) prior to determination of NMDA. We report here a one-step

derivatization procedure with *N*-α-(2,4-dinitro-5-fluorophenyl)-D-valine amide (FDNP-Val-NH_2, a close analogue of Marfey's reagent but having a much higher extinction coefficient) followed by chromatography on an inexpensive ODS-Hypersil reversed phase HPLC column with a 2 min isocratic elution from 0.1% TFA/water to 0.1% TFA/MeCN (90:10), followed by a linear gradient up to 70% of 0.1% TFA/MeCN. The derivatives have a UV absorption maximum at 340 nm, permiting detection levels in the range of 50-100 pmol. The NMDA peak does not interfere with those of any primary or secondary amino acids (or GABA and taurine); hence, the method does not require their removal by pretreatment with OPA. Our method is highly reliable and fast (overall run time of 50 min). After extensive studies with 20 natural amino acids, samples of biological tissues (brain, optical lobe, and bucchal mass of octopus) were analyzed successfully. NMDA was found in all of these octopus samples, confirming the results of other researchers. (Supported by NIH-MBRS-SCORE Grant GM-45455.)

192. Stability of benzotriazole derivatives with free Cu, Zn, Co, and metal-containing enzymes: Binding and interaction with superoxide dismutase and vitamin B_{12}. Muna Abu-Dalo and **Mark T Hernandez**. Department of Civil, Environmental and Architectural Engineering, University of Colorado, 1111 Engineering Dr. #441, Boulder, CO 80309 [fax (303) 492-7317, muna.abu-dalo@colorado.edu, mark.hernandez@colorado.edu]

There are many large environmental sources of benzotriazoles because high concentrations are universally included in aircraft and roadway deicing fluids, antifreeze, brake fluids, lubricating oils, and industrial cooling systems. Some benzotriazole derivatives have been implicated as hormone regulators, which also carry the ability to induce uncoupling responses or otherwise inhibit respiration processes in some ecosystem receptor models. However, the mechanisms associated with benzotriazole toxicity and inhibition are as yet unknown. Using differential pulse polarography, the stability constants of commercially significant corrosion inhibitors, 4- and 5-methylbenzotriazole, coordinated with free Cu(II) and Co(III), were determined to be greater than 10^8. Polarographic analyses were extended to confirm that methybenzotriazole also binds the copper center(s) in the ubiquitous enzyme superoxide dismutase, and the corrin site in the coenzyme cobalamin (vitamin B_{12}). These results suggest that the metal chelating ability of this unique class of compounds may confer inhibition to certain enzyme systems.

193. Stochastic sensing of 2,4,6-trinitrotoluene. Xiyun Guan and Hagan P. Bayley. Department of Medical Biochemistry and Genetics, Texas A&M University System Health Sciences Center, 440 Joe H. Reynolds Medical Building, College Station, TX 77843 (xyguan@medicine.tamu.edu)

2,4,6-Trinitrotoluene (TNT) is an explosive mainly used in military and industrial applications, as well as a priority pollutant listed by the U.S. Environmental Protection Agency. A stochastic sensing method for TNT has been developed using a protein pore constructed by engineering staphylococcal α-hemolysin. This method employed single-channel recording with the planar lipid bilayer technique, where current modulation represents individual binding events. In the absence of target compounds, no binding events were detected. However, in the presence of TNT, binding events with reduced current were identified, and the concentration of TNT could be related to the event frequency. The method could detect TNT at micromolar levels. In addition, the sensing method was selective for TNT. Compounds such as 2,6-dinitrotoluene, 2,4-dinitrotoluene, and 4-nitrotoluene did not interfere with TNT detection. The method has a potential application in identifying and quantifying TNT in an aqueous environment.

194. Using liquid crystals and nanostructured surfaces to detect regulatory proteins involved in cell signaling pathways. Yan-Yeung Luk,[1] Nicholas L. Abbott,[1] Paul J. Bertics,[2] Matthew L. Tingey,[1] and David J Hall.[3] [1]Department of Chemical Engineering, University of Wisconsin, 1415 Engineering Dr., Madison, WI 53706-1691 [fax (608) 262-5434, yluk@che.wisc.edu], [2]Department of Biomolecular Chemistry, University of Wisconsin, and [3]Department of Chemistry, Lawrence University

This presentation reports oriented immobilization of protein receptors on gold films possessing nanometer-scale topographies, and detection of protein binding to these receptors by using liquid crystals. We use self-assembled monolayers (SAMs) formed from nitrilotriacetic acid (NTA)-terminated alkanethiols (**1**) and tri(ethylene glycol)-terminated alkanethiols (**2**). Mixed SAMs formed from **1** and **2** bind the hexahistidine-tagged protein MEK via specific complexation of the hexahistidine tags of MEK to the Ni(II)−NTA complexes on the surface. When gold films are prepared by oblique deposition at an angle of 30° from the normal, we measured the level of bound MEK to disrupt the uniform orientation of 4-cyano-4′-pentylbiphenyl (5CB), thus leading to an easily visualized change in the optical appearance of the liquid crystal. However, on gold films deposited at an angle of 40° from the normal, bound MEK does not disrupt the alignment of the liquid crystal, whereas binding of an antibody specific for MEK does lead to a nonuniform alignment. These results suggest that nanostructured surfaces presenting NTA and ethylene glycol-terminated SAMs form a useful interface for imaging proteins bound to histidine-tagged, surface-immobilized receptors.

195. Detection of chirality in solid-state amino acids using Fourier transform vibrational circular dichroism. Rosina A. Lombardi,[1] Xiaolin Cao,[1] Soon Sam Kim,[2] Rina K. Dukor,[3] and Laurence A. Nafie.[1] [1]Department of Chemistry, Syracuse University, 1-014 Center for Science and Technology, Syracuse, NY 13244-4100 (ralombar@syr.edu), [2]Jet Propulsion Laboratory, and [3]BioTools Inc.

The search for life on Mars has led many researchers involved in NASAs Martian exploration to focus on the development of technology that can detect and describe indicators of life. Amino acids, which serve as the basic building blocks of life, can be used as biomarkers, if detected, for the proof of existence of life on solar system bodies. For the purpose of developing a technique that can elucidate the life issue, current research is focused on the improvement of solid-state analysis for the measurement of vibrational

circular dichroism (VCD) in the mid-IR region, where chiral signatures can be detected and interpreted with the most certainty. Solid-state sampling methods employed thus far include mulls, pellets, and a spray technique that allows for the deposition of finely divided particles to create a thin film. The spray method has greatly improved VCD sensitivity, causing signal intensities to approach those of their IR counterparts.

196. HPLC separations using circular dichroism and fluorescence detection. Richard A. Larsen[1] and Amanda L. Jenkins.[2] [1]Special Applications, Jasco Inc., 8649 Commerce Dr., Easton, MD 21601 [fax (410) 822-7526, larsen@jascoinc.com], and [2]Applications Development, Jasco Inc.

Quantitative and qualitative detection of HPLC separations is generally accomplished using UV−vis detectors; however, this type of detector is not capable of differentiating between enantiomers. These detectors also do not have the sensitivity required for extremely small amounts of sample. This paper will discuss the use of circular dichroism (CD) detectors for the detection and differentiation of chiral compounds. The high sensitivity of fluorescence detection will also be discussed and examples presented illustrating the applicability of fluorescence detection for small sample aliquots. CD detectors can be used to detect incomplete chiral separation of compound enantiomers, and quantitative results for the amounts of enantiomeric excess can be obtained. Fluorescence detection can provide highly sensitive quantitative analysis for molecules containing a fluorophore, increasing the detection range afforded by standard UV−vis detectors.

197. Oxidation of conjugated linoleic acid by oat seed peroxygenase: Identification of products. George J. Piazza, Thomas A. Foglia, and Alberto Nunez. Fats, Oils, and Animal Coproducts Research Unit, Eastern Regional Research Center, United States Department of Agriculture, 600 E. Mermaid Ln., Wyndmoor, PA 19038 [fax (215) 233-6559, gpiazza@arserrc.gov]

Conjugated linoleic acid (CLA) is a term that refers to the positional and geometric conjugated dienoic isomers of linoleic acid. In the past two decades, studies have shown that CLA isomers can suppress cancer development, are antiantherogenic, and have both growth-promoting and body fat-reducing properties. The physiological mode of action of CLA is currently under intense scrutiny, and there is interest in generating chemical derivatives to determine if these have biological activity. Recently, we have used oat seed peroxygenase, an iron heme enzyme that participates in oxylipin metabolism in plants, to form epoxides on unsaturated fatty acids and esters, as well as alkenes. We have extended our studies to include the effect of peroxygenase on one CLA isomer, 9(*Z*),11(*E*)-octadecadienoic acid. After product methylation, the acid sensitive monoepoxide, methyl 9,10(*Z*)-epoxy-11(*E*)-octadecenoate (**1**), was found to be the primary oxidation product produced in aqueous media, although methyl 9,10(*E*)-epoxy-11(*E*)-octadecenoate and methyl 11,12(*E*)-epoxy-9(*Z*)-octadecenoate were produced in minor amounts. Epoxide **1** and its free acid are allylic unsaturated epoxides, and are therefore somewhat unstable. Upon hydrolysis, several polar compounds were produced, and their structural assignments were complicated by reactions that occurred directly in the mass spectrometer. These could be inhibited by catalytic hydrogenation of the double bonds before mass spectrometric analysis. The hydrolysis products were identified as methyl 9,10(*threo*)-dihydroxy-11(*E*)-octadecenoate, methyl 9,10(*erythro*)-dihydroxy-11(*E*)-octadecenoate, methyl 9,12(*erythro*)-dihydroxy-10(*E*)-octadecenoate, and methyl 9,12(*threo*)-dihydroxy-10(*E*)-octadecenoate. Evidence for the structural assignments of the hydrolysis products will be presented.

