Abstracts, Division of Biological Chemistry, 222nd National Meeting of the American Chemical Society, August 26−29, 2001

Richard B. Silverman, Program Chair

General Oral Session−Sunday Morning

1. Solid-state NMR structural studies of the membrane-bound HIV-1 fusion peptide. Jun Yang, Rong Yang, Charles M. Gabrys, and **David P. Weliky**. Department of Chemistry, Michigan State University, East Lansing, MI 48824 (fax: 517-353-1793, yangjun1@msu.edu, weliky@cem.msu.edu)

Fusion peptides representing the 23 N-terminal residues of the HIV-1 envelope protein gp41 were synthesized and structurally characterized with solid-state NMR. For the HIV-1 virus, this sequence is critical for viral fusion with target host cells. The free fusion peptide also induces fusion of liposomes and erythrocytes, and the site-directed mutagenesis/fusion activity relationship of the free peptide is comparable to that of the intact virus. A variety of solid-state NMR measurements demonstrated that the peptide has a predominantly extended membrane-bound conformation. Internuclear distance measurements were then applied to deduce an intermolecular beta sheet structure, which is consistent with the hypothesis that peptide oligomerization is a structural requirement for fusion peptide-induced membrane fusion. Additional solid-state NMR measurements demonstrated that the lipid bilayer structure remains intact at high concentrations of fusion peptide. This observation is consistent with a peptide catalytic rather than equilibrium effect on the bilayer structure.

2. Structure of an aflatoxin B_1 deoxyguanosine adduct mismatched with deoxyadenosine. Indrajit Giri, Mark D. Jenkins, and Michael P Stone. Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235

The G → T transversion is the dominant mutation induced by aflatoxin B_1, a potent fungal mutagen. The solution structure of d(ACATC^{AFB}GATCT)·(AGATAGATGT) was refined using molecular dynamics calculations restrained by NOE data and dihedral restraints. The structure models the putative intermediate in a G to T transversion. This mismatched structure is compared with the unmodified GA mismatch and with the ^{AFB}GC adduct. In the mismatched structure, sequential ^{1}H NOEs were interrupted between C^5 and $^{AFB}G^6$, but intrastrand NOEs were traced through the aflatoxin moiety, via H6a of aflatoxin and H8 of $^{AFB}G^6$. Opposite the lesion, NOEs between mismatched A^{15} and the neighboring nucleotides were either missing or weak. A total of 42 NOEs were observed between DNA and aflatoxin. Molecular dynamics calculations using 308 NMR derived NOE restraints and other experimental and empirical restrained yielded refined structures of d(ACATC^{AFB}GATCT)·(AGATAGATGT) with pairwise rmsd < 1 Å. (Supported by NIH Grant CA55678.)

3. Crystal structures of an anti-single-stranded DNA antibody fragment reveal a conserved DNA recognition module and an induced fit binding mechanism. John J. Tanner. Department of Chemistry, University of Missouri−Columbia, 125 Chemistry Building, 601 S. College Ave., Columbia, MO 65211 (fax: 573-882-2754, tannerjj@missouri.edu)

Anti-DNA antibodies are important because they have been implicated in the autoimmune disease systemic lupus erythematosus. They also serve as models for understanding protein−DNA recognition. The focus of this presentation is a recombinant antigen-binding fragment (Fab) called "DNA-1" that was isolated from a bacteriophage display library. DNA-1 is an anti-single-stranded DNA Fab that binds specifically to homothymine polymers with an equilibrium dissociation constant of 150 nM. Three crystal structures of this Fab will be presented: a 2.1 Å structure of a DNA-1/oligo(dT) complex, and two other structures of the unliganded Fab. The DNA-1/oligo(dT) structure reveals that the antibody recognizes DNA by sandwiching the thymine bases between tyrosine side chains, which perfectly positions the DNA bases to make sequence-specific hydrogen bonds with the protein. Few interactions are formed with the DNA backbone, and no protein−DNA ion pairs are observed. Comparison of this structure to other anti-nucleic acid antibodies reveals a DNA−antibody recognition module that is hypothesized to be a fundamental structural element responsible for antibody recognition of ssDNA. Structures of the unliganded Fab show that DNA binding is accompanied by both global and local conformational changes in the protein.

4. A generic complementation assay for protein evolution and proteomics. Sonja Krane,† Colleen Bleczinski,† Kathleen Baker,† Gilda Salazar-Jimenez, Hening Lin, Debleena Sengupta, and **Virginia W. Cornish**. Department of Chemistry, Columbia University, New York, NY 10027

Reaction-independent assays for enzymatic activity hold tremendous promise for protein evolution and proteomics. The difficulty is to design a method that is high-throughput and that can readily be adapted to a variety of different chemical reactions. Here we describe an approach, based on the yeast two-hybrid assay, where we make transcription of a reporter gene dependent on enzymatic turnover. Specifically, dimerization of the DNA-binding and activation domains of a transcriptional activator is made dependent on a dimeric ligand, or chemical inducer of dimerization (CID), and then the bond between the two ligands is replaced with the chemistry of interest. Thus, enzyme-catalyzed cleavage or formation of the bond between the two ligands controls

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reconstitution of the transcriptional activator and transcription of an engineered reporter gene. As a proof of principle, we have used a well-studied enzymatic reaction, cephem hydrolysis by a cephalosporinase, to demonstrate this strategy. First we synthesized a dexamethasone−methotrexate (Dex-Mtx) CID with a cleavable cephem linker (Dex-cephem-Mtx) and showed that this molecule retained the ability to activate transcription in the yeast two-hybrid assay. Then we introduced the cephalosporinase enzyme into the yeast cells and developed conditions where we can detect enzyme-catalyzed cleavage of the cephem linker as disruption of the transcriptional read-out. Finally, in a mock screen, we have distinguished active and inactive cephalosporinase variants based on their effect on the levels of transcription of a *lacZ* reporter gene. (†These three authors contributed equally to this work.)

5. Oligonucleotide-based cocaine sensors. Milan N. Stojanovic and Donald W. Landry. Department of Medicine, Columbia University, Box 84, 630 W. 168th St., New York, NY 10032 (fax: 212-305-3475, mns18@columbia.edu)

We developed simple, rational approach to aptameric sensors. A one-step procedure converts an anti-cocaine aptamer with one phosphorothioate-substituted phosphodiester bond into a fluorescent sensor. A 3-fold increase in fluorescence was observed upon cocaine binding, and the dynamic range is from 32 to 4000 μM cocaine. The mechanistic details of the sensor will be discussed, and compared to previous designs based on secondary structure formation.

6. Characterizing universal bases for hybridization dependent processes: Betting on wildcards for sequencing by hybridization. John S. Oliver, Kathy A. Parker, and William J. Suggs. Department of Chemistry, Brown University, Providence, RI 02912 (fax: 401-863-2594, John_Oliver@Brown.edu)

In recent years the use of oligonucleotides containing nondiscriminate or universal bases, such as 3-nitropyrrole and 5-nitroindole, has been proposed for applications such as pcr or as sequencing primers. In general these efforts have not met with much success. In large part this is due to insufficient characterization of the effect the universal base has on hybridization in a variety of sequence contexts. As a result, universal bases are viewed as a curiosity rather than a useful tool. We are interested in applying universal bases as wildcards in a new and improved method of Sequencing By Hybridization (SBH) with microarrays. The successful application of universal bases to "patterned" probes will increase the information content of oligonucleotide microarrays for sequencing by orders of magnitude. Our investigations have revealed that universal bases affect the behavior of neighboring bases. The use of multiple and different universal bases can modify the behavior of each universal base. Recent progress in the determination of thermodynamic parameters of universal bases in DNA and PNA will be discussed in the context of gapped probes.

7. Using model substrates to study the influence of affinity on cell adhesion. Mihoko Kato and Milan Mrksich. Department of Chemistry, University of Chicago, 5735 S. Ellis Ave., Chicago, IL 60637 (mkato@midway.uchicago.edu)

We investigated the influence of ligand affinity on cell adhesion using self-assembled monolayers presenting either a low- or a high-affinity peptide ligand for the integrin receptor. Cell adhesion through interaction of integrin receptors with extracellular matrix (ECM) results in the clustering of receptors into aggregates known as focal adhesions, which are important for cell anchorage and signaling. The complexity of the ECM necessitates well-defined model systems for mechanistic studies of adhesion. Fibroblasts attached to model substrates and displayed morphologies, focal adhesions, actin stress filaments, and FAK signaling that were characteristic of cells adherent to fibronectin. Cells adhering to the high-affinity ligand had approximately twice as many focal adhesions as did cells adhering to the low-affinity ligand, and the focal adhesions were overall smaller on the former substrate. These results suggest that the rate of nucleation relative to the rate of growth of focal adhesions is greater for high-affinity integrin−ligand interactions.

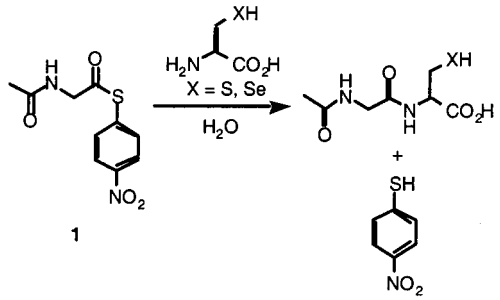
8. Molecular recognition of the cholanic acids by human ileal lipid binding protein. Gregory P. Tochtrop, David P. Cistola, and Douglas F. Covey. Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 S. Euclid, St. Loius, MO 63110 (fax: 314-362-7058, gptochtr@artsci.wustl.edu)

Human bile acid-binding protein (I-BABP) is a member of the intracellular lipid binding protein family. It is thought to function in the transcellular transport and enterohepatic circulation of bile salts. Bile salts are amphipathic steroids synthesized from cholesterol in the liver. In the GI tract, bile salts act as detergents that aid in the absorption of dietary lipids. Unlike other family homologues, I-BABP binds two bile acids with weak intrinsic affinity but a high degree of positive cooperativity. To identify the structural correlates of the cooperativity and identify the location of the two binding sites, we synthesized selectively ^{13}C- and ^{15}N-enriched bile salts to study a ternary complex of I-BABP with glycocholate and glycochenodeoxycholate. The experimental strategies include: (i) isotope-edited/filtered NOESY experiments using ^{13}C/^{15}N-enriched protein and unenriched bile acids, and (ii) isotope-edited NOESY experiments using selectively ^{13}C- or ^{15}N-enriched bile acids and unenriched protein. The latest results focusing on bile acid−protein interactions will be presented.

9. Selenocysteine in native chemical ligation and expressed protein ligation. Robert J. Hondal,[1] Bradley L. Nilsson,[2] and Ronald T. Raines.[3] [1]Department of Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr. (rhondal@biochem.wisc.edu), [2]Department of Chemistry, University of Wisconsin−Madison, and [3]Departments of Biochemistry and Chemistry, University of Wisconsin−Madison, Madison, WI 53706

L-Selenocysteine (Sec or U) has been called the "21st amino acid". Like the 20 common amino acids, selenocysteine is inserted during the translation of *m*RNA and has its own *t*RNA^{Sec} and codon, UGA. This codon also serves as the *opal* stop codon. Decoding a UGA codon as one for selenocysteine requires a special *m*RNA called a selenocysteine insertion sequence (SECIS) element. Because eukary-

otic and prokaryotic cells use a different SECIS element to decode UGA as selenocysteine, the production of eukaryotic selenocysteine-containing proteins in prokaryotes is problematic. Here, we describe a general semisynthetic route to produce proteins containing selenocysteine. This method makes use of replacing selenocysteine in place of cysteine in native chemical ligation (scheme below) or expressed protein ligation. The high nucleophilicity of selenium and the low pK_a of the selenol group enable this reaction to proceed ca. 1000-fold faster at pH 5.0. We have demonstrated that selenocysteine can replace a cysteine residue of ribonuclease A to produce a semisynthetic selenium-containing variant that shares the same catalytic activity as the wild-type enzyme. Creation of other naturally occurring selenoproteins using this methodology will be discussed.



10. Design of an artificial enzyme system employing an open, solvent exposed cavity; examination of catalysis in aqueous and organic media. Dongfeng Qi,[1] Cheng-Min Tann,[1] David P. Cistola,[2] and Mark D. Distefano.[1] [1]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, and [2]Department of Biochemistry & Molecular Biophysics, Washington University, School of Medicine, St. Louis, MO 63110

Intestinal fatty acid binding protein (IFABP) is a protein consisting of two orthogonal β-sheets and an α-helical lid. Helixless (hs) IFABPV60C was prepared by deleting the helical region using site-directed mutagenesis. hsIFABP-PX60 was constructed by attaching a pyridoxamine (PX) cofactor to Cys60 within the cavity. Using α-keto glutarate and Phe as substrates, this catalyst produced L-Glu with 37% *ee* and had 0.76 turnover after 24 h. Compared to the results obtained with IFABP-PX60 ($\sim$94% *ee* for L-Glu and 3.9 turnovers in 24 h), hsIFABP-PX reduces the reaction rate and gives poorer enantioselectivity. After attaching a more reactive cofactor, *N*-methyl pyridoxamine, to hsIFABPV60C, hsIFABP-MPX60 was produced. Under the same conditions, this catalyst showed increased reaction efficiency (6.0 turnovers in 24 h) but even poorer enantiomer selectivity (17% *ee* for L-Glu). Significantly, the hsIFABP-MPX60 conjugate showed transamination reactivity in organic solvents such as toluene with 30% conversion of Glu along with 40% *ee* for L-Glu in 24 h. For the production of Val, 95% conversion and 93% *ee* for D-Val were obatined. These results suggest that the α-helical lid that creates a closed cavity is required for high enantioselectivity in aqueous solution; however, the open cavity in organic solvent provides the direct access to the substrate, thus making it the first transamination biocatalyst active in organic solvents.

11. Antibodies catalyze the oxidation of water. Paul Wentworth, Jr., Lyn H. Jones, Anita D. Wentworth, Kim D. Janda, and Richard A. Lerner. Department of Chemistry, The Scripps Research Institute (BCC582), 10550 N. Torrey Pines Rd., La Jolla, CA 92037 (fax: 858-784-2590, paulw@scripps.edu)

Recently we reported that antibodies, regardless of source or antigenic specificity, generate hydrogen peroxide (H_2O_2) in a photooxidative process involving singlet molecular oxygen ($^1O_2$), thereby potentially aligning recognition and killing within the same molecule. Mechanistic studies now lead us to propose that antibodies and the $\alpha\beta$ T-cell receptor (TCR), but not other proteins, generate H_2O_2 via catalytic oxidation of water by $^1O_2$, producing up to 500 mol equiv of H_2O_2 in the absence of any discernible cofactor and electron donor. Based on isotopic labeling and kinetic data, we propose that antibodies are capable of facilitating an unprecedented nucleophilic addition of H_2O to $^1O_2$ to form H_2O_3 as the first intermediate in a reaction cascade that eventually leads to H_2O_2. X-ray crystallographic studies with Xe point to conserved oxygen binding sites within the antibody fold where this chemistry could be initiated. Our findings point to a unique protective function of immunoglobulins against $^1O_2$ and raise the question of whether the need to detoxify $^1O_2$ has played a decisive role in the evolution of the immunoglobulin fold.

12. Screening combinatorial libraries for optimal enzyme substrates by mass spectrometry. Dehua Pei, Peng Wang, Donald F. Snavley, Hua Fu, and Michael A. Freitas. Department of Chemistry, The Ohio State University, 100 W. 18th Ave., Columbus, OH 43210 (fax: 614-292-1532, pei.3@osu.edu)

A method has been developed for the rapid identification of optimal enzyme substrates from combinatorial libraries by mass spectrometry. The method was validated by screening a 361-member N-formylated tripeptide library, f-XXR (X = 19 different amino acids), for optimal substrates of *Escherichia coli* peptide deformylase (PDF). The library was synthesized on the solid phase, and the N-terminal formyl group was added using a 1:1 (mol/mol) mixture of HCO_2H and DCO_2D. In a mass spectrum, each member of the starting library produced a doublet peak (separated by 1.0063 Da). Limited treatment of this library with *E. coli* PDF resulted in the deformylation of those peptides that are the most efficient substrates of PDF. The deformylated products, which appeared as singlets in the mass spectrum, were readily identified and sequenced via tandem mass spectrometry. The specificity data obtained by this method are in excellent agreement with literature data, demonstrating it as an effective approach to the identification of optimal enzyme substrates. Application of this method to other enzymes will also be discussed.

Olin Award Symposium—Sunday Afternoon

13. Protein kinases: One step at a time. Joseph A. Adams. Department of Pharmacology, University of California, San Diego, 331 CRB, MC 0506, 9500 Gilman Dr., La Jolla, CA 92093-0506 (fax: 858-534-8248, j2adams@ucsd.edu)

Protein kinases can finely alter cellular processes in both beneficial and harmful ways.The latter, in particular, has sparked widespread interest in therapeutic strategies for

disease control. Both, however, underscore the need for understanding the molecular details of activity regulation within this enzyme family. X-ray diffraction methods have provided three-dimensional frameworks for understanding the protein kinase family. However, elucidating the molecular origins for activity regulation requires solution techniques. We have shown that the key to understanding regulation and catalysis mandates a careful union of three-dimensional structure and enzyme dynamics. We will discuss the approaches for constructing catalytic mechanisms for protein kinases using fast mixing (rapid quench flow and stopped-flow) and solvent perturbation (viscosity) methods. These techniques demonstrate that most protein kinase deliver the phosphate of ATP to substrates at high rates despite low turnover numbers. Conformational changes play a large role in limiting overall substrate phosphorylation even in simple protein kinases. Using hydrogen−deuterium exchange and mass spectrometric techniques, it was shown that nucleotides induce long-range effects on the kinase structure. These solution-derived methods indicate that movements in domains and secondary structural elements may provide the source for these slow conformational changes that limit substrate processing.

14. Changes in protein conformational mobility upon activation of ERK2 as detected by hydrogen exchange. Andrew Hoofnagle,[1] Katheryn Resing,[1] Elizabeth J. Goldsmith,[2] and **Natalie G. Ahn**.[1] [1]Department of Chemistry and Biochemistry, HHMI, University of Colorado, Boulder, CO 80309, and [2]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX

Changes in protein mobility likely accompany changes in conformation during the trans-activation of enzymes; however, few studies exist that validate or characterize this behavior. In this study, amide hydrogen/deuterium exchange mass spectrometry was used to probe the conformational flexibility of extracellular signal-regulated protein kinase-2 (ERK2) before and after activation by phosphorylation. The exchange data indicated that ERK2 activation caused altered backbone flexibility in addition to the conformational changes previously established by X-ray crystallography. The changes in flexibility occurred in regions involved in substrate binding and turnover, suggesting their importance in enzyme regulation.

15. Structural analysis of DNA polymerase processivity clamp loading. John Kuriyan, David Jeruzalmi, and Mike O'Donnell. Laboratories of Molecular Biophysics, Rockefeller University, Howard Hughes Medical Institute, 1230 York Ave., New York, NY 10021-6399 (fax: 212-327-8618)

Chromosomal replicases in bacteria, archaebacteria, and eukaryotes achieve high processivity by attachment of the catalytic subunits of the polymerase to circular ring-shaped clamps that slide along DNA. Because these ring-shaped proteins do not readily bind to DNA by themselves, the polymerases include multi-protein clamp-loader assemblies (g-complex in bacteria, RFC in archaebacteria and eukaryotes) that utilize ATP binding and hydrolysis to load the processivity clamps onto DNA. We have made significant progress toward a structural understanding of DNA polymerase clamp loading by obtaining two new structures. We have determined structures of a subunit of *E. coli* g-complex bound to an open form of the b-ring (at 2.5 Å) which reveals how the clamp-loader breaks the sliding clamp circle. We have also determined the structure (at 2.8 Å) of an intact and active 5-protein clamp-loader assembly from *E. coli*, which is an exciting development for understanding the mechanism of this complex motor protein.

16. Reversible phosphorylation: The role of protein tyrosine phosphatases in signal transduction and disease. Jack E. Dixon. University of Michigan Medical School, Ann Arbor, MI 48109

Protein tyrosine phosphatases play critical roles in regulating numerous intracellular signal pathways. In addition, phosphoinositides play an integral role in a diverse array of cellular signaling processes. Although considerable effort has been directed toward the kinases that produce inositol lipid second messengers, the study of phosphatases that oppose these kinases remains limited. Current research is focused on the identification of novel lipid phosphatases such as PTEN and myotubularin and their physiologic substrates, signaling pathways, and links to human diseases. The use of bioinformatics in conjunction with genetic analyses in model organisms will be essential in elucidating the roles of these enzymes in regulating PI-mediated cellular signaling. In addition, our laboratory recently utilized a yeast two-hybrid screen employing the *Drosophila* PTPase 61F to identify a novel adaptor protein similar to the mammalian Nck which we have called Dock. A *Drosophila* homologue of human Down syndrome cell adhesion molecule (Dscam), an immunoglobulin superfamily member, was isolated by its affinity to Dock, an SH3/SH2 adaptor protein required for axon guidance. Dscam binds directly to both Dock SH2 and Dock SH3 domains. Genetic studies revealed that Dscam, Dock, and Pak, a serine/threonine kinase, act together to direct pathfinding of Bolwig's nerve, containing a subclass of sensory axons, to an intermediate target in the embryo. Dscam also is required for the formation of axon pathways in the embryonic central nervous system; cDNA and genomic analyses reveal the existence of multiple forms of Dscam with a conserved architecture containing variable Ig and transmembrane domains. Alternative splicing can potentially generate more than 38 000 Dscam isoforms. This molecular diversity may contribute to the specificity of neuronal connectivity.

17. Dynamics of signaling by PKA. S. S. Taylor, P. Banky, D. Johnson, and L. Burns. Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of California, San Diego, 9500 Gilman Dr., MC 0654, La Jolla, CA 92093-0654

PKA, one of the simplest members of the protein kinase superfamily, serves as a prototype for understanding structure and function. The proteins that contribute to PKA signaling are diverse in their global architecture and their biophysical and dynamic properties. The highly conserved catalytic (C) subunit is focused on phosphoryl transfer with an extensive and integrated network of signaling throughout the molecule. The dynamic movements of the protein are an essential driving force for catalysis. The regulatory (R) subunits and the small protein kinase inhibitors (PKIs) bind with high

affinity to C and suppress catalytic activity. Both R subunits and PKI are multifunctional and contain significant regions of plasticity. R subunits target PKA to specific sites by docking to A Kinase Anchoring Proteins (AKAPs) through their N-terminal dimerization/docking domains. The AKAPs also are modular with regions of disorder. The structures, dynamic properties, and localization of these proteins will be discussed. (Supported by funding from the NIH and the HHMI.)

General Poster Session—Sunday Evening

18. Rapid detection and identification of microorganisms by multiwavelength spectroscopy. Luis H. Garcia Rubio,[1] **Xiaojuan (Judy) Fu**,[1] Jose A. Olivares,[2] Catalina Alupoaei,[1] and Adrian Smith.[1] [1]Department of Chemical Engineering, University of South Florida, 4202 E. Fowler Ave., Tampa, FL 33620 (fax: 813-974-3651, fu@grad.csee.usf.edu), and [2]Bioscience Division, P.O. Box 1663, Los Alamos National Laboratory, Los Alamos, NM 87545

Ultraviolet−visible (UV−Vis) multiwavelength transmission spectroscopy is a rapid method that can provide quantitative information on particle size, particle size distribution, and chemical composition for submicron and micron size particles. In this paper, spectroscopy fingerprints of different selected microorganisms have been demonstrated. The spectral differences are explained in terms of the differences in the size, shape, and chemical composition. It is also shown that the complete analysis over the UV or near-UV region provides information on the nucleic acid and protein content which can be applied in discriminating different microorganisms. Advances in the development of miniaturized spectrometers with long optical cells increase the potential of this technique as a rapid, reliable, and efficient biosensor for detection and identification of microorganisms in water and other media.

19. Beta amyloid peptides on lipid membranes. Canay Ege and Ka Yee C. Lee. Department of Chemistry & Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637

The beta amyloid peptide (Aβ), a 40−43 residue peptide, is the major component found in the amyloid aggregates in Alzheimer's disease patients. In vitro cell culture studies have shown that Aβ is toxic to neurons and that toxicity is associated with the β-structured aggregates. However, such in vitro studies have to employ supraphysiological concentrations of up to millimolar Aβ for short periods of time, whereas the in vivo deposition of Aβ evolves over long periods of time by the production of nanomolar concentrations in the brain. It is more informative, therefore, to look at the interactions of the peptide with lipid membranes, since the presence of lipids can induce the aggregation of Aβ at micromolar concentrations. We have studied the interaction between Aβ1−40 and Aβ1−42 with phospholipid molecules using Langmuir monolayers and fluorescence microscopy to address the role of cell membranes in the pathology of the disease.

20. Aversive diterpenes of *Euphorbia esula*. Fathi T. Halaweish,[1] James A. Rice,[1] and Scott Kronberg.[2] [1]Department of Chemistry & Biochemistry, South Dakota State University, 121 Shepard Hall, Box 2202, Brookings, SD 57007 (fathi_Halaweish@sdstate.edu), and [2]USDA, Agriculture Research Service, Mandan, ND 58554

Leafy spurge (*Euphorbia esula* L.), a plant introduced into the Great Plains of North America from Europe, has become a serious economic and ecological threat to the productivity of agricultural and natural areas. Cattle, the predominant livestock species on the Great Plains, as well as common wild ruminant species in this region appear to consume little if any leafy spurge. This is likely because they experience a toxic response after they consume small amounts of this plant and consequently learn to avoid it. Domestic sheep and goats can consume considerable amounts of leafy spurge and are used to help control it, but even these species appear to suffer a toxic response at high levels of intake. Toxic diterpene ingenols have been isolated from leafy spurge tissues, but compounds in this plant have not been evaluated with respect to their capacity to induce food aversion learning in mammals. We conducted bioassay-guided fractionations of leafy spurge in an attempt to isolate toxic and aversive compounds in the plant. These bioassay-guided fractionations led to spectroscopic (NMR, IR, and MS) identification of ingenol and one of its diesters as two cytotoxic toxic and aversive compounds in leafy spurge.

21. Formation of nanoscale compartments in phospholipid membranes. Adrian S. Muresan[1] and Ka Yee C. Lee.[2] [1]Department of Physics, The University of Chicago, 5735 S. Ellis Ave., Chicago, IL 60637 (fax: 773−702-0805, a-muresan@uchicago.edu), and [2]Department of Chemistry & Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637

We explored the morphology and dynamics of domain growth resulting from lateral phase separation on the nanometer length scale in a phospholipid membrane. Planar supported bilayers composed of mixtures of distearoylphosphatidylcholine (DSPC)/dimyristoylphosphatidylcholine (DMPC) were imaged in fluid using temperature- and time-dependent atomic force microscopy (AFM). We find that both isolated lipid domains and networks can be formed in these model bilayers. For a binary lipid mixture, the phase-separated domains can be compact or branched depending on the relative lipid ratio. The compact structure leads to the formation of isolated domains, while the branched aggregates connect, eventually giving rise to a network. These structures enable membrane compartmentalization on a nanometer scale. The fact that the phase-separated morphology is tunable with lipid content points to the possibility that the cell can attain the desired surface topology by regulating its lipid composition.

22. How do lung surfactant peptides affect 2d−3d transitions in lipid monolayers? Ajaykumar Gopal and Ka Yee C. Lee. Department of Chemistry and Institute for Biophysical Dynamics, University of Chicago, 5735 S. Ellis Ave., Chicago, IL 60637

Lung surfactant (LS) is a complex film of lipids and proteins that operates at low surface tension at the air−water interface in the lungs. Recent electron microscopy studies suggest that this film may actually be a monolayer of material in equilibrium with multilayered 3d reservoirs. Three-

dimensional reversible folding in surfactant protein containing lipid monolayers was reported recently and may provide a possible mechanism for the coexistence of reversible reservoirs with monolayers. We observe that such folding transitions coexist with other 2d−3d transitions (e.g., vesicle formation) and do not necessitate the presence of surfactant proteins in the system. In 7:3 mixtures of phosphatidylcholine (PC) and phosphatidylglycerol (PG) molecules, 2d−3d transition is temperature-modulated. Reversible folding dominates below 30 °C and vesicle formation above 33.5 °C, and both transitions are observed at intermediate temperatures. We have, herein, reexamined the role of the peptide SP-B_{1-25} in influencing morphology and 2d−3d transitions in lipid monolayers.

23. In vitro synthesis of eIF-5A-deoxyhypusine and partial purification of deoxyhypusine hydroxylase. **J.-K. Huang**, W. Cheng, P. G. Gowda, K. J. Liudahl, A. M. Walzer, and L. Wen. Department of Chemistry, Western Illinois University, One University Circle, Macomb, IL 61455 (fax: 309-298-2180, mfjkh@wiu.edu)

Mature eIF-5A is the only protein known to contain the unusual amino acid hypusine. Hypusine synthesis in eIF-5A is a unique two-step posttranslational modification involving two enzymes, deoxyhypusine synthase (DS) and deoxyhypusine hydroxylase (DH). Inhibitors of either DS or DH have been shown to exert antiproliferative and antiretroviral effects. Since DH has not yet been purified, our objective is to purify and characterize the enzyme. A knowledge of the structure−function relationship of this enzyme will help in designing better inhibitors. DS and eIF-5A precursor proteins are essential in producing eIF-5A-deoxyhypusine that will serve as a substrate for DH. We have constructed, overexpressed, and partially purified functional recombinant human eIF-5A and hDS proteins. Using the two proteins and ^{3}H- or ^{14}C-labeled spermidine, we have synthesized labeled eIF-5A-deoxyhypusine. The labeled eIF-5A-deoxyhypusine has been used as a substrate to monitor bovine DH activity during purification. (Supported in part by a grant from the NIH, 1R15 GM60266-01A1.)

24. Investigation of solvent-induced structural changes in ferrocytochrome *c*. Sivashankar G. Sivakolundu and Patricia Ann Mabrouk. Department of Chemistry, Northeastern University, 112 Hurtig Hall, Northeastern University, Boston, MA 02115 (phone: 617-373-5432, ssivakol@lynx.neu.edu)

Structural changes in ferrocytochrome *c* (ferrocyt c) in 30% acetonitrile (ACN)−water solution were examined using 2D NMR techniques. Oxidized cytochrome *c* in 30% ACN solution undergoes alkaline transition and exhibits dynamic conformational equilibrium between the different conformers formed. On the other hand, ferrocyt c remains as a single conformer. Comparative analysis of the proton chemical shifts of ferrocyt c in aqueous solution and 30% ACN solution indicates that the overall structure of the protein and the heme ligation are preserved. However, distinct changes in the secondary and tertiary structure were observed. For example, the M80 residue, which is the sixth axial ligand, exhibits structural perturbations as evidenced by significant differences in the proton chemical shifts. These changes may shed light in understanding the nature of protein−solvent interactions in general, and the isomerization of oxidized cytochrome *c* in 30% ACN solution and at alkaline pH.

25. Modification of protein byproducts of lipid peroxidation increases binding of redox active transition metals. Gang Sun and Lawrence M. Sayre. Department of Chemistry, Case Western Reserve University, Cleveland, OH 44106 (fax: 216-368-3006, gxs52@po.cwru.edu)

Covalent modification of protein byproducts of lipid peroxidation during oxidative stress may create new binding sites for transition metals in a manner that the coordinated metals retain redox activity. We found that modification of the model proteins ribonuclease A (RNase) and beta-lactoglobulin by glyoxal and by 4-hydroxy-2-nonenal (HNE) resulted in a more than 2-fold increase in dialysis-resistant binding of Cu(II). Using catalysis of aerobic oxidation of ascorbate as an assay of redox activity of dialysis-resistant bound metal, pretreatment of protein with glyoxal or HNE resulted in a 4-fold increase in oxygen consumption compared to control, indicating that the modification-dependent bound copper was particularly redox-active. Although prior methylation of RNase lysines itself resulted in increased binding of Cu(II), there was now very little change produced by treatment with glyoxal or HNE, suggesting lysines as the major targets for modification leading to increased metal ion binding. Prior treatment of protein with diethylpyrocarbonate to block histidines did not alter the increase in binding and redox activity induced by modification with glyoxal or HNE. Very small effects were seen for iron compared to copper.

26. Phenotypic reversion of transformed fibroblasts by suramin and elevated lysyl oxidase expression. Amitha H. Palamakumbura and Philip C. Trackman. Boston University Goldman School of Dental Medicine, Division of Oral Biology, Boston, MA 02218-2392

Suramin is an anti-cancer drug. Lysyl oxidase (LO) catalyzes the final enzymatic step of collagen and elastin cross-linking, and has tumor suppressor activity. This study investigates suramin-induced phenotypic reversion of *c*-H-*ras*-transformed murine NIH3T3 cells (RS485 cell line) and regulation of LO biosynthesis and activity. The role of LO activity in phenotypic reversion was determined utilizing a LO inhibitor, β-aminopropionitrile. Suramin (0−150 μM) changed cell morphology and decreased the growth rate by up to 45%, and cell cycle analysis showed a decreased S phase to 16.3%. Suramin (150 μM) increased LO mRNA levels by 50-fold and increased production of pro-LO protein. LO enzyme activity was elevated by 2.5-fold. Growth of RS485 cells in the presence of both 150 μM suramin and 400 μM β-aminopropionitrile did not prevent suramin-induced changes. Suramin-induced phenotypic reversion of RS485 cells is independent of increased LO enzyme activity, but may depend on other LO functions.

27. Spectroelectrochemical studies of cross-linked hemoglobins. Simona A. Dragan, Alanah Fitch, and Ken. W. Olsen. Department of Chemistry, Loyola University Chicago, 6525 N. Sheridan Rd., Chicago, IL 60626 (fax: 773-508-3086, sdragan@mail.owc.net)

The electron transfer in alpha99 and beta82 cross-linked hemoglobins with bis(3,5-dibromosalicyl) fumarate compared

to unmodified hemoglobin is studied using spectroelectrochemical methods. A rigorously oxygen-free, rapid-equilibrium experimental system was designed to determine the formal redox potential of the heme group as well as to measure full oxidation and reduction rates. Formal redox potentials as well as full oxidation and reduction rates were determined in the presence of hexaammineruthenium(III) as mediator. The formal redox potential was found more positive for the alpha99 and more negative for the beta82 cross-linked hemoglobin compared to the unmodified hemoglobin. For all three compounds, the formal redox potentials were found sensitive to electrolyte concentration, which suggests an additional stabilization of a quaternary conformation, by salt bridges between subunits. By modulating the electrolyte concentration, the reduction rate constants could be measured as a function of driving force. Self-exchange rate constants were determined via Marcus theory. Preliminary data show that the self-exchange rate of autoxidation differs between native and cross-linked hemoglobins independent of the thermodynamic driving force. Both modified compounds exhibit faster autoxidation rates than hemoglobin, a fact that suggests structure and accessibility of the heme group may influence the rates observed.

28. Substrate nucleates the folding of its own active site as shown in the crystal structures of both bound and unbound 1L-*myo*-inositol-1-phosphate synthase. Adam J. Stein, John W. Frost, and James H. Geiger. Department of Chemistry, Michigan State University, East Lansing, MI 48824 (fax: 517-353-1793, stein@cem.msu.edu)

1L-*myo*-Inositol-1-phosphate synthase (MIP synthase) catalyzes the conversion of D-glucose 6-phosphate to 1L-*myo*-inositol 1-phosphate. This represents the first step in the biosynthesis of all inositol-containing compounds. The conversion involves a complex series of transformations including oxidation, intramolecular aldol cyclization, and reduction, all of which occur in the same active site of the enzyme. We have determined the crystal structure of MIP synthase bound to NAD and bound to an inhibitor, 2-deoxyglucitol 6-phosphate. While 60 amino acids are disordered in the unbound form of the enzyme in the vicinity of the active site, the inhibitor nucleates the folding of this domain, serving to completely encapsulate it within the enzyme. Three helices and a long beta strand are formed in this process. A mechanism for this binding is proposed that first incorporates the binding of the inhibitor to the ordered part of the enzyme followed by nucleation and folding of the disordered region. We further postulate a mechanism for the conversion based on the structure of the inhibitor-bound complex.

29. Thyroid physiology of male and female Heteropneustes fossiles. Revathi Kasturi. Ethiraj College, No. 6/8, 10th St., Nandanam Extension, Madras 600035, India (fax: 763-764-3865)

Thyroid physiology of male and female Heteropneustes fossiles was studied. The effect of light on thyroid metabolism is studied by exposing fish to light for different durations of time. These changes are monitored using the radioimmunoassay (RIA) kit for iodine 131. The uptake of iodine 131 is monitored using easy and simple methods: UV−Vis spectrometry and gamma counting. Photoperiod-induced thyroid changes were studied from the serum triiodothyronine (T3) level, serum thyroxine (T4) level, and protein binding iodine 131 uptake. The RIA kit for iodine 131 is used, and the serum triiodothyronine (T3) level and the serum thyroxine (T4) level were monitored using the gamma counter. The protein binding iodine 131 uptake was measured by a UV−Vis spectrometer at 420 nm. Heteropneustes fossiles were exposed to light and darkness for different lengths of time. Iodine 131 uptake was not affected during short and long photoperiods. The duration of a short photoperiod is exposure of light on fish for 8 h and darkness for 16 h. A long photoperiod is exposure of light for 16 h and darkness for 8 h. The fish exposed to light had significantly high levels of protein-bound iodine. The serum T3 and T4 levels were reduced in the darker environment. The serum T3 and T4 levels were increased in the fish, which were exposed to light. The method and the mechanism will be discussed in the presentation. Thyroid metabolism was thus studied in the male and female Heteropneustes fossiles by a UV−Vis spectrometer and a gamma counter. The chemistry and the mechanism will be presented in detail.

30. X-ray structure of a transcription signaling complex containing the Oct-1 POU domain, SNAP190, and DNA. Stacy L. Hovde,[1] R. William Henry,[2] and James H. Geiger.[1] [1]Department of Chemistry, Michigan State University, East Lansing, MI 48824 (fax: 517-353-1793, dewees@cem.msu.edu), and [2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824

We are studying the transcription of the human small nuclear (sn) RNA gene family to understand mechanisms of transcription activation. These genes are among the most actively transcribed in the cell, and, interestingly, transcription occurs by either RNA polymerase (Pol) II or III, depending upon promoter architecture. Both Pol II- and III-transcribed snRNA promoters have a distal sequence element (DSE), which is recognized by the activator protein Oct-1, and a proximal sequence element (PSE), which is recognized by the general transcription factor SNAPc. SNAPc is composed of five proteins and functions to nucleate preinitiation complexes for transcription. Transcriptional activation of human snRNA genes is increased by direct protein interactions between Oct-1 and the SNAP190 component of SNAPc. To understand the nature of these interactions at the molecular level, the crystal structure of the Oct-1 POU protein/U1 octamer site/SNAP190 peptide ternary complex has been solved to 2.3 Å resolution.

31. Abstract withdrawn.

32. *Rhodobacter capsulatus* DXP synthase: Steady-state kinetics and substrate binding. Lisa M. Eubanks and C. Dale Poulter. Department of Chemistry, University of Utah, Salt Lake City, UT 84112

1-Deoxy-D-xylulose-5-phosphate synthase (DXP synthase) catalyzes the first 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. DXP synthase catalyzes the decarboxylation of

pyruvate and the subsequent condensation of the thiamin-bound two-carbon intermediate with GAP to yield 1-deoxy-D-xylulose 5-phosphate (DXP) and CO_2 in a reaction similar to those catalyzed by transketolases. Numerous studies with transketolases have demonstrated that the enzymatic reaction proceeds through a ping-pong mechanism. Therefore, studies are presented which provide evidence for a similar thiamine diphosphate (TPP)-dependent mechanism in DXP synthase wherein pyruvate binds first, CO_2 is released, GAP binds second, and ultimately DXP is released from the enzyme. Steady-state kinetic studies in which both substrates are varied gave rise to the characteristic parallel double-reciprocal plots consistent with a ping-pong mechanism. Kinetic studies with dead-end reversible inhibitors for pyruvate and GAP provided inhibition patterns also consistent with this type of mechanism. Finally, $^{14}CO_2$-trapping experiments with [1-^{14}C]pyruvate and TPP demonstrated the enzymatic activation of pyruvate and release of CO_2 in the absence of the second substrate.

33. Chemoselective method for site-specific immobilization of peptides via aminooxy group. Zhiguang Yu,[1] Maciej Adamczyk,[2] John C. Gebler,[2] and Rajarathnam E. Reddy.[2] [1]Department of Chemistry, Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, IL 60064 (fax: 847-938-5188, george.yu@add.ssw.abbott.com), and [2]Department of Chemistry (D9NM), Diagnostics Division, Abbott Laboratories, Abbott Park, IL 60064

Site-specific modification of peptides and proteins is an important area of basic research for preparation of well-defined biosensors and probes. The unique properties of the aminooxy group present an opportunity for chemoselective immobilization of peptides. We have prepared FLAG peptide derivatives containing L-aminooxylysine and L-lysine units in their sequence at the C- and N-terminals via solid-phase synthesis. Site-specific modification of peptides through the aminooxy group was demonstrated in the preparation of biosensors and selective conjugation in the preparation of biotinylated probes. The effect of the incorporation of L-aminooxylysine into the FLAG sequence and its subsequent labeling on the FLAG epitopic character was measured using a surface plasmon resonance detector. It was found that incorporation of L-aminooxylysine into the FLAG peptide and immobilization through aminooxy group preserved the integrity of FLAG epitope.

34. A new assay for protein disulfide bond isomerization. Elizabeth A. Kersteen,[1] Seth R. Barrows,[1] and Ronald T. Raines.[1,2] [1]Department of Biochemistry and [2]Department of Chemistry, University of Wisconsin−Madison, Madison, WI 53706

Protein disulfide isomerase (PDI) catalyzes the formation of native disulfide pairings in eukaryotic cells. The essential function of PDI in vivo is the isomerization of nonnative disulfide bonds. Obtaining information about the catalytic mechanism has been hindered by the complexity of the substrates used in previous isomerization assays. Here, a simple two-disulfide substrate is developed for use in an isomerization assay. Two potential substrates, each containing two stabilizing disulfide bonds, are under investigation. Tachyplesin I is a 17-residue antimicrobial peptide isolated from horseshoe crabs; μ-conotoxin SI is a 13-residue neurotoxin isolated from conus snails. By selective oxidation of the four cysteine residues in these peptides, three different fully oxidized forms of each can be synthesized and distinguished by HPLC. Using nonnative versions of these small peptides as substrates enables the dissection of the catalytic mechanism of PDI, its variants, and other enzymic and small-molecule catalysts.

35. Chemical inducers of dimerization in *E. coli*. Eric A. Althoff and Virginia W. Cornish. Department of Chemistry, Columbia University, 3000 Broadway, Havemeyer Hall, MC 3153, New York, NY 10027 (fax: 212-932-1289, eaa33@columbia.edu)

Small molecules which control the dimerization of two proteins provide an extremely useful tool with which to study proteins and biological systems. So-called chemical inducers of dimerization, or CIDs, have been observed in yeast and other eukaryotes; however, a generally applicable CID system has not yet been observed in bacteria. The use of CIDs in bacteria would have several advantages particularly in the size of DNA libraries one is able to screen. To this end, we report significant advances toward a generally applicable bacterial chemical inducer of the dimerization system. Using heterodimerizers of Dex, Mtx, and AP755 and their receptor proteins of rGR, DHFR, and FKBP12, we have shown progress toward a CID in *E. coli*. We have adapted a bacterial protein−protein interaction assay, reconstitution of *Bordetella pertussis* adenylate cyclase from two functional complements, as the in vivo reporter. These studies show important advances toward the use of CID technology in *E. coli*.

36. Bioconversion of oleic acid to 7-hydroxy-8-octadecenoic and 7,10-dihydroxy-8-octadecenoic acids by *Pseudomonas aeruginosa* was enhanced in the presence of a yeast. Jenq-Kuen Huang,[1] Valerie C. Sershon,[1] Keven D. Wells,[2] Robert V. Gessner,[2] Kenneth C. Keudell,[2] and Lisa Wen.[1] [1]Departments of Chemistry and [2]Biological Sciences, Western Illinois University, 1 University Circle, Macomb, IL 61455 (fax: 309-298-2180, mfjkh@wiu.edu)

Some microorganisms convert oleic acid to its corresponding hydroxy or keto fatty acid derivatives. During screening of yeasts for bioconversion of oleic acid to useful compounds, Son observed that cocultivation of *Kluyveromyces marxianus* NRRL Y-8281(NRRL Y-8281) with *P. aeruginosa*, WIU-JS, enhanced the bioconversion of oleic acid to 10-HOD and a polar compound (M.S. Thesis, WIU). The synergistic effect was further studied by including *P. aeruginosa*-2HS, -2Hsp, and WIU-JP known to produce 10-HOD. The polar compound was identified as 7,10-dihydroxy-8-octadecenoic acid (7,10-DOD). Our conclusion is that NRRL Y-8281 enhanced the conversion of oleic acid to 10-HOD and 7,10-DOD by other *P. aeruginosa* as well. The effector came from yeast, and is heat stable. (Supported in part by grants from the University Research Council, Western Illinois University, and USDA CSREES 99-35501-8321.)

37. Biosynthesis of the thiazole moiety of thiamin in *Escherichia coli*: Identification of a novel acyl disulfide

linked protein−protein conjugate that is functionally analogous to the ubiquitin/E1 complex. Jun Xi, Ying Ge, Cynthia Kinsland, Fred W. McLafferty, and Tadhg P. Begley. Department of Chemistry and Chemical Biology, Cornell University, 120 Baker Lab, Ithaca, NY 14853 (fax: 607-255-4317, jx18@cornell.edu)

The recent study of thiamin biosynthesis will be described including the identification and characterization of a covalently linked protein−protein conjugate between ThiF and ThiS thiocarboxylate by Fourier transform mass spectrometry combined with mutagenesis and chemical modification. As an essential intermediate involved in the biosynthesis of the thiazole moiety of thiamin, this ThiF/ThiS conjugate is the first characterized example of an acyl disulfide containing intermediate in a biosynthetic system. This is also the first example of an ubiquitin-E1-like protein−protein conjugate in prokaryotes, suggesting a strong evolutionary link between thiamin biosynthesis and the ubiquitin conjugating system.

38. Chloroplast glyceraldehyde-3-P dehydrogenase contains a single disulfide bond located in the C-terminal extension to the B subunit. Jianfeng Qi,[1] Michail N. Isupov,[2] Jennifer A. Littlechild,[2] and **Louise E. Anderson**.[3] [1]Department of Chemistry, Michigan State University, East Lansing, MI 48824, [2]Department of Chemistry and Biological Sciences, University of Exeter, Stocker Rd., Exeter, U.K. EX4 4QD, and [3]Department of Biological Sciences, University of Illinois−Chicago, Chicago, IL 60607 (louise@uic.edu)

Mass mapping analysis based on cyanylation and CN-induced cleavage indicates that the two cysteine residues in the C-terminal extension of the B subunit of the light-activated pea leaf chloroplast glyceraldehyde-3-P dehydrogenase form a disulfide bond. No evidence was found for a disulfide bond in the A subunit, nor was there any indication of a second disulfide bond in the B subunit. The availability of the structure of the extended glyceraldehyde-3-phosphate dehydrogenase from the archaeon *Sulfolobus solfataricus* allows modeling of the B subunit. As modeled, the two cysteine residues in the extension are positioned to form an interdomain disulfide cross-link.

39. Affinity binding of mannosylerythritol lipids toward human immunoglobulin G. Jae Hong Im, Hiroshi Yanagishita, Toru Ikegami, and Dai Kitamoto Green. Processes Group, Green Technology Institute, National Institute of Advanced Industrial Science and Technology, 1-1 Higashi, Tsukuba-shi, Ibaraki 305-8565, Japan

Some glycolipids such as gangliosides G_{M1}, G_{D1a}, etc. exhibit a high affinity toward immunoglobulins. The possibility of developing these glycolipids into ligand for immunoglobulins, however, is far from straightforward due to their limited amounts and heterogeneity. Mannosylerythritol lipid (MEL), a yeast glycolipid biosurfactant, is abundantly produced from vegetable oils by *Candida* strains at a yield of 100 g L^{-1}. It also shows remarkable cell differentiation activities against human leukemia (HL-60), rat pheochromocytoma (PC-12), and mouse melanoma cell lines (B-16). MEL was investigated as the new ligand for human immunoglobulins (HIgG), and the binding mechanism of MEL and HIgG was also studied. In ELISA assay, MEL showed nearly the same binding affinity toward the protein as that of bovine G_{M1}. MEL, noncovalently attached onto poly(2-hydroxyethyl methacrylate) beads, exhibited a significant affinity constant of 1.43×10^6 M^{-1} for the protein, which is 4-fold greater than that of the immobilized protein A reported.

40. Collective motions of neutral and low pH forms of influenza virus hemagglutinin. Basak Isin,[1] Ivet Bahar,[2] and **Pemra Doruker**.[1] [1]Polymer Research Center and Chemical Engineering Department, Bogazici University, Bebek 80815, Istanbul, Turkey, and [2]Center for Computational Biology & Bioinformatics and Department of Molecular Genetics & Biochemistry, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213

Influenza virus hemagglutinin (HA) is an integral membrane glycoprotein involved in virus attachment to target cells and in the fusion of viral and endosomal membranes. Membrane fusion is mediated by the low pH environment of endosomes. The Gaussian network model (GNM) and its extension to anisotropic networks (ANM) are utilized for elucidating the dynamics of both neutral and low pH forms of HA. Correlated domain motions underlying membrane fusion are identified by focusing on a subset of cooperative motions extracted by a normal-mode analysis. In the slowest modes, a hinge bending motion is observed for both low and neural pH forms. This motion can be related to the association of endosomal and viral membranes. Another slow mode of motion is the twisting of the neutral pH form along its longitudinal axis, which may be important in the clustering of HA molecules, and in the pore opening process.

41. Conformation inversion of inositol phosphates. Carla J. Volkmann,[1] Ginger M. Chateauneuf,[1] Jyotsna Pradhan,[2] Andrew T. Bauman,[1] Richard E. Brown,[1] and Pushpalatha P. N. Murthy.[1] [1]Department of Chemistry, Michigan Technological University, 1400 Townsend Dr., Houghton, MI 49931 (fax: 906-487-2061, cjvolkma@mtu.edu), and [2]Department of Natural Sciences, University of North Florida, Jacksonville, FL 32224

Compounds that can rapidly change conformation with temperature or pH show great potential as molecular switches, sensors, transport carriers, and allosteric receptors. The highly phosphorylated derivatives of inositol undergo conformational inversion with pH. The conformational inversion of phytic acid has previously been studied. In this study, we plan to investigate the affects of stereochemistry and position of phosphate groups on the conformational inversion process. The *scyllo* isomer of inositol hexakisphosphate and two isomers of inositol pentakisphosphate were investigated. Dynamic NMR techniques were employed to analyze conformational inversion. The coalescence temperatures were measured experimentally, and activation energies of each inversion process were determined. Energy values of the alternate conformations were calculated by molecular modeling. The results show interconversion from the sterically unhindered form to the sterically hindered form. NMR spectroscopic and molecular modeling data of the conformational inversion of inositol phosphates will be presented.

42. Contribution of individual modules of a leucine-rich repeat protein to its affinity for other proteins. Sunil S.

Chandran,[1] Kimberly M. Taylor,[1] and Ronald T. Raines.[1,2] [1]Department of Biochemistry and [2]Department of Chemistry, University of Wisconsin−Madison, Madison, WI 53706

Ribonuclease inhibitor (RI) is a 50 kDa, leucine-rich repeat protein regulating the cytosolic activity of ribonuclease A (RNase A) and its homologues. Analysis of the genetic sequence for RI has established its modular structure with the presence of well-defined domains. An investigation involving the systematic deletion of each domain has been undertaken. Examination of the capability of modified RI to bind to RNase A provides a rational understanding of tight protein−protein interactions. These studies could lead to the development of a "minimal" RI encompassing all the interactions necessary to achieve tight binding to RNase A.

43. Control of Lucina pectinata hemoglobin I redox properties through site-directed mutagenesis: cDNA library characterization. Ruth G. León, Yamil López, Jorge L. Colón, and Fernando A. Gonzalez. Department of Chemistry, University of Puerto Rico, PO Box 23346, Río Piedras, PR 00931 (fax: 787-759-6885)

The clam Lucina pectinata contains a sulfide-reactive hemoglobin (HbI) that exhibits several unique properties. The ligand binding properties of HbI are influenced by an array of aromatic residues near the heme. The electron-transfer properties are also believed to be controlled by this array. The aim of this project is to investigate the effect of specific amino acid substitutions on HbI redox properties through site-directed mutagenesis and subsequent protein expression. A cDNA library has been constructed and screened in preparation for this study. The HbI gene was isolated and amplified with PCR, for which short degenerate oligonucleotide primers were synthesized. The amplified gene was inserted and ligated into a plasmid vector from which it was trasnformed into ultracompetent *E. coli* bacteria for expression. Several problems were encountered during the procedures which were solved using modified and special protocols.

44. Covalent modification of lysine residues in coproporphyrinogen oxidase. Jamal H. Momani, Timothy D. Lash, and Marjorie A. Jones. Department of Chemistry, Illinois State University, Normal, IL 61790-4160 (fax: 309-438-5538, jhmoman@ilstu.edu)

Coproporphyrinogen oxidase, an enzyme in the heme biosynthetic pathway, catalyzes the oxidative decarboxylation of coproporphyrinogen-III to protoporphyrinogen-IX by an unknown mechanism. A simple model for the enzyme active site (Lash et al., 1999) suggests at least two regions for substrate binding and one for the catalytic process. However, the amino acids involved in both substrate−enzyme binding as well as the catalytic mechanism are not known. Medlock and Dailey (1996) reported that two lysine residues are highly conserved between different organisms' version of this enzyme. Thus, covalent modification of the epsilon amine group of lysine should be useful to probe the active site. The purified enzyme has been treated with a highly selective reagent, 2,4,6-trinitrobenzenesulfonic acid, which modifies lysine amino acids. Product was measured using HPLC and UV spectroscopy. Enzyme activity decreased in a dose-dependent way, suggesting lysine is critical for substrate binding or catalysis. (Supported by NIH Grant 1R15GM/OD52687-01A1 and by a Pfizer Summer Undergraduate Research Fellowship.)

45. Cytotoxic folate-targeted ribonuclease variants. Bryan D. Smith,[1] Joshua J. Higgin,[2] and Ronald T. Raines.[1,2] [1]Department of Biochemistry and [2]Department of Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706

Variants of ribonuclease A are toxic to cancer cells because they can enter the cytoplasm, evade the endogenous ribonuclease inhibitor protein, and digest cellular RNA. The ribonuclease internalization pathway is not known, but the effectiveness of a cytotoxic ribonuclease is limited by the concentration of the enzyme that reaches the cytoplasm. Certain cancer cells overproduce the folate receptor on their surface, and protein−folate conjugates are efficiently internalized into these cancer cells. To target more ribonuclease to the cytoplasm of cancer cells, we have engineered ribonuclease−folate conjugates that can enter cells via folate receptor-mediated endocytosis. To evade ribonuclease inhibitor, we specifically coupled folate to residue 88 of ribonuclease A. We also randomly attached multiple folate moieties to K41R/G88R ribonuclease A, a potently cytotoxic ribonuclease. The cytotoxicity of these conjugates is described, along with future directions.

46. Enzymatic hydrolysis of cellulose to glucose: Reaction kinetics and pathways. Muzaffer Yasar, Solmaz Akmaz, and Ismet Gürgey. Department of Chemical Engineerıng, University of Istanbul, Avcılar, Istanbul 34850, Turkey

Biomass such as forestal, agricultural, and domestic wastes contain an important amount of cellulose. The importance of cellulose as the earth's most abundant renewable resource has been recognized as alternative fuels and chemicals. Chemical or biochemical conversation is required to transform this insoluble, polymeric form of glucose into useful materials. The enzymatic hydrolysis of cellulose for sugar production has received a great deal of attention in recent years. Due to the crystalline structure of cellulose, the cellulosic biomass cannot be readily saccharifed by enzymes without pretreatment. Various pretreatment techniques have been used to enhance the susceptibility of the substrate to enzmes. These include mechanical and chemical methods as well as a steam explosion pretreatment mainly used in this study. The aim of this study was to investige the effect of the steam hydrolysis on the saccharification of bioamass by using pure α-cellulose as a model compound. Steam hydrolysis of pure cellulose and its subsequent saccharification by *Trichoderma reesei* cellulases were investigated. First, the cellulose was steam-hydrolyzed in a 10 mL stainless batch-reactor at reaction temperatures of 180, 190, 205, and 220 °C for holding times ranging from 0 to 360 min. Residual fractions were recovered by filtration sequence. The variations of residual yields were used to calculate the reaction rate constants and the activation energies for cellulose steam hydrolysis. Second, reaction products obtained at 180, 190, 205, and 220 °C and 4 h were subjected to enzymatic hydrolysis. Enzymatic hydrolysis of steam-pretreated cellulose was carried out in 1 L flasks at 30, 40, and 50 °C agitated at 350 rpm. The pH was adjusted to 4.7 with 0.1 M

sodium acetate buffer. The reactivity of enzymatic reaction was strongly affected by the pretreatment of cellulose. Experimental results provided insight into steam hydrolysis and enzymatic reaction kinetics of cellulosic biomass.

47. Dependence of cholesterol oxidase activity on lipid membrane structure. KwangWook Ahn and Nicole S. Sampson. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794

Wild-type cholesterol oxidase activity with membranes of defined lipid phase is under investigation. Using a binary system of phospholipid and cholesterol, the initial velocities of cholesterol oxidase have been determined as a function of phospholipid and mole fraction of cholesterol. Assays were carried out at two temperatures: 10 °C above and 10 °C below the T_m of each phospholipid used. Dioleoylphosphatidylcholine, the T_m of which is −18 °C, was used for the normalization of temperature effects on rate data.

48. Design of an semisynthetic oxidoreductase using a protein cavity as a scaffold. Cheng-Min Tann, Dongfeng Qi, and Mark D. Distefano. Department of Chemistry, University of Minnesota, Minneapolis, MN 55414

Proteins are attractive as scaffolds for the design of molecular catalysts. Intestinal fatty acid binding protein (IFABP) consists primarily of two orthogonal β-sheets surrounding a large cavity (ca. 600 Å^3) into which ligands bind. It also contains an α-helical lid that covers one end of the protein cavity. Helixless (hs) IFABPV60C was prepared by replacing Val60 by Cys and deleting the helical region using site-directed mutagenesis. Flavin cofactors are catalytically very versatile, and they catalyze many of the same reactions promoted by naturally occurring flavoenzymes. Covalent modification of hsIFABPV60C with a flavin analogue at Cys60 results in the formation of conjugate (hsIFABP-FL60) with oxidoreductase activity. hsIFABP-FL60 was characterized by thiol titration, UV−vis, fluorescence spectroscopy, and electrospray MS to confirm the attachment of the flavin cofactor. Kinetic studies monitoring the rate of dihydronicotinamide and thiol oxidation by hsIFABP-FL60 will be discussed in this presentation.