198. Preparation of boiling-stable granular resistant starch with enzymes and heat-moisture treatment. Zhigang Luo,[1] Qunyu Gao,[2] and Liansheng Yang.[3] Carbohydrate Lab, South China University of Technology, Wushan, Guangzhou 510640, China (fax 86-20-87111725, luozhig75@sohu.com), [2]Carbohydrate Lab, Food College, South China University of Technology, and [3]Food and Biological Engineering College, South China University of Technology

The purpose of the paper was to examine whether enzymatic hydrolysis of high-amylose corn starch (Hylon) would enhance the effect of heat-moisture treatment to produce boiling-stable granular resistant starch (RS). Native starch was prepared with α-amylase and pullulanase and then subjected to heat-moisture treatment under 30% moisture and 100 °C for 12 h. The amount of RS that survives boiling during analysis was determined by the AOAC method of determining the total amount of dietary fiber. It has been demonstrated that when α-amylase was added at 60 °C and pH 7.2 for 80 min with 2 MWU/g of dry starch(s) and pullulanase hydrolysis carried out at 55 °C and pH 5.3 for 24 h with 3.2 ASPU/g of ds, after heat-moisture treatment the highest yield of RS (60.2%) could be achieved.

199. Study on the biochemical treatment of acrylic fiber wastewater. Chaocheng Zhao, Haihong Liu, and Zhiwei Wang. Department of Environmental Science and Engineering, University of Petroleum, 271#Beier Rd., Dongying 257061, China (fax 86-5468393524, zhaochch@mail.hdpu.edu.cn)

The active sludge method and some strengthening techniques of it on acrylic fiber wastewater treatment were studied. These data showed that it is very difficult to make acrylic fiber wastewater reach the discharge standard using only the aerobic active sludge method. Fenton reagent, ozone, and the anaerobic acidification method as pretreatment had little effect on improving the removal rate of COD. Adding an abundance of nutriment or domestic sewage has little effect on cometabolization. Therefore, it is necessary to add a physical or chemical method as a subsequent treatment technique after the active sludge method.

200. Effect of steam explosion on cellulose degradation by *Trichoderma reesei* cellulase. Solmaz Akmaz,[1] Ismet Gurgey,[1] and Muzaffer Yasar.[2] [1]Department of Chemical Engineering, University of Istanbul, Avclar, Istanbul, Turkey (solmaz@istanbul.edu.tr), and [2]Department of Chemical Engineering, University of Istanbul

The enzmatic hydrolysis of cellulose for sugar production has received a great deal of attention in recent years. Enzymatic hydrolysis is an interesting way to produce sugars

from cellulosic wastes because of its mild operating conditions and the absence of byproducts. Pretreating cellulosic substrates before enzymatic hydrolysis renders the material maximally susceptible to attack by the polysaccharide degrading enzymes. In this study, we investigated the effect of high-temperature hydrolysis on the saccharification of biomass by using pure α-cellulose as a model compound. Hydrolysis of pure α-cellulose and its subsequent saccharification by *Trichoderma reesei* cellulases was investigated. Firstly, the cellulose was hydrolyzed by water in a 10 mL stainless batch reactor at 180, 190, 205, and 220 °C for up to 6 h. Solid and dissolved fractions were separated by filtration. The solid residual fractions were used to calculate the reaction rate constants and the Arrhenius parameters for hydrolysis of cellulose at 180, 190, 205, and 220 °C. The reaction products obtained at 220 °C and 4 h were subjected to a further enzymatic hydrolysis in 1 L flasks at 30, 40, and 50 °C agitated at 350 rpm for up to 24 h. The pH was adjusted to 4.7 with 0.1 M sodium acetate buffer. Experimental results provide insight into hydrolysis of cellulosic biomass by water at high temperatures and by enzymatic reaction.

201. Enzymatic transesterification for biodiesel production with a novel route from renewable oils in a solvent-free medium. Wei Du, Yuanyuan Xu, Dehua Liu, and Jing Zeng. Department of Chemical Engineering, Tsinghua University, Beijing 100084, China (fax 0086-10-62785475, duwei@tsinghua.edu.cn)

A novel route for biodiesel production has been developed in this paper, in which methyl acetate was adopted as a novel acyl acceptor, and it has been demonstrated that this novel acyl acceptor showed no negative effect on enzyme activity. And in this novel route, there is no glycerol produced in the process so it is very convenient to recycle the lipase without any extra treatment, and this new route seems to be very promising for enzymatic transesterification for large-scale production of biodiesel. Novozyme435 (immobilized *Candida antarctica* lipase) was screened from several lipases, giving the highest methyl ester (ME) yield of 92%. The optimum conditions of the transesterification were as follows: 30% enzyme based on oil weight, methyl acetate:oil molar ratio of 12:1, temperature of 40 °C, and reaction time of 10 h. Since no glycerol was produced in the process, it is very convenient for recycling the catalyst, and lipase retained high activity after being used repeatedly for a long period of time.

202. Enzymatic transesterification of soybean oil for biodiesel production with different acyl acceptors in a solvent free medium. Yuanyuan Xu, **Wei Du**, Dehua Liu, and Jing Zeng. Department of Chemical Engineering, Tsinghua University, Beijing 100084, China (fax 0086-10-62785475, duwei@tsinghua.edu.cn, duwei@tsinghua.edu.cn)

Lipase-catalyzed transesterification of renewable oil is very promising for biodiesel production. With some short chain alcohols as the acyl acceptor, a high yield of 95% could be obtained; however, byproduct glycerol was found to have some negative effect on enzymatic activity. Though 2-propanol was effective for glycerol removal during the repeated use of lipase when short chain alcohols were adopted as the acyl acceptor, it is complicated especially for large-scale production. Some other acyl acceptors have been explored in this paper, and methyl acetate seems to be a promising acyl acceptor for biodiesel production since no glycerol is produced in the process. The methyl ester (ME) yield of 92% could be achieved, and lipase expressed good stability in the reaction system.

203. System configurations for processes based on enzymatic hydrolysis of lignocellulosic biomass. Kiran L Kadam, Ali Mohagheghi, and James D McMillan. Biotechnology Division for Fuels and Chemicals, National Renewable Energy Laboratory, 1617 Cole Blvd., Golden, CO 80401 [fax (303) 384-6877, kiran_kadam@nrel.gov]

By development of a common platform for fermentable biomass sugars, a number of products, including ethanol, can be produced from lignocellulosic feedstocks (e.g., agricultural, forestry, and other plant-based residues). This facilitates diversification beyond biomass-to-ethanol processes into an integrated biomass refinery or biorefinery concept. In this context, the integration issues in a process based on enzymatic hydrolysis of lignocellulosic biomass, with ethanol as a model product, will be discussed. The ethanol yield and productivity in a biomass-to-ethanol process are limited by the extent and rate of cellulose conversion. Therefore, the mode by which fermentation and saccharification are integrated is dictated by the characteristics of the cellulase system used to hydrolyze cellulose. Cellulase enzymes are typically more thermostable than ethanologenic microorganisms but are also inhibited by the accumulation of hydrolysis products (cellobiose and glucose). In simultaneous saccharification and fermentation (SSF), sugars from lignocellulosic biomass are produced and converted in situ to ethanol, thereby eliminating or mitigating sugar−product inhibition of enzymatic cellulose conversion. In a separate or sequential hydrolysis and fermentation (SHF) process that decouples these two operations, an intermediate stream of fermentable sugars is produced, which then can be fermented to a variety of value-added products, including ethanol. The disadvantage of SSF is that cellulose hydrolysis must be carried out under conditions that are compatible with the fermentative microorganism. In contrast, a hybrid hydrolysis and fermentation (HHF) process configuration enables most of the cellulose conversion to be carried out at higher temperatures which are optimal for enzyme action, which is followed by fermentation conducted at lower temperatures which are suitable for the microbial catalyst. Although HHF is an excellent compromise for currently available enzymes and microorganisms, second-generation cellulases with higher resistance to end-product inhibition and potential thermophilic ethanologens would change the dynamics. We will illustrate the relative merits and demerits of these process configurations in greater detail and propose a rational framework for determining how to best integrate cellulose conversion and fermentation steps based on the characteristics of the enzyme and microbial systems.

204. Association of scrapie prion protein with well-defined soil constituents. Joel A. Pedersen,[1] **Kristen E. Phillips**,[1] Christopher J. Johnson,[2] Debbie I. McKenzie,[3] and Judd M. Aiken.[3] [1]Molecular and Environmental Toxicology Center and Department of Soil Science, University of Wisconsin−

Madison, 1525 Observatory Dr., Madison, WI 53706-1299 [fax (608) 265-2595, joelpedersen@wisc.edu, kephillips2@wisc.edu], [2]Program in Cellular and Molecular Biology, Department of Animal Health and Biomedical Sciences, University of Wisconsin—Madison, and [3]Department of Animal Health and Biomedical Sciences, University of Wisconsin—Madison

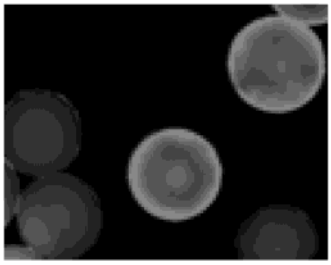
Compelling circumstantial evidence suggests soil may serve as an environmental reservoir for the infectious agent in transmissible spongiform encephalopathies (TSEs) of ovids and cervids. Sorption to specific soil constituents likely contributes to the preservation of the TSE agent in the environment. We investigated the association of the scrapie-specific isoform of the prion protein (PrP^{Sc}) with well-defined soil constituents as an initial step in determining the influence of sorption on the environmental stability of the infectious agent. Batch sorption experiments were conducted with smectitic clay and quartz sand to determine the extent of association with these common soil minerals. Nonsorbed and mineral surface-sorbed PrP^{Sc} were separated by centrifugation through a sucrose cushion, and the amount of PrP^{Sc} sorbed was determined by Western blot analysis. Initial results indicate the PrP^{Sc} sorption capacity of clay minerals substantially exceeds that of quartz sand. These results have important implications for the mobility and bioavailability of PrP^{Sc} in soil environments.

205. PAMAM dendrimer-modified paramagnetic beads: Improved solid support for bead-based immunoassays. Svetlana Farrell,[1] Shobha Purushothama,[2] H. Brian Halsall,[1] and William R. Heineman.[1] [1]Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221-0172 (farrellsvm@zoomtown.com), and [2]Efoora/Virotech

An enzyme-labeled sandwich immunoassay with dendrimer-modified beads as a solid support has been developed for the artificial microorganism, Bugbead. Paramagnetic beads were functionalized sequentially with G0.5, G2.5, G5.5, or G6.5 Starburst PAMAM dendrimers, and then neutravidin. The effect of the dendrimers on the bead capacity for capture antibody and on the immunoassay was evaluated by comparison to the control assays with neutravidin attached directly to the beads. Detection was carried out with a rotating disk electrode in a 40 μL drop. The capacity of the beads increased when going from the G0.5 to G5.5 dendrimer and then decreased when going to G6.5. The G5.5 bead capacity for the antibody was 15 times higher than the control, and these beads also exhibited increased assay sensitivity and analyte capture in the Bugbead immunoassay. The detection limit was 700 and 1500 Bugbeads for the G5.5 dendrimer and the control assay, respectively.