49. Determination of the important interactions for binding of cholesterol oxidase to lipid vesicles. David E. Wolfgang and Nicole S. Sampson. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400 (wolfgang@linus.chem.sunysb.edu)

Cholesterol oxidase catalyzes the conversion of cholesterol to cholest-4-en-3-one. This water-soluble enzyme must bind to the membrane as a prerequisite to catalysis. The specific binding interactions have not been characterized. Cholesterol oxidases from both *Brevibacterium* and *Streptomyces* have exposed Met and Trp residues on the same face of the enzyme as the entrance to the active site. We hypothesize that these residues insert into the lipid. A Leu at the active site entrance has been mutated to Cys and conjugated to acrylodan. Using fluorescence spectroscopy, binding affinities for lipid vesicles have been measured. The contributions to binding of the surface-exposed Met and Trp residues are measured using acrylodan-labeled double mutants. Moreover, the lipid headgroups of the vesicles are varied to determine their importance for binding. Our results thus far indicate that hydrophobic interactions are the most important.

50. Effects of fluorine substitution on the mechanism of medium-chain acyl-CoA dehydrogenase. **E. M. Holt**, L. Luo, and M. T. Stankovich. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

The β-oxidation cycle produces up to 40% of the energy needed by the human body. Medium-chain acyl-CoA dehydrogenase (MCAD) is one of the enzymes that catalyze the first step in this process by oxidizing saturated fatty acyl-CoA substrates to *trans*-enoyl-CoA products. Deficiencies in MCAD have been linked to Sudden Infant Death Syndrome (SIDS) and other metabolic disorders. Redox studies of ACDs have provided insight into the overall energetics of the reaction and have revealed that substrate/product binding plays a key role in the regulation of fatty acid metabolism. Relatively little is known about the thermodynamic contribution of the E/S complex to regulation due to its transient nature. To better understand the catalytic mechanism of MCAD, 2-fluoroacyl-CoA (*S* and *R* isomers) will be used as a slow substrate and a reversible inhibitor. Redox characterization and computational studies will be presented. (Supported by NIH Grant GM29344.)

51. Mutagenesis and characterization of red fluorescent protein. A. Nolan, R. Naik, and M. Stone. AFRL/MLPJ, Wright-Patterson Air Force Base, Dayton, OH 45433-7102

Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has widely been used in cell biology over the past decade. When excited with near-ultraviolet (UV) light (395 nm), wild-type GFP emits green light at 509 nm. Since the cloning and expression of the gene encoding GFP, this molecule has been studied extensively and utilized for various biomedical applications. Mutagenesis of the gene encoding GFP has also proven itself to be a valuable tool. For example, mutagenesis of wild-type GFP has improved properties such as photostability and quantum efficiency. Mutagenesis has also yielded GFP variants with fluorescent hues ranging from blue to yellow. Recently, a red fluorescent protein (RFP) has been isolated from the coral *Discosoma*. The protein is homologous to GFP in structure, and it has a large degree of amino acid conservation. However, when compared to GFP, RFP takes nearly twice as long to fold and has a much lower quantum yield. Through mutagenesis, we attempted to create variations of RFP with improved folding efficiency and a higher quantum yield.

52. Effects of the unnatural enantiomer of cholesterol on EGF receptor signaling. Emily J. Westover,[1] Linda J. Pike,[2] and Doublas F. Covey.[3] [1]Bioorganic Chemistry Program, [2]Department of Biochemistry and Molecular Biophysics, and [3]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 S. Euclid, Campus Box 8103, St. Louis, MO 63110

Membrane rafts, domains enriched in cholesterol and sphingolipids, serve as specialized platforms for cellular signal transduction. Cholesterol is critical to raft formation and function. However, it is unclear whether cholesterol acts indirectly by modulating membrane properties, such as thickness and fluidity, or directly via specific chiral interac-

tions with lipids or proteins. To address this issue, we have synthesized the unnatural enantiomer of cholesterol (ent-cholesterol). Ent-cholesterol has physical properties identical to natural cholesterol (nat-cholesterol), but interacts differently with chiral molecules. We have replaced nat-cholesterol with ent-cholesterol in cultured mammalian cells and assessed the effects of this substitution on raft-based signal transduction. We report the effects of ent-cholesterol on signaling through the epidermal growth factor (EGF) receptor, a peptide hormone receptor that partitions into membrane rafts. [Supported by NIH Grant GM 47969 and by a Lucille P. Markey Predoctoral Fellowship (E.J.W.).]

53. Electrostatic control of protein−protein docking and electron transfer. Zhao-Xun Liang,[1] Judith M. Nocek,[1] A. Grant Mauk,[2] and Brian M. Hoffman.[1] [1]Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208 (zxliang@chem.nwu.edu), and [2]Department of Biochemistry and Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada

Most of the cell functions are regulated by protein−protein interactions with delicately balanced binding affinity and specificity. Due to the weak affinity and transient nature of many of the protein complexes involved in those protein−protein interactions, both structural and dynamic information is still elusive. In this study, with a model system consisting of zinc-substituted myoglobin (ZnMb) and cytochrome b_5, we demonstrate that the modification of the surface electrostatics of ZnMb by means of chemical modification and site-directed mutagenesis causes profound changes in the protein−protein docking specificity and electron-transfer (ET) rate. With the help of electrostatic calculations and Brownian dynamic simulations, we show that the increase of the ET rate and the improvement of the docking specificity result from the optimization of the electrostatic interactions at the docking interface. This work suggests that optimal local electrostatic interactions are critical for protein−protein docking to achieve the high specificity and modest binding affinity required for the fast association/dissociation rate. Meanwhile, this study also suggests that photoinduced ET can be used as a practical tool for studying transient protein−protein interactions.

54. Rapid sequencing of library-derived peptides by partial Edman degradation and mass spectrometry. Peng Wang, Gulnur Arabaci, and Dehua Pei. Department of Chemistry, The Ohio State University, 100 W. 18th Ave., Columbus, OH 43210 (fax: 614-292-1532, wang.524@osu.edu)

In this report, we have adapted a protein ladder sequencing method to rapidly sequence support-bound peptides derived from combinatorial libraries. In this method, an N-formylated tetrapeptide library, f-XXXXBBRM-resin (X = Ala, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Nle, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; B = β-alanine), is synthesized on a solid support without partial chain termination (except for Ile and Nle), and, therefore, all of the library beads carry an equal amount of full-length peptides. After screening against a molecular target (e.g., deformylase) and removal of the formyl group, the peptide on a selected bead is subjected to multiple cycles of partial Edman degradation to generate a peptide ladder, and its sequence is determined by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. The ~100 selected peptides bounded to the resin were quickly and accurately sequenced by this method with a 90% success rate.

55. Enolpyruvate protonation by yeast pyruvate kinase. Delia Susan-Resiga and Thomas Nowak. Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556

Pyruvate kinase (PK) catalyzes the phosphorylation of ADP by phosphoenolpyruvate (PEP) and subsequent protonation of enolpyruvate. Protonation is catalyzed and stereospecific. X-ray diffraction of muscle and yeast PK enzymes indicates T298 (yeast PK) as the putative proton donor. T298S, T298C, and T298A were constructed and purified. T298S has 50% V_{max} relative to wild-type PK, and T298A has 2% activity. Similar kinetic properties to wt PK are observed with Mg^{2+}, but with Mn^{2+} there is no positive cooperativity without fructose 1,6-bisphosphate (FBP). T298C requires FBP with Mg^{2+} and has 20% V_{max}. The T298A has lost a p$K_{a,2}$ = 6.4, and T298C shows a shift of p$K_{a,2}$ to 5.7. Proton inventory studies indicate solvent isotope effects on k_{cat} and $k_{cat}/K_{m,PEP}$. Fractionation factors are dependent on the metal and are significantly <1. The data suggest water as a proton donor. A temperature dependence of the water relaxation rates indicates outer-sphere effects. $^{205}Tl^+$ NMR studies, where Tl^+ and Mn^{2+} are activators, indicate conformational differences for PEP at the catalytic site between wild-type, T298S, and T298A PK complexes.

56. Evaluation of the vinyl sulfoxide class of *anti*-juvenoids as inhibitors of prenyltransferase. Sara M. Jull,[1] Stephanie E. Sen,[1] and Gregory J. Ewing.[2] [1]Department of Chemistry, Indiana University−Purdue University Indianapolis, 402 North Blackford St., Indianapolis, IN 46202, and [2]Applied Biosystems, Foster City, CA

Several vinyl sulfoxides are known to possess topical insecticidal activity that is selective for animals of the order Lepidoptera, Coleoptera, and Diptera. SAR studies indicate that in addition to the sulfoxide functionality, potency requires the presence of a hydrophobic tail, allylic (*E*)-olefin geometry, and an electron-withdrawing and/or polar group at the C-3 and C-1 positions of the molecule, respectively. Insecticidal activity is due to inhibition of juvenile hormone biosynthesis, at one or more steps prior to farnesoic acid formation. Despite their potential utility as insecticidal agents, the specific mode of action of these compounds is not known. An examination of the most active compound (1) would indicate that it is a potential transition-state analogue of the isoprenoid-forming enzyme prenyltransferase, being a mimic of the condensation of allylic and nonallylic diphosphate substrates (2). To test this hypothesis, a series of allylic vinyl sulfoxides were prepared. Since metabolism to another (active) structure may occur upon topical application, compounds possessing alcohol, diphosphate, and acetate functionalities were tested for in vitro activity against JH-producing prenyltransferase and the related enzyme isopentenyl diphosphate isomerase from the lepidopteran insect, *Manduca sexta*. The corresponding sulfide and sulfone

derivatives were also prepared and tested for inhibitory potency. Results of these studies will be presented.

57. Evidence for utilization of an exogenous carboxylic acid as a catalytic proton donor by pyrrole-2-carboxylate decarboxylase. Jeffrey A. Smiley,[1] Akira Uchida,[2] and Toru Nagasawa.[2] [1]Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH 44555 (fax: 330-742-1579, jasmiley@cc.ysu.edu), and [2]Department of Biomolecular Science, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan

Pyrrole-2-carboxylate decarboxylase from *Bacillus megaterium* has previously been shown to have a requirement for added acetate, to catalyze the decarboxylation reaction. We postulated that the requirement for the added acetate might actually be a requirement for acetic acid, present at low concentration at neutral pH, and that the added COOH form might act as the proton donor in an acid-catalyzed reaction. If the COOH form is indeed the active "co-factor" in the reaction, then the measured K_m of 42 mM for total acetate at pH 7.0 corresponds to a K_m of 0.23 mM for acetic acid. In decarboxylase assays using fluoroacetate substituting for acetate, only a slight hint of saturation by fluoroacetate is observable with added concentrations up to 800 mM; a rough estimate of the K_m for total fluoroacetate is 10 M, corresponding to a K_m for fluoroacetic acid of 0.3 mM, consistent with the model of the exogenous carboxylic acid as the active cofactor in catalysis. Other carboxylic acids acting as putative proton donors will be examined.

58. Structure of green fluorescent protein chromophores probed by Raman spectroscopy. **Alasdair F. Bell**,[1] Xiang He,[1] Rebekka M. Wachter,[2] and Peter J. Tonge.[1] [1]Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794, and [2]Institute of Molecular Biology and Department of Physics, University of Oregon, Eugene, OR 97403

The green fluorescent protein (GFP) from *Aequorea victoria* has become a valuable tool in molecular and cell biology because it can be used to genetically encode for fluorescence. Any application of GFP requires irradiation with light to produce the fluorescence signal. Wild-type GFP is known to be converted by UV or visible light between protonation states with different absorption and fluorescence spectra. Our goal is to examine the effect that irradiation has on the structure and consequently the properties of GFP chromophores. Our approach is to apply the sensitivity of Raman spectroscopy to changes in the ground-state structure of GFP chromophores both for model compounds and within proteins. Importantly, we can monitor the protonation state and cis/trans isomerization of the chromophore, both of which have a profound effect on the absorption and fluorescence spectra of GFPs.

59. Expression and studies of the substrate specificity of pikromycin/methymycin polyketide synthase modules 5 and 6. David E. Cane and Yifeng Yin. Department of Chemistry, Brown University, Box H, Providence, RI 02912-9108

Pikromycin/methymycin polyketide synthase is a modular, multifunctional protein whose individual modules are each responsible for a single cycle of polyketide chain extension and functional group modification. Two of these modules, module 5 and module 6, were separately expressed in the heterologous host *E. coli* with the pikromycin thioesterase domain fused to the C-terminus. The substrate specificity of each purified module has been investigated and provides interesting comparisons with the previously studied homologous modules 5 and 6 of the erythromycin PKS, deoxyerythronolide B synthase.

60. Expression of $P2Y_2$ nucleotide receptor in human 1321N1 and U937 cells. Elsa V. Arocho, Jr., Nataliya E. Chorna, and Fernando A. Gonzalez. Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, San Juan, P. R. 00931, PR (fax: 787-758-5612, elsie16@hotmail.com)

It is recognized that the nucleotides ATP and UTP mediate with equal potency the activation of the $P2Y_2$ G-protein coupled receptor. The activation of $P2Y_2$ receptors by these nucleotides has been shown to induce phenotypic differentiation of human promonocytic U937 cells, and also to induce mitogenic effects in human astrocytoma 1321N1 cells. The purpose of this research is to investigate the mechanism of $P2Y_2$ receptor signaling in 1321N1 human astrocytoma and U937 human promonocytic cell populations after agonist stimulation. Both control and UTP-treated U937 cells were used to study the role of the $P2Y_2$ receptor in the cell cycle and the monocyte-to-macrophage cell differentiation. Different combinations of primers were used for the RT-PCR amplification of the $P2Y_2$ cDNA derived from human 1321N1 astrocytoma cell transfectants, and changes in the expression level of the $P2Y_2$ receptor after stimulation with UTP were monitored in order to understand their relation to the signal transduction pathways of the cells.

61. Identification and functional assigment of an active site serine in mevalonate kinase. Sandra E. Ríos, Yong K. Cho, and Henry M. Miziorko. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (fax: 414-456-6570, srios@mcw.edu)

In phosphotransferase reactions, there are many precedents for participation of serine/threonine residues in substrate binding or in enhancement of catalytic efficiency. Alignment of >20 mevalonate kinase sequences indicates that, among the residues containing side chain alcohols, only S145, S146, S201, and T243 are strictly conserved. These residues have been individually mutated to alanine. Structural integrity of the mutants has been evaluated by binding studies using the fluorescent ATP analogue trinitrophenyl-ATP (TNP-ATP) as well as a spin-labeled ATP analogue (ATPγSAP). TNP-ATP binding stoichiometries for the mutants vary little from the wild-type mevalonate kinase value. Scatchard analyses of ATPγSAP binding indicate that K_d changes ≤3-fold and binding stoichiometries by <40% in comparison with wild-type values. Kinetic characterization of the mutants indicates only modest changes in $K_{m(ATP)}$. K_m for mevalonate increases by ≈20-fold for S146A, by ≈40-fold for T243A, and by 100-fold for S201A. V_{max} changes for S145A, S201A, and T243A are ≤3-fold. V_{max} for S146A is diminished by 4000-fold. In terms of V/K_{MVA}, this substitution produces a

100 000-fold effect, suggesting an active site location and catalytic role for S146. $K_{d(Mg-ATP)}$ increases by almost 40-fold for S146A, indicating a specific role for S146 in liganding Mg-ATP. The V_{max} effect for S146A is too large to be totally explained by cation liganding. H-bonding of S146's hydroxyl group to ATP's phosphoryl chain could contribute to the large observed V_{max} effect. S146 does not map within a proposed C-terminal ATP binding motif. Instead, S146 is situated in a more centrally located motif which characterizes the mevalonate kinase, phosphomevalonate kinase, galactokinase, and homoserine kinase protein family, suggesting that this region constitutes part of the active site in all of these phosphotransferases.

62. Implication by site-directed mutagenesis of Arg314 and Tyr316 in the coenzyme site of pig mitochondrial isocitrate dehydrogenase. Peychii Lee and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 (fax: 302-831-6335, pclee@Udel.edu)

Sequence alignment of pig heart NADP-dependent isocitrate dehydrogenase (IDH) with eukaryotic (human, rat, yeast) and *E. coli* IDHs reveals that Tyr316 is completely conserved and is equivalent to the *E. coli* Tyr345, which interacts with the 2′-phosphate of NADP in the crystal (Hurley et al., 1991). Lys321 is also completely conserved in the five IDHs. The positive charge is maintained at amino acid 314, while Arg323 is only found in some species. The importance of these four amino acids to IDH function was studied by site-directed mutagenesis. Mutants (R314Q, Y316F, Y316L, K321Q, and R323Q) were generated by the polymerase chain reaction. Wild-type (WT) and mutant enzymes were expressed in *E. coli* as a maltose binding fusion protein, cleaved by thrombin, and purified to homogeneity using amylose affinity and anion exchange chromatography. The kinetic parameters (V_{max} and K_Ms) of K321Q and R323Q are similar to those of WT, which indicates that Lys321 and Arg323 are not involved in IDH function. R314Q exhibits a 10-fold increase in the K_M for NADP to 91.9 μM as compared to that of WT (8.8 μM), while they have comparable V_{max} values (37.8 μmol of NADPH min^{-1} mg^{-1} for R314Q and 41.3 μmol of NADPH min^{-1} mg^{-1} for WT). These results suggest that R314Q participates in NADP binding to the enzyme. The K_i for NAD of R314Q (8 mM) is comparable to that of WT (11 mM), indicating that the positive side chain of Arg314 may interact specifically with the 2′-phosphate of NADP. The hydroxyl group of Tyr316 is not required for IDH function since Y316F exhibits similar kinetic parameters to those of WT. Y316L shows a 4-fold increase in the K_M for NADP (31.8 μM) and a decrease in the V_{max} (9.4) as compared to WT. The results suggest that the aromatic ring of the Tyr316 of IDH contributes to binding and to catalysis. (Supported by NIH Grant DK39075.)

63. Improved enzymatic assay for and photoaffinity labeling of β-carotene 15,15′-dioxygenase. Babak Borhan, **Montserrat Rabago-Smith**, and Pulgam Veera Reddy. Department of Chemistry, Michigan State University, East Lansing, MI 48824 (fax: 517-353-1793, borhan@cem.msu.edu, rabago@cem.msu.edu)

We are interested in developing a rapid and efficient assay for the enzymatic assay of β-carotene 15,15′-dioxygenase (BCDOX), the enzyme responsible for conversion of β-carotene to retinal. Utilizing the difference in polarities of β-carotene and retinal, we plan to develop a partition assay that will quantify the progress of enzymatic reaction by a simple extraction and liquid scintillation measurement of the aqueous phase (using radiolabeled β-carotene). Our progress toward synthesis of a radioactive photoaffinity probe and photolabeling studies with BCDOX will also be presented.

64. Intermembrane transfer of various cholesterol hydroperoxide species. Andrew Vila and Albert W. Girotti. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (fax: 414-456-6510, avila@mcw.edu)

Generation of lipid hydroperoxides (LOOHs) under conditions of oxidative stress may be especially damaging to cell membranes if iron-catalyzed chain peroxidation is initiated. Being more polar than parent lipids, LOOHs may translocate more readily from one membrane to another, thereby broadening the range of potential peroxidative injury. We have tested this hypothesis by comparing the spontaneous intermembrane transfer of cholesterol (Ch) and various cholesterol hydroperoxides (ChOOHs), using photoperoxidized [^{14}C]Ch-labeled erythrocyte ghosts as donors and small unilamellar liposomes (SUVs) as acceptors. Adopting normal-phase HPTLC with phosphorimaging to analyze the different sterol populations, we found that ChOOHs as a group translocated much faster than parent Ch at 37 °C ($k = 1.35$ h^{-1} vs 0.02 h^{-1}). The initial ChOOH transfer rate (measured either as uptake by SUVs or as loss from ghosts) was invariant upon increasing the acceptor:donor lipid ratio from 1:1 to 15:1 (mol/mol), consistent with desorption from the donor membrane being rate-limiting. Transfer kinetics of individual ChOOH isomers (singlet oxygen-derived 5α-OOH, 6α-OOH, 6β-OOH; and free radical-derived 7α/7β-OOH) were examined by means of reverse-phase HPLC with electrochemical detection. Transfer rate constants were found to decrease with decreasing polarity in the following order: 7α/7β-OOH > 5α-OOH > 6α-OOH > 6β-OOH. The same trend was observed when L1210 cells were used as acceptors, implying that highly mobile 7α- and 7β-OOH are potentially the most dangerous of these species under conditions of inadequate detoxification. It is apparent from these results that intermembrane translocation of ChOOHs and other LOOHs (either between cells or within cells) could result in highly disseminated peroxidative injury, a prospect not well recognized heretofore. (Supported by NIH Grant CA72630 and NRSA Predoctoral Fellowship F31-CA85171.)

65. Investigating the regulation of human liver short-chain acyl-CoA dehydrogenase. A. K. Saenger,[1] J. Vockley,[2] and M. T. Stankovich.[1] [1]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, and Department of Medical Genetics, Mayo Clinic and Mayo Foundation, Rochester, MN 55905

Acyl-CoA dehydrogenases (ACDs) are flavoproteins that catalyze the oxidation of saturated acyl-CoA thioesters. Substrate/product binding shifts the midpoint potential of the ACD up to +120 mV, making electron transfer thermodynamically favorable. Deficiencies in the short-chain acyl-CoA dehydrogenase (SCAD) lead to serious metabolic

disorders. Two nucleotide variations in the human SCAD (hSCAD) gene, C511T and G625A, have been identified and associated with SCAD deficiency. Disease caused by these mutations may be the result of inefficient electron transfer, which can be probed through spectroelectrochemistry. Human liver SCAD, only recently cloned and expressed, also appears to have similar properties to two well-studied enzymes: bacterial short-chain and pig medium-chain acyl-CoA dehydrogenases. The differences in redox properties between the various ACDs may be attributed to chain-length specificity or to variations occurring between bacterial and mammalian systems. Redox characterization of wild-type hSCAD and the naturally occurring disease-causing mutants is presented. (Supported by NIH Grant GM29344.)

66. Investigation of the oligomeric structure of HMG-CoA lyase isoforms. Robbyn Tuinstra and Henry M. Miziorko. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (fax: 414-456-6570, rtuinstr@mcw.edu)

HMG-CoA lyase catalyzes the cleavage of 3-hydroxy-3-methylglutaryl-CoA into acetyl-CoA and acetoacetate, a reaction involved both in ketogenesis and in leucine catabolism. The deduced amino acid sequence contains an N-terminal mitochondrial signal sequence as well as a putative C-terminal peroxisomal targeting motif. Thus, two isoforms of HMG-CoA lyase may exist in vivo, namely, a mature mitochondrial isoform, and a peroxisomal isoform that retains the mitochondrial leader. The mitochondrial isoform appears to be a dimer. On the basis of gel permeation chromatography elution, the peroxisomal isoform of HMG-CoA lyase has been reported to be a monomer. To investigate whether the N-terminal leader would alter the oligomeric state of HMG-CoA lyase, gel permeation and protein cross-linking studies have been conducted using the two isoforms. Gel permeation chromatography confirms the low molecular weight estimate for the peroxisomal isoform. However, upon treatment with *N*,*N*′-*o*-phenylenedimaleimide (PDM), enzyme will form a covalent dimer, as visualized by SDS−PAGE and Coomassie blue staining. Both mitochondrial and peroxisomal HMG-CoA lyases exhibit identical gel permeation elution times whether not the enzyme has been pretreated with cross-linking reagent. Cross-linking of both isoforms involves C323 as a C323S variant of either isoform will not form cross-linked species. Pretreatment with diamide to reversibly form an intersubunit disulfide linkage involving C323 blocks irreversible cross-linking with PDM. Cross-linking is not simply a consequence of sequential modification of two monomers. Variation of enzyme concentrations over a 50-fold range indicates no difference in the extent or rate of cross-linking. Moreover, inclusion of a large excess of carrier protein (ovalbumin) does not block complete cross-linking of HMG-CoA lyase. These results suggest that although both isoforms elute on gel permeation chromatography with an apparent monomeric molecular weight, cross-linking studies support a dimeric quaternary structure which is not dependent on the presence of the mitochondrial targeting sequence. (Supported by NIH Grant DK21491 and by the American Heart Association/Northland Affiliate.)

67. Kinetic mechanism of mitochondrial phosphoenolpyruvate carboxykinase. Maria Metchkarova and Thomas Nowak. Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

Avian mitochondrial phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reversible reaction that converts oxaloacetate (OAA) and GTP to phosphoenolpyruvate (PEP), GDP, and CO_2. It is known that each substrate in this Bi-Ter reaction binds to PEPCK-Mn, but the kinetic competence is unclear. Initial velocity studies in the direction of PEP carboxylation were performed. Each substrate was varied at fixed, variable concentrations of the second substrate, and the third substrate was constant and saturating. Velocity responses with PEP as the variable substrate and Mn-IDP as the fixed variable at saturating CO_2 and with CO_2 as the variable substrate with Mn-IDP as the fixed variable at saturating PEP result in Lineweaver−Burk plots intersecting in the second quadrant. Parallel patterns are obtained when PEP is the variable and CO_2 is the fixed variable at saturating and subsaturating Mn-IDP. These steady-state kinetics, combined with product inhibition data, suggest an ordered addition of PEP, followed by Mn-IDP and subsequently CO_2.

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

The thioesterase (TE) domain of the pikromycin synthase, one of the polyketide synthases, was overexpressed in *Escherichia coli*. Different *N*-acetyl cysteamine thioesters were tested as substrates, and their kinetic parameters were determined. The results indicate that the TE domain prefers substrates that mimic the important features of its natural substrate. The ability of the TE domain to catalyze polyketide cyclizations was also tested.

69. Location of the coenzyme binding site of pig heart NADP-dependent isocitrate dehydrogenase by site-directed mutagenesis of His^{309}, His^{315}, and His^{319}. Y. C. Huang and R. F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 (fax: 302-831-6335, 19038@Udel.edu)

^{1}H NMR studies of pig heart NADP-dependent isocitrate dehydrogenase (IDH) in complex with NADP, NADPH, and coenzyme fragments indicated that 1−2 histidines are in the coenzyme site (Ehrlich and Colman, 1985), while affinity cleavage of the enzyme by Fe^{2+}−isocitrate suggested His^{309} as a coordination site for metal−isocitrate (Soundar and Colman, 1993). Sequence alignment predicts that His^{309} of pig heart IDH is equivalent to His^{339} of *E. coli* IDH, which interacts with the coenzyme in the crystal structure of the bacterial enzyme (Hurley et al., 1991), and porcine His^{315} and His^{319} are close to that site. The mutant porcine enzymes H309Q, H309F, H315Q, and H319Q were prepared by site-directed mutagenesis using a megaprimer PCR method. The wild-type (WT) and mutant enzymes were expressed in *E. coli* and purified, yielding WT enzyme with $V_{max} = 37.8$ μmol of NADPH min^{-1} (mg of protein)$^{-1}$. The H319Q mutant has K_m values for NADP, isocitrate, and Mn^{2+}similar to those of WT, and $V_{max} = 20.1$ μmol of NADPH min^{-1} (mg of protein)$^{-1}$. Thus, His^{319} is not involved in coenzyme binding and has a minimal effect on catalysis. In contrast, H315Q exhibits a K_m for NADP 40 times that of WT and

$V_{max} = 16.2$ μmol of NADPH min^{-1} (mg of protein)$^{-1}$, with K_m values for isocitrate and Mn^{2+} similar to those of WT. These results implicate His^{315} in the region of the NADP site. Replacement of H309 by Q or F yields enzyme with no detectable activity. The H309 mutants fail to bind NADPH, under conditions in which WT and H319Q bind 1.0 mol of NADPH/mol of subunit, indicating that His^{309} is essential for the binding of coenzyme. The H309 mutants still bind isocitrate stoichiometrically, as do WT and the other mutant enzymes. However, the H309 mutants are not cleaved by metal−isocitrate, implying that the metal ion is not bound normally. Since circular dichroism spectra are similar for WT and all mutant enzymes, these amino acid substitutions do not cause major conformational changes. We propose that His^{309} plays an important role in the binding of coenzyme, and also contributes to the proper coordination of divalent metal ion in the presence of isocitrate. (Supported by NIH Grant DK39075.)

70. Mechanism of reaction of a class C β-lactamase with submicromolar substrates/inhibitors: Acyl phosph(on)ates. Kamaljit Kaur and Rex F. Pratt. Department of Chemistry, Wesleyan University, Hall-Atwater Laboratories, Lawn Avenue, Middletown, CT 06459 (fax: 860-685-2211, kkaur@wesleyan.edu)

The interactions of a series of acyl phosph(on)ates with *Enterobacter cloacae* P99 β-lactamase have been investigated. These compounds are both substrates and irreversible inhibitors of this enzyme. Turnover involves fast acylation of the enzyme followed by a slow deacylation, whereas inactivation involves phosphylation. The most potent of these compounds, benzo[*b*]thiophene-2-carbonyl 2′-naphthyl phosphate, rapidly acylates the enzyme (1.25×10^6 M^{-1} s^{-1}) and forms a tight complex ($K_m = 0.15$ μM). Since the acyl enzyme has a half-life of 4 s, the enzyme is also transiently inhibited during the turnover process. Mechanistically, these compounds can be seen as substrate analogues of the aryl boronate inhibitors described by Shoichet and co-workers [(1998) *J. Med. Chem. 41*, 4577]. Models of these compounds at the active site, based on the available crystal structures of class C β-lactamases, suggest the most probable mode of binding and reaction. These models provide insight into the design of potentially more effective β-lactamase inhibitors and, perhaps, antibiotics.

71. Mechanism of ribonuclease cytotoxicity. Kimberly A. Dickson and Ronald T. Raines. Department of Biochemistry and Department of Chemistry, University of Wisconsin−Madison, Madison, WI

Onconase, a homologue of bovine pancreatic ribonuclease A (RNase A), is isolated from the Northern leopard frog (*Rana pipiens*) and is toxic to human tumor cells. Onconase is now in Phase III clinical trials for the treatment of malignant mesothelioma. The requirements for ribonuclease cytotoxicity include the abilities to evade the cytoplasmic ribonuclease inhibitor (RI) and to degrade cellular RNA. RNase A binds RI and is not cytotoxic, but variants of RNase A that do not bind to RI possess cytotoxic activity. The antitumor activity demonstrated by cytotoxic ribonucleases varies depending on the type of tumor. Factors that could determine susceptibility are the ability of ribonucleases to bind to the cell surface and the efficiency of their internalization. The goal of this project is to characterize the interactions of ribonucleases at the surface of human cells and to reveal the pathway of their internalization.

72. Mechanistic and structural diversity in the 4-oxalocrotonate tautomerase (4-OT) family. R. M. Czerwinski,[1] J. J. Almrud,[2] A. D. Kern,[2] M. L. Hackert,[2] and **C. P. Whitman**.[1] [1]College of Pharmacy and [2]Department of Chemistry and Biochemistry, The University of Texas, Austin, TX 78712

4-OT, a plasmid-encoded hexameric enzyme consisting of small monomers (62 amino acids), catalyzes the isomerization of β,γ-unsaturated enones to their α,β-isomers. Pro-1 functions as a general base catalyst to abstract the α-proton in conjunction with Arg-39, which functions as a general acid catalyst. Phe-50 maintains the hydrophobic environment of the active site [(1984) *Biochemistry 23*]. To determine how 4-OT evolved, several members of the family have been identified and characterized. Among these are chromosomal homologues from *B. subtilis*, *E. coli*, and *P. pavonaceae*, a bacterium that utilizes 1,3-dichloropropene. The three proteins share low sequence identity (<36%), but all retain Pro-1. Crystallographic studies show that the *E. coli* homologue, which lacks a critical GXGG motif, is a dimer, where each monomer has 76 amino acids. The *P. pavonaceae* homologue is a heterohexamer and functions as a dehalogenase. This diversity in the 4-OT family suggests that nature used these short sequences as building blocks to create new structures and activities.

73. Mechanistic studies and selectivity of phosphopantothenoylcysteine decarboxylase. Erick Strauss and Tadhg P. Begley. Department of Chemistry and Chemical Biology, Cornell University, 120 Baker Laboratory, Ithaca, NY 14853 (fax: 607-255-4137, es71@cornell.edu)

Phosphopantothenoylcysteine decarboxylase (PPC-DC) of *E. coli* is part of a bifunctional enzyme (CoaBC, previously Dfp) that also catalyzes the formation of phosphopantothenoylcysteine from phosphopantothenate and cysteine mediated by activation with cytidine triphosphate (CTP). This enzyme is directly involved in the biosynthesis of coenzyme A. The discovery that PPC-DC is a flavoenzyme allows a number of mechanisms to be proposed for the decarboxylation reaction. Based on the results of solvent exchange and primary deuterium isotope effect experiments, the effect of substrate and product on the UV/visible spectrum of the enzyme-bound flavin, and experiments with substrate analogues, we propose a mechanism for decarboxylation based on the formation of an initial adduct between the substrate thiol and C(4a) of the oxidized flavin. The results of experiments probing the selectivity of the coupling enzyme, which will only incorporate L-cysteine even in the presence of an excess of various cysteine analogues, are also presented.

74. Medium effects on phosphoryl transfer reactions and their possible applicability to enzymatic phosphoryl transfer. Alvan C. Hengge, **Piotr Grzyska**, Przemyslaw G. Czyryca, Justin Golightly, Kelly Small, Paul Larsen, and Richard H. Hoff. Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300

The addition of DMSO to aqueous solutions of phosphate esters increases the rate of hydrolysis of phosphate monoesters with good leaving groups. Mechanistic analysis shows that these reactions proceed by the same mechanism and with a similar transition state as the aqueous reaction. The rate acceleration disappears for leaving groups having a basicity of phenol or higher. Computational and experimental studies were carried out to discover the origin of the rate accelerations, and to determine whether transfer of a phosphate ester substrate from water to the active site of alkaline phosphatase has a similar effect on the substrate. Computational results indicate that solvation of the phosphoryl group has a significant effect on the scissile P−O ester bond, and that this effect is most pronounced in esters with good leaving groups. Phosphate esters modeled into the active site of alkaline phosphatase show similar destabilization relative to aqueous solution.

75. Modification of the catalytic histidine in serine proteases: testing the ring-flip mechanism. Kristin Coffman Haddad, Jim L. Sudmeier, David G. Sanford, and William W. Bachovchin. Department of Biochemistry, Tufts University Medical School, Boston, MA 02111

The catalytic triad of serine proteases significantly accelerates the rate of proteolysis. Despite intensive study, the mechanism of this acceleration is not fully understood. Recently, our lab has proposed a reaction-driven ring-flip mechanism to help explain how the catalytic triad promotes peptide cleavage over peptide bond formation. The proposed movement of the imidazole ring could spatially redirect the proton transfer, in turn activating the nucleophilic serine for formation of the tetrahedral intermediate and then water for attack and resolution of the peptide−enzyme complex. We tested this by examining the susceptibility of the histidine side chain to chemical modification. The pattern of covalent modification was examined using several different agents in the presence and absence of substrates using the prototypical serine protease, alpha lytic protease. Differential susceptibility of the catalytic histidine to reversible modification by diethyl pyrocarbonate upon addition of a substrate analogue is consistent with the ring-flip hypothesis.

76. Molecular basis of substrate specificity in medium chain acyl-CoA dehydrogenase: Thermodynamic contributions of added methylene groups of increasing chain length enoyl-CoAs upon binding to the enzyme. D. K. Srivastava and **K. V. Gopalan**. Department of Biochemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105

The steady-state kinetic data of human liver medium chain acyl-CoA dehydrogenase (HMCAD) reveal that the K_m for acyl-CoA drastically decreases (from 450 to 6.8 μM) upon increase in its carbon chain length from C_4 to C_6, but it remains less variant between C_6 to C_{14}. With the assumption that such a disparity is intrinsic to the molecular basis of substrate specificity, we performed detailed thermodynamic studies for the binding of increasing chain lengths of aliphatic CoA derivatives to the enzyme site via isothermal titration microcalorimetry. The experimental data revealed that the $\Delta G°$, $\Delta H°$, and $\Delta C_p°$ values are linearly dependent between enoyl-CoA chain lengths of C_6−C_{12}, yielding the magnitudes of $\Delta\Delta G°/CH_2$, $\Delta\Delta H°/CH_2$, and $\Delta\Delta C_p°/CH_2$ to be −149 cal/mol, −472 cal/mol, and −24 cal mol^{-1} K^{-1}, respectively. Of these, the latter value was found to be different than that derived from the changes in the polar and nonpolar surface areas (calculated from the X-ray crystallographic data) upon binding of similar ligands to the enzyme site, suggesting that solvation and desolvation of the enzyme and ligand site phases are not the exclusive contributors of the experimentally determined $\Delta C_p°$ values.

77. *N*-Hydroxyureas as new nitric oxide donors. Zhou Zou, Jinming Huang, and S. Bruce King. Department of Chemistry, Wake Forest University, Salem Hall, Winston-Salem, NC 27109 (fax: 336-758-5728, zouz01g@wfu.edu)

Oxidation of *N*-hydroxyurea produces nitric oxide (NO), an important biological messenger molecule. Increasing evidence suggests that the biological effects of *N*-hydroxyurea may be medicated by NO. Our previous results indicated that the heme-containing proteins and enzymes including hemoglobin and horseradish peroxidase oxidize *N*-hydroxyurea with NO release. To investigate whether NO release is a general chemical property of the hydroxyurea functional group, simple substituted *N*-hydroxyureas were synthesized and reacted with hemoglobin. Absorption spectroscopy indicates that these substituted *N*-hydroxyureas react with hemoglobin in a similar fashion as *N*-hydroxyurea. Electron paramagnetic resonance measurements show that the reactions of these compounds with hemoglobin produce iron nitrosyl hemoglobin, evidence for NO release. Chemiluminescence NO measurements provide evidence for both nitrite and nitrate formation during these reactions. Based upon these results, more complicated *N*-hydroxyureas derived from L-amino acids and carbohydrates will be prepared and evaluated as nitric oxide releasing agents.

78. Nanoscale patterning of self-assembling protein fibers on chemically tailored monolayers. Christine M. R. Clancy,[1] Jennifer C. Smith,[1] Thomas Scheibel,[2] Susan L. Lindquist,[2] Norbert F. Scherer,[3] and Milan Mrksich.[1] [1]Department of Chemistry, University of Chicago, 5735 S. Ellis Ave., Chicago, IL 60637 (fax: 773-702-0805, cclancy@midway.uchicago.edu), [2]Departments of Chemistry and of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, and [3]Department of Chemistry, James Franck Institute, and Institute for Biophysical Dynamics, University of Chicago, Chicago, IL 60637

The controlled design and production of nanoscale materials is a field of intense research. The combination of "physical materials" and surface functionalization methods with biological materials is an especially promising area of investigation. A truncated form of yeast Sup35p, which is known to self-assemble into fibers ∼10 nm wide and micrometers in length, has been used as a building block for creating nanoscale protein patterns. Microcontact printing techniques were used to prepare patterned surfaces of spatially well-defined and chemically distinct self-assembled monolayers (SAMs). The chemical natures of the SAM surfaces were used to guide the deposition of previously assembled protein fibers. The kinetics of adsorption were studied by surface plasmon resonance measurements for different SAMs. The spatial distribution of protein on the

patterned surfaces was characterized by atomic force microscopy. The chemical nature of the surface influences the structure of the fiber or protein material that does adsorb to the surface.

79. Photoconversion studies of green fluorescent protein and its model compounds. Xiang He, Alasdair F. Bell, and Peter J. Tonge. Department of Chemistry, SUNY at Stony Brook, Stony Brook, NY 11794-3400

The green fluorescent protein (GFP) from *Aequorea victoria* has developed into an extremely valuable research tool in molecular and cell biology. It has been shown that light absorption causes structural changes in the GFP chromophore as well as its fluorescent emission. It is of great importance to analyze the changes in the chromophore structure due to light absorption and to determine the spectroscopic properties of the different chromophore structures. Raman spectroscopy has provided detailed information on the structure of GFP, and it has been utilized to study the photoconversion of GFP and its model compounds. To elucidate the GFP protein environment modulation, the model chromophore ethyl 4-(4-hydroxyphenyl)methylidene-2-methyl-5-oxoimidazolacetate and its derivatives have been synthesized. The crystal structure of the model chromophore confirmed that the ethylenic proton on the exocyclic double bond is trans to the imidazolinone. NMR and Raman spectroscopy were used to characterize the changes of the model chromophore upon light absorption.

80. Preliminary studies of 4-hydroxyphenylpyruvate dioxygenase from *Streptomyces avermitilis*. Vincent M. Purpero, Tamara N. Nelson, and Graham R. Moran. Department of Chemistry, University of Wisconsin−Milwaukee, Milwaukee, WI 53211

4-Hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27) is an Fe(II)-dependent mononuclear non-heme dioxygenase that converts 4-hydroxyphenylpyruvate (HPP) into homogentisate (2,5-dihydroxyphenylacetate, HG). This reaction is the second step of the pathway for tyrosine catabolism that is ubiquitous in aerobic organisms. In mammals, in born mutations the gene for fumarylacetoacetase (FAA), the enzyme to catalyze the final step in this pathway, can give rise to the disease type 1 tyrosinemia (T1Y). In humans, T1Y leads to cirrhosis and primary cancer of the liver, resulting in death typically by the age of 5. Our objective is to alleviate the symptoms of T1Y by specifically inhibiting HPPD and hence shutting down the tyrosine catabolism pathway. Our encompassing objective is to characterize the reaction coordinate of HPPD in sufficient detail to identify reasonable transition state geometries for the design of transition state analogues. Initially we have overexpressed HPPD from *Streptomyces avermitilis* to 35% of total cell protein in *Escherichia coli*. The enzyme in its apo- or holo-form can readily be isolated using a combination of anion exchange, size exclusion chromatography. The purified oxidized holoenzyme has a weak absorbance band at 600 nm. Under reducing conditions in atmospheric oxygen, the enzyme has an apparent turnover number of 4 s^{-1} and exhibits significant substrate inhibition. The substrate, HPP, has keto and enol tautomeric forms of which HPPD exclusively uses the keto form as a substrate. The rate of tautomerization from enol to keto forms is general base catalyzed, and HPLC analysis of the mixture at equilibrium in aqueous solution shows that the substrate exists as a mixture of at least eight components. Inhibition studies indicate that a variety of divalent metals compete with iron for occupancy of the active site. Steady-state measurements show a K_m for HPP under atmospheric conditions of 27 μM and a K_i of 590 μM. The K_m for oxygen is 10.1 μM while the apparent K_d for Fe(II) measured by its steady-state dependence is 0.5 μM.

81. Reactions of hydroxyurea with hemoglobin. Jinming Huang,[1] S. Bruce King,[1] and Dany Kim-Shapiro.[2] [1]Department of Chemistry and [2]Department of Physics, Wake Forest University, Salem Hall, Winston-Salem, NC 27109 (fax: 336-758-4656, huangj@wfu.edu)

Hydroxyurea represents a new treatment for sickle cell disease. Recent evidence indicates that a portion of hydroxyurea's beneficial actions may be mediated by nitric oxide. Using absorption and electron paramagetic resonance spectroscopy, we demonstrate that the reaction of hydroxyurea and oxy-, deoxy-, or methemoglobin produces nitric oxide that binds to the heme iron. Chemiluminescence experiments did not indicate the formation of any *S*-nitrosohemoglobin in these reactions. Chemiluminescense measurements also revealed the amount of nitrite and nitrate produced in these reactions. Similar reactions in the presence of potassium cyanide and carbon monoxide provide important information regarding the mechanism of the reaction of hydroxyurea with these hemoglobins. Reactions of hydroxyurea and synthetic hydroxyurea derivatives also provide mechanistic information regarding these nitric oxide liberating reactions.

82. Probe the conformation of flavin in flavoproteins using Raman spectroscopy. Yuangang Zheng and Paul R. Carey. Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44016

Flavoproteins contain FAD or FMN as the cofactor in the active site; both the electronic properties and the structural flexibility of bound flavin are crucial to the physiological function of the flavoproteins. For example, FADs in *p*-hydroxybenzoate hydroxylase (PHBH) and phenol hydroxylase (PHHY) switch between "buried" and "exposed" conformation during the catalytic process or while different substrates bind. Using Raman spectroscopy, we have developed several Raman marker bands for the alternate conformation of bound flavin in PHBH and PHHY. Further, we have characterized the conformations of the bound flavin in dozens of flavoproteins using Raman marker bands, and the findings are highly consistent with the structures determined by X-ray and NMR. Therefore, Raman spectroscopy provides a novel technique to rapidly probe the conformational changes within the flavoproteins.

83. Probing subunit interactions of rat glutathione *S*-transferase 1-1. Melissa A. Vargo, Lucia Nguyen, and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 (fax: 302-831-6335, mvargo@Udel.edu)

Glutathione *S*-transferases constitute a family of detoxification enzymes that catalyze the conjugation of glutathione

to various xenobiotic substrates. Alpha class gluathione *S*-transferase isozyme 1-1 is a dimer (51.0 kDa) of identical subunits with a complete active site within each monomer. From the crystal structure, two of the main areas of interaction between the subunits are (1) a hydrophobic region resembling a ball-and-socket joint with F52 from one subunit fitting into a pocket formed by F136, V139, and M94 of the other subunit and (2) an electrostatic region involving R69 and E97 from both subunits. Mutations were constructed in these regions to determine whether monomeric enzymes could be generated by a single mutation. Each of the four amino acids contributing to region 1 was replaced by alanine. The molecular mass of F52A, determined by light scattering, was 22.3 kDa, and the enzyme retains detectable activity. In contrast, the other three alanine mutant enzymes were dimers. When retaining the aromaticity of the side chain as in F52Y, the activity and dimeric molecular mass remained unchanged. In region 2, removing the charge of one amino acid in the mutations E97Q and R69Q causes no change in oligomeric state. Among the single mutant enzymes studied, only the F52A enzyme was a monomer, suggesting that the hydrophobic contribution of F52 is the major contributor to dimer stabilization but the monomeric enzyme retains some catalytic activity. (Supported by NIH Grant CA66561.)

84. Profiling the specific reactivity of the proteome with nondirected chemical libraries. Gregory C. Adam,[1] Benjamin F. Cravatt,[2] and Erik J. Sorensen.[1] [1]Department of Chemistry and Skaggs Institute for Chemical Biology and [2]Departments of Cell Biology and Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037

Through screening the proteome with chemical probes bearing functionalities common to organic synthesis but underutilized in biology, proteins or classes of proteins susceptible to new forms of inactivation may be discovered. A library of biotinylated sulfonates was synthesized, and its members were applied to complex proteomes under conditions that distinguish patterns of specific protein reactivity. Individual sulfonates exhibited unique profiles of proteome reactivity that in extreme cases appeared completely orthogonal to one another. Targets of the tagged sulfonate library include members of multiple structurally and mechanistically distinct enzyme families. Progress toward understanding the mechanisms by which the sulfonate probes react with their discrete enzyme targets will be reported. These data reveal that a nondirected approach toward probing the chemical reactivity of the proteome can readily identify compounds possessing selective and unanticipated biological activities.

85. Protein kinase peptide substrate and small molecule inhibitor discovery in the post genomic sequence era: Proof of principle with a death associated kinase. Anastasia Velentza,[1] Andrew M. Schumacher,[1] Curtis Weiss,[1] Anu Sawkar,[1] Magdalena Zasadzki,[1] Martin Egli,[2] and D. Martin Watterson.[1] [1]Department of Molecular Pharmacology and Biochemistry, Northwestern University, Chicago, IL 60611, and [2]Vanderbilt University, Nashville, TN 37232

Death associated protein kinase (DAPK) is a multidomain, serine/threonine protein kinase (PK) implicated in apoptosis, susceptibility to human disease, and ischemia-induced neuronal cell death. Protein or peptide substrates and small molecule inhibitors for DAPK have not been identified. We report here an approach that combines positional scanning peptide substrate library synthesis, activity screens, high-resolution protein crystallography, and molecular modeling to discover DAPK peptide substrates and small molecule inhibitors. The results allow the quantitative assay of DAPK activity in biological samples, the purification of DAPK from vertebrate tissues, and the initiation of small molecule inhibitor discovery. The results provide a firm foundation for current inhibitor discovery and biology investigations, and raise interesting questions about the control of DAPK activity and cell death.

86. Purification and characterization of 2-(2′-hydroxyphenyl)benzenesulfinate desulfinase. L. M. Watkins, R. C. Rodriguez, and M. Cody. Department of Chemistry and Biochemistry, Southwest Texas State University, San Marcos, TX 78666

The enzyme 2-(2′-hydroxyphenyl)benzenesulfinate desulfinase (HPBS desulfinase) catalyzes the cleavage of the carbon−sulfur bond of 2-(2′-hydroxyphenyl)benzenesulfinate (HPBS) to form 2-hydroxybiphenyl (HBP) and sulfite. HPBS desulfinase was purified from *Rhodococcus* strain sp. IGTS8. The purification was monitored using a spectrofluorimetric assay and assessed by SDS−polyacrylamide gel electrophoresis. The p*I* of the enzyme is 5.5, the temperature optimum is 35 °C, and the pH optimum is 7.0. The K_m for HPBS is 0.90 ± 0.15 μM, and the V_{max} is 55.6 ± 2.3 μM min^{-1}. The products HBP and sulfite are not inhibitory. None of the analogues tested were substrates for the enzyme, and very few were able to act as inhibitors of the enzyme. The activity of the enzyme is decreased in the presence of Cu^{2+} and Zn^{2+}. The enzyme was susceptible to tyrosine, tryptophan, and cysteine specific modification agents.

87. Purification and immunodetection of alkaline phytase from lily pollen. Barry G. Garchow,[1] Shoichiro Ozaki,[2] Glenn Prestwich,[2] and Pushpalatha Murthy.[1] [1]Department of Chemistry, Michigan Technological University, 1400 Townsend Dr., Houghton, MI 49931, and [2]Department of Medicinal Chemistry, University of Utah, 30 S. 2000 East, Salt Lake City, UT 84112

A number of inositol phosphates, including phytic acid (*myo*-inositol hexakisphosphate; $InsP_6$), Ins(1,3,4,5,6)P_5, Ins(1,3,4,5)P_4, and Ins(1,4,5)P_3, play critical roles in signal transduction and cellular calcium signaling. $InsP_6$ is the major storage form of phosphate in cereal grains and legumes (1−5% dry weight). Livestock, poultry and swine, lack the necessary enzymes to hydrolyze $InsP_6$ and absorb inositol and phosphorus. Thus, most of the $InsP_6$ present in feed grains is excreted as waste, leading to environmental contamination. Phytases are the principal enzymes responsible for the hydrolysis of phytic acid. Acid phytases show optimal activity at pH 5−6, and an alkaline phytase has optimal activity at pH 8. Acid phytases have been extensively studied, but alkaline phytase has received relatively little attention. This poster describes our attempts at the purification of alkaline phytase from lily pollen. In addition, the development of an immunodetection protocol for the screening of alkaline phytase will be presented.

88. Purification of prenyltransferase from the lepidopteran insect *Manduca sexta*. Stephanie E. Sen, Andrea E. Sperry, and **Jessica L. Jordan**. Department of Chemistry, Indiana University−Purdue University Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202

In insects, juvenile hormone (JH) helps to regulate entrance into the pupal stage, and also is required for adult female oogenesis and the maturation of the adult male accessory sex gland in some insects. JH is synthesized in the corpora allata, a small retrocerebral endocrine gland composed of several hundred cells. We have previously demonstrated that prenyltransferase, a key enzyme in this biosynthetic pathway, is unique to the insect order Lepidoptera because of its ability to differentiate and couple homologous isoprenoid compounds. To allow for more detailed structural analysis of this system, prenyltransferase was purified from both the whole body and corpora allata of the tobacco hornworm, *Manduca sexta*. Using whole body larvae as our initial tissue source, prenyltransferase was purified by isoelectric focusing, following by ion exchange, hydroxyapatite, and size exclusion chromatography. Utilizing a pH 5−7 gradient, farnesyl diphosphate synthase activity was separated from geranylgeranyl diphosphate synthase, isopentenyl diphosphate isomerase, and endogenous phosphatases. The active fractions containing farnesyl disphosphate synthase were subjected to ion exchange chromatography using a DEAE Sepharose column and a combined phosphate and pH gradient that avoided excessive exposure to high salt concentrations, which irreversibly inhibited the prenyltransferase. The purified material requires nonionic detergent for stability and divalent manganese (0.5 mM) or magnesium (1 mM) for catalytic activity. This purification strategy has been applied to JH-producing prenyltransferase of the corpus allatum of larval *M. sexta*. Initial results indicate the presence of a farnesyl diphosphate synthase having substrate specificity that is related to JH homologue production.

89. Random mutagenesis of cytochrome P450 2A6 and screening with indole oxidation products. F. Peter Guengerich, Katsunori Nakamura, and Martha V. Martin. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232

Human P450 2A6 is involved in the oxidation of some drugs and particularly several procarcinogens. We initiated studies with mutants selected from randomized libraries generated in SRS regions 2 and 3, screening on the basis of indole 3-hydroxylation. 3-Hydroxyindole yields several colored products, including indigo and indirubin. Screening of *Escherichia coli*-based SRS2 and -3 libraries yielded colonies that produced indigo as well or better than wild-type (WT) P450 2A6. Isolated F209T showed indole 3-hydroxylation less than WT but had a k_{cat} for coumarin 7-hydroxylation 13× greater than WT. The double mutant L240C/N297Q consistently produced very blue colonies. The L240C change alone did not show enhanced activity, but both N297Q and N297H did. These five mutants yielded different mixtures of pigments from indole than WT, as judged by visible spectra and HPLC of products. These studies indicate the potential value in understanding P450 2A6 function and also generating new products for practical applications.

90. Redirecting the specific reactivity of a natural product and its application to functional proteomics. Junko Tamiya,[1] Benjamin F. Cravatt,[2] and Erik J. Sorensen.[1] [1]Department of Chemistry and Skaggs Institute for Chemical Biology and [2]Departments of Cell Biology and Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037

Activity-based protein profiling aims to create chemical agents to profile changes in enzyme activity in complex proteomes. Combining this methodology with a natural product scaffold, a library of biotinylated analogues of the natural product fumagillin was constructed and tested against complex proteomes. Fumagillin is an angiogenesis inhibitor, which contains an electrophilic spiroexpoxide and a hydrophobic side chain. The spiroepoxide covalently modifies the metalloprotease methionine aminopeptidase-2 (MetAp-2). Variation of the side chain to both hydrophobic and hydrophilic moieties redirected this natural product, facilitating the specific labeling of a diverse number of proteins directly in complex proteomes.

91. Regioselective reduction of steroids with assistance from bovine serum albumin. Lauren E. Kayser and X. C. Liu. Department of Chemistry, Indiana State University, Terre Haute, IN 47809 (lauren_kayser@yahoo.com)

Recently, proteins have been the focus of numerous research endeavors. An innovative approach is the use of proteins as an auxiliary for bioorganic synthesis. Currently, bovine serum albumin (BSA), a protein with multiple binding domains, is being used to regioselectively reduce steroids, particularly 4-androstene-3,17-dione, as a model system. Ketone groups on both the C3 and C17 locations are difficult to differentiate in standard chemical reductions. However, in the presence of BSA, the B, C, and D rings of the steroid are involved in the steroid/BSA binding, leaving only the C3 ketone, located on the A ring, available for reduction. TLC results show that in the presence of BSA, 4-androstene-3,17-dione is monoreduced, while a control reaction gives a mixture of mono- and direduced products. Products from the BSA studies are being analyzed with HPLC and carbon and proton NMR.

92. Ribonuclease A zymogen as a chemotherapeutic for malaria. Parit Plainkum,[1] Stephen M. Fuchs,[2] and Ronald T. Raines.[2] [1]Department of Microbiology, Faculty of Science, Mahidol University, Rama VI Rd., Payathai, Bangkok 10400, Thailand, and [2]Department of Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706

Ribonuclease A (RNase A) catalyzes the cleavage of RNA. This activity can make RNase A cytotoxic. Here, RNase A was engineered to combat *Plasmodium falciparum* malaria. To make the toxicity of RNase A specific to cells infected with the parasite, a loop of residues was inserted as a bridge across the enzymic active site. These residues connect the N- and C-termini and contain a recognition sequence for the *Plasmodium falciparum* protease, plasmepsin II. New N- and C-termini were created in an existing surface loop by means of a circular permutation. The resulting RNase A zymogen was thus designed to show no ribonucleolytic activity unless activated by plasmepsin II, as can occur only in an infected

cell. Analogous RNase A zymogens could be used in the treatment of other parasites, as well as viruses. (Supported by NIH Grant GM44783, Mahidol University, and the Asian Partnership Initiative.)

93. Ribonuclease internalization. Marcia C. Haigis[1] and Ronald T. Raines.[2] [1]Department of Biochemistry and [2]Departments of Biochemistry and Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (fax: 608-262-3453, hebert@biochem.wisc.edu)

Several members of the bovine pancreatic ribonuclease (RNase A) superfamily have antitumor properties. Onconase, a homologue from *Rana pipiens*, shows marked toxicity to cancer cells, but RNase A does not. Variants of RNase A with lowered affinity for ribonuclease inhibitor (RI) demonstrate antitumor properties. The mechanism of cellular internalization for onconase and G88R RNase A was investigated using chemical, pharmacological, and genetic approaches. Microscopy studies using K-562 cells indicate that ribonucleases readily bind the cell surface and are internalized via acidic vesicles. Cytotoxicity assays performed in a HeLa cell line that overproduces a mutant form of dynamin indicate that receptor-mediated endocytosis is not required for internalization. To probe the internalization pathway(s), three agents, NH_4Cl, monensin, and brefeldin A, were used to perturb acidic vesicles and the Golgi apparatus. The data suggest that onconase and G88R RNase A are not internalized via the same pathway. These results provide new insight on the mechanism of ribonuclease-mediated toxicity.

94. Role of lysine residues in the active site of onconase. Jinhwan Eugene Lee[1] and Ronald T. Raines.[2] [1]Department of Biochemistry and [2]Departments of Biochemistry and Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (jhlee@biochem.wisc.edu)

Onconase is a member of the bovine pancreatic ribonuclease A (RNase A) superfamily produced in the eggs and skin of the Northern leopard frog, *Rana pipiens*. Despite its 10^4-fold lower enzymatic activity than RNase A, onconase shows toxicity to tumor cell lines by effectively evading the cytosolic ribonuclease inhibitor protein. In RNase A, Gln11 and Lys41 form part of the enzymic active site, and enhance catalysis by preventing nonproductive substrate binding and stabilizing negative charge created during catalysis, respectively. In onconase, the corresponding residues are Lys9 and Lys31. To explore the role of Lys9 and Lys31, and to reveal the origin of the low activity of onconase, we made several variants at both residues. The enzymatic activity and the cytotoxicity of these variants are described herein.

95. Search of a secondary alcohol dehydrogenase gene from a *Micrococcus luteus*. L. Wen, M. J. Quigle, S. J. Haskett, G. H. Huang, and J.-K. Huang. Department of Chemistry, Western Illinois University, One University Circle, Macomb, IL 61455 (fax: 309-298-2180, mflw@wiu.edu)

Nocardia cholesterolicum NRRL 5767 can convert oleic acid to 10-hydroxystearic acid (10-HSA), an industrial useful product, with high yields. However, a side-product 10-ketostearic acid (10-KSA) is also produced which complicates downstream separation. The conversion of oleic acid to 10-HSA and subsequently to 10-KSA is catalyzed by oleate hydratase and secondary alcohol dehydrogenase (2°-ADH), respectively. Our long-term objective is to engineer *NC* NRRL 5767 so it possesses only oleate hydratase by blocking its 2°-ADH. Blocking the expression of 2°-ADH by antisense technology requires studies of this gene. Based on the partial amino acid sequence of 2°-ADH from *Micrococcus luteus* (WIU JH-20), oligonucleotide probes were deduced and custom-synthesized. The probes have been used to hybridize to a Southern genomic blot. The three hybridizing bands of 0.5, 1.6, and 3 kb have been gel-purified and cloned into pBluescript. Putative clones containing 2°-ADH have been identified by colony hybridization. (Supported by grants from the University Research Council and by USDA CSREES Grant 99-35501-8312.)