206. Quantum dots for simultaneous dual fluorescence on-bead assay for monitoring the capture of proteins Hernando J. Olivos, **Kiran Bachhawat-Sikder**, and Thomas J. Kodadek. Center for Biomedical Inventions and Departments of Internal Medicine and Molecular Biology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75390-9185 [fax (214) 648-4156, hernando.olivos@utsouthwestern.edu, kiran.sikder@utsouthwestern.edu]

A new methodology has been developed to screen combinatorial libraries on resin for protein ligands with the aid of a red-emitting quantum dot. This methodology supports a two-color fluorescence assay in which "hits" from a library screen show a red emission while beads that do not carry a protein-binding compound show a green emission. This is due to the intrinsic fluorescence of the polystyrene bead. This methodology is sensitive and allows the detection of proteins with greater efficiency than traditional organic chromophores used in fluorescence assays, which do not allow the simultaneous dual fluorescence detection.



207. Selection of molecular beacons. Manjula Rajendran and Andrew D. Ellington. Department of Chemistry and Biochemistry, University of Texas at Austin, 1 University Station A5300, Austin, TX 78712 [fax (512) 471-7014, manjulaj@mail.utexas.edu]

Nucleic acid binding species (aptamers) that have high affinities and specificities for target molecules can be selected from combinatorial oligonucleotide libraries. Aptamers that signal the presence of their cognate ligands in solution by virtue of appended fluorophores in conformationally labile positions have been termed "signaling aptamers". We have developed a method that directly couples selection for ligand binding to a nucleic acid conformational change that engenders signaling. As a proof of principle, we have selected signaling aptamers that are responsive to oligonucleotide effectors. The selected signaling aptamers function as molecular beacons, but utilize a novel mechanism for signaling that is different from a canonical molecular beacon. The direct selection method can potentially be applied to a wide variety of target molecules, and may thereby allow the development of numerous reagentless biosensors.

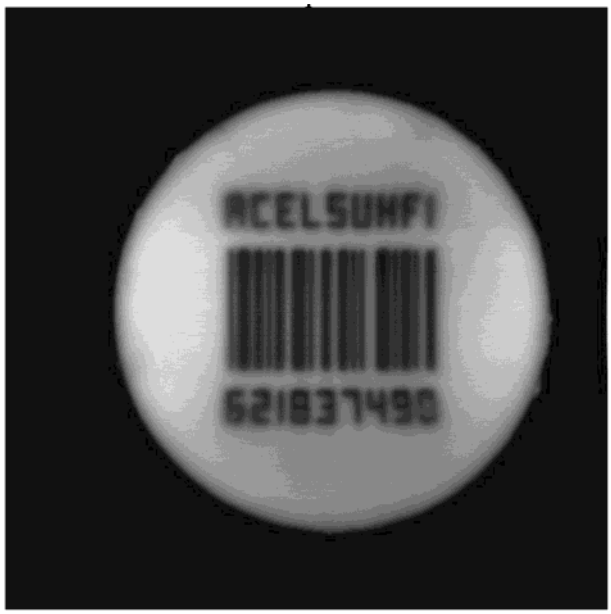
208. Solution phase combinatorial libraries for whole-cell selection assays. Diana Samaroo and Charles M. Drain. Department of Chemistry and Biochemistry, Hunter College and The Graduate Center of the City University of New York, 695 Park Ave., New York, NY 10021 [fax (212) 772-5332, dsamaroo@hunter.cuny.edu]

Photodynamic therapeutics (PDT) use light to illicit a toxic response of an otherwise nontoxic molecule such as a dye, or to cause the dye to activate endogenous molecules in the cell or tissue, mostly by the photosensitized formation of singlet oxygen. Several labs have demonstrated by in vitro experiments that PDT may be effective for antiviral and antibacterial applications. Combinatorial chemistry allows for the synthesis of large libraries of molecules in few reaction steps. Our work is on solution-phase libraries so that whole cells may be used to discover active agents. This mode of discovery, coupled with the power of modern

analytical methods, is especially useful for therapeutics wherein the entire cell, virus, or virus-infected cell is the target. The reason for this is that there may be a cadre of compounds that have affinity for different parts of the cell that act in concert to illicit a synergistic effect and more efficiently kill the cell. The general development of solution phase combinatorial libraries of chromophores such as porphyrins, porphyrazines, and corroles will be presented. Preliminary data on whole-cell selection assays using cancer cell lines will also be presented, as will testing for activity as photodynamic therapeutics (PDT).

209. New generation of encoded microcarriers. Kevin Braeckmans,[1] **Stefaan C. De Smedt, Jr.**,[1] Jo Demeester,[1] Marc Leblans,[2] Chris Roelant,[2] and Emmanuel Gustin.[2] [1]Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium (fax +32-9-264.81.89, kevin.braeckmans@rug.ac.be, stefaan.desmedt@rug.ac.be), and [2]Tibotec BVBA

Performing bead-based assays on large numbers of molecules in drug screening requires labeling of each of the microspheres according to the particular ligand bound to its surface. This allows mixing of the uniquely encoded microspheres and simultaneous assaying of them. Those microcarriers that show a positive reaction may then have their code read, thereby leading to the identity of the ligand. We present a new way of optically encoding such microcarriers for screening purposes (e.g., polystyrene microspheres). In a fluorescently dyed microcarrier, a pattern can be written by "spatial selective photobleaching" of the fluorescence, a photoinduced process through which certain regions of a fluorescent sample lose their fluorescent color. Any geometry can be bleached, e.g., a symbol or bar code, at a certain depth in the microcarrier by using a modified confocal laser scanning microscope. This technique provides for a virtually unlimited number of unique codes.



210. Chemical genetic studies of cyclin degradation. Gregory P. Tochtrop[1] and Randall W. King.[2] [1]Institute of Chemistry and Cell Biology, Harvard Medical School, 250 Longwood Ave., SGM 604, Boston, MA 02115 [fax (617) 432-3702, gregory_tochtrop@hms.harvard.edu], and [2]Harvard Institute of Chemistry and Cell Biology, Harvard Medical School

To identify small molecules that inhibit known or novel proteins required for cyclin B proteolysis, we screened for compounds that blocked the degradation of a cyclin B-luciferase fusion protein in *Xenopus* cell cycle extracts. After screening 108 000 compounds, we identified 29 that reproducibly inhibited degradation. By assessing the state of mitotic phosphorylation in treated extracts, we found that 19 inhibitors blocked mitotic entry whereas 10 compounds blocked mitotic exit. Counterscreening with a β-catenin−luciferase fusion protein allowed us to discriminate specific inhibitors of cyclin proteolysis from broad inhibitors of ubiquitin-dependent degradation. Our subsequent studies have focused on the identification of the targets of inhibitors that block exit from mitosis. Characterization of one inhibitor using biochemical reconstitution and affinity depletion experiments suggests that it inhibits the activity of a novel component required for cyclin B proteolysis in *Xenopus* extracts.

211. Combinatorial artificial receptor arrays. Robert E. Carlson. RECEPTORS LLC, Suite 510B/MD 23, 1107 Hazeltine Blvd., Chaska, MN 55318 [fax (952) 448-1651, ecochem@mn.rr.com]

Artificial receptors are applicable by design to any target and application environment. High-priority artificial receptor applications include proteome analysis, pharmaceutical development, and bioterrorism defense. The development of useful artificial receptors has been an elusive goal. The application of combinatorial chemistry and microarray technology to receptor development has the potential to revolutionize the quest for practical artificial receptors. The combinatorial artificial receptor array (CARA) platform is an integrated system for the rapid identification and optimization of artificial receptors to any target. The CARA approach is based on (1) the design of molecular recognition-focused receptor subunits which are (2) displayed using a microarray format for (3) the high-throughput identification of artificial receptors to any target. The development of prototype CARA arrays will be presented along with a discussion of progress toward the preparation of the single-slide Discovery100 ReceptorArray of 100 000 candidate receptors.

212. Electrostatic interaction in the denatured state of protein. Jaehyun Cho[1] and Daniel P. Raleigh.[2] [1]Graduate Program in Biochemistry and Structural Biology, State University of New York at Stony Brook, 100 Nicolls Rd., Stony Brook, NY 11790 [fax (631) 632-7960, jacho@ic.sunysb.edu], and [2]Department of Chemistry, State University of New York at Stony Brook

The denatured states of the wild-type N-terminal domain of L9 and a surface mutant, K12M, were investigated by studying the pH-dependent stability. Mutation of K12 to M12 increases the stability of the protein by 1.7 kcal/mol. K12 is highly mobile and fully solvent exposed, and does not participate in any salt bridge interactions in the native state. Structural and energetic analysis of the wild type and K12M

shows that the increase in stability is not due to a native state effect. Analysis of pH-dependent stability profiles clearly shows that the mutation causes changes in the denatured state. Analysis of the same type of mutation for other lysine residues near K12 indicates the existence of nonrandom residual electrostatic interaction in a denatured state. Our results suggest that electrostatic interactions between distant residues occur in the denatured state and can be as important as native state interactions in determining stability.

213. Probing the folding of the N-terminal domain of L9 using unnatural amino acids. Burcu Anil and Daniel P. Raleigh. Department of Chemistry, State University of New York at Stony Brook, Graduate Chemistry Building, Room 643, Stony Brook, NY 11794 [fax (631) 632-7960, banil@ic.sunysb.edu]

The transition state for folding of the N-terminal domain of ribosomal protein L9 has been previously characterized by ϕ value analysis. The ϕ values have been found to be fractional and very small (between 0 and 0.4). Further mutations were introduced into the domain using the unnatural amino acids 2-aminobutyric acid and norvaline. Residues selected for substitution, the side chains of which are in the hydrophobic core, were the ones for which the ϕ values have been the highest of all in the previous study. Substitutions with unnatural amino acids removed the possible interactions made by individual methyl groups of these residues. Thermodynamic and kinetic analyses of these mutants showed that interactions are weakly formed in the transition state upon folding.