96. Searching for gamma-aminobutyric acid aminotransferase inhibitors: A synthetic and computational approach. Yue Pan and Richard B. Silverman. Department of Chemistry, Northwestern University, Evanston, IL 60208-3113

Low level of gamma-aminobutyric acid (GABA) in the brain is associated with epilepsy, a central nervous system disease characterized by recurring convulsive seizures. Gamma-aminobutyric acid aminotransferase (GABA-AT) degrades GABA in the brain and therefore is the target for inhibition. 4-Amino-5-hexenoic acid (vigabatrin) is a drug on the drug market (except in the U.S.) that irreversibly inhibits GABA-AT. Based on its structure, a conformationally rigid compound was designed and synthesized. It turned out to be even more potent ($k_{inact}/K_I = 52\ mM^{-1}\ min^{-1}$) than vigabatrin ($k_{inact}/K_I = 0.35\ mM^{-1}\ min^{-1}$). The inactivation mechanism needs further investigation. Presumably it inactivates GABA-AT through the Michael addition pathway. We also synthesized (3*R*)- and (3*S*)-3-amino-1-cyclopentene-1-carboxylic acid as GABA-AT inhibitors. The software DOCK discovered some promising molecules that may inhibit the enzyme. The biological test is under way.

97. Semisynthesis of proteins containing nonnatural modules. Ulrich Arnold,[1] Matthew P. Hinderaker,[2] Bradley L. Nilsson,[2] Bayard R. Huck,[2] Samuel H. Gellman,[2] and Ronald T. Raines.[1,2] [1]Department of Biochemistry and [2]Department of Chemistry, University of Wisconsin−Madison, Madison, WI 53706 (uarnold@biochem.wisc.edu)

The introduction of nonnatural modules into proteins provides a new means to explore the basis for conformational stability, folding/unfolding pathways, and biological function. Here, the method of intein-mediated protein ligation was used to introduce a nonnatural module into ribonuclease A (RNase A). Semisynthetic wild-type (wt) RNase A produced by this means and full-length RNase A produced by intein-mediated protein expression had the same ribonucleolytic activity as did wt-RNase A. A variant was produced in which *R*-nipecotic acid−*S*-nipecotic acid replaces the beta-turn at residues Asn113-Pro114. This variant showed ribonucleolytic activity comparable to that of the wt-RNase A, indicating that it is folded properly. The effect of the beta-turn mimic

on the conformational stability and folding/unfolding rate of the protein was investigated as well.

98. Stabilization of semi-synthetic ribonuclease A with covalent mimics of disulfide bonds. Matthew P. Hinderaker,[1] Ulrich Arnold,[2] and Ronald T. Raines.[1,2] [1]Department of Biochemistry and [2]Department of Chemistry, Univesity of Wisconsin−Madison, Madison, WI 53706

We are evaluating novel methods to introduce covalent mimics of disulfide bonds into the C-terminal region of ribonuclease A. C-terminal disulfide bonds are critical to the stability of the related and more cytotoxic protein onconase. Expressed protein ligation readily allows for the incorporation on nonnatural moieties into proteins. The C-terminal region of ribonuclease is chemically synthesized on the solid phase, incorporating the synthetic nonnatural amino acids required for the formation of disulfide mimics. After cyclization, the peptide is then ligated to its N-terminal sequence which is produced recombinantly as a fusion protein in *E. coli*, resulting in a full-length active protein.

99. Serine-1176 alanine mutation of neuronal NOS−Implications in catalysis. Satya P. Panda, Jonathan S. Nishimura, Linda J. Roman, and Bettie Sue Masters. Department of Biochemistry, University of Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78229

Three isoforms of nitric oxide synthsases (NOS), neuronal, endothelial, and inducible, are bidomain enzymes with an N-terminal oxygenase domain and a C-terminal reductase domain, joined by a calmodulin binding site. The reductase domains of NOSs share ∼58% sequence homology with NADPH−cytochrome P450 reductase. Since the global architectures of the crystal structures of the heme domains of NOS isoforms reveal very minor differences, this work focuses upon regulation mediated through the reductase domain. The crystal structure of the neuronal NOS reductase domain, residues 936−1412 [the FAD-containing domain; Masters et al. (1999) *Flavins and Flavoproteins* (Ghisla, S., et al., Eds.) pp 845−852], shows that Ser1176 is positioned near the N5 atom of the FAD isoalloxazine ring. Mutation of this residue to an alanine decreases the rate of electron transfer to external acceptors as well as to the heme domain necessary for NO production.

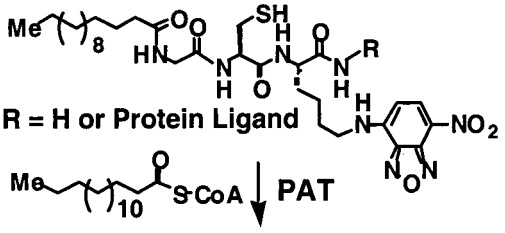
100. Single molecule investigation of the folding of C-domain P RNA: *Bacillus subtilis* RNase. Z. Xie, X. Fang, T. Sosnick, T. Pan, and N. F. Scherer. Departments of Chemistry, Biochemistry, and Molecular Biology, University of Chicago, and Institute for Biophysical Dynamics, 5735 S. Ellis Ave., Chicago, IL 60637

The folding thermodynamics and kinetics of a 255-nucleotide ribozyme, the catalytic domain of *Bacillus subtilis* RNase P RNA, have been extensively studied previously using ensemble techniques [Fang, X., Pan, T., and Sosnick, T. R. (1999) *Biochemistry 38*, 16840−16846; Fang, X., Pan, T., and Sosnick, T. R. (1999) *Nat. Struct. Biol. 6*, 1091−1095]. Mg^{2+}-induced folding of this ribozyme is a highly cooperative process free of kinetic traps. Multiple thermodynamic and kinetic intermediates are present along the folding pathway. Biological processes such as folding and transcription are very likely to involve a rich set of subpopulation kinetic paths and transient states, whose existence will be difficult to establish by ensemble measurement techniques. This information can, however, be obtained by examining the dynamics of single molecules [Zhuang, X., Bartley, L. E., Chu, S., et al. (2000) *Science 288*, 2048−2051]. We use single molecule fluorescence spectroscopy to address the folding of this ribozyme with the objectives: (1) to obtain kinetic constants from individual C-domain P RNA molecules equilibrating between unfolded, intermediate, and native states; (2) to identify folding/unfolding pathways at subpopulation levels; (3) to explore the structural aspects of the intermediates and directly reveal the Mg^{2+} binding sites that are responsible for the folding cooperativity. The study utilizes single-fluorophore polarization and fluorescence resonance energy transfer (FRET), the former as a probe of ribozyme orientational dynamics and the latter as a probe of the distance and orientational changes between pairs of fluorescent donor and acceptors bound to RNA. Depending on which specific sites are labeled on the host molecules, allows FRET probing of either local or global dynamics. In our experiment, the ribozyme molecules were labeled with Alexa488 (donor) and Cy3 (acceptor). The molecules are immobilized on a coverslip surface via a standard streptavidin−biotin protocol and kept in a sample chamber where the buffer (i.e., thermodynamic) condition can be altered. An epi-fluorescence confocal microscopy configuration with 3-dimensional sample scanning capability is employed. The single molecule fluorescence time-trajectories are recorded and analyzed by auto- and cross-correlation approaches to extract the characteristic time scales of structural fluctuations for different thermodynamic states of the ribozyme. The results of the study will be reported in the poster.

101. Solid-phase synthesis and plasma membrane localization of nonnatural palmitoyl acyltransferase substrates: Strategies for controlling gene expression by altering protein subcellular localization. Steffen P. Creaser, Stephen L. Hussey, Smita S. Muddana, and Blake R. Peterson. Department of Chemistry, Pennsylvannia State University, University Park, PA 16802 (spc10@psu.edu)

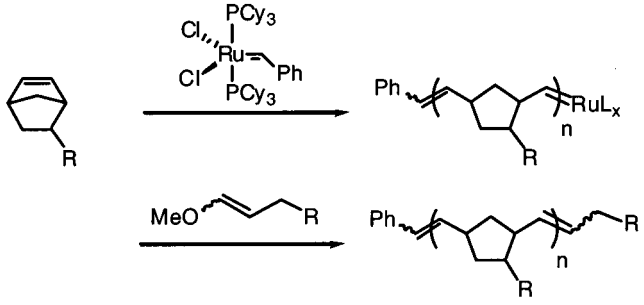
Gene expression in eukaryotic cells is regulated by intrinsic signal transduction pathways that relay extracellular stimuli from the plasma membrane to the nucleus via protein signaling cascades. Two important mediators of these signaling pathways are members of the Ras and Src families of proteins, which reside at the inner leaflet of the plasma membrane due to posttranslational lipid modification by enzymes including palmitoyl acyltransferase (PAT). We are investigating minimalist synthetic substrates of PAT that localize at the plasma membrane of whole cells. When coupled to cell-permeable protein ligands, these compounds may recruit intracellular proteins to the plasma membrane and influence gene expression. We report herein the solid-phase synthesis of fluorescent PAT substrates covalently linked to synthetic derivatives of the steroid hormone β-estradiol. Fluorescence microscopy was employed to analyze the subcellular distribution of these compounds in

Jurkat lymphocytes. Preliminary efforts and related strategies to influence cellular signal transduction will be described.



102. Specifically end-labeled polymers for probing multivalent interactions. Robert M. Owen,[1] Jason E. Gestwicki,[2] Travis Young,[1] Christopher W. Cairo,[1] and Laura L. Kiessling.[3] [1]Department of Chemistry, [2]Department of Biochemistry, and [3]Departments of Chemistry and Biochemistry, University of Wisconsin−Madison, 1101 University Ave., Madison, WI 53706 (rowen@chem.wisc.edu)

Multivalent interactions play an important role in a wide range of biological processes, including inflammation, viral infection, fertilization, and the immune response. Synthetic, linear polymers have proven to be powerful tools for both mimicking and inhibiting natural multivalent interactions. Work in our research group has utilized ring-opening metathesis polymerization (ROMP) for the synthesis of defined polymeric displays. To extend the utility of these polymers, we have explored methods for site-specific end labeling of ROMP polymers. We have developed a series of substituted vinyl ethers that terminate ROMP in a controlled fashion and lead to the incorporation of the desired functionality at the end of polymer chain in high yields. This functionality was utilized to incorporate fluorescent tags and to attach polymers to a surface. The resulting conjugates were used to examine cellular and multivalent binding events.



103. Spectroelectrochemical study of myoglobin in DMSO. Qiu Ci Li and Patricia Ann Mabrouk. Department of Chemistry, Northeastern University, 112 Hurtig Hall, Boston, MA 02115 (fax: 617-373-8795, quttie@hotmail.com)

The first example of direct electron transfer between an intact metalloprotein and solid electrode in nonaqueous media will be presented for the case of horse myoglobin (H Mb) in dimethyl sulfoxide (DMSO), containing 1.3−7.5% water. Mb exhibits stable and well-defined cyclic voltammetric responses that are not affected by the water content when varied between 1.3 and 7.5%. The electrochemistry characteristics are consistent with a one-electron, quasi-reversible, diffusion-controlled charge-transfer process at Au. The formal heterogeneous electron-transfer rate constant, calculated from ΔE_p at 20 mV/s, is $(1.7 \pm 0.5) \times 10^{-4}$ cm/s. It is competitive with that previously reported in aqueous solution. UV−vis and resonance Raman (RR) spectroscopy, which has been proven to be an extremely powerful probe of heme active site structure, were used to probe Mb in the relevant redox active forms, specifically metmyoglobin (metMb), deoxymyoglobin (deoxyMb), and the carbon monoxide adduct of Mb (Mb-CO).

104. Spectroscopic, EPR, and structural studies of 5-methyltrihydro-l-biopterin function in NO synthase during a single catalytic turnover. C. C. Wei,[1] Z. Q. Wang,[1] A. S. Arvai,[2] C. Hemann,[3] R. Hille,[3] E. D. Getzoff,[2] and D. J. Stuehr.[1] [1]Department of Immunology, Cleveland Clinic Foundation, Cleveland, OH, [2]Department of Molecular Biology, The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, and [3]Department of Cellular and Molecular Biology, The Ohio State University, Columbus, OH

Nitric oxide synthase (NOS) catalyzes two consecutive reactions to generate NO and citrulline from L-arginine (Arg), with *N*-hydroxy-L-arginine (NOHA) formed as a bound intermediate. We are utilizing stopped-flow spectroscopy, stopped quench, and rapid freeze EPR methods to investigate the mechanism of NO synthesis during a single catalytic turnover. The NOS heme binds and activates O_2 in both steps of NO synthesis. 6*R*-Tetrahydrobiopterin (H4B) is required for activity and has both allosteric and redox roles. Using the oxygenase domain of inducible NO synthase ($iNOS_{oxy}$), our recent single turnover study demonstrated that H4B radical formation (11 s^{-1}) is coupled to the disappearance of an initial heme $Fe^{II}O_2$ intermediate (12.5 s^{-1}) as well as to Arg hydroxylation (9 s^{-1}). In this report, we extended these studies using 5-methyltrihydrobiopterin (5-methyl-H4B), a H4B analogue that we expected would stabilize the radical. In the presence of Arg, rapid-scanning stopped-flow spectroscopy revealed that the $Fe^{II}O_2$ intermediate in 5-methyl-H4B-bound $iNOS_{oxy}$ reacted at a rate of 34.6 s^{-1}, which is 3-fold faster than with H4B. This rate was coupled to a faster rate of 5-methyl-H4B radical formation (40 s^{-1}) from rapid-freeze EPR data, and correlated with a faster rate of Arg hydroxylation. The EPR spectra of enzyme-bound 5-methyl-H4B radical had a different hyperfine structure than the bound H4B radical, and were more stable, with a decay rate of 0.2 s^{-1} compared with 0.7 s^{-1} for H4B. A crystal structure of 5-methyl-H4B-bound NOS_{oxy} showed no conformational changes compared to H4B-bound enzyme, indicating the factors causing the rate changes are inherent to 5-methyl-H4B itself. Our studies suggest that H4B electron transfer to the heme $Fe^{II}O_2$ intermediate is critical for Arg hydroxylation and is rate-determining for Arg hydroxylation once ferrous heme binds O_2. In addition, the rate of electron transfer depends on stabilization of the resulting pterin radical.

105. Structural analysis of cyclic peptide ligands for $\alpha_6\beta_1$ integrin. Haishan Li and Nicole Sampson. Department of Chemistry, SUNY Stony Brook, Stony Brook, NY 11794-3400

Fertilin is a membrane-bound, heterodimeric (α/β) protein present on the equatorial region of the sperm head where

initiation of fusion events occurs and is believed to bind to an egg plasma membrane receptor (α_6/β_1), triggering fusion. Fertilin β is a member of the still expanding family of ADAM (A Disintegrin And Metalloprotease) proteins that are related to the soluble snake venom metalloproteases (SVMPs). Sequence alignments of the putative binding loop of the disintegrin domains of ADAMs and SVMPs highlighted that for ADAMs a consensus ECD sequence is the binding sequence and for SVMPs a conserved RGD sequence is the minimum recognition element. Many studies have been carried out for the structural characterization of local conformations of the RGD motif for understanding the interactions between SVMPs and integrin receptors. However, little is yet known about the biological properties and structural features of the disintegrin-like domains of ADAM protein. In this study, we determined the solution conformations of ECD peptide inhibitors of mammalian fertilization by NMR spectroscopy and molecular modeling.

106. Structural analysis of rabbit phosphoglucose isomerase complexed D-arabinose 5-phosphate. Vishal P. Patel, Kathy Z. Chang, and Constance J. Jeffery. Department of Biological Sciences, University of Illinois at Chicago, Laboratory for Molecular Biology, 900 S. Ashland Ave., Chicago, IL 60607

The second reaction of glycolysis, the aldose−ketose isomerization of D-glucose 6-phosphate to D-fructose 6-phosphate, is catalyzed by the enzyme phosphoglucose isomerase (PGI, EC 5.3.1.9). We have determined the X-ray crystal structure of rabbit PGI complexed with the competitive inhibitor D-arbinose 5-phosphate. The structure was solved at a resolution of 1.9 Å and has an *R*-factor of 22.4% and an *R*-free of 19.5%. A molecule of D-arbinose 5-phosphate is found bound in each active site pocket.

107. Structure and function of glutamate receptors. Vasanthi Jayaraman, **Qing Cheng**, and Shalita Thiran. Department of Chemistry, Marquette University, 535 N. 14th St., Milwaukee, WI 53202 (fax: 414-288-7066, vasanthi.jayaraman@marquette.edu, qing.cheng@marquette.edu)

Fourier transform infrared spectroscopy was used to investigate ligand−protein interactions in the GluR4 glutamate receptor subunit. Specifically, the SH stretching mode of the single non-disulfide-bonded cysteine residue and the amide vibrations of the protein were used to study the structural changes induced in the protein due to ligand binding. These studies indicate that full agonists such as glutamate induce more extensive secondary structural changes in the ligand-binding domain than do partial agonists such as kainate or antagonists such as quinoxaline diones. Full agonists also alter the hydrogen-bonding strength of the single free cysteine side chain in the domain, while the partial agonists and antagonists do not. In addition to the changes in the protein, specific ligand−protein interactions were also probed using isotopically labeled ligands. These investigations provide a detailed picture of the specific interactions such as hydrogen bond strength and ionic interactions at the carboxylates, nitro, and carbonyl moieties of the ligand. These results identify chemical and structural differences that may explain the different functional characteristics of the two agonists acting on ionotropic glutamate receptors.

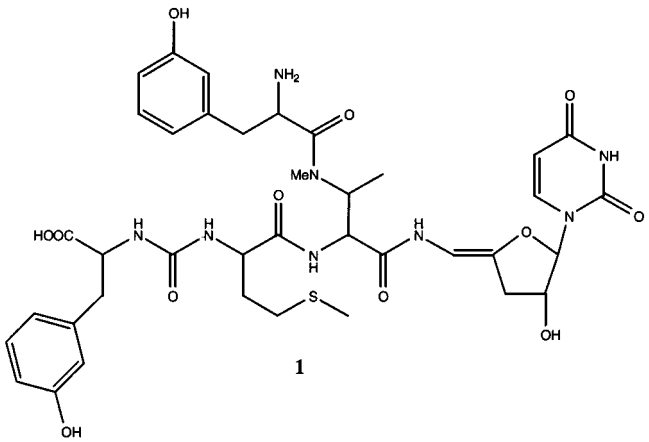
108. Substrate polarization by medium-chain acyl-CoA dehydrogenase as revealed by Raman spectroscopic study. Jiaquan Wu, Alasdair F. Bell, and Peter J. Tonge. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400

Medium-chain acyl-CoA dehydrogenase (MCAD) (EC 1.3.99.3) is the first enzyme in the fatty acid β-oxidation pathway. It is well established that the reaction catalyzed by MCAD involves abstraction of the α-proton from substrate by the enzyme and a hydride transfer from the β-carbon of the substrate to the FAD cofactor of the enzyme. Previous studies have revealed that these two processes might be thermodynamically unfavorable considering that the α-proton of a fatty-acyl-CoA is around 21 while that of the active site residue is about 6.5, and that the electro-potential ($E^{0'}$) of free enzyme is −136 mV and that of the free substrate is −26 mV. Nevertheless, the dehydrogenation rate of *n*-octanoyl-CoA catalyzed by MCAD is ca. 320 s^{-1} at 5 °C and larger than 1000 s^{-1} at 25 °C. Many spectroelectrochemical studies have been done to reconcile the apparent conflict in the unfavorable thermodynamic properties with the distinct dehydrogenation rate. Studies have revealed that binding of a substrate to the enzyme is able to change electronic conformations of both the enzyme and the substrate and thus change thermodynamic properties of the system. It was hypothesized that the dramatic change of the substrate thermodynamic properties indicates that the substrate has been polarized. Raman spectroscopy is very sensitive in detecting the fine electronic conformation changes of substrates in biosystems. To verify the substrate polarization in MCAD-catalyzed reaction, the behavior of a product analogue, hexadienoyl-CoA (HD-CoA), complexed with MCAD was subject to the Raman study. HD-CoA was labeled with ^{13}C in the HD moiety in order to explain the Raman spectra. To identify the source of the substrate polarization, the putative H-bonding residues were tested. On one hand, MCAD enzyme was reconstituted with 2′-deoxy-FAD to remove one of the H-bonding groups, FAD ribityl 2′-OH. On the other hand, the E376 residue was mutated to a proline to remove the other H-bonding source, the backbone amide H from E376.

109. Synthesis of novel inhibitors of phospho-*N*-acetylmuramylpentapeptide translocase (mraY). Nigel Howard and Timothy D. H. Bugg. Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom (fax: +44 (024) 765 24112, n.i.howard@warwick.ac.uk)

To overcome the growing problem of increased bacterial resistance to the current range of antibiotics, new targets for antibacterial agents are required. Enzymes involved in the biosynthesis of the peptidoglycan component of the cell wall (the sugar/peptide network which helps keep the bacterial cell intact) provide suitable targets. Phospho-*N*-acetylmuramyl pentapeptide translocase, the first enzyme in the membrane step of peptidoglycan biosynthesis, has been chosen, as it has been shown to be essential, and inhibited a family of natural uridyl peptide antibiotics, including mureidomycins, napsamycins, and pacidamycins. One of these, Mureidomycin A (Mrd A) (**1**), isolated from *Streptomyces flavidoviridens* SANK 60486, was chosen as the basis of a series of peptidylnucleosides (**2**) synthesized in an attempt

to determine a structure−activity relationship for the "top chain" of the natural product.



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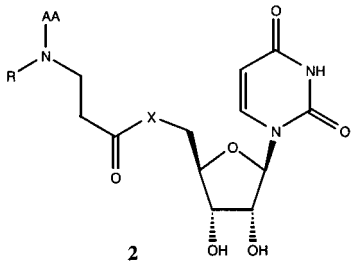


2

R = H or Me
X = O or NH
AA = L-Phe, D-Phe, Tyr, β-Ala, Ala, Gly

110. Synthesis, characterization and utilization of UDP-glucose derivatives: Mechanistic probes for studying the glucosyltransferase activity of *C. difficile* Toxins A and B. Sudeep Bhattacharyay and Andrew L. Feig. Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300, sbhattac@indiana.edu)

A common form of severe antibiotic-associated diarrhea is caused by *C. difficile* infection. The toxicity is mediated by two enzymes, Toxin A and Toxin B. Both toxins are metal ion-dependent glucosyltransferases that utilize UDP-Glc as their glucose donor. We have synthesized several analogues of UDP-Glc for use in mechanistic studies of these enzymes. Methylanthraniloyl derivatives of the sugar−nucleotide complexes have allowed us to use fluorescence spectroscopy to probe the binding of these substrates to the toxin. The analogues bind approximately 2.5-fold weaker than the native substrate and thus are useful mechanistic probes. Phosphorothioate analogues of UDP-Glc have been used to study the interaction of the metal ion cofactors with the substrate during the glucosyltransfer reaction. The mechanistic insights gained from the use of these substrate analogues will be presented.

111. The effect of PEG on cyt c. Wei Guo and Patricia Ann Mabrouk. Department of Chemistry, Northeastern University, 112 Hurtig Hall, Boston, MA 02115 (fax: 617-373-8795, guo_wei@hotmail.com)

We used UV−vis, circular dichroism (CD), and cyclic voltammetry (CV) measurements on cytochrome *c* (cyt c) in the presence and absence of added 0.01 M poly(ethylene glycol) (PEG) to determine whether PEG changes the structure and function of cyt c. Addition of PEG-8000 to ferri- and ferro-cyt c produces a increase in the intensity of both the Soret and Q-band spectral features. The secondary structure of cyt c is unchanged by addition of PEG. The redox potential, E^o, was not affected measurably. Cyt c remained chemically reversible at bis(4-pyridyl)disulfide (BPD)-modified gold in the presence of added PEG. The diffusion coefficient, D_o, decreased by a factor of 50 when PEG was added. The addition of PEG decreased the heterogeneous electron transfer rate constant by a factor of 3. Therefore, PEG does not appear to deleteriously affect either the structure or the electrochemical behavior of cyt c in aqueous solution.

112. The synergy peptide PHSRN and the adhesion peptide RGD mediate cell adhesion through a common mechanism. Yuezhong Feng. Department of Chemistry, University of Chicago, Chicago, IL 60637 (mmrksich@midway.uchicago.edu, yfeng@harper.uchicago.edu)

This work compares the attachment of BHK cells and 3T3 Swiss fibroblasts to model substrates presenting either the peptide GRGDS or the peptide PHSRN. The work used self-assembled monolayers of alkane thiolates on gold presenting the peptide ligands mixed with tri(ethylene glycol) groups because these substrates permit rigorous control over the structures and densities of peptide ligands while they prevent nonspecific interactions with adherent cells. Both cell types attached efficiently to monolayers presenting either RGD or PHSRN. The degree of cell spreading, however, was substantially higher on substrates presenting RGD relative to PHSRN. Staining of fibroblasts with anti-vinculin and phalloidin revealed clear cytoskeletal filaments and focal adhesions for cells attached by way of either RGD or PHSRN. Inhibition experiments showed that the attachment of fibroblasts to monolayers presenting RGD could be inhibited completely by a soluble RGD peptide and partially by a soluble PHSRN peptide. This work demonstrates unambiguously that PHSRN alone can support cell attachment and that the RGD and PHSRN bind competitively to the integrin receptors.

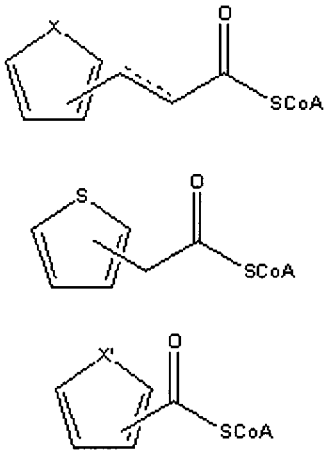
113. Theoretical study of the role of cation−Π interactions in the biological activity of AChE. Kiruba S. M. George and Ming Wah Wong. Department of Chemistry, National University of Singapore, 3, Science Dr. 3, Singapore 117 543

The enzyme acetylcholine esterase (AChE) catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) at the cholinergic synapse. The active site of the enzyme is located at the bottom of a 20 Å deep gorge. It is suspected that the interaction of the aromatic-Π subunits of amino acids such as phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), and histidine (His) with the positively charged ammonium head of acetylcholine helps it push itself down the 20 Å deep gorge to reach the deep-seated active site of AChE. Such interaction between a cation and an aromatic-Π system is referred to as "Cation−Π" interaction. To shed light on the importance of such interactions in the biological function of AChE, we

modeled a series of acetylcholine−benzene and acetylcholine−phenylalanine complexes using ab initio calculations. All the structures were optimized at the B3LYP/6-31G* level. Since acetylcholine is a very flexible molecule, we performed a systematic conformation search to locate all possible conformers of acetylcholine. All the unique conformers were optimized both in the gas phase and in solution at the B3LYP/6-31G* level. Solvent calculations were performed using SCRF and SCIPCM models. In addition, G3(MP2) calculations were performed on all the important conformers of acetylcholine.

114. Thermodynamic regulation of medium-chain acyl-CoA dehydrogenase. Teresa R. Lamm, Theresa Kohls, and Marian T. Stankovich. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

MCAD belongs to the family of enzymes which catalyze the first step of β-oxidation, the two-electron reduction of acyl-CoAs to α,β-*trans*-enoyl-CoAs. Deficiencies of these enzymes prevent the body from fully utilizing energy stored as fat and can lead to serious metabolic disorders. For example, nearly 15% of all previously diagnosed Sudden Infant Death Syndrome (SIDS) cases are due to a point mutation in MCAD. Substrate/product binding is known to shift the enzyme redox potential 100 mV positive, allowing electron transfer from the substrate to the enzyme-bound flavin to become thermodynamically favorable. New spectrally active analogues (see structures below) will be used to investigate binding-induced changes within the substrate/product couple. Polarization and redox effects on the ligand itself will be presented. (Supported by NIH Grant GM29344.)



Spectrally-Active Analogs
X = S, O, or NH, X' = S or O

115. Toward the synthesis and biological characterization of artificial micelles. Sarah A. Webb, Lewis J. Belcher, Falk Eike Flach, Yehia Mechref, Milos V. Novotny, and Martha G. Oakley. Department of Chemistry, Indiana University, 800 East Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300)

Molecular recognition at the surfaces of membranes is critical for many signal transduction pathways. To study the specificity of these interactions at a molecular level, we have developed a strategy for the synthesis of polymeric mimics of phosphatidylinositol 4,5-bisphosphate (PIP_2). We describe the synthesis and characterization of our polymeric derivatives and studies of their biological activity.

116. Toward an in vivo selection for antiparallel coiled coils. Jessica J. Hollenbeck, Byung-Moon Kim, Jang Lee, and Martha G. Oakley. Department of Chemistry, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405 (fax: 812-855-8300, jjhollen@indiana.edu)

Basic region leucine zipper (bZip) proteins have a bipartite DNA-binding domain consisting of a coiled-coil dimerization domain and a highly charged basic region that directly contacts DNA. Using a model antiparallel coiled coil and the basic region of the naturally occurring bZip protein GCN4, we have designed a model bZip heterodimer that binds to DNA specifically and with high affinity only when the α-helices of the coiled coil are aligned in an *antiparallel* relative orientation. Using this functional bZip domain, we have developed an in vivo transcription interference assay for selecting antiparallel coiled coils from a randomized pool of model coiled-coil heterodimers.

117. Transmembrane protein packing motifs and inter-helical domain analysis. Dr. Joseph Orgel and Constance J. Jeffery. Department of Biological Sciences, University of Illinois, Laboratory for Molecular Biology, 900 S. Ashland Ave., Chicago, IL 60607 (fax: 312-413-2691, jorgel@tigger.cc.uic.edu)

Transmembrane spanning proteins are predicted to make up over 25% of most genomes, often fulfilling roles such as pores, ion channels, and receptors. A great deal of interest has been generated due to their importance to cellular functions, as possible targets for drugs, and also because of their central role in many diseases, such as cystic fibrosis. Because of the profound difficulty in purification and crystallization of these proteins, increased emphasis is being made upon alternative disciplines for structural determination, such as bioinformatics. The majority of transmembrane proteins are predicted to have at least one or more α-helical domains that span the lipid membrane. In the process of developing predictive algorithms for the packing of these α-helical domains, we have conducted a review of specific amino acid sequence data and structural models of the few high-resolution crystal structures available for these important proteins. We have found a prevalence of particular amino acid sequences, or motifs that may relate to the topology of the α-helices, but more interestingly the manner in which they pack. In addition, by carefully defining and classifying differences in inter-helix connecting loops, we are able to narrow the possible helix packing angles and helix neighbors.

118. Ultra-high-resolution structures of a bridged bimetallohydrolase. William Desmarais, Dagmar Ringe, and Greg Petsko. Biophysics and Structural Biology Department, Brandeis University, Rosenstiel Basic Medical Sciences Research Center, P.O. Box 549110, Waltham, MA 02454 (fax: 630-252-0443, desmar@auriga.rose.brandeis.edu)

Bimetallohydrolases are a class of enzymes that utilize two metals connected by a bridging ligand in its active site

to catalyze hydrolytic reactions. Their ability to catalyze such diverse reactions as the degradation of DNA, RNA, phospholipids, and polypeptides makes them key players in carcinogenesis, tissue repair, protein maturation, cell cycle control, hormone-level regulation, and protein degradation. These enzymes are also involved in the degradation of agricultural neurotoxins, urea, antibiotics, and several phosphorus(V) materials used in chemical weaponry. As a model for bimetal enzymes and for enzymes that cleave the N-terminus from peptides, we have been studying the aminopeptidase from *Aeromonas proteolytica* (AAP). AAP is a small, secreted, dizinc monomeric enzyme (32 000 Da) that is thermostable for several hours at 70 °C. The active site of AAP is composed of a (μ-aqua)(μ-carboxylato)dizinc(II) catalytic core. It is important to note that although the bridging oxygen has been described previously as a water molecule (H_2O), the protonation state of it is unknown. It is possible that the bridging oxygen is actually a bridging hydroxy anion (OH^-) or an oxygen dianion (O^{2-}). Each metal ion is coordinated to a terminal carboxylate and a histidine residue, and both metals are required for full catalytic activity. Since AAP is 80% active when only one metal is present, it has been proposed that the second metal is utilized to lower the pK_a of the bridging water species and to stabilize an intermediate in the chemical reaction pathway. To completely understand the exact roles of the two metal ions in the reaction pathway of AAP, the precise position of every atom in the active site, including hydrogens, must be determined, and the protonation state of the bridging oxygen must be identified. Recent advances in X-ray crystallography have made it possible to observe electron density that corresponds to hydrogens in protein crystal structures with resolutions higher than 1.2 Å. This poster will show the 1.2 Å and the 0.95 Å structures of native AAP and describe the mechanistic information that one can glean from ultra-high-resolution X-ray data.

119. Understanding enzyme motion: Using protein splicing to synthesize specifically labeled protein hinge mutants. Juyeon Jung and Nicole S. Sampson. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400

The loop of TIM (triosephosphate isomerase) is very important for proper catalytic function because it affects substrate binding and subsequent catalysis. The objective of this study is to develop a specific labeling method that will allow us to directly analyze the rate of loop movement and the conformation of the loop of TIM during catalysis by NMR spectroscopy. The general strategy to introduce specific isotopic labels is solid-phase peptide synthesis and recombinant protein expression followed by thioester-meditated protein ligation. The N-terminal and C-terminal fragments of TIM are prepared by in vivo protein expression using inteins, and the central part of TIM that contains the loop of interest is prepared by chemical synthesis to incorporate. Two cysteine mutations were introduced at the junctions for protein ligation. The double mutant was expressed and purified; k_{cat} and K_m were determined to be 59.12 s^{-1}, 0.384 mM for wild-type and 53.00 s^{-1}, 1.062 mM for double mutant. The thioester-mediated synthesis of radiolabeled TIM will be presented.

Pfizer Award Symposium—Monday Morning

120. Catalytic function of metals in protein farnesyltransferase. Katherine E. Bowers,[1] Jennifer Pickett,[1] Kendra E. Hightower,[2] and **Carol A. Fierke**.[2] [1]Department of Chemistry, University of Michigan, 930 N. University, Ann Arbor, MI 48109 (fax: 734-647-4865, kebowers@umich.edu, fierke@umich.edu), and [2]Department of Pharmacology and Cancer Biology, Duke University Medical School, Durham, NC

Protein farnesyltransferase (FTase) is a zinc metalloenzyme that catalyzes the transfer of a farnesyl group to a cysteine residue of several signal transduction proteins and is a target enzyme for antitumor therapies. Substrate specificity is integral to the function of FTase; however, nonpeptidic thiol compounds are substrates with decreased binding affinity and farnesylation rate constants. Substitution of other metals for the active site zinc or fluorine at C3 of farnesyl diphosphate (FPP) also decreases the farnesylation rate constant. Catalysis requires formation of a zinc—thiolate nucleophile and magnesium ion coordination of the diphosphate leaving group. These data suggest that FTase catalyzes protein farnesylation with an "exploded" transition state where the metal-bound sulfur has a partial negative charge, the C1 of FPP has a partial positive charge, and the diphosphate leaving group has a negative charge. The functional roles of amino acids in the diphosphate binding pocket are being explored by mutagenesis.

121. How new enzymes evolve from old ones. Gregory A. Petsko and Jennifer Collins. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, PO Box 549110, Waltham, MA 02454 (petsko@brandeis.edu)

Complete genome sequencing for both prokaryotes and eukaryotes has established that gene duplication is the clay that evolution uses to mold new enzymes out of old ones. Two models may be considered: in one, recruitment of preexisting chemistry with later alteration of substrate specificity places the difficulty of chemical catalysis ahead of that of substrate binding. In the other, the product of one reaction is recruited as the substrate of the next one in the pathway, or vice-versa. These models make different and testable predictions about the structural organization of metabolic pathways. We will also present new genetic and biochemical evidence in yeast for the evolution of a new mini-superfamily by sub-telomeric recombination and promoter mutation.

122. Mechanistic enigmas: Dopamine β-monooxygenase and peptidyl-glycine α-amidating enzyme. J. P. Klinman. Department of Chemistry and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Dopamine β-monooxygenase (DβM) and peptidyl-glycine α-amidating enzyme (PAM) belong to a small class of copper proteins that catalyze an ascorbate- and oxygen-dependent hydroxylation of either the α-carbon of phenethylamine derivatives (DβM) or the β-carbon of C-terminally glycine extended peptides (PAM). These enzymes show considerable sequence and mechanistic homology, and are considered to operate by identical chemical mechanisms despite the differences in substrate specificity. The published X-ray struc-

ture for the hydroxylating domain (PHM) of PAM indicates two copper atoms that are ca. 10 Å apart and are at the solvent interface, making it difficult to postulate a mechanism for the reductive activation of dioxygen. In this presentation, recent data for PHM and DβM will be integrated with previously published results to implicate a new chemical mechanism. (Supported by a grant from the NIH: GM 25765.)

123. Radical peregrinations catalyzed by B_{12} enzymes. Ruma Banerjee. Department of Biochemistry, University of Nebraska, N133 Beadle Center, Lincoln, NE 68588-0664 (fax: 402-472-7842, rbanerjee1@unl.edu)

Coenzyme B_{12} or AdoCbl-dependent isomerases involve the 1,2 interchange of a variable group and a hydrogen atom on vicinal carbons. These chemically difficult reactions are enabled by the deployment of radical chemistry with the cofactor serving as a latent free radical reservoir. A subject of enduring debate in the field is how these enzymes affect the trillion-fold rate acceleration of Co−carbon bond homolysis and how the reactivity of the cofactor is contained. We have been examining these issues using methylmalonyl-CoA mutase, which catalyzes the 1,2 rearrangement of methylmalonyl-CoA to succinyl-CoA. Our results demonstrate that a key to controlling Co−carbon bond homolysis is kinetic coupling of this step to generation of a substrate radical by H-atom abstraction. Both the rate and amount of cob(II)alamin formation have been analyzed as a function of temperature with the protiated substrate. These studies yield the following activation parameters for the homolytic reaction at 37 °C with the protiated substrate: $\Delta H^\circ = 18.8 \pm 0.8$ kcal/mol, $\Delta S^\circ = 18.2 \pm 0.8$ cal mol^{-1} K^{-1}, and $\Delta G^\circ = 13.1 \pm 0.6$ kcal/mol. Our results reveal that the enzyme lowers the reaction barrier ($\Delta\Delta G^\circ = 17$ kcal/mol). The magnitude of the primary deuterium isotope effect on the coupled Co−carbon bond homolysis−hydrogen atom abstraction step (35.6 at 20 °C) suggests the involvement of quantum mechanical tunneling in this reaction.

Repligen Award Symposium—Monday Afternoon

124. The 5′-deoxyadenosyl radical as a discrete enzymatic intermediate in *S*-adenosylmethionine-dependent and adenosylcobalamin-dependent reactions. Perry A. Frey. Institute for Enzyme Research, University of Wisconsin−Madison, 1710 University Ave., Madison, WI 53705

The deoxyadenosyl free radical, 5′-deoxyadenosine-5′-yl, has long been postulated to initiate free radical-based mechanisms in reactions of adenosylcobalamin (AdoCbl)-dependent enzymes. It has also been postulated as an intermediate in the actions of enzymes that depend on *S*-adenosylmethionine (SAM) to initiate free radical chemistry. Until recently, no evidence for the formation of this radical as a discrete entity has been obtained. Two methods have been developed to search for this species: the use of coenzyme analogues that would produce a stabilized analogue of the deoxyadenosyl radical, and the application of a stereochemical approach to detect its fleeting existence. The SAM-dependent lysine 2,3-aminomutase (LAM) accepts 3′,4′-anhydroadenosylmethionine (anSAM) as a coenzyme. Reaction mixtures containing LAM, anSAM, and L-lysine frozen in the steady state at 4 K display the EPR spectrum of the corresponding anhydrodeoxyadenosyl radical, an allylic analogue of the deoxyadenosyl radical. The analogue is kinetically competent as an intermediate. 3′,4′-Anhydroadenosylcobalamin (anAdoCbl) activates the AdoCbl-dependent dioldehydrase (DDH). EPR analysis of mixtures of DDH and anAdoCbl frozen in the steady state at 4 K reveals the presence of the anhydrodeoxyadenosyl radical spin-coupled with cob(II)alamin. The AdoCbl-dependent ribonucleoside triphosphate reductase (RTPR) reacts with AdoMet in the presence of the allosteric activator dGTP to produce cob(II)alamin, 5′-deoxyadenosine, and a thiyl radical on Cys 408 at at the active site. This is presumably the mechanism by which RTPR catalyzes the dGTP-dependent exchange of deuterium from D_2O into the 5′-methylene carbon of AdoCbl. C408S- and C408A-RTPR are inactive and do not catalyze the exchange reaction. However, both variants catalyze the dGTP-dependent epimerization of the 5′-methylene group of chirally labeled [5′-deutero]AdoCbl. dGTP-dependent homolytic scission of the Co−C5′ bond to cob(II)alamin and the deoxyadenosyl radical accounts for epimerization at C5′. (Supported by Grant DK 28607 from the National Institute of Diabetes and Digestive and Kidney Diseases.)

125. On DNA replication by the T4 replisome. Stephen J. Benkovic. Department of Chemistry, The Pennsylvania State University, 414 Wartik Laboratory, University Park, PA 16802

The assembly and disassembly of the T4 replisome from a collection of eight different proteins has 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.

126. Enzymatic assembly of hybrid polyketide/nonribosomal peptide natural products. C. T. Walsh. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 45 Shattuck St., Boston, MA 02115 (fax: 617-432-0438, christopher_walsh@hms.harvard.edu)

Polyketide and nonribosomal peptide natural products are biosynthesized by multimodular enzymes acting in assembly line fashion. Monomers are activated as acyl-*S*-enzyme intermediates, and chain growth proceeds from the N- to the C-terminus of the assembly line via a cascade of elongating acyl-*S*-enzyme intermediates. In polyketide chain elongation, C−C bond formation is catalyzed by ketosynthase (KS) domains while C−N bond formation in peptide chain elongation is catalyzed by condensation (C) domains. We have examined the interfaces between polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) in formation of hybrid acyl chains to evaluate the action of the KS and C domains during PK/NRP chain assembly. Examples to be presented include the EpoA/B subunit interface in methylthiazole ring formation in epothilone biosynthesis, the HMWP2/HMWP1 interface in yersiniabactin biosynthesis, and the loading module/PKS1 module interface at the start of rifamycin biosynthesis.

127. Cobalamin-dependent methyl transfers. Rowena G. Matthews. Biophysics Research Division & Department of

Biological Chemistry, University of Michigan, 930 N. University Ave., 4028 Chemistry, Ann Arbor, MI 48109-1055 (fax: 734-764-3323, rmatthew@umich.edu)

Cobalamin cofactors and their close relatives the corrinoids are involved in the catalysis of two broad categories of reactions. The role of adenosylcobalamin in generating radical intermediates in rearrangement reactions is well understood. In contrast, the rationale for the use of cobalamin to facilitate methyl transfers is much less clear. Cobalamin-dependent methionine synthase (MetH) is a prototype for the broad class of corrinoid-dependent methyl transferases, many of which catalyze reactions that are central for energy generation in Archaea. A universal feature of corrinoid-dependent methyl transferases is that the cobalamin cofactor is bound with its nucleotide substituent (dimethylbenzimidazole in the case of cobalamin) displaced from the cobalt. In most cases, the nucleotide ligand is replaced by a histidine ligand from the protein. A central issue is the role of the histidine ligand in facilitating methyl transfers to and from cobalamin. Our recent research on MetH suggests that ligand replacement is esssential for efficient methyl transfer to and from cobalamin. An equally important role for the histidine ligand and its associated network of hydrogen-bonded residues is to control access to the cobalamin by the methyl-donating and methyl-accepting substrates.

Lilly Award Symposium—Tuesday Morning

128. Structural investigation of the HCV IRES RNA by NMR. Peter Lukavsky, Geoff Otto, Insil Kim, Alyssa Lancaster, Peter Sarnow, and **Joseph D. Puglisi**. Department of Structural Biology and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5126

Hepatitis C virus mRNA uses a novel mechanism of translational initiation, which avoids normal cap-dependent processes. The HCV mRNA contains an important regulatory element in the 5′-untranslated region called an internal ribosome entry site (IRES). 40S ribosomal subunits bind directly to this region of conserved secondary structure. We have investigated the mechanism of HCV-IRES-mediated translational inititation using a combination of biochemical and biophysical approaches, including NMR spectroscopy. We will present an overview of this work, and how it suggests a role for RNA structure in HCV translation initiation.

129. Building an RNA active site from the inside-out. Peter M. Gordon and **Joseph A. Piccirilli**. Departments of Biochemistry & Molecular Biology and of Chemistry, University of Chicago, HHMI, 5841 S. Maryland Ave., MC1028, Chicago, IL 60637 (fax: 773-702-0271, jpicciri@midway.uchicago.edu)

Divalent metal ions are essential for both the folding and catalysis of the group II ribozyme. Using atomic-level substitutions of potential metal ion ligands and metal specificity switch experiments, we previously demonstrated that divalent metal ions provide transition state stabilization of the 3′-oxyanion leaving group during both steps of splicing. To extend this analysis and to define further the active site, we have been searching for the ligands that bind and position these catalytic metal ions. Domain 5 (D5) of the group II intron is essential for catalysis, and extensive biochemical, phylogenetic, and genetic analyses suggest that this domain forms part of the catalytic center. Thus, we examined the reactivity of D5 constructs containing S_p phosphorothioate substitutions. Positions tested included sites at which the R_p phosphorothioate was shown previously to inhibit the reaction and highly conserved positions that are sensitive to mutations and believed to be located at the active site. The S_p phosphorothioate substitutions had a variety of effects on splicing efficiency, but only the *pro*-S_p oxygen of A in the conserved AGC triad at the stem of D5 exhibited a switch in metal specificity upon sulfur substitution. Phosphorothioate substitution at this position significantly reduced the ability of D5 to catalyze 5′ splice site hydrolysis in Mg^{2+}, but could be rescued upon the addition of more thiophilic metal ions such as Cd^{2+} or Mn^{2+}. Additional metal specificity switch experiments combined with quantitative approaches for distinguishing specific metal ions suggest that this metal ion, which has not been identified previously, significantly contributes to catalysis but is distinct from the metal ion responsible for leaving group stabilization at the 5′ splice site.

130. Channels in the RNA folding landscape. **Daniel Herschlag**. Department of Biochemistry, Stanford University, B400 Beckman Center, Stanford, CA 94305 (fax: 650-723-6783, herschla@cmgm.stanford.edu)

Results from exploration of the earliest events in RNA folding and from initial exploration of distinct regions of the RNA folding landscape will be presented. Small-angle X-ray scattering (SAXS) experiments have provided a time-resolved picture of the folding process of the *Tetrahymena* group I RNA enzyme over a time window of more than 5 orders of magnitude. Upon addition of Mg^{2+} to initiate folding, a substantial phase of compaction is observed on the low millisecond time scale, and the overall compaction and global shape changes are largely complete within 1 s. This process occurs faster than formation of the first stable tertiary structure, identifying new folding intermediates and indicating that this RNA searches for its tertiary contacts within a highly restricted subset of conformational space. We suggest that rapid electrostatic collapse is likely to be a general feature of the folding of structured RNAs. Structured RNAs, like proteins, achieve their active states by traversing complex energetic landscapes that are only beginning to be explored. We have followed the folding of the *Tetrahymena* ribozyme beginning from distinct regions within the landscape. This was accomplished by varying the solution conditions present prior to folding, with subsequent rapid buffer exchange to allow Mg^{2+}-induced folding to occur under constant conditions. Folding of individual ribozyme molecules was followed by single molecule fluorescence resonance energy transfer (smFRET). SAXS experiments and chemical protection experiments showed that changes in solution conditions give distinct starting ensembles. These ensembles then give distinct folding properties under identical conditions, strongly suggesting that folding proceeds through distinct channels. These observations begin to quantitatively map additional dimensions in a rugged energy landscape for folding of this RNA.

131. Factor-independent ribosomal translocation. Daniel Southworth, Anthony Cukras, Julie Brunelle, and **Rachel Green**. Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD 21205

During translation, the translocation of the tRNA−mRNA complex with respect to the ribosome is promoted by EF-G and is coupled to the energy of GTP hydrolysis. Interestingly, factor-independent translation has been observed in a number of biochemically modified ribosomal systems. First, factor-independent translation has been observed with salt-washed ribosomes that apparently lack the small ribosomal subunit protein S12 [Gavrilova, L. P., et al. (1974) *FEBS Lett. 45*, 324−328]. Ribosomes that have been modified with the thiol-specific reagent pCMB (*p*-chloromercuribenzoate) catalyze factor-independent translation [Gavrilova, L. P., and Spirin, A. S. (1971) *FEBS Lett. 17*, 324−326]. Last, streptomycin-resistant ribosomes carrying specific mutations in protein S12 have slightly enhanced rates of factor-independent translation [Asatryan, L. S., and Spirin, A. S. (1975) *FEBS Lett. 138*, 315−321]. The observation of factor-independent translation is thought to represent enhanced rates of spontaneous (EF-G independent) translocation in these systems. These data then suggest that specific ribosomal elements (such as protein S12) present barriers that prevent translocation from occurring spontaneously, thus coupling this step in translation to the energy of GTP hydrolysis. We are using biochemical approaches to characterize these systems more extensively. First, we are measuring the rates of translocation of the modified ribosomes in specific functional assays and are using translocation-specific antibiotics to confirm the authenticity of the translocation event. Further experiments are aimed at identifying the components in the ribosome critical to the complex movements of translocation. The identification of ribosomal proteins that are critical in modulating translocation may lead us to rRNA elements that are also involved in this event.

132. Structural basis for stability and activity of large RNAs. Jennifer Doudna. Department of Molecular Biophysics and Biochemistry, Yale University, Bass Building, Room 334, 266 Whitney Ave., New Haven, CT 06520 (fax: 203-432-3104, jennifer.doudna@yale.edu)

Many cellular RNAs form specific structures essential for function, often in complex with proteins or other ligands. Analysis of transfer, catalytic, in vitro selected, and ribosomal RNAs revealed that helical packing predominantly involves the interaction of single-stranded adenosines with a helix minor groove. The thermodynamic basis for the unusual stability of this motif stems from the near-perfect shape complementarity between the adenosine base and the minor groove. Recent crystal structures show how this mode of helix packing enables close approach of adjacent RNA strands while maintaining standard backbone geometries. These results provide a functional explanation for the noted abundance of conserved adenosines within the unpaired regions of RNA secondary structures.

Divergent Evolution of Enzyme Function Symposium—Tuesday Afternoon

133. Different reactions catalyzed by the same or similar active sites. John A. Gerlt. Departments of Biochemistry and Chemistry, University of Illinois, Urbana, IL 61801

We are studying the members of three mechanistically diverse groups of enzymes: the enolase superfamily, the crotonase superfamily, and the orotidine 5′-phosphate decarboxylase (OMPDC) suprafamily. Within each group, a conserved active site architecture is used to catalyze different reactions. The reactions catalyzed by the members of the enolase superfamily share stabilization of an enolate anion derived from a carboxylate anion, the members of the crotonase share stabilization of an enolate anion derived from a coenzyme A thioester, and the members of the OMPDC suprafamily catalyze reactions that do not share any mechanistic attribute. Recent results in our studies of these groups of enzymes will be discussed.

134. Evolution and global distribution on triazine-catabolic enzymes. Lawrence P. Wackett. Departments of Biochemistry, Molecular Biology, and Biophysics and The Biological Process Technology Institute, University of Minnesota, 140 Gortner Laboratory, St. Paul, MN 55108

Triazine compounds are used in industry and agriculture. Their fate in the environment is predicated on the presence microbial catabolic enzymes. The common triazine herbicide atrazine was considered to be poorly biodegradable until recently. Atrazine is catabolized via an initial dechlorination reaction catalyzed by atrazine chlorohydrolase. The chlorohydrolase is a member of the amidohydrolase superfamily which includes adenosine deaminase and urease. Recently, the triazine melamine was shown to be catabolized by melamine deaminase, an enzyme with 98% amino acid sequence identity to atrazine chlorohydrolase. Yet the enzymes show no overlap in substrate specifity. Identical atrazine-catabolic genes have now been isolated from four continents, further suggesting a recent evolutionary origin and spread of the atrazine chlorohydrolase gene. Random and directed mutagenesis studies have been conducted to investigate the evolutionary potential of proteins similar to atrazine chlorohydrolase and melamine deaminase.

135. Structural studies of enzymes belonging to the crotonase superfamily. Hazel M. Holden. Department of Biochemistry, University of Wisconsin, 433 Babcock Dr., Madison, WI 53706 (fax: 608-262-1319, Hazel_Holden@biochem.wisc.edu)

The crotonase (or enoyl-CoA) hydratase superfamily contains members that catalyze a wide range of metabolic reactions, including hydration, dehalogenation, and isomerization. Other members of the group have been implicated in carbon−carbon bond formation and cleavage as well as the hydrolysis of thioesters. While seemingly unrelated mechanistically, the common theme among members of the superfamily is the need to stabilize an enolate anion intermediate derived from an acyl-CoA substrate. This is accomplished by two structurally conserved peptidic NH groups, which provide hydrogen bonds to the carbonyl moieties of the acyl-CoA substrates and form an "oxyanion" hole. This laboratory has solved the three-dimensional structures of a number of members of this superfamily, including 4-chlorobenzoyl-CoA dehalogenase, methylmalonyl-CoA decarboxylase, and 2-ketocyclohexanecarboxyl-CoA hydrolase. The structures of these enzymes will be described and compared.

136. From sequence space to mechanism space in a metalloenzyme superfamily. Richard N. Armstrong. Department of Biochemistry, Vanderbilt University, Nashville, TN 37232 (r.armstrong@vanderbilt.edu)

The relationships of proteins—particularly enzymes—within a protein superfamily can be understood not only in terms of the sequence and conformational space they occupy but also by chemical threads that relate their functional attributes. This latter aspect we now refer to as mechanism space. This lecture will present an analysis of a metalloenzyme superfamily, the members of which catalyze a very diverse set of reactions with unrelated transition states but a more general common mechanistic imperative. The vicinal oxygen chelate (or VOC) superfamily are structurally related proteins with paired babbb motifs that provide a metal coordination environment with two or three open or readily accessible coordination sites to promote direct electrophilic participation of the metal ion in catalysis. The known types of reactions catalyzed include isomerizations, epimerizations, oxidative cleavage of C−C bonds, and nucleophilic substitutions. The remarkable access to mechanism space that is provided by the VOC superfamily appears to derive from a simple, pseudo-symmetric structural fold that maximizes the catalytic versatility of the metal center. (Supported by NIH Grant AI42756.)

137. Mapping active site space and reactivity in complex proteomes. Benjamin F. Cravatt,[1] Gregory C. Adam,[2] Yongsheng Liu,[3] and Erik J. Sorensen.[4] [1]Departments of Cell Biology and Chemistry and Skaggs Institute for Chemical Biology, [2]Department of Chemistry, [3]Department of Cell Biology, and [4]The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd., BCC 128, La Jolla, CA 92037 (fax: 858-784-2345, cravatt@scripps.edu)

The field of proteomics aims to characterize dynamics in protein function on a global scale. However, several classes of enzyme are subject to posttranslational forms of active site-directed regulation, limiting the utility of conventional proteomics techniques for the characterization of these proteins. Recently, we have initiated a research program aimed at generating chemical probes that interrogate the status of enzyme active sites in crude proteomes, thereby facilitating the functional characterization of enzymes in samples of high complexity. We will describe our efforts to map active site space and structure for several enzyme classes that collectively segregate into two general categories: (1) enzymes for which proteomics-compatible, active site-directed affinity agents are well-defined, and (2) enzymes for which proteomics-compatible, active site-directed affinity agents are currently lacking.

Protein Biosynthesis at High Resolution Symposium—Tuesday Afternoon

138. Exploring the chemical mechanism of ribosomal RNA catalyzed protein synthesis. Scott A. Strobel. Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114

Ribosomes are responsible for converting all coding information contained within the DNA genome into functional proteins. The ribosomal machinery is comprised of RNA and protein components, but recent structural evidence demonstrates that the ribosomal RNA comprises the ribosome's catalytic or peptidyl transferase center (*3*, *5*). Crystallographic and biochemical evidence suggests that a single adenosine (A2451) within the ribosomal active site is the most likely candidate for participation in RNA catalysis of protein synthesis. To explore the mechanism of this reaction, we screened for nucleotides in *E. coli* 23S rRNA that may have a perturbed pK_a based upon the pH dependence of dimethyl sulfate modification. We found that a single universally conserved A (2451) within the central loop of domain V has a near-neutral pK_a of 7.6 ± 0.2, which is approximately the same as that reported for the peptidyl transferase reaction. This is the same residue that is closest to the tetrahedral intermediate within the ribosome crystal structure. These results are consistent with a mechanism wherein the nucleobase of A2451 could serve as a general acid−base during peptide bond formation. Similar analyses of ribosomes from yeast show that the unusual acidity of the active site adenosine is a conserved feature of the ribosome. To investigate this mechanism in chemical detail, we have developed an improved version of the classical fragment assay and are working to establish a kinetic profile for the reaction. The assay utilizes two synthetic substrates, a biotinylated P-site substrate and a puromycin-derived A-site substrate. Unlike the previous version of the fragment assay, this reaction proceeds efficiently at 37 °C in the absence of methanol. The reaction is dependent upon the A-site and P-site substrates and intact 50S ribosomal subunits. It is inhibited by the antibiotic chloramphenicol. Under single turnover conditions, the reaction follows a single exponential decay with an endpoint of less than 2% unreacted. These simple substrates make it possible to kinetically dissect the steps involved in peptide bond formation.

139. Structure and function in the large ribosomal subunit. J. Hansen,[1] D. Klein,[1] M. Schmeing,[1] J. Ippolito,[1] P. Nissen,[1,†] N. Ban,[1,‡] **P. B. Moore**,[1,2] and T. A. Steitz.[1,2,3] [1]Department of Molecular Biophysics and Biochemistry, [2]Department of Chemistry, and [3]Howard Hughes Medical Institute, Yale University, New Haven, CT 06520-8107

In 2000, an atomic resolution structure for the large subunit from *Haloarcula marismortui* was published that included 2833 of its 3045 nucleotides and 27 of its 31 proteins [Ban et al. (2000) *Science 289*, 905−920], and results reported on large subunit complexes with peptidyl transferase substrate and transition state analogues showed that the ribosome is a ribozyme [Nissen et al. (2000) *Science 289*, 920−930]. Refinement has now revealed the positions of more than 7000 water molecules and about 200 cations that associate with the subunit. The free-*R* factor of the structure is now about 21%. The structure is stabilized by RNA/cation interactions, RNA/RNA interactions, and protein/RNA interactions. A new RNA/RNA interaction, called the "A-minor motif", stabilizes 23S rRNA tertiary structure in many places. The cross-linking of 23S rRNA sequences by the extended and highly basic tails of several ribosomal proteins also makes an important contribution. The binding sites of nine ribosomal proteins include a novel RNA secondary structure motif called the "kink-turn". In addition, structures have been obtained for complexes of the large subunit with several new

peptidyl transferase substrates and substrate analogues. The data indicate that these substrates react to form peptide bonds in the crystals under investigation. Finally, substantial progress has been made in our crystallographic study of the interactions of antibiotics with the large ribosomal subunit, many of which bind in the neighborhood of the peptidyl transferase site. (†Present address: Department of Molecular and Structural Biology, Aarhus University, Aarhus, DK-8000, Denmark. ‡Present address: Institute for Molecular Biology and Biophysics ETHZ, ETH Honggerberg, CH-8093, Zurich, Switzerland.)