214. High-pressure refolding of bikunin protein aggregates: efficacy and thermodynamics. Matthew B. Seefeldt,[1] Jun Ouyang,[2] Wayne A. Froland,[2] and Theodore W. Randolph.[1] [1]Department of Chemical Engineering, University of Colorado, Campus Box 424, Boulder, CO 80503 (seefeldt@colorado.edu), [2]Bayer Corp.

Purpose. The purpose of this research was to determine if high hydrostatic pressure could be successfully used to refold placental bikunin, a therapeutic protein which aggregates during eukaryotic expression. Second, thermodynamic parameters were obtained to gain insight into the fundamentals of pressure-modulated refolding.

Results. Recovery yields of 78% (reverse phase) were obtained in solutions containing refolding buffer and protein (0.5 mg/mL) that were pressurized to 2000 bar at 25 °C. Yields from pressure-modulated refolding were significantly higher than those obtained from a conventional chaotrope refolding process (55% RP). The $\Delta V_{\text{refolding}}$ was found to be -28 ± 5 mL/mole, whereas refolding pressures maintained the native monomer conformation.

Conclusions. We determined that hydrostatic pressure can be more effective for refolding a model pharmaceutical protein than the conventional chaotrope-based process. Secondly, this study verified that high pressure destabilizes the aggregate while favoring the native conformation, enabling pressure to be an effective refolding tool.

215. Production of native protofibril structures from aggregation of α-synuclein in methanol/water solutions. Mihaela M. Apetri,[1] Rekha Srinivasan,[1] Nakul C. Maiti,[2] Vernon E. Anderson,[2] and Michael G. Zagorski.[1] [1]Chemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106 (mma19@po.cwru.edu), and [2]Biochemistry, Case Western Reserve University

α-Synuclein is a presynaptic protein and is the major fibrillar component of Lewy bodies and Lewy neurites, which are fibrous cytoplasmic inclusions and are the pathological hallmarks of Parkinson's disease (PD). α-Synuclein is natively unfolded in aqueous solution at neutral pH, while changes in the solution conditions (such as solvent) can promote either α-helical or β-sheet structure. A limiting factor with kinetic and biophysical studies of α-synuclein relates to its high water solubility and the long time periods required for observation of natively folded β-sheet aggregated structure. We report here that methanol/water solutions accelerate the random $\rightarrow$ β-sheet conformational conversion that occurs during the production of Lewy bodies in PD. Using circular dichroism, thioflavin T fluorescence, atomic force microscopy, and native-PAGE, we studied the effect of a cosolvent, methanol, in inducing β-sheet aggregation. We show that the secondary structure and aggregation are very sensitive to the mole fraction of methanol, and that at 15−25% methanol a partially folded intermediate with enhanced propensity for protofibril formation is observed. These protofibrils do not assemble into mature fibrils. At higher methanol concentrations, the protein is folded showing a well-developed β-sheet secondary structure with a weaker propensity for protofibril formation. Thus, methanol/water solutions may provide a medium that permits easier analysis of the conversion of random α-synuclein to protofibril and may also provide information about inhibiting fibrillation to Lewy bodies.

216. High-temperature protein nanopore. Xiaofeng Kang, Liqun Gu, Stephen Cheley, Lakmal Jayasinghe, and Hagan P. Bayley. Department of Medical Biochemistry and Genetics, Texas A&M University System Health Sciences Center, 440 Joe H. Reynolds Medical Building, College Station, TX 77843 (xfkang@medicine.tamu.edu)

Single-channel measurements were used to study the thermal stability of the nanopores formed by α-hemolysin and leukocidin as representative β-barrel transmembrane proteins. The channel currents change linearly and reversibly with temperature over a wide temperature range. To our surprise, the protein nanopores are still very stable at a high temperature. The possible effects of temperature-dependent factors such as the solution conductivity, pH, and electrode potential on the nanopore current were investigated. The experimental results demonstrated that the temperature dependence of the channel current could be ascribed to the changes in solution conductivity and pore size with temperature. High-temperature nanopores will be very useful both in basic science and biotechnology, including sensing technologies.

217. Reduced dynamic behavior upon positively cooperative binding. Nichola L. Davies[1] and Dudley H. Williams.[2] [1]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom (fax +44-1223-336913, nld24@cam.ac.uk), and [2]Cambridge Centre for Molecular Recognition, University of Cambridge

Cooperativity is the phenomenon through which one set of binding interactions can change the binding energy of another, with consequences in biological events such as ligand binding, protein folding, and enzyme catalysis. We define a binding event as being positively cooperative with respect to a second interaction when its affinity is increased by the presence of the second interaction. The dynamic behavior of the complex will be reduced, with a benefit in enthalpy and a cost in entropy. The positively cooperative binding of a series of ligands (bacterial cell wall analogues) to an asymmetric receptor (the ristocetin A dimer) has been studied. The population of dimeric species relative to the monomer population falls because in the dimer, a second binding site with lower affinity must be occupied. We also observe an increase in the barrier to dimer dissociation upon the binding of these ligands, indicating reduced dynamic behavior.

218. Characterization of peptide fragments derived from the villin headpiece subdomain: Evidence for nonrandom structure in the unfolded state. Yuefeng Tang[1] and and Daniel P. Raleigh.[2] [1]Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794 (yuetang@ic.sunysb.edu), and [2]Department of Chemistry, State University of New York at Stony Brook

A set of peptide fragments derived from villin headpiece 36 (HP36), a three-α-helix protein, were characterized in an effort to study the conformation of the unfolded state. Peptides derived from three isolated helices were largely unstructured. A longer peptide fragment which contains the first and second helix exhibits a much more α-helical formation as judged by CD. The observation of an upfield-shifted methyl proton resonance from V50 and one-dimensional NOE experiments show that the structure is formed by tertiary interactions between aromatics and the methyl group on V50. This work strongly suggests that there is structure in the unfolded state of HP36.

219. Characterization of α-synuclein oligomerization. Keqian Liu and Vernon E. Anderson. Department of Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4935

In Parkinson's disease, the presence of α-synuclein (αS) aggregates within Lewy body (LB) occlusions represents a hallmark of this disorder. αS is a 140-residue presynaptic protein of unknown function. In aqueous solution, αS is found to be natively unfolded with a largely random coil extended structure without a hydrophobic core. *In vitro*, αS is capable of self-aggregation into fibrils via oligomeric intermediate forms. There is significant controversy over whether the fibrils of αS, an oligomeric form, or the formation of LBs is primarily responsible for the nerve cell death in the *substantia nigra*. This uncertainty in the identity of the toxic structure(s) underlines the necessity of characterizing the structure of αS in its monomeric, oligomeric, and fibrillar forms and the progress of aggregation to understand the etiology of α-synucleinopathies. The aggregates generated in monomeric αS ammonium sulfate precipitation under frozen conditions have been characterized by light scattering. The formation of soluble oligomeric species in the initial steps of αS aggregation, which cannot enhance the excitation fluorescence of historical dye ThT, is monitored by native gel electrophoresis. Kinetic studies indicate that these oligomeric intermediates are committed to forming fibrils but do not significantly dissociate to free monomer. The presence of these kinetically competent intermediates requires the aggregation process under physiological conditions to be more complex than a simple nucleation polymerization model.

220. Surface interface effects upon the conformational stability of a peripheral membrane protein. Scott A. Hocker,[1] Keith M. Gligorich,[1] Katie Brocksmith,[1] Yo-Yuan Cheng,[2] Shen-Hsien Lin,[2] Huan-Cheng Chang,[2] Meng-Chih Su,[1] and Geoffrey C. Hoops.[1] [1]Department of Chemistry, Butler University, Indianapolis, IN 46208 [fax (317) 940-8434], and [2]Institute of Atomic and Molecular Sciences, Academia Sinica

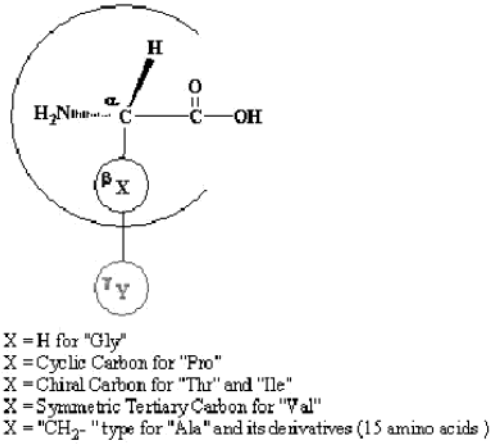
Cytochrome *c* served as a model peripheral membrane protein in an investigation of the effect of the membrane surface upon polypeptide conformation. Because of its similar pI, and hence similar surface charge distribution, a fused silica surface was employed as a mimic of the negatively charged phospholipid cellular membrane. The conformational stability of the protein adsorbed to this surface was characterized by attenuated total internal reflection (ATIR) absorption spectroscopy, using the Soret band as a probe. These surface absorption data were compared to absorption data obtained in solution under the same conditions to identify effects that can be attributed to the protein−surface interaction. Changes in the folding state of the protein were induced by employing several different alcohols at varying concentrations and pHs. The surface was found to stabilize the presumably native conformation of cytochrome *c* under conditions that were denaturing for the protein in solution.

221. Structural classification of amino acids and *β*-C stereochemistry. Chi Ming Yang. Neurochemistry and Chemical Biology, Institute for Life Science and Health, San Diego, CA, and Nankai University, Tian Jin 300071, China (fax 011 86 22 23503863)

The standard amino acids selected by Nature constitute a paradigm of complexity in Nature's integrity. Although these amino acids can traditionally be grouped according to their side chain physicochemical properties (hydrophilic, hydrophobic, neutral, nonpolar, polar uncharged, polar negatively charged or acidic, polar positively charged or basic, aromatic, and nonaromatic), their ambiguous physicochemical properties have since inspired a tremendous amount of excellent research and speculation.

By re-examining the stereochemistry in standard amino acids [$NH_2CH(Xy)COOH$] using computer modeling, we develop a structural recategorization of the standard amino acids according to their *β*-carbon stereochemistry, resulting in five groups of stereostructurally distinctive amino acids, which are Pro, Val, Gly, Thr, Ile, and Ala with 14 other derivatives. Consider the current chemical biology effort on incorporation of Ala derivatives such as aminobutyrate, Tyr, and Phe derivatives as unnatural amino acids into protein molecules; this type of amino acid structural classification

may have important implications in current molecular biology.