140. Testing a possible origin for translation/coded protein biosynthesis. Michael Yarus. Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347

On the hypothesis that an ancestral era existed when RNAs were the principal biocatalysts (an RNA world), one inevitably predicts the performance of the reactions of translation by RNAs. It is now possible to experimentally test this possibility. In fact, RNAs that carry out all four required translational reactions (coding, activation, aa-RNA synthesis, and peptidyl transferase) can be selected from pools of randomized oligoribonucleotide sequences. Therefore, the invention of protein biosynthesis in an RNA world has strong experimental support. Notable among these RNA reactions is peptidyl transferase, which, uniquely, is still a ribozyme reaction in modern biology. Even more significantly for its origins, most of the universal peptidyl transferase active site can apparently be (re)derived by a modern selection from randomized RNA sequences.

Chemical and Biochemical Aspects of Proteins Involved in Cell Signaling Symposium—Wednesday Morning

141. Chemical approaches to tyrosine phosphorylation. Philip A. Cole. Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205 (pcole@jhmi.edu)

Protein tyrosine kinases catalyze the transfer of a phosphoryl group from ATP to the tyrosine side chains of proteins and play important roles in signal transduction. We will report on our progress to apply a variety of chemical synthesis and kinetic methods to elucidate the structure, function, and regulation of tyrosine kinases and the substrates that they phosphorylate. Relevance of our findings to cell signaling and/or drug design will be discussed.

142. Signaling from Rho GTPases to actin: Insights from NMR into biology. Annette S. Kim, Matthias Buck, Norzehan Abdul-Manan, and **Michael K. Rosen**. Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021

Many GTPases in the Ras superfamily transmit signals through allosteric activation of downstream effector molecules. The Rho-family GTPase, Cdc42, can signal to the actin cytoskeleton through effectors in the Wiskott−Aldrich Syndrome Protein (WASP) family. Interaction with Cdc42 appears to relieve an autoinhibitory contact between the GTPase binding domain (GBD) and C-terminal region of WASP family members, enabling them to stimulate the Arp2/3 actin nucleating complex. We have determined the structures of both autoinhibited WASP (a GBD−VCA complex) and activated WASP (GBD in complex with Cdc42). Comparison of the structures indicates that the hydrophobic core of the autoinhibited GBD fold must be destroyed to bind the GTPase. This dramatic conformational change causes release of the C-terminus, separating it from the GBD, and forms the basis of WASP activation by Cdc42. The structural model is confirmed by the ability of Cdc42 to catalyze exchange of amide protons from the autoinhibited GBD−VCA complex, and by thermodynamic measurements of GTPase binding. Activation mechanisms involving disruption of folded domains may be widely used by GTPase effectors, with implications in biophysics, signal transduction, and medicine.

143. Signal transduction from cytosolic calcium to nuclear chromatin remodeling. Jun O. Liu. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The second messenger calcium plays a pivotal role in transmitting signals from the cytosol into the nucleus to regulate gene expression in a variety of physiological processes including T cell receptor-mediated T lymphocyte apoptosis. How calcium signal is transmitted to activate the transcription of the critical genes involved in T cell apoptosis has remained largely unknown. Recently, we found that calcium signal is transmitted via at least two parallel pathways. One pathway comprises the calcium-dependent protein phosphatase calcineurin and its substrate NFAT. And the other involves a transcriptional corepressor, Cabin1 (Cain), and its associated histone deacetylases. The two pathways are integrated in the nucleus by a transcription factor named MEF2, leading to the full activation of a proapoptotic target gene. Some recent insights into the mechanism of regulation of chromatin remodeling by calcium will be discussed.

144. Structural and computational studies of the inactivation mechanisms of protein kinases. John Kuriyan. Howard Hughes Medical Institute, The Rockefeller University, 1230 York Ave., New York, NY 10021 (kuriyan@earthlink.net)

The Src family tyrosine kinases are controlled by phosphorylation on two distinct tyrosine residues. Phosphorylation on one, Tyr 527, reduces the activity of the catalytic tyrosine kinase domain, whereas phosphorylation on the other (Tyr 416) stabilizes the active conformation of the kinase. Tyr 527 is located within the C-terminal tail of the kinase, and it interacts with the protein's SH2 domain when it is phosphorylated. This interaction between the SH2 domain and the phosphorylated tail somehow changes the structure of the active site such that the centrally located "activation loop" bearing the unphosphorylated Tyr 416 folds into the catalytic center, leading to inactivation. We have used X-ray crystallography, molecular dynamics simulations, and mutagenesis to study the nature of the coupling between the C-terminal tail and the active site of the Src kinases. An important component of the regulatory mechanism operating in these proteins is the ability of the linker between the SH2

and SH3 domains to act as an "inducible snaplock". When the SH2 and SH3 domains are bound internally, they are coupled together dynamically so that they act as a relatively rigid unit that impedes activating conformational transitions in the catalytic domain. However, when released from their internal engagement, the linker between them melts and allows domains to move flexibly with respect to each other. The importance of flexibility in the catalytic domain of protein kinases for the development of specific inhibitors will also be discussed, using the mode of action of the Novartis inhibitor of the Abelson tyrosine kinase as an example.

Chemical Mechanisms of Oxidative DNA Damage and Repair Symposium—Wednesday Morning

145. Mechanisms of oxidative DNA damage by the antitumor agent 3-amino-1,2,4-benzotriazine 1,4-dioxide (tirapazamine): A lesson in how to kill hypoxic tumor cells. Kent S. Gates. Department of Chemistry, University of Missouri, Columbia, MO 65211 (fax: 573-882-2754, gatesk@missouri.edu)

Agents that cause oxidative DNA damage (e.g., ionizing radiation, bleomycin) have proven useful in cancer therapy. The recently developed antitumor agent 3-amino-1,2,4-benzotriazine 1,4-dioxide (tirapazamine) causes oxidative DNA damage selectively in oxygen-poor (hypoxic) tumor cells. Here we will consider the chemical properties that endow tirapazamine and other heterocyclic *N*-oxides with their useful medicinal activities.

146. Biochemical characterization of DNA containing C-1′ oxidized abasic site lesions. Hamilton J. Lenox and **Terry L. Sheppard**. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113

Damage of DNA by reactive oxygen species (ROS) produces nucleobase and sugar lesions that compromise the coding potential and strand integrity of DNA. Hydrogen abstraction at C-1′ of the sugar portion of nucleotides produces deoxyribonolactone (or oxidized abasic site) lesions in DNA. The biological effects of these sugar lesions remain largely unexplored. To gain insight into the chemical biology of these oxidative DNA damage events, a method for the site-specific generation of oxidized abasic sites within DNA strands has been developed. A stable, photochemically caged form of the lactone lesion was introduced into DNA by solid-phase DNA synthesis. Photolytic decaging was used to produce DNA oxidized abasic sites with high efficiency. Using this approach, the biochemical properties of the deoxyribonolactone lesion, such as biological half-life, have been explored and will be described.

147. Probing the effects of oxidative stress on DNA using chemically synthesized modified oligonucleotides. Marc M. Greenberg. Department of Chemistry, Colorado State University, Fort Collins, CO 80523

Exposure of DNA to a variety of oxidants produces damaged nucleotides and strand breaks. DNA damage is involved in aging and in a number of disease states, such as the etiology and treatment of cancer. A variety of DNA damage processes involve radical intermediates. Some DNA damaging agents (e.g., ionizing radiation) generate a myriad of radical intermediates. In turn, multiple lesions can be formed from individual reactive intermediates. To study these important processes, we probe the reactivity of individual reactive intermediates, as well as the structural and functional effects of lesions that are produced or incorporated at defined sites, respectively, in chemically synthesized oligonucleotides. Recent advances and discoveries in the area of DNA damage chemistry using this approach will be described.

148. Preventing mutagenesis associated with 8-oxoguanine: Recognition and repair properties of the base-excision repair enzymes MutY and Fpg. S. S. David, M. D. Leipold, R. P. Hickerson, C. L. Chepanoske, S. D. Williams, J. G. Muller, and C. J. Burrows. Department of Chemistry, University of Utah, Salt Lake City, UT 84112

The base-excision repair (BER) pathway recognizes and repairs aberrant DNA bases. Two *E. coli* BER enzymes, MutY and Fpg, play an important role in preventing DNA mutations caused by the oxidatively damaged lesion 7,8-dihydro-8-oxo-2′-deoxyguanosine (OG). Fpg catalyzes the removal of OG while MutY removes misincorporated adenines from OG:A mispairs. We have found that Fpg will remove products arising from oxidation of OG, and is quite sensitive to the base opposite the oxidized OG base. In contrast, MutY does not remove adenine opposite these oxidized OG lesions. This suggests that these lesions may contribute to the mutagenesis associated with OG. The ease of oxidation of OG can also be used as a tool to probe protein—DNA interactions. Indeed, oxidation of OG in the presence of MutY provides a specific MutY—DNA cross-link that has implicated a specific lysine residue in OG recognition. These and other results will be further discussed.

149. Unraveling the complexity of oxidative DNA damage: Formation of base adducts from products of deoxyribose oxidation. Peter C. Dedon. Bioengineering and Environmental Health, MIT, 56-787, Cambridge, MA 02139 (fax: 617-258-0225, pcdedon@mit.edu)

Free radicals attack both the nucleobases and deoxyribose in DNA, with the strand breaks arising from the latter generally viewed as terminal products. However, oxidation of each position in deoxyribose yields a unique spectrum of aldehyde- and ketone-containing species that, by analogy to other endogenous and exogenous electrophiles, should be capable of forming adducts with nucleobases. In support of this hypothesis, we demonstrated that the base propenal arising from 4′-oxidation of deoxyribose reacts with guanine to form the pyrimidopuranone adduct, M1G, while oxidation of the 5′-position generates butenedialdehyde that reacts with cytosine to form a bicyclic adduct. More recent studies have revealed that deoxyribose oxidation by Fe-EDTA results in the formation of 3′-phosphoglycolaldehyde residues. This electrophile then undergoes a novel phosphate—phosphonate rearrangement to form glyoxal and, subsequently, the 1,N^2-glyoxal adduct of guanine. The phenomenon of strand breaks leading to base adducts suggests a novel source for endogenous DNA adducts as well as a complicating factor in understanding lesion-clustering associated with radiation-induced DNA damage.

Mechanistic and Structural Studies of Cofactor Biosynthetic Enzymes Symposium—Wednesday Afternoon

150. The mechanistic enzymology of vitamin B6 (pyridoxine) biosynthesis. David E. Cane. Department of Chemistry, Box H, Brown University, Providence, RI 02912-9108

Vitamin B6 is a relatively simple molecule, with a single heterocyclic ring, no more than seven carbon atoms, and no chiral centers, which plays a central role in amino acid metabolism. Although the biochemical details of B6-dependent reactions have been the subject of intensive study, the details of the biosynthesis of B6 itself have remained obscure until recently, with little or no information about the enzymology of the pyridoxine ring formation, or even the precise structure of the main building blocks. We have recently shown that in *Escherichia coli* the product of the *pdxA* gene catalyzes the NAD^+-dependent oxidative decarboxylation of 4-hydroxythreonine phosphate (4-HTP) to a compound tentatively identified as aminohydroxyacetone phosphate (AHAP). A second enzyme, pyridoxine synthase, encoded by the *pdxJ* gene, then catalyzes the condensation of AHAP with 1-deoxyxylulose phosphate (dXP) to generate pyridoxol phosphate and 1 equiv of inorganic phosphate. Further experiments have established the origin of the oxygen atoms of vitamin B6 and other details of the ring-forming mechanism.

151. *Escherichia coli* LipA is a lipoyl synthase. J. R. Miller,[1] R. W. Busby,[1] S. W. Jordan,[2] J. Cheek,[3] T. F. Henshaw,[3] G. W. Ashley,[3] J. B. Broderick,[3] J. E. Cronan, Jr.,[2] and **M. A. Marletta**.[1,4] [1]Howard Hughes Medical Institute, [4]Department of Biological Chemistry, and [4]Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109-0606, [3]Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322, and [2]Departments of Microbiology and Biochemistry, University of Illinois at Urbana−Champaign, Urbana, IL 61801

The *Escherichia coli lipA* gene product has been genetically linked to carbon−sulfur bond formation in lipoic acid biosynthesis [Vanden Boom, T. J., Reed, K. E., and Cronan, J. E., Jr. (1991) *J. Bacteriol. 173*, 6411−6420] although in vitro lipoate biosynthesis with LipA has never been observed. The *lipA* gene and a hexahistidine-tagged *lipA* construct (LipA-His) were overexpressed in *E. coli* as soluble proteins. Electron paramagnetic resonance spectroscopy and iron and sulfur stoichiometry data indicate that the proteins contain a mixture of [3Fe-4S] and [4Fe-4S] clusters. Reduction of LipA with sodium dithionite results in small quantities of an $S = 1/2$ $[4Fe\text{-}4S]^{1+}$ cluster with the majority of the protein containing a species consistent with an $S = 0$ $[4Fe\text{-}4S]^{2+}$ cluster. LipA was assayed for lipoate biosynthesis using octanoyl acyl carrier protein and *E. coli* lipoyl-[acyl carrier-protein]-protein-*N*-lipoyltransferase (LipB) to lipoylate apopyruvate dehydrogenase complex (apo-PDC) [Jordan, S. W., and Cronan, J. E. (1997) *Methods Enzymol. 279*, 176−183]. When sodium dithionite reduced LipA was incubated with octanoyl-ACP, LipB, apo-PDC, and *S*-adenosylmethionine (AdoMet) formation of lipoylated PDC, methionine, and 5′-deoxyadenosine was observed. Similar results were obtained when octanoylated PDC was incubated with reduced LipA, suggesting that the substrate of LipA is octanoylated E2 protein. Confirmation that LipA catalyzes formation of lipoylated E2 protein was obtained by MALDI mass spectrometry of a recombinant PDC lipoyl-binding domain that had been lipoylated in a LipA reaction. These results provide information about the mechanism of LipA catalysis and place LipA within the family of iron−sulfur proteins that utilize AdoMet for radical-based chemistry.

152. Functional genomics and enzymology of NAD biosynthesis. Andrei Osterman and Oleg Kurnasov. Integrated Genomics Inc., Chicago, IL

Comparative analysis of genome sequences reveals significant variation of NAD biosynthetic pathways among different organisms. For example, out of the four *E. coli* pathways: (i) de novo biosynthesis; (ii) niacin salvage; (iii) common steps (from quinolinate to NAD); and (iv) NMN salvage, only the latter is present in *H. influenzae*. Reconstruction of NAD metabolism from genomic data suggests that almost all types of living cells require nicotinate/nicotinamide mononucleotide adenylyltransferase (NMNAT) for the completion of NAD biosynthesis. When this study was initiated, only the archaeal version of the NMNAT gene was identified. We used the analysis of chromosomal clustering to predict the gene encoding bacterial NMNAT. This prediction was verified by expression, purification, and characterization of NMNAT orthologs from diverse bacterial species. All bacterial NMNATs display strong preference (up to 15 000-fold) for the deamidated form of the substrate (NaMN) over NMN. The reverse substrate preference is characteristic of archaeal NMNATs. We have also identified and characterized genes encoding two isoforms of human NMNAT. Both human NMNATs show no preference for either form of the substrate. Comparison of 3D structures of archaeal and bacterial enzymes sheds light on the structural basis of substrate specificity in the NMNAT superfamily. The central domain of the multifunctional NadR protein from *E. coli* represents another diverse NMNAT subfamily with strong preference (>200-fold) for NMN over NaMN. We have shown that the C-terminal domain of NadR constitutes a highly specific nicotinamide ribose kinase. This enzymatic activity was previously described, but a corresponding gene was unknown. Analysis of NadR domain organization allows us to suggest a new interpretation of the unique NAD metabolism in *H. influenzae*. We used gene deletion and disruption techniques to probe for the essentiality of various types of NMNAT genes in various metabolic contexts including *E. coli*, *H. influenzae*, *S. aureus*, and *Synechocystis* sp. Along with a verification of NAD biosynthetic pathways, these experiments provide a validation for our ongoing search of NMNAT inhibitors, potentially leading to a development of new broad-spectrum anti-infectives.

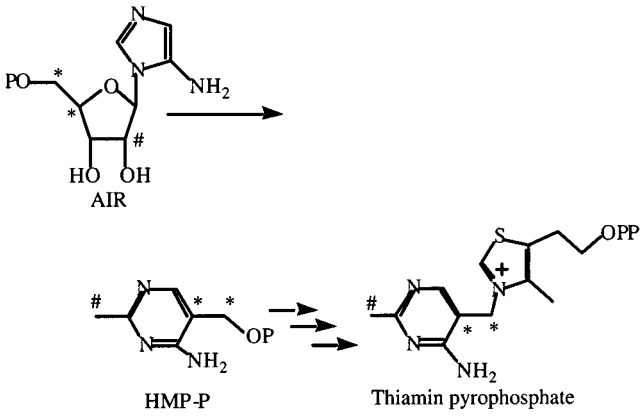
153. Synthesis and use of 5,6-dimethylbenzimidazole during adenosylcobalamin (coenzyme B_{12}) synthesis in *Salmonella enterica*. J. C. Escalante-Semerena. University of Wisconsin, Madison, WI 53706

5,6-Dimethylbenzimidazole (DMB) is the lower ligand base of cobalamin (Cbl, B_{12}). Although it has been known for over 30 years that DMB can be derived from FMN, the metabolic route explaining this conversion has remained speculative. Evidence is presented in support of the hypothesis that the conversion of FMN to DMB proceeds largely via an abiotic pathway. Under physiological conditions (pH

7, 37 °C), 4,5-dimethylphenylenediamine reacted with ribose 5-phosphate to yield DMB. The reaction required molecular oxygen, and product was detectable within 1 h. Results from labeling studies using [^{14}C, C-1]ribose indicated that the C-1 of ribose was the sole source of the C-2 carbon of DMB. The conversion of FMN to DMB may require the activity of a single FMN-dependent oxygenase enzyme. In vitro and in vivo evidence suggests that DMB is activated into a new dinucleotide referred to as α-DMB, adenine dinucleotide, or α-DAD.

154. Thiamin biosynthesis in bacteria. Tadhg Begley and Ryan A. Mehl. Department of Chemistry and Chemical Biology, Cornell University, 120 Baker Laboratory, Ithaca, NY 1485 (tpb2@cornell.edu)

The formation of the pyrimidine moiety of thiamin involves a very complex rearrangement of aminoimidazole ribotide (AIR) to give 2-methyl-4-amino-5-hydroxymethylpyrimidine phosphate (HMP-P). This lecture will describe the first successful reconstitution of this reaction in a cell-free system.



155. Menaquinone and ubiquinone biosynthesis. R. Meganathan. Department of Biological Sciences, Northern Illinois University, Castle Dr., DeKalb, IL 60115 (rmeganathan@niu.edu)

Menaquinone (MK) and ubiquinone (Q) are derived from the shikimate pathway. MK is considered a vitamin (vitamin-K2) since it cannot be synthesized by mammals whereas Q is not considered a vitamin as it can be synthesized from the essential amino acid tyrosine. In the formation of the quinonoid nuclei, the pathways diverge at the chorismate branch point of the shikimate pathway. The MK biosynthetic pathway is: chorismate→isochorismate→SHCHC→*o*-succinylbenzoate (OSB)→OSB-CoA→DHNA-CoA→DHNA→DMK→MK. Orthologs of *o*-succinylbenzoate synthase (MenC) from different organisms show very little homology. The three-dimensional stucture of MenC from *E. coli* has been determined. Two conserved catalytically important lysine residues have been identified by site-directed mutagenesis. Bacteria form the 4-hydroxybenzoate (4-HB) required for Q biosynthesis directly from chorismate whereas in eukaryotes tyrosine is the precuror. The reactions in the tyrosine→4-HB conversion are not known. The 4-HB undergoes a prenylation, a decarboxylation, three hydroxylations, and three methylations resulting in Q.

156. Biosynthesis of methanogenic cofactors. Robert H. White. Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061 (fax: 540-321-9070, rhwhite@vt.edu)

The current state of our work on establishing the pathways and enzymes/genes involved in the biosynthesis of the methanogenic coenzymes will be presented. The coenzymes to be discussed will include coenzyme M, methanofuran, methanopterin, coenzyme F420, and coenzyme B. The study of the biosynthesis of each these coenzymes has revealed new biochemistry both in terms of the reactions catalyzed as well as in the identification of new genes/enzymes involved in the biosynthetic pathways. In the coenzyme M pathway, we have characterized the enzyme that catalyzed the Michael addition of bisulfite to phosphoenolpyruvate and an enzyme that is a member of a new class of magnesium-dependent acid phosphatases that hydrolyzed the product of this reaction. In the F420 pathway, we have identified four new enzymes, one of which is the first example of a nucleotidyltransferase that proceeds through a phosphorylated serine intermediate. These as well as other examples will be discussed.

157. Biosynthesis of the molybdenum cofactor: a conserved pathway with interesting evolutionary relationships. Michael W. Lake,[1] Michael J. Rudolph,[1] Nils Schrader,[1] Guenter Schwarz,[1] Song Xiang,[1] Margot M. Wuebbens,[2] K. V. Rajagopalan,[2] and **Hermann Schindelin**.[1] [1]Department of Biochemistry and Center for Structural Biology, SUNY Stony Brook, Stony Brook, New York 11794-5115, and [2]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

The molybdenum cofactor (Moco) is an essential component of a diverse group of enzymes catalyzing important redox transformations in the global carbon, nitrogen, and sulfur cycles. Moco consists of a mononuclear molybdenum coordinated by the dithiolene moiety of a family of tricyclic pyranopterins containing a *cis*-dithiolene group in their pyran ring. This tricyclic pyranopterin is commonly referred to as molybdopterin (MPT) and the Mo−MPT complex as Moco. Biosynthesis of the Mo/W-cofactor is an evolutionarily conserved pathway, and genes involved in Moco biosynthesis have been identified in eubacteria, archaea, and eukaryotes. Although some details of Moco biosynthesis are still unclear at present, the pathway can be divided into three steps. (i) Early steps in which a guanosine derivative, most likely GTP, is converted into precursor Z. (ii) Transformation of precursor Z into molybdopterin. This process generates the dithiolene group responsible for coordination of the molybdenum atom in the cofactor. (iii) Metal incorporation into the apo-cofactor. We have determined the high-resolution crystal structures of most of the proteins involved in Moco biosynthesis in *Escherichia coli*. The resulting models have provided important insights into the function of these proteins. Of particular importance are the studies on the second and third steps in this pathway, which transcend the field of Moco biosynthesis and provide valuable information about the mechanisms of ubiquitin activation and anchoring of inhibitory neuronal receptors, respectively.

Significant Contributions to Biological Chemistry over the Last 125 Years Symposium—Wednesday Afternoon

158. Landmark discoveries in the trail from chemistry to cellular biochemistry (1876−ca. 1950). Howard Gest. Departments of Biology and of History & Philosophy of Science, Indiana University, Bloomington, IN 47405

Molecular oxygen was discovered in 1775 by the self-taught English polymath Joseph Priestley, who emigrated to America and settled in Northumberland, PA, in 1794. In addition to numerous other contributions to chemistry, Priestley made the important observations that O_2 is produced by plants and that gases mediate the interdependence of plant and animal life. The O_2 generated by green plant photosynthesis is required for energy-yielding animal respiration, and the latter process furnishes a major requirement for photosynthesis, CO_2. In 1874, 77 chemists met at Priestley's PA home to commemorate Priestley's discoveries, and this led to the establishment of the ACS in 1876. Priestley's biological experiments dealt with aerobes, the most advanced forms of life. It was the research of Louis Pasteur, however, on the fermentation of sugars by anaerobic microbes (1861−1876) that provided a focus on systems that were the first to reveal significant clues to the biochemical basis of bioenergetics. Analysis of the detailed mechanism of anaerobic sugar fermentation by yeast during the first third of the 20th century, and the later study of energy-yielding respiration with O_2, required use of many new techniques. The latter became the tools that were exploited to yield the basic outlines of cell biochemistry. These included: chromatography, metabolic gas manometry, spectrophotometry, the use of stable and radioactive isotopes as tracers of intermediary metabolism, and procedures for purification of proteins and other macromolecules. Our knowledge of cellular biochemistry increased dramatically during the period 1925−1950. Noteworthy advances included discovery and characterization of: the (Krebs) citric acid cycle, "activated" intermediates such as acetyl coenzyme A, electron carriers and coenzymes necessary for energy conversion and reductive biosynthesis, ATP—the universal energy "currency", and a multitude of enzymes involved in catabolic and biosynthetic metabolism. In this lecture, a "time-line" of pioneering researches will be presented.

159. Biochemistry in the United States in the first half of the twentieth century. Mildred Cohn. Department of Biochemistry and Biophysics, University of Pennsylvania, 242 Anat/Chem, Philadelphia, PA 19104-6059 (fax: 215-898-4217)

By the 1930s, biochemical research in the United States witnessed a transition from emphasis on nutrition and analytical methods in medicine to chemistry of proteins and intermediary metabolism. Research in the 30's and 40's focused on the transformations of small molecules, including the elucidation of glycolysis, the urea and citric acid cycles, and biological oxidations. Outstanding influences on current research include isotopic tracers, X-ray crystallography of proteins, amino acids, and small peptides, synthesis of a macromolecule, glycogen, in a cell-free system, and isolation of active and inactive forms of glycogen phosphorylase which led later to the discovery of protein phosphorylation and of cyclic AMP. Interest in nucleic acid began when its genetic role was established in 1944. The origins before 1950 of some currently important methodologies including chromotographic and electrophoretic separations, isotopes, mass spectrometry, and computer simulation will be discussed.

160. Surprise! Biochemistry is simpler and more complex than we thought. Perry A. Frey. University of Wisconsin−Madison, 1710 University Ave., Madison, WI 53705

Biochemical knowledge in 1950 will be described in general terms and contrasted with the current state of the field. Major advances in nucleic acid and protein chemistry, as well as complex carbohydrates will be outlined. Advancement of the field proceeded both incrementally and with great bounds and not a few surprises. This lecture will focus on the surprising turns in biochemistry over the past 50 years. These developments were brought about by the scholarship of visionary scientists, whose work fueled the growth of biochemical knowledge. It began with the elucidation of the astonishing double helical structure of DNA, which immediately changed the course of chemistry and genetics and all allied fields. Other surprises in nucleic acid and protein chemistry potentiated the growth of biochemistry and brought the science into the fields of pharmacology, physiology, immunology, microbiology, and medicine and led to the creation of biotechnology.

161. Structural enzymology over the last 40 years. Gregory A. Petsko. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, PO Box 549110, Waltham, MA 02454 (petsko@brandeis.edu)

A hallmark of biochemical research during the past 40 years has been the use of three-dimensional structure information, from NMR or, more commonly, X-ray crystallography, as a guide to understanding how enzymes function and for the planning and interpretation of other experiments. The field of structural enzymology began in the 1960s when David C. Phillips and his associates determined the three-dimensional structure of hen-egg-white lysozyme and its complexes with inhibitors and from that structural information deduced the factors responsible for the enzyme's catalytic power. I will review that seminal research and then discuss the current state of the field, which has grown to encompass enzymes as large as the ribosome and is able to determine the structures of productive enzyme−substrate complexes and catalytic intermediates by time-resolved techniques.

General Poster Session—Wednesday Evening

162. 2′-Mercaptonucleosides as RNA structure/function probes. Jason P. Schwans, Cecilia N. Cortez, and Joseph A. Piccirilli. Department of Biochemistry & Molecular Biology and Chemistry, HHMI, University of Chicago, Chicago, IL 60615

The unique physiochemical properties of sulfur render it one of the most powerful tools for exploring the structure and function of nucleic acids. To exploit these properties in probing the role of the 2′-hydroxyl group in RNA-mediated processes, we have synthesized a series of 2′-mercapto-5′-*O*-(1-thio)-nucleoside triphosphates for use in nucleotide analogue interference mapping (NAIM) experiments. The 2′-deoxy-2′-*tert*-butyl disulfide protected nucleosides of cytidine, uridine, adenosine, and inosine were synthesized and

converted to their α-thiotriphosphates in a one-pot, two-step reaction. Reduction of the disulfide by DTT provided the α-thiotriphosphates. The analogues are substrates for the Y639F mutant T7 RNA polymerase, providing the potential to replace every 2′-hydroxyl group with a mercapto group in an individual RNA. Using the *Tetrahymena* ribozyme as a model system, we incorporated these nucleoside derivatives randomly by in vitro transcription. NAIM experiments have revealed sites of interference that in some cases are distinct from those arising from 2′-deoxy and 2′-methoxy substitution. We are currently investigating the molecular basis of this interference.