222. HSP70 in the gill and mantle of the oyster *Crassostrea virginica*. Mary G. Hamilton and Margarita Oks. Department of Natural Sciences, Fordham College at Lincoln Center, New York, NY 10023 [fax (212) 636-7217, hamilton@fordham.edu]

Heat shock proteins (HSPs), also known as stress proteins, are present constitutively in all life forms but may be induced when the organism undergoes a stress in its environment such as excessive heat. We incubated organ cultures of gill and mantle tissues for 2 h at 35 °C to see if induction of Hsp70 would occur. Analyses were first carried out on homogenates using a Western blot of an SDS−PAGE gel with anti-bovine Hsp70 (Sigma-Aldrich) that recognizes both constitutive and induced Hsp70. Discrete bands with molecular masses of ca. 70 kDa were observed, but there were no significant differences between the control (13 °C) and heated (35 °C) tissues. In a second experiment, the homogenates were analyzed in an ELISA (Stressgen Bioreagents) that employed anti-mouse inducible Hsp70. A significant increase in the level of Hsp70 in the heated samples confirmed the presence in oyster of an HSP-regulated stress system.

223. Interfacial aggregation of amyloid-β peptide. Michael R. Nichols and Terrone L. Rosenberry. Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Rd., Jacksonville, FL 32224 [fax (904) 953-7370, nichols.michael@mayo.edu]

Accumulation of aggregated amyloid-β peptide (Aβ) in the brain is a pathological hallmark of Alzheimer's disease (AD). In vitro experiments indicate Aβ monomer undergoes noncovalent self-aggregation to form soluble oligomers and ultimately highly ordered insoluble fibrils. Since Aβ is an amphiphile and Aβ assembly is highly concentration-dependent, we sought to determine whether Aβ aggregation would be enhanced at a liquid−liquid interface. Aβ introduced to a two-phase chloroform/aqueous system aggregated ~100 times more rapidly than Aβ in the aqueous system alone. Thioflavin T binding and dynamic light scattering measurements suggested that interfacial Aβ aggregation involved a dynamic equilibrium between the monomer and oligomer. A sharp decrease in the level of aggregation was noted at 48−72 h, which coincided with the appearance of a fibril meshwork at the interface. Cholesterol further accelerated aggregation, and inclusion of the peptide KLVFF-K_6 dramatically increased the kinetics of interfacial fibril formation. Understanding biophysical features influencing Aβ aggregation may provide therapeutic insights.

224. Comparative study of the core domain of the molten globule state of the α-lactalbumin−lysozyme family. Farhana A. Mamun and Daniel P. Raleigh. Department of Chemistry, State University of New York at Stony Brook, Graduate Building, Stony Brook, NY 11794 [fax (631) 632-7960]

Developing an understanding of protein folding and stability is a major area of research in structural biology. An area of intense interest is the characterization of partially folded states (often called molten globules). The *c*-type lysozymes and the α-lactalbumins form a structurally similar class of proteins that exhibit different folding behaviors. All α-lactalbumins form a stable molten globule state at equilibrium, while most lysozymes do not. The Ca^{2+} binding lysozymes are the exception, and among these, equine lysozyme and canine milk lysozyme form a particularly stable partially structured state. The α-lactalbumin−lysozyme fold is made up of two subdomains, the α-subdomain and the β-subdomain. The α-subdomain contains five helices, namely, helices A−D and a 3_{10} helix. We have previously developed a protein dissection approach for studying the molten globule state formed by α-lactalbumin. This work describes the application of this approach to equine lysozyme, bovine α-lactalbumin, and canine milk lysozyme. Peptide constructs were synthesized which contain helices A and B cross-linked with the D and 3_{10} helices by a native disulfide bond. The peptide constructs were derived from the region of these proteins that corresponds to the region of human α-lactalbumin that has previously been identified as being critical for formation of the molten globule state. The peptides are monomeric and form partially structured states in the absence of the rest of the protein as judged by CD and fluorescence. They are, however, less structured than the corresponding construct from human α-lactalbumin.

225. Measurement of oligomeric structure formation in phycocyanin using stopped flow kinetics. Katelyn B. Connell and Yvonne M. Gindt. Department of Chemistry, Lafayette College, Easton, PA 18042 (connellk@lafayette.edu)

Understanding protein folding mechanisms would provide the tools necessary to combat a significant number of diseases. The kinetics of quaternary structure formation were studied using phycocyanin, a phycobiliprotein isolated from cyanobacteria. Phycocyanin contains a chromophore that reports its quaternary structure. Using a stopped flow mixer, the absorption was monitored with a UV−vis spectrometer as it changed the quaternary structure. The kinetic traces were fit from reactions carried out at several concentrations to multiexponential equations to discriminate tertiary folding from oligomerization. In particular, the pathway from trimeric to hexameric heterodimeric forms was studied in depth, and concentration and temperature dependence have been explored. The results were then used to create possible folding mechanisms. The formation of oligomeric structure was dominated by a very fast second-order step.

226. Monitoring the effective hydrodynamic radii of denatured ensembles of proteins by pulse field gradient NMR techniques. Ying Li and Daniel P. Raleigh. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794 (yingli@ic.sunysb.edu)

The characterization of the denatured states of proteins is a key problem in studies of protein folding. Pulse field gradient NMR (PFG-NMR) methods have been used to determine the effective hydrodynamic radii of native and nonnative protein conformations. The isolated C-terminal domain of the ribosomal protein (CTL9) has been studied as a function of pH using this method. CTL9 contains three histidine residues, and their ionization states significantly affect the folding rate and equilibrium stability of the protein. The protein is more stable and folds faster at a higher pH. Hydrodynamic radii of CTL9 were measured using PFG-NMR with increasing urea concentrations at different pHs. It is found that the denatured protein expands as the urea concentration increases. For each set of data at a fixed pH, the extrapolated curve of the posttransition region represents the denatured ensemble hydrodynamic radii as a function of urea concentration. This value can be extrapolated to 0 M urea to estimate the hydrodynamic properties of the denatured state under native conditions.

227. Determination of membrane protein stability via thermodynamic coupling of folding to thiol−disulfide interchange. Lidia Cristian, James D. Lear, and William F. DeGrado. Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, 1010 Stellar Chance Building, 422 Curie Blvd., Philadelphia, PA 19104

While progress has been made in understanding the thermodynamic stability of water-soluble proteins, our understanding of the folding of membrane proteins is at a relatively primitive level. A major obstacle to understanding the thermodynamics of membrane protein folding is the discovery of systems in which the folding is in thermodynamic equilibrium, and the development of methods for quantitatively assessing this equilibrium in detergent micelles and lipid bilayers. In this work, we describe the application of disulfide cross-linking in quantitatively measuring the thermodynamics of membrane protein association in detergent micelles and phospholipid bilayers. The method involves initiating disulfide cross-linking of a protein under reversible redox conditions in a thiol−disulfide buffer and quantitative assessment of the extent of cross-linking at equilibrium. The model system for this study is the homotetrameric M2 proton channel protein from the influenza A virus. Previously, it has been shown that transmembrane peptides from this protein specifically self-assemble into tetramers that retain the ability to bind to the drug amantadine. The disulfide-coupled equilibrium method was used to quantitatively measure the thermodynamics of this folding interaction in detergent micelles and phospholipid bilayers. The effects of phospholipid acyl chain length and cholesterol on peptide association were investigated. The association of the helices is modulated by the thickness of the bilayer and is also dependent on cholesterol levels present in the phospholipid bilayer. Physiologically relevant variations in the cholesterol level are sufficient to strongly influence the association. Evaluation of the energetics of peptide association in the presence and absence of cholesterol showed a significantly tighter association upon inclusion of cholesterol in the phospholipid bilayers.

228. Metal ion-mediated protein assembly. Lindsey M. Gottler,[1] Omar M. Yaghi,[1] and E. Neil G. Marsh.[2] [1]Department of Chemistry, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109 (lgottler@umich.edu), and [2]Department of Chemistry, University of Michigan

Metal ions play an important structural role in many proteins. We are exploring whether coordination of metals such as mercury, silver, and cadmium by surface thiol groups can be exploited in assembling protein complexes of defined geometries. We are examining the ability of various metal ions to cross-link the B12-binding subunit of glutamate mutase. This small protein contains a single reactive thiol on a flexible surface loop, and thus provides an attractive model system for testing this concept.

229. Fluorous proteins: Consequences of the incorporation of trifluoromethyl groups in a globular protein. Jia-Cherng Horng, Viktor Moroz, James Marecek, and Daniel P. Raleigh. Department of Chemistry, State University of New York at Stony Brook, 100 Nicolls Rd., Stony Brook, NY 11794 [fax (631) 632-7960, jhorng@ic.sunysb.edu]

Knowledge of protein folding mechanisms has been derived from studies of the effects of point mutations upon folding. Such experiments are usually interpreted using Φ values, the ratio of the effect of the mutation on the stability of the transition state, $\Delta\Delta G^{\ddagger}$, to the effect on the stability of the protein, $\Delta\Delta G^{\circ}$. Unnatural amino acids offer the possibility of making conservative changes that could not be contemplated using the 20 coded amino acids. Amino acids containing trifluoromethyl groups are an attractive choice since they are reasonably isosteric to their nonfluorinated counterparts and should cause a significant change in ΔG°. Here we use trifluorovaline as a substitute for valine in a 56-residue globular protein, the N-terminal domain of L9. We introduced trifluorovaline separately at two positions. The variants are more stable by 0.8 and 1.4 kcal/mol and fold faster than the wild type. The Φ values for these substitutions are 0.16 and 0.11.

230. Design and characterization of a homodimeric antiparallel coiled coil. Daniel Gurnon[1] and Martha G. Oakley.[2] [1]Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (dgurnon@indiana.edu), and [2]Department of Chemistry, Indiana University

We report the first successful design of a self-associating antiparallel coiled coil, APH. The simultaneous application of Coulombic and hydrophobic components results in a decided preference for the antiparallel alignment as judged by HPLC, sedimentation equilibrium, and chemical denaturation data. The designed peptide is comparable in stability to naturally occurring leucine zipper peptides and can be expressed in bacteria. These properties of APH suggest potential in vivo protein fusion and biomaterials applications.

231. Design and characterization of artificial integral membrane proteins for vectorial electron transfer across

soft interfaces. Shixin Ye,[1] Joseph Strzalka,[1] Bohdana M. Discher,[2] Dror Noy,[2] Chris C. Moser,[2] P. Leslie Dutton,[2] and J. Kent Blasie.[3] [1]Chemistry Department, University of Pennsylvania, 231 S. 34th St., Philadelphia, PA 19104 [fax (215) 573-2112], [2]Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania, and [3]Department of Chemistry, University of Pennsylvania

One approach to creating novel "biomolecular" materials is to utilize artificial peptides designed with a simple, stable structural motif to exhibit a particular biological functionality at the molecular level. Importantly, the motif must also be designed to facilitate their organization into ordered ensembles at the macroscopic level. Thus, the designed molecular functionality can be translated into a macroscopic material property. Here, we have built on previously designed four-helix bundle motifs to redesign the bundle to render it amphiphilic. The latter would provide for favorable nonpolar interactions with the hydrocarbon chains of phospholipids and thereby serve to align the bundle perpendicular to the water−hydrocarbon interface in phospholipid monolayers and bilayers analogous to the transmembrane domain of integral membrane proteins. The prosthetic groups could then be located within the core of the bundle in both the hydrophilic and hydrophobic ends, and separated by a defined distance, thereby providing for the possibility of controlled electron transfer across the hydrocarbon−water interface to create a macroscopic transmonolayer or transbilayer electric potential. We have designed and characterized a series of amphiphilic four-helix bundle peptides utilizing selected portions of the previously designed bundle and extending the sequence of each helix such that the extension of the bundle possessed a hydrophobic exterior with either a polar or nonpolar interior. These extension sequences were derived from the transmembrane domains of membrane proteins, including the M2 influenza proton channel, a synthetic proton channel analogue thereof, and cytochrome *b*. The α-helical content of the peptides in detergents and phospholipids was ascertained by circular dichroism, and the oligomeric state of the helices in detergents was determined by analytical ultracentrifugation. The K_d's for stoichiometric metalloporphyrin binding were in the range of 10−100 nM, and the midpoint potentials for their oxidation and reduction were in the range of −140 to −100 mV. The amphiphilic four-helix bundle peptides were also shown to orient perpendicular to the air−water interface in Langmuir monolayers of the pure peptides and in mixtures with phospholipids at higher surface pressures via X-ray reflectivity. The orientation of these peptides in phospholipid vesicles is being investigated by small-angle X-ray scattering.

232. Probing azido sugar metabolism *in vivo* using the Staudinger ligation. Danielle H. Dube, **Jennifer A. Prescher**, and Carolyn R. Bertozzi. Department of Chemistry, University of California, Berkeley, CA 94720 (ddube@uclink.berkeley.edu, prescher@uclink.berkeley.edu)

Decorating cell surfaces with functional groups that react selectively with small chemical probes is useful for a variety of imaging and drug delivery strategies. Our laboratory has previously demonstrated that cells in tissue culture incorporate the abiotic azide group into cell surface glycoconjugates via metabolism of unnatural carbohydrate precursors. Moreover, these cell surface azides can be selectively reacted with phosphine-based probes in a process termed the Staudinger ligation. This chemoselective reaction forms a covalent link between the cell surface azide and the exogenously delivered phosphine reagent. We report here the extension of this work *in vivo*. Specifically, mice were administered an azide-bearing carbohydrate precursor, and the metabolic incorporation of the unnatural functional group was observed in various murine organs by treating isolated cell populations or tissue homogenates with a phosphine probe. The presence of azide groups on murine splenocytes was also detected by administering the phosphine probe to mice previously treated with the unnatural azido sugar, and our results demonstrate that the Staudinger ligation proceeds selectively *in vivo*. We are currently exploring the *in vivo* Staudinger ligation with radiolabeled phosphine reagents for noninvasive imaging of metabolically incorporated azide groups in mice.

233. Stereochemical control of cell adhesion on self-assembled monolayers presenting organized saccharides: Potential effects of templated water structure. Yan-Yeung Luk[1] and Milan Mrksich.[2] [1]Department of Chemical Engineering, University of Wisconsin, 1415 Engineering Dr., Madison, WI 53706-1691 [fax (608) 262-5434, yluk@che.wisc.edu], and [2]Department of Chemistry, The University of Chicago

Self-assembled monolayers (SAMs) of alkanethiolates on gold films are important model substrates for biological studies. These surfaces have potential in biochip development and the integration of electric circuitry with cell biology. Both technologies require that the monolayers be bio-inert in preventing protein denaturation, nonspecific adsorption, and cell adhesion for bio-specific interactions. In this presentation, we demonstrate that mannitol-terminated SAMs are highly effective at preventing the adsorption of proteins and the attachment of cells. This class of monolayers has demonstrated unprecedented control of the period of time over which cells are confined within patterned regions on a substrate, and still remain viable. Furthermore, control of stereochemistry of the open saccharides groups at the interface biases the degree of cell attachment and the extent of cell proliferation. Mechanistic studies strongly correlate its surface structure and bio-inertness, mediated by a possible water structure templated at the interface. This work finds immediate application in biotechnology, and promises to be useful in cancer research and tissue engineering.

234. Study of a preparative method on dermatan sulfate from porcine intestinal mucosa. Wenjun Mao[1] and Robert J. Linhardt.[2] [1]Marine Drugs and Foods Institute, Ocean University of China, 5 Yushan Rd., Qingdao 266003, China (wenjunmqd@hotmail.com), and [2]Chemistry, Medicinal and Natural Products Chemistry, and Chemical and Biochemical Engineering, University of Iowa

The dermatan sulfate is a polydisperse, micro-heterogeneous sulfated copolymer of *N*-acetyl-D-galactopyranose (*N*-acetylgalactosamine, D-Galp-NAc) and primarily L-idopyranosyluronic acid (iduronic acid, L-IdoAp) having an average molecular weight of 20000−30000. Dermatan sulfate exhibits venous antithrombotic activity and has been studied clinically. Some interaction specificity has been demonstrated

for the dermatan sulfate, with the serine proteinase inhibitor, heparin cofactor II, and with annexins and other proteins. The unique conformational and flexible features of the iduronic acid residue make dermatan sulfate a particularly interesting target for glycosaminoglycan−protein interaction studies. Dermatan sulfate is found in a wide variety of animal tissues, but is prepared commercially from either porcine or bovine mucosa. This study investigated an effective preparative method for the isolation of dermatan sulfate from porcine intestine mucosa. Dermatan sulfate was purified from porcine intestine mucosa, and its structure was characterized. The purity and identity of the dermatan sulfate were determined following treatment with chondroitin lyase B by using polyacrylamide gel electrophoresis. The disaccharide compositional analysis on the dermatan sulfate, determined by treatment with chondroitin lyase ABC, was followed by high-resolution capillary electrophoresis. One-dimensional ^{1}H NMR spectroscopy was used to elucidate the structural characteristics of the dermatan sulfate.

235. Studies of the interaction between heparin and proteins of biological importance using surface plasmon resonance. Fuming Zhang,[1] Sybil C. Lang Hrstka,[1] Haining Yu,[1] Eric Edens,[1] and Robert J. Linhardt.[2] [1]Department of Chemistry, Division of Medicinal Chemistry, College of Pharmacy, and Department of Chemical and Biochemical Engineering, University of Iowa, Iowa City, IA 52242 (fzhang@icaen.uiowa.edu), and [2]Departments of Chemistry, Chemical Engineering, and Biology, Rensselaer Polytechnic Institute

Heparin and heparan sulfate have been shown to interact with a number of biologically important proteins, thereby playing an essential role in the regulation of various physiological processes. The understanding of these interactions at the molecular level is important for the design of new highly specific therapeutic agents. In addition, an understanding of the specificity of heparin and heparan sulfate will be necessary to understand normal physiologic and pathophysiologic processes. Surface plasmon resonance (SPR) spectroscopy has been successfully used for quantitative modeling of heparin−protein interactions. In natural biological systems, heparan sulfate is found immobilized on the cell surface through its core protein, and captures heparin-binding proteins that flow over the cell surface. In this study, we report interactions between heparin and different proteins, including Circumsporozoite (CS) protein, heparin cofactor II, TNF, IL-10, and IFN, using a heparin biochip on which surface heparin was covalently immobilized through a preformed albumin−heparin conjugate.

236. Spectroscopic evidence for multiple T states in hemoglobin: A sol−gel study. Uri Samuni,[1] David Dantsker,[1] Laura Juszczak,[1] Stefano Bettati,[2] Andrea Mozzarelli,[2] and Joel M. Friedman.[1] [1]Department of Physiology and Biophysics, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 [fax (718) 430-8819, samuni@aecom.yu.edu], and [2]Department of Biochemistry and Molecular Biology, University of Parma

The addition of CO to sol−gel encapsulated human deoxyhemoglobin (HbA) triggers a progression of conformational and functional changes. Visible resonance Raman spectra of the CO photoproduct reveal at least four species based on the Fe−His frequency: 215, 222, 227, and 230 cm^{-1}. The lowest and highest values correspond to the initial deoxy T state and liganded R (R2) state species, respectively. The 222 and 227 cm^{-1} species are generated using encapsulation protocols that give rise to low- and high-affinity T states (LA and HA, respectively). UV resonance Raman spectra indicate that the progression from deoxy T to LA to HA is associated with a progressive loosening of T state constraints within the hinge and switch regions of the $\alpha1\beta2$ interface. The ability to access LA and HA is attributed to the sol−gel making the T to R transition much slower than the deoxy T to LA or HA transition.

237. Applications of the quartz crystal microbalance/heat conduction calorimeter (QCM/HCC) in measuring the thermodynamic and rheological hydration effects on a myoglobin−buffer thin film. Allan L. Smith[1] and **Sister Rose Mulligan**.[2] [1]Department of Chemistry, Drexel University, and Masscal Corp., and [2]Department of Chemistry, Immaculata University, P.O. Box 660, 1145 King Rd., Immaculata, PA 19345 [fax (610) 993-8550, rmulligan@immaculata.edu]

The quartz crystal microbalance/heat conduction calorimeter (QCM/HCC) was used to study the hydration of myoglobin films (1−2 μm). The QCM/HCC provides three, simultaneous measurements characterizing the process of protein hydration: (1) mass changes upon (de)hydration (10 ng/cm^2), (2) corresponding thermal changes ($\pm$100 nW), and (3) thin film motional resistance ($\pm$0.5 Ω) changes as a function of water vapor activity. These isothermal (25 °C) measurements provide a thermodynamic analysis of hydration, including $\Delta H_{\text{hydration}}$ of myoglobin, hydration isotherms, and kinetic information for the rate of hydration. The motional resistance as a function of water vapor activity will be shown as a way to probe the energy loss and damping effects. The resistance measurements provide insight into the mechanical properties of the protein film, namely, the complex shear modulus G and its two components: the storage modulus, G', and the loss modulus, G''. The hydration effects of the sodium phosphate buffer will be shown. Two appealing aspects of using the QCM/HCC are the direct calorimetric measurements on the sample and the small sample size that is needed (0.5−3 mg).

238. Structure and function of mutant fatty acid-binding proteins. Rima E. Estephan,[1] Xiaomin Yang,[2] Hsin Wang,[2] Sarala Kodukula,[3] Judith Storch,[3] and Ruth E. Stark.[2] [1]Department of Chemistry, City University of New York Graduate Center and College of Staten Island, 2800 Victory Blvd., Staten Island, NY 10314 (rima_joe@hotmail.com), [2]Department of Chemistry, College of Staten Island, and [3]-Department of Nutrition, Rutgers University/Cook College

Liver fatty acid-binding protein (LFABP), unlike other FABP family members, has two FA binding sites and transfers these molecules through a diffusion-controlled mechanism. To gain further insight into the nature of ligand interactions and the impact of key residues on LFABP structure, the K31L and R122L/S124A mutants were constructed. The K31L mutation caused a localized structural perturbation in the helical region near the mutation site

compared with wild-type LFABP, whereas the R122L/S124A mutation produced more pervasive conformational changes as judged by NMR-based chemical shift perturbation analysis. Moreover, titration studies showed that unlike K31L LFABP, the R122L/S124A LFABP mutant exhibited a single magnetic environment for oleate, suggesting the possibility that only a single molecule of FA was bound at a site near the protein surface. Further studies are underway to determine the stoichiometry and affinity of these mutants for bound oleate and to elucidate possible alterations in the transfer mechanism.

239. His93Gly cavity mutant of sperm whale myoglobin as a template for ferric, ferrous, and ferryl heme states of mixed ligand complexes. Roshan Perera, Masanori Sono, and John H. Dawson. Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter St. GSRC, Columbia, SC 29208 (roshan@mail.chem.sc.edu)

The general problem associated with the heme iron systems in organic solvents has been the generation of ferric, ferrous, and ferryl heme state model complexes as metalloprotein active site mimics. The coordination of thiol, thioether, and amine ligands in the ferric, ferrous, and oxoferryl heme states of proteins has been relatively unexplored. In the presence of excess amine, for example, ferric porphyrin model systems are reduced to bis(amine)iron(II) complexes. In contrast, our investigation with the H93G Mb cavity mutant has revealed that ferric as well as ferrous H93G Mb can form both mono/bis-amine-ligated heme states and coordination structures depending on the type and size of the amine ligand. This presentation will further address important new information regarding the ligation by neutral cysteine thiol in ferrous heme proteins that has not been documented previously. In particular, to establish spectroscopic signatures for heme systems, we have prepared five-coordinate adducts of the ferrous myoglobin H94G cavity mutant with neutral thiol and thioether sulfur donors as well as six-coordinate derivatives as with CO, NO, O_2, and bis-ligated complexes. A novel thiol-ligated oxyferrous complex is reported for the first time. The complexes have been characterized by comparison of the electronic absorption and magnetic circular dichroism spectra of the complexes under examination with those of structurally defined naturally occurring heme iron centers. The ability to prepare a variety of heme iron ligand adducts with H93G Mb demonstrates its versatility as a template for the preparation of heme protein model complexes. (Supported by NIH Grant GM 26730.)

240. Electron transfer in proteins: A comparison between the renormalized perturbation expansion and the pathway model. Sean Laing, David Justice, and Valentin Gogonea. Department of Chemistry, Cleveland State University, 2121 Euclid Ave., SI 413, Cleveland, OH 44115 [fax (216) 687-9298, s.laing@csuohio]

The algorithm, for determining the element of electronic coupling between electron donor and acceptor sites, implements two methods: (a) the pathways model uses an empirical "decay per bond" parameter, and (b) the second method uses a tight binding Hamiltonian approach (Green's function matrices). Green's function matrices are evaluated using a renormalized perturbation expansion. The new methodology leads to self-energy for each atom in the electron transfer pathway and gives a better estimation of the electronic coupling. Three search algorithms are implemented for the pathway model: (a) the shortest path, (b) the *k*-shortest paths, and (c) an all-pathways search. The shortest path and *k*-shortest paths are used to determine the contribution to the electronic coupling from the shortest pathways. The performance of these two methods is evaluated by comparing the pathways and the magnitude of the electronic coupling obtained using myoglobin and cytochrome *c* proteins.

241. Enhanced vibrational circular dichroism in metal complexes and metalloproteins with low-lying electronic states. Yanan He, Laurence A. Nafie, and Teresa B. Freedman. Department of Chemistry, Syracuse University, 1-014 CST, Syracuse, NY 13244 [fax (215) 443-4070, yhe02@syr.edu]

Enhanced vibrational circular dichroism (VCD) intensities are observed for open-shell metalloproteins and metal complexes with low-lying magnetic-dipole-allowed d−d transitions occurring in the infrared region. We have extended recent studies of sparteine (sp) bound to Co(II), Ni(II), and Zn(II) to chiral catalysts, including (*R,R*)-(−)-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II), which exhibits nearly monosignate positive VCD intensities 100 times larger than those of closed shell complexes and 10 times larger than those of $Co(sp)Cl_2$. We have also made the first observations of enhanced VCD in Co(II)-substituted carbonic anhydrase with bound cyanate ion, and cytochrome *c* with bound azide and cyanide ions. For these metalloproteins, ligand stretches are observed with *g* values similar to those previously measured for azide and cyanide bound to low-spin metmyoglobin and methemoglobin.

242. Dendrimeric hemoglobins: Cooperativity in a cluster. Jie Zhang[1] and Ronald Kluger.[2] [1]Department of Chemistry, University of Toronto, 80 St. George St., Toronto, ON M5S 3H6, Canada (jzhang@chem.utoronto.ca), and [2]Department of Chemistry, Universty of Toronto

Isolated hemoglobin binds four oxygens cooperatively due to interactions between subunits. It has been proposed that hemoglobins in the proximity of one another have altered properties in the cell and in red cell substitutes. To study the interprotein interactions, we created a stable, functional cluster of cross-linked hemoglobins with a starburst dendrimer as a core. The effects of the core were calibrated in a 1:1 conjugate with cross-linked hemoglobin. This conjugate has reduced oxygen affinity while maintaining significant cooperativity. The assembled cluster of up to five cross-linked tetramers per dendrimer is functional and also binds oxygen with similar cooperativity. However, detailed functional differences between the clusters and the dendrimer−hemoglobin single conjugate are indicative of significant protein−protein interactions.

243. Study of biomedical processes. Les loran. Department of Physics, University of Puerto Rico, Natural Sciences, P.O. Box 23343, Rio Piedras, PR 00931

Theoretical methods were used to study the mechanism and kinetics of relevant biomedical processes.

244. Subcritical damped oscillatory behavior of reactions in circle. Kal Renganathan Sharma. Department of Chemical Engineering and Biotechnology, Sakthi Engineering College, Anna University, Oragadam, Kancheepuram, Chennai 602105, India (fax 2526019, jyoti_kalpika@yahoo.com)

A system of reactions in circle is modeled using the methods of Laplace transforms and the method of the Jacobian matrix. The conditions of reaction rate constants when the damped oscillatory kinetics may be observed are derived for three reactants, four reactants, eight reactants, and the general case. For the case of reactions in circle with three reactants, the kinetics will exhibit damped oscillatory behavior when $k_3 < k_2 + k_1 + 2\sqrt{k_2 k_1}$ or when any one of the rate constants is less than the square of the sum of the square root of the other two rate constants. For the case of reactions in circle with four reactants, the kinetics will exhibit damped oscillatory behavior when $B^3/27 + C^2/4 > 0$ where $B = \beta - \alpha^2/3$) and $C = \chi + 2\alpha^3/27 - \alpha\beta/3$. In terms of the rate constants, α is given by the sum of all four reaction rate constants, β represents the sum of all the possible binary products of rate constants, and χ is the sum of all the possible triple products of rate constants in the system in circle. For the general case, the characteristic equation whose solution will determine the exact solution of the system is written. The cases where complex roots result will represent the conditions of the rate constants where damped oscillatory behavior can be expected. We attempted to treat reactions of a class such as those involved in the Krebs cycle (1,2) consisting of the essential steps of oxalaacetic acid (A), citric acid (B), isocitric acid (C), α-ketogluaric acid (D), succyl-CoA (E), succinic acid (F), fumaric acid (G), maleic acid (H), and other examples such as those in metabolic pathways as reactions in circle, and the kinetics were studied.

245. Using predicted subcellular localization to reexamine the annotation of the enzyme encoding *Arabidopsis* genes. Peter M. Palenchar,[1] Dennis E. Shasha,[2] and Gloria M. Coruzzi.[1] [1]Department of Biology, New York University, Waverly Place, New York, NY 10012 (pmp5@cs.nyu.edu), and [2]Courant Institute of Math and Computer Sciences, New York University

Identifying enzymes that are evolutionarily related but catalyze different reactions provides information about the flexibility of catalytic and binding motifs. Because of their rich secondary metabolism, plants are an abundant source of closely related enzymes with different functions. On the basis of sequence information alone, however, it is frequently difficult to distinguish homologous enzymes that remain involved in established reactions from closely related enzymes that serve other functions, and misannotation of the function of such homologues frequently results. Using information about organellar transporters and the predicted subcellular localization of enzymes from *Arabidopsis thaliana*, we reexamined the annotation of its genome to see if it is likely that each enzyme is localized with its purported substrate(s). If colocalization is unlikely, we predict those enzymes are likely misannotated. For example, our analysis suggested that At3g61440, which encodes a mitochondrially localized *O*-acetylserine lyase, is never localized with its purported substrates *O*-acetylserine and sulfide. Instead, a review of the literature suggests to us that this enzyme is a cyanoalanine synthase [Warrilow, A. G., and Hawkesford, M. J. (2000) *J. Exp. Bot.*, 985−993].

246. Modeling the hydrodynamics of cerebrospinal fluid flow in the human brain. Andreas Linninger,[1] Christian Tsakiris,[1] Alexander Munoz,[1] Richard Penn,[2] and Max Lee.[2] [1]Laboratory for Product and Process Design, Department of Chemical Engineering, University of Illinois at Chicago, 810 S. Clinton, Chicago, IL 60612 (Linninge@uic.edu), and [2]Department of Neurosurgery, University of Chicago

Models and experiments stress the pulsating character of the cerebrospinal fluid flow in the human brain and the fluid structure interaction between the cerebrospinal fluid (CSF) flow and the elastic tissues of the brain parenchyma. Disturbance in the CSF flow in the ventricles can lead to a condition known as hydrocephalus, which has led to considerable controversy over fluid and pressure dynamics, occurring in choroid plexus. In our new hypothesis, arterial blood flow through the choroid plexus brings about an oscillatory motion of the CSF in the ventricular spaces. The dynamic interaction between the pulsating CSF flow and the elastic brain tissue is described mathematically by means of the continuity equation, macroscopic energy balance for the fluid, and Newton's law for the motion of the elastic brain matter. Fluid properties, inertia of the brain tissue, as well as elastic and dissipative counterforces caused by the tissue displacements are considered.

247. Comparison of the binding activity of randomly oriented and uniformly oriented proteins immobilized by chemoselective coupling to a self-assembled monolayer. Yan-Yeung Luk,[1] Nicholas L. Abbott,[1] Ronald T. Raines,[2] Matthew L. Tingey,[1] and Kimberly A. Dickson.[3] [1]Department of Chemical Engineering, University of Wisconsin, 1415 Engineering Dr., Madison, WI 53706-1691 [fax (608) 262-5434, yluk@che.wisc.edu], [2]Department of Biochemistry and Department of Chemistry, University of Wisconsin—Madison, and [3]Department of Biochemistry, University of Wisconsin—Madison

The orientation of a protein immobilized on a surface determines the exposure and presentation of the active site of the protein to a contacting phase, and thus, orientation is thought to play a central role in determining the binding activity of an immobilized protein. In this presentation, we will report on the immobilization of ribonuclease A (RNase A) on a chemically well-defined surface via two different immobilization schemes. Whereas one scheme leads to a unique orientation of RNase A, the other leads to a random orientation of RNase A on the surface. We will compare the binding activity of the ribonuclease inhibitor to RNase A immobilized via each scheme. Because both schemes for immobilization host the RNase A in the same interfacial microenvironment, these results permit assessment of the role of the orientation of an immobilized protein on its binding activity.

248. A molecular-level thermodynamic switch controls chemical equilibrium in biological systems: Why does the human body maintain a constant temperature of 37 °C? Paul W. Chun. Department of Biochemistry and

Molecular Biology, University of Florida College of Medicine, 1600 S.W. Archer Rd., Box 100245, JHMHC, Gainesville, FL 32610-0245 [fax (352) 392-2953, pwchun@biochem.med.ufl.edu]

The possibility of the existence of life processes is not a clear and urgent demand of the physical universe. In fact, life exists only over a limited temperature range when the balance of energy and entropy demands is favorable. There is a lower cutoff point, T_h, and an upper limit, T_m, above which enthalpy is favorable but entropy is unfavorable. Only between these two temperature limits, where $\Delta G°(T) = 0$, is the net chemical driving force favorable for biological processes such as protein folding, protein−protein, protein−nucleic acid, or protein−membrane interactions, and protein self-assembly. In the human body, this point is 37 °C. The nature of the biochemical thermodynamic compensation between T_h and T_m may be characterized by evaluating the inherent chemical bond energy and heat capacity integrals. All interacting biological systems examined using the Planck−Benzinger methodology have shown such a thermodynamic switch at the molecular level, suggesting that its existence may be universal.

249. Osmotic observations on soft gels and biological tissue samples. Ferenc Horkay,[1] Adam S. Berman,[1] Iren Horkayne-Szakaly,[2] and Peter J. Basser.[1] [1]Laboratory of Integrative and Medical Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, 13 South Dr., Bethesda, MD 20892 [fax (301) 435-5035, horkay@helix.nih.gov], and [2]Department of Pathology, Washington Hospital Center

The knowledge of hydration of biological tissue samples and understanding the relationship among hydration, structure, and function are important in biology as well as in many biotechnological applications. An apparatus has been developed to determine the effect of controlled changes in water vapor pressure on the equilibrium swelling properties and the osmotic swelling pressure of small (<1 μg) tissue specimens. The water uptake of aggrecan−hyaluronan aggregates and cartilage−hydrogel constructs has been assessed by a modified quartz crystal microbalance (QCM). A novel method was developed to overcome uncertainties caused by nonuniform coupling of biological samples to the quartz crystal. The tissue is suspended in a dilute poly(vinyl alcohol) solution prior to deposition on the surface of the quartz electrode. The polymer solution stabilizes the tissue structure and prevents dehydration. It is demonstrated that the QCM method is well suited to the study of the osmotic properties of soft biological samples.

Thursday Morning: Frontiers of Enzymology

Stephen Benkovic, Organizer

250. DNA replication by the T4 replisome. Stephen J. Benkovic,[1] Ann M. Valentine,[2] Jingsong Yang,[1] Rosa Maria Roccasecca,[1] Michael Trakselis,[3] and Xi Jun.[1] [1]Department of Chemistry, The Pennsylvania State University, 414 Wartik Laboratory, University Park, PA 16802 [fax (814) 865-2973, sjb1@psu.edu], [2]Department of Chemistry, Yale University, and [3]MRC/Cancer Cell Unit

The assembly and disassembly of the T4 replisome from a collection of eight different proteins have been studied by a variety of biophysical methods. The nature of the protein−protein interactions that serve to coordinate the synthesis of the leading and lagging strands in DNA replication, the molecular events that accompany the progression of the replication fork, and the overall strategy evolved to create molecular machines will be discussed. Particular emphasis will be placed on the assembly and operation of the primosome, the subassembly that primes Okazaki fragment synthesis, and the various physical chemical methods.

251. Transition state analogue complexes of purine nucleoside phosphorylase from humans and *Plasmodium falciparum*. Vern L. Schramm,[1] Wuxian Shi,[1] Andrzej Lewandowicz,[1] Peter C. Tyler,[2] Gary B. Evans,[2] Richard H. Furneaux,[2] and Steve C. Almo.[3] [1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 [fax (718) 430-8565, vern@aecom.yu.edu], [2]Carbohydrate Chemistry, Industrial Research Ltd., and [3]Center for Synchrotron Biosciences, Department of Biochemistry, Albert Einstein College of Medicine

Inhibition of human purine nucleoside phosphorylase (huPNP) induces apoptotic death in rapidly dividing T-cells. Transition state analogues for huPNP have been designed from transition state principles and synthesized. These inhibitors are being explored for the control of T-cell cancers and suppression of autoimmune states. NMR and crystallographic studies reveal the mechanism of slow-onset tight-binding inhibition. Immucillin-H is a first-generation analogue with electrostatics and geometry resembling a point on the reaction coordinate just prior to transition state formation. DADMe-Immucillin-H is a second-generation transition state analogue with features that resemble a point on the reaction coordinate just past transition state formation. Neither is a perfect transition state analogue, but both are sufficiently related to the transition state to bind with picomolar dissociation constants. The PNP from *Plasmodium falciparum* (pfPNP) is required for purine salvage in parasites cultured in human erythrocytes. Human erythrocytes can be cured of *P. falciparum* infections by brief treatments with PNP inhibitors in the absence of added hypoxanthine. The enzyme from pfPNP differs considerably from huPNP in structure and exhibits an expanded substrate specificity. This difference has been exploited in designing and synthesizing transition state analogues with specificity for pfPNP. Crystallography reveals the basis for the differences in substrate specificity. (Supported by research grants from the NIH.)

252. Conformational changes in enzyme catalysis: Single-molecule and ensemble kinetics of dihydrofolate reductase. Gordon G. Hammes,[1] Zhiquan Zhang,[1] and P. T. Ravi Rajagopalan.[2] [1]Department of Biochemistry, Duke University, Box 3711, Durham, NC 27710 [fax (919) 684-9709, hamme001@mc.duke.edu], and [2]Department of Chemistry, Pennsylvania State University

The role of conformational changes in enzyme catalysis has been extensively studied but is still not fully understood in molecular terms. To assess the role of conformational changes in individual reaction steps and in individual molecules, stopped flow and single-molecule fluorescence microscopy were used to probe the interactions of methotrexate, NADPH, and dihydrofolate with dihydrofolate re-

ductase (DHFR). DHFR was labeled with the fluorescent probe Alexa 488 on the protein "flap" that closes over the active site when ligands are bound. The enzyme is fully active when attached to the microscope slide through a biotin tail at the amino terminus. Fluorescent changes are observed due to substrate and inhibitor binding and to hydride transfer. Multiple conformational changes are found with both methods. The nature of these changes will be discussed, as well as the general role of conformational changes in enzyme catalysis.

253. In search of the active site in RNA catalysis. Daniel Herschlag. Department of Biochemistry, Stanford University, Beckman Center B400, Stanford, CA 94305-5307 (herschla@cmgm.stanford.edu)

How do enzymes achieve their enormous rate enhancements and exquisite specificity? Comparing the behavior and properties of the two different classes of biological catalysts, proteins and RNA, has been extremely valuable in revealing fundamental features of enzymatic catalysis. One critical step toward a complete understanding of catalysis is identification of all transition state interactions with active site groups. For many RNA enzymes, such as the group I ribozoyme from *Tetrahymena*, divalent metal ions play an integral role in catalysis. However, the assignment of functional interactions in phosphoryl transfer reactions had been surprisingly intractable for both RNA and protein enzymes. We therefore extended the classical "metal ion specificity switch" approach, allowing assignment of interactions with three active site metal ions. We are now further extending this approach to identify the RNA ligands for these metal ions. Our progress toward this goal and the general implications for RNA function will be described.

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