163. Binding studies of bulged DNAs with an antitumor drug. Patricia Jackson and Catherine Yang. Department of Chemistry, Rowan University, Bosshart Hall, 201 Mullica Hill Rd., Glassboro, NJ 08028

The binding of the isolated Neocarzinostatin derivative to bulged DNAs results in alterations in ellipsicity of both the DNAs and the drug. Spectroscopic evidences suggest that the drug specifically recognizes DNA bulges by using a combination of conformational selection and induced fit, not by binding to a preorganized site. Analysis of the circular dichroism spectrum indicates that the degree of induced fit observed is primarily due to an optimizing of van der Waals contacts with the walls of the bulge cavity. This effective recognition of bulge DNA appears to depend, to a significant extent, on the bent narrow space being flexible enough to adopt a geometrically optimal conformation that is compatible with the wedge-shaped drug molecule, rather than involving 'lock and key' recognition. This process is truly interactive in that the conformation of the drug also changes as it adapts to the conformation of the bulged DNA. Spectroscopic studies with various bulged DNAs also reveal that binding strength directly correlates with bulge stability.

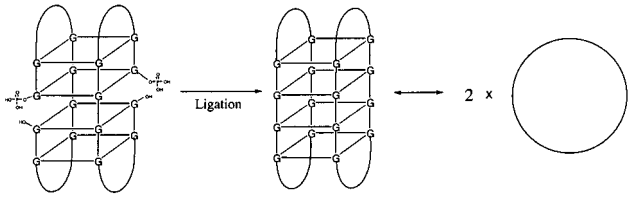
164. Chemoenzymatic synthesis of DNA−carbohydrate conjugates. Yingli Wang and Terry L. Sheppard. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113

Cell surface glycoconjugates play a central role in cellular recognition of exogenous proteins, neighboring cells, and pathogens. The specific binding of glycoconjugates by carbohydrate binding proteins, called lectins, mediates diverse processes ranging from fertilization to inflammation. Glycoconjugates such as glycolipids and glycoproteins are synthesized in the cell with controlled stereochemistry and regiochemistry by glycosyl transferase enzymes. Previous studies have demonstrated that enzymatic glycosylation of synthetic peptides provides a powerful approach for glycopeptide synthesis. We extended this approach by developing a chemoenzymatic method for the synthesis of complex DNA glycoconjugates. Monosaccharides were conjugated to the 5′-end of DNA oligonucleotides through phosphoramidite chemistry. β-1,4-Galactosyl transferase attaches a galactose residue to the sugar portion of DNA conjugates bearing either a glucose or an *N*-acetylglucosamine. Further enzyme-catalyzed glycosylations were used to synthesize complex oligosaccharides covalently linked to DNA. The characterization, detection, and biochemical recognition properties of these novel hybrid biomaterials will be reported.

165. Circular oligonucleotides constructed on the structural basis of G-quadruplex. Jian Chen. Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong

Circular oligonucleotides possess many distinctive properties from their linear counterparts, such as a higher DNA-binding affinity, a greater sequence selectivity, and a better resistance to degradation by nuclease. Two types of methodologies for constructing circular oligonucleotides besides solid-phase synthesis have been well established during the past years: (1) via the formation of double-helical complexes; and (2) through the transition of triple helical conformation. Unlike these previous approaches relying on the structural features of double and triple helices, construction of circular oligonucleotides on a new structural basis, G-quadruplex, has recently been achieved in our laboratory (see Scheme 1 below). The new circularization processes exhibited high sequence specificity and high efficiency. Conformational analysis of the linear precursors, physical and biological confirmation of the circular structures, and the factors affecting the circularization processes will be presented and discussed.

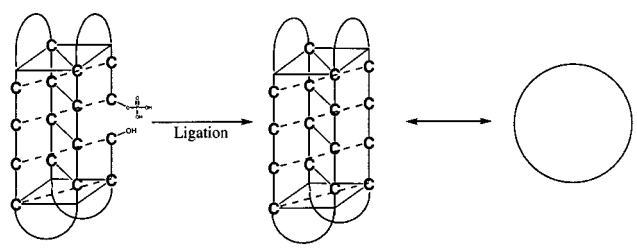
Scheme 1. Preparation of circular deoxyribonucleotide based on G-Quadruplex



166. Construction of circular oligodeoxynucleotides on the structural basis of *i*-motif. Dongsheng Liu. Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong

Oligodeoxynucleotides possessing four repeats of d(CCCTAA) can form intramolecular tetraplex structures, namely, *i*-motif, under slightly acidic condition. Such C-rich sequences are often found in the C-strand region of the vertebrate telomeric end, biological functions of which are commonly connected to cancer and aging. Based on the currently available information on the physical properties of *i*-motif, certain circular oligodeoxynucleotides were recently designed and constructed in our laboratory (see Scheme 1 below). Besides solid phase synthesis, the methods for constructing circular oligonucleotides via the formation of double and triple helices have been well established in recent years. Unlike these double and triple helical strategies, the

Scheme 1. Preparation of circular deoxyribonucleotide based on the structural feature of *i*-motif



circular oligodeoxynucleotide newly prepared in our laboratory is the first example of its kind constructed on the structural basis of *i*-motif. Conformational analysis of these circular oligodeoxynucleotides and sequence selectivity of the circularization process will be discussed.

167. Defining the mode of DNA repair by apurinic/apyrimidinic endonucleases using MASIA sequence decomposition, MD-simulations, and structure-driven protein design. Numan Özgün,[1] **Catherine H. Schein**,[1] Mathura Venkatarajan,[1] Tadahide Izumi,[2] and Werner Braun.[1] [1]Computational Biology, Sealy Center for Structural Biology, and [2]Sealy Center for Molecular Science, HBC&G, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1157 (fax: 409-747-6850, numan@bohr.utmb.edu, cathy@newton.utmb.edu)

Apurinic/apyrimidinic endonuclease (APE1) is essential for base excision repair of damaged DNA. Despite several crystal structures, the mechanism of cleavage by APE1 is still open to debate. The sequence of human APE1 was decomposed into motifs, using multiple sequence alignment, a novel principle component vector analysis, and the consensus macro of our MASIA program. Most of these motifs are at the protein/DNA interface. The structure of the polar N-terminal extension, necessary for reduction of transcription factors, in the presence of DNA may clarify its biological function. Homology modeling of APE-related proteins (to relate sequence changes to structure) and molecular dynamics (MD) simulations (to assess side chain and metal ion mobility) are being used to determine the role of these motifs in nucleic acid binding and cleavage. Analysis of designed mutants of APE1 in in vitro assays and an in vivo complementation assay is used to test predictions.

168. Design of potent telomerase inhibitors using 2′-*O*-(2-methoxyethyl) RNA. Anissa N. Elayadi. Departments of Pharmacology and Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390

Telomerase is a ribonucleoprotein that is upregulated in many types of cancer. Its potential as a target for chemotherapy awaits the development of potent and selective inhibitors. One of the leading candidates at this time is 2′-*O*-(2-methoxyethyl) (2′-MOE) RNA, which binds to the template region of telomerase in a complementary fashion. 2′-MOE RNA oligomers ranging in length from 13 to 20 nucleotides were examined in cell extract with IC_{50} values ranging from 5 to 10 nM at 37 °C. Upon transient transfection into DU 145 prostate cancer cells, inhibition of telomerase activity persisted for up to 7 days. The selectivity of inhibition was determined to be dependent on both oligomer length and the number of phosphorothioate substitutions. The ability of 2′-*O*-(2-methoxyethyl) RNA oligomers to inhibit telomerase in cells over time suggests that they are excellent lead compounds for in vivo testing and therapy.

169. Participation of Arg143 of DNA repair enzyme MutY in damage recognition and removal. O. A. Lukianova, S. L. Chepanoske, M.-P. Golinelli, and **S. S. David**. Department of Chemistry, University of Utah, Salt Lake City, UT 84112

E. coli MutY is an adenine glycosylase involved in DNA repair that recognizes and removes an adenine base mispaired with 8-OG and G. MutY contains a unique DNA binding motif referred to as the iron−sulfur cluster loop (FCL) motif, which has been shown to be intimately involved in specific damage recognition and removal. We have employed site-specific cross-linking using a photoreactive nucleotide, 4-thiothymidine, located 5′ of the mispaired adenine to investigate the DNA−protein interface of MutY. This study revealed a conserved Arg143 as the site of modification. We also demonstrated that R143A mutant exhibits altered DNA binding properties toward OG:A and G:A mispairs, as well as the adenine glycosylase activity toward a G:A mispair. The X-ray crystal structure of MutY showed that Arg143 donates a hydrogen bond to Cys192, which is located inside the FCL. These results indicate a subtle, yet significant role for Arg143 in substrate recognition and removal.

170. Reconstructing the substrate for uracil DNA glycosylase: Tracking the transmission of binding energy in catalysis. James T. Stivers and Yu Lin Jiang. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 (fax: 410-955-3023, jstivers@jhmi.edu)

The DNA repair enzyme uracil DNA glycosylase (UDG) is a powerful *N*-glycohydrolase that cleaves the glycosidic bond of deoxyuridine in DNA. We have investigated the role of substrate binding energy in catalysis by systematically dismantling the optimal substrate $Ap^{+1}\mathbf{U}pA^{-1}pA^{-2}$ by replacing the nucleotides at the +1, −1, or −2 positions with a tetrahydrofuran abasic site nucleotide (D), 3-hydroxypropyl phosphodiester spacer (S), phosphate monoester (p), or a hydroxyl group (h). Contrary to previous reports, the minimal substrate for UDG is 2′-deoxyuridine (h**U**h). UDG has a significant catalytic efficiency (*CE*) for h**U**h of 4×10^7 M^{-1} [$CE = (k_{cat}/K_m)(1/k_{non})$, where k_{non} is the rate of the spontaneous hydrolysis reaction of h**U**h at 25 °C]. Addition of +1 and −1 phosphate monoanions to form p**U**p increases k_{cat}/K_m by 45-fold as compared to h**U**h. Addition of flexible hydroxypropyl groups to the +1 and −1 positions to make Sp**U**pS increases k_{cat}/K_m by over 10^5-fold as compared to h**U**h, which is a 20-fold greater effect than observed with rigid D substituents in these positions (i.e., Dp**U**pD). The −2 phosphoester or nucleotide is found to increase the reactivity of trimer substrates with rigid furanose rings or nucleotides in the +1 and −1 positions by 1300−270 000-fold (i.e., Dp**U**pD → Dp**U**pDpA or Ap**U**pA → Ap**U**pApA). In contrast, the −2 nucleotide provides only an 8-fold rate enhancement when appended to the substrate containing the more flexible +1 and −1 S substituents (Sp**U**pS → Sp**U**pSpA). These context-dependent effects of a −2 nucleotide are interpreted in terms of a mechanism in which the binding energy of this "handle" is used to drive the rigid +1 and −1 A or D substituents into their binding pockets, resulting in a net catalytic benefit of −4.3 to −7.5 kcal/mol. Taken together, these results systematically track how UDG uses distant site binding interactions to produce an overall *four billion-fold* increase in *CE* as compared to the minimal substrate h**U**h.

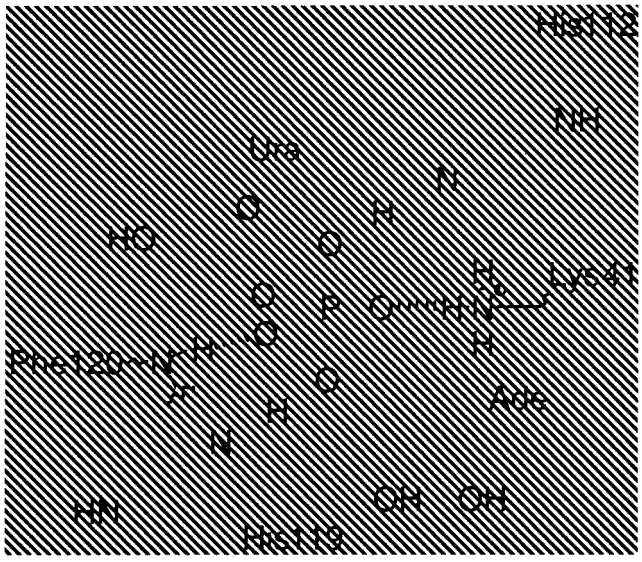
171. Removal of 8-oxoguanine and hydantoin products by the DNA repair enzyme Fpg. M. D. Leipold, J. G.

Muller, C. J. Burrows, and S. S. David. Department of Chemistry, University of Utah, Salt Lake City, UT 84112

7,8-Dihydro-8-oxo-2′-deoxyguanosine (OG) is highly reactive toward further oxidation by a variety of cellular oxidants. Recent work has identified two new DNA lesions, guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp). The presence of Gh and Sp lesions in DNA templates has been shown to result in misinsertion of G and A by DNA polymerases, and, therefore, both are potentially mutagenic DNA lesions. The Fpg protein was found to remove Gh and Sp efficiently opposite all four of the natural DNA bases. The rate of damaged base excision by Fpg was found to be highly dependent upon the base opposite the OG, Gh, or Sp lesion. Fpg acting on Gh and Sp lesions may dramatically influence their mutagenic potential; these lesions should be considered when studying oxidative DNA damage and its associated effects on DNA mutagenesis. Insights into the ability of eukaryotic enzymes to recognize and excise Gh and Sp lesions will be discussed.

172. Stereochemical consequences of catalysis by ribonuclease A: Nonnatural variants and phosphorothioate substrates. Robert J. Hondal, Richelle L. Abel, and Ronald T. Raines. Department of Biochemistry and Department of Chemistry, University of Wisconsin−Madison, Madison, WI 53706

The reaction mechanism of bovine pancreatic ribonuclease A (RNase A) continues to provide insight into the mechanism of phosphodiester cleavage. To clarify the roles of active-site residues in catalysis, nonnatural variants of RNase A were constructed using the technique of expressed protein ligation. The main chain nitrogen of Phe120 donates a hydrogen bond with one of the two nonbridging oxygens of the scissile phosphoryl group in the transition state. To determine the value of this interaction to catalysis, the main chain nitrogen of Phe120 was replaced with an oxygen, creating an ester linkage. The effect of this substitution on catalysis was determined with the two diastereomeric phosphorothioate analogues of UpA and UpU. In addition, the methylimidazole side chain of His119, which acts as an acid during catalysis, was replaced with a methylthiazole and an ethylamino group. Together, the data enable us to refine the mechanism of catalysis of phosphodiester cleavage by RNase A.

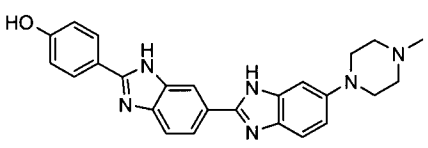


173. Structural investigations of SV40 T-antigen DNA-binding domain interacting with DNA. Elizabeth Bradshaw, Xuelian Luo, David G. Sanford, James Sudmeier, Peter A. Bullock, and William W. Bachovchin. Department of Biochemistry, Tufts University, 136 Harrison Ave., Boston, MA 02111 (fax: 617-636-2409, ebradsha@opal.tufts.edu)

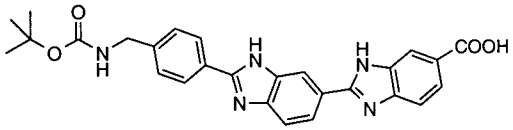
SV40 is used to study DNA replication, because T-antigen is the only viral protein required for replication, all other proteins are supplied by the cell. T-antigen's DNA-binding domain (TBD) recognizes GAGGC in the SV40 origin of replication. An 11mer of DNA containing the recognition sequence was added to TBD, and the assignments of Ca, N, ^{N}H, and most of the Cb have been made. Binding to the DNA causes large chemical shifts for residues in two regions of TBD, A1 and B2. These regions have been implicated in binding to the DNA by mutagenesis. Dynamic light scattering indicates that the addition of this DNA causes dimerization of TBD. NMR suggests that the regions involved in the dimerization are B3 and residues 167−169. This interaction is likely to be functionally relevant in the hexamer−hexamer interaction at the origin of replication by T-antigen.

174. Synthesis and biological evaluation of a novel Hoechst 33258 amino acid analogue for direct incorporation of a high affinity DNA binding motif into peptides. Carsten Behrens,[1,†] Niels Harrit,[1] and Peter E. Nielsen.[2] [1]Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100, Copenhagen, Denmark, and [2]Center for Biomolecular Recognition, Department of Biochemistry B, The Panum Institute, Blegdamsvej 3c, DK-2200, Copenhagen, Denmark

Protein DNA recognition plays a key role in cellular gene regulation, transcription, and duplication, and structural knowledge on the specific amino acid−nucleotide interactions underlying these processes can be valuable input for de novo design of new DNA binding drugs. However, specific amino acid−nucleotide interactions are often difficult to predict having only the primary peptide sequence of a DNA binding protein on hand. To facilitate DNA binding studies on peptide fragments of larger DNA-recognizing proteins, as well as studies of small peptide−DNA interactions in general, we have synthesized a novel Hoechst 33258 amino acid analogue (**7**) for direct installation of a DNA minor groove binding motif into peptides, using solid-phase



Hoechst 33258



7

synthesis. DNase I footprinting and spectroscopic studies reveal that both the strong DNA binding affinity of Hoechst 33258 and the characteristic fluorescence properties associated with DNA binding are maintained in peptide derivatives of **7**. (†Present address: Novo Nordisk A/S, Novo Nordisk Park 1, DK-2760 Måløv, Denmark.)

175. Synthesis and characterization of RNA containing 8-chloroadenosine, a potential chemotherapeutic agent. Lisa Chen and Terry L. Sheppard. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113 (fax: 847-491-7713, lisachen@chem.nwu.edu)

8-Chloroadenosine (8-Cl-Ado) induces apoptosis in several cancer cell lines. Upon entering cells, the modified nucleoside undergoes enzymatic conversion to the corresponding 5′-triphosphate derivative, 8-Cl-ATP. Treated cells show preferential incorporation of 8-Cl-Ado into messenger RNA over other forms of RNA, with a simultaneous decrease in total RNA levels. To study the biochemistry of these processes, 8-Cl-Ado was introduced chemically into RNA sequences. Starting from adenosine, a phosphoramidite derivative of 8-Cl-Ado was synthesized and was used to incorporate the modified base into internal sites of RNA oligonucleotides using solid-phase RNA synthesis. A controlled-pore glass solid support of 8-Cl-Ado was prepared and used to introduce the nucleoside at 3′-terminal positions in RNA sequences. The effects of 8-Cl-Ado substitution on RNA biosynthesis, structure, and function were explored using a combination of biochemical and biophysical methods. 5′-Phosphorylated derivatives of 8-Cl-Ado were synthesized to study the structural features and enzymatic substrate specificity of 8-Cl-Ado nucleotides.

176. Unwinding of the putative DNA binding domain of Polk by *cis*-diamminedichloroplatinum(II). Erika Volckova and Rathindra Bose. Department of Chemistry, Kent State University, Kent, OH 44240 (erika@Platinum.kent.edu)

The widely used anticancer drug *cis*-diamminedichloroplatinum(II) (cis-DDP) readily binds a number of eukaryotic and prokaryotic DNA polymerases, and inhibits their polymerase activities. Since several new polymerases are thought to be activated and participate in replication of damaged DNA, the interaction of cis-DDP with the putative DNA binding domain of Polk was investigated. The DNA binding zinc finger with 35 amino acids exhibits substantial helical structure. Fluorescence spectroscopic measurements revealed a binding constant of 2.2×10^5 M. The platinum anticancer drug almost completely unwinds the zinc finger structure as evidenced by the absence of characteristic CD peaks. The NMR structures of the 35-mer Zn finger along with the dynamics of the cis-DDP reaction will be reported.

177. Pyrene nucleotide and *N*-methyl thymine derivatives as probes for investigating the mechanism of thymine photodimer bypass by yeast polymerase η. Liping Sun,[1] Kaijiang Zhang,[1] Lily Zhou,[1] Fenghua Yuan,[2] Eric T. Kool,[3] Zhigang Wang,[2] and John-Stephen A. Taylor.[1] [1]Department of Chemistry, Washington University, One Brookings Dr., St. Louis, MO 63130 (fax: 314-935-4481), [2]Graduate Center for Toxicology, University of Kentucky, Lexington, KY, and [3]Department of Chemistry, Stanford University, Stanford, CA

Yeast polymerase η is a member of the class of DNA damage bypass polymerases that can bypass cis-syn thymine dimers in a nonmutagenic fashion by preferentially inserting A's opposite each T of the dimers. To better understand the mechanism of this error-free bypass reaction by pol η, we have determined the selectivity of pyrene and adenine nucleotide incorporation opposite a normal TT site and its thymine dimer, as well as their N3-methyl derivatives. Nucleotide insertion selectivity was determined by direct competition assays as well as by steady-state kinetics analysis of the nucleotide incorporation efficiency. Pyrene nucleotide was chosen as a probe because it has been previously shown to be incorporated opposite abasic sites in preference to adenosine by pol I family polymerases and thus can in principle be used to determine whether a template base is in the active site or not. The N3-methyl analogues of T were chosen because the methyl group should interfere with base pairing with adenosine if the template T is in the active site. Our results show that pol η has an unusual preference for incorporating pyrene nucleotide over adenine opposite both unmodified and modified T's and has an exceptionally high preference for incorporating pyrene nucleotide opposite the N3-methyl analogues of the 3′-T of a TT site or the thymine dimer. Whereas the efficiency of pyrene nucleotide insertion opposite the 3′-T was similar for all substrates, the efficiency of adenosine incorporation was greatly diminished by the presence of a methyl group on the N3 of T. The implications of these results and others for the mechanism of DNA synthesis and thymine dimer bypass by pol η will be discussed.

178. Solution structure of 3-methyladenine DNA glycosylase I reveals a novel fold for removal of 3-methyl purine bases from DNA. Alexander C. Drohat and James T. Stivers. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205 (fax: 410-955-3023, adrohat@jhmi.edu)

The integrity of an organism's genetic information is under constant threat from both metabolic and environmental compounds that can modify DNA, and all cells have enzymatic pathways devoted to the repair of damaged DNA. One common type of damage is that inflicted by alkylating agents, which react with the electron-rich sites of purine bases to form 3-alkyl or 7-alkyl base adducts. Of these, 3-methyladenine (3-MeA) is the most deleterious since it blocks DNA replication, and its removal from DNA is thus a critical function for all species. The 3 methyladenine DNA glycosylase I gene of *E. coli* (TAG) codes for a 189 residue protein that shows strong sequence identity (37−43%) with open reading frames in other Gram-negative bacteria and *Arabidopsis*, suggesting that a TAG activity exists in these organisms. However, TAG has no sequence homology to other DNA glycosylases, or any other distant proteins as determined from a BLAST search. TAG does not contain the consensus helix−Hairpin−helix (hHh) motif that is characteristic of the hHh superfamily of DNA glycosylases, and shows a high selectivity for the removal of 3-MeA, which distinguishes it biochemically from other alkyl purine specific DNA glycosylases that have been structurally characterized. These findings suggest that TAG may represent a novel glycosylase fold that is highly specific for

3-MeA. To answer these questions, we have assigned the ^{13}C, ^{15}N, and ^{1}H NMR chemical shifts of TAG using the standard suite of high-field NMR experiments, and determined the three-dimensional structure on the basis of NMR-derived distance restraints. The structure of TAG is essentially all alpha-helical, and thus differs from the $\alpha + \beta$ folds of 3-MeA DNA glycosylase II of *E. coli* (AlkA) and the alkyl adenine DNA glycosylase from humans (AAG). We have identified the substrate binding residues of TAG by chemical shift perturbation mapping upon addition of the reaction products 3-MeA and abasic DNA, and many of these residues are absolutely conserved in TAG from several species. This represents the first NMR structure of a DNA glycosylase, and will contribute significantly to our understanding of the specificity and mechanisms of these important enzymes.

179. A study of the effects of modified bases on the structure of the 1920-region rRNA hairpin. Christine S. Chow, Helen M.-P. Chui, May Meroueh, and John Santa-Lucia, Jr. Department of Chemistry, Wayne State University, Detroit, MI 48202 (fax: 313-577-8822, csc@chem.wayne.edu, cmp@chem.wayne.edu)

Pseudouridine and its analogues, such as 3-methylpseudouridine and 3-methyluridine, have been synthesized and converted to their corresponding phosphoramidites. A series of 19-nucleotide RNAs corresponding to the 1920-region of 23S ribosomal RNA have been synthesized. The structures and stabilities of these RNAs have been examined by thermal melting, circular dichroism spectroscopy, and 1D and 2D NMR spectroscopy. Combined, these biophysical methods reveal unique effects on the RNA structure for certain pseudouridine modifications. Sequence and structural contexts are important and appear to determine whether the modifications will have stabilizing or destabilizing effects. In contrast, methylation at position 1915 has a minimal effect on the RNA stability and structure.

180. Ability of DNA polymerase to distinguish dibasic oligonucleotides. Estela Alvarez, Alan Ewing, J. P. Phan, Jim Kirchner, Muzong Cheng, and Jen-i-Mao. Lynx Therapeutics Inc., 25861 Industrial Blvd., Hayward, CA 94545 (fax: 510-670-9302, ealvarez@lynxgen.com)

The ability to determine DNA sequence by high-throughput techniques has become important in genomics. Pyrosequencing allows sequencing of DNA using as substrate a template and a series of enzymatic reactions. In this paper, the ability of Klenow DNA polymerase to select the correct nucleotide was quantified by sequencing a short DNA stretch resulting from annealing a 28-mer top strand and a 14-mer bottom strand. Two double-stranded DNA substrates were prepared, one with (normal) and the other without (dibasic) an adenine at the C-1 ribose on the end nucleotide of the 28-mer strand. Mixtures of templates containing different ratios of the dibasic to normal site were generated, analyzed by LC, and pyrosequenced. Our results indicated that the peak height of the last peak decreased in direct proportion to the amount of the dibasic template. In addition, parameters such as precision, limit of quantitation, and linearity were determined to validate this technique.

181. Active-site variants of ribonuclease A. Richele Abel,[1] Robert J. Hondal,[1] and Ronald T. Raines.[2] [1]Department of Biochemistry and [2]Department of Biochemistry and Department of Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (rabel@biochem.wisc.edu)

Ribonuclease A (RNase A) is a model enzyme for studying the mechanism of catalysis. In one proposed mechanism, two histidines are thought to play an important role in catalyzing the degradation of RNA. His12 acts as a catalytic base by deprotonating the 2′-hydroxyl group of the ribose, which in turn attacks the phosphoryl group. His119 acts as a catalytic acid and protonates the 5′-hydroxyl leaving group. The pH−rate profile of RNase A supports this mechanism; the maximum rate of RNase A is at pH 6, and the enzyme loses activity at higher or lower pH. Here, we investigate the pH−rate profile of two active-site variants, H12Q RNase A and H119N RNase A, which lack the catalytic base and acid, respectively.

182. Discrimination between thymine dinucleotides and photodamaged products using surface-enhanced Raman spectroscopy. K. K. Caswell and C. J. Murphy. Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter St., GSRC, Columbia, SC 29208 (phone: 803-576-5955, fax: 803-777-9521, caswell@mail.chem.sc.edu)

Cyclobutane pyrimidine dimers, particularly thymine dimers, are the predominant UV-induced photoproducts and have been associated with skin cancer. These DNA lesions are the result of a [2+2] photocycloaddition between adjacent thymine bases on the same polynucleotide strand. The unique molecular vibrations between damaged and undamaged adjacent thymine bases, as a signature, can be exploited by surface-enhanced Raman spectroscopy (SERS). SERS involves the Raman scattering of the molecular vibrations that are enhanced when a molecule, such as DNA, adsorbs to a nanosized metal surface. Silver colloids, ∼44 nm spheres, have been employed as SERS substrates to greatly enhance thymine's weak Raman signal. This method of detection has provided distinct spectra that can discriminate between thymine dinucleotides and UV-induced photodamaged products.

183. Identifying ligands within the *Tetrahymena* ribozyme that bind and position the catalytic metal ions. James L. Hougland,[1] Alexander V. Kravchuk,[2] Dan Herschlag,[2] and Joseph Piccirilli.[1] [1]HHMI and Departments of Biochemistry & Molecular Biology and Chemistry, University of Chicago, 5841 S. Maryland Ave., MC1028, Chicago, IL 60637 (jpicciri@midway.uchicago.edu, j-hougland@uchicago.edu), and [2]Department of Biochemistry, Stanford University, Stanford, CA

Metal ions are essential to the phosphoryl transfer reactions catalyzed by the *Tetrahymena* ribozyme. However, the ligands for these catalytic metal ions within the ribozyme core are not known. Atomic level substrate modifications coupled with quantitative analysis has identified a unique constellation of three metal ions in the active site that stabilize the transition state by coordinating to the nucleophile, leaving group, and *pro*-S_p oxygen of the scissile phosphate. Now that the metal−substrate interactions have been elucidated, we have shifted our focus toward identifying the ligands within the ribozyme core that bind and position the catalytic metal ions. To accomplish this, we have introduced site-

specific phosphorothioate modifications into the ribozyme phosphate backbone. By analyzing the effect of these modifications on the affinities of the catalytic metal ions for the ribozyme, we hope to link specific phosphate oxygens to each of the three metal ions, thus revealing the locations of the metal ions within the ribozyme.

184. In vitro expansions of long repetitive DNA sequences. Wirote Tuntiwechapikul and **Miguel Salazar**. College of Pharmacy, Division of Medicinal Chemistry, University of Texas at Austin, Austin, TX 78712

We have undertaken an in vitro study of DNA expansions with short duplexes containing repeats of four, six, and eight bases using Taq DNA, Klenow Pol I, and T4 DNA polymerases. Studies with Taq DNA polymerase show that short duplexes consisting of repetitive DNA sequences can be effectively expanded regardless of the repeat sequence. Expansions occur more readily as the length of the repeat sequence decreases but are generally more efficient at reaction temperatures closer to the melting point of the starting duplex. There is no evidence that structure is involved in the expansion process since even DNA duplexes consisting of random repetitive DNA sequences can be expanded in like manner. Studies with Klenow Pol I and T4 DNA polymerases indicate that the strand displacement activity of the enzyme used could play an important role in the observed in vitro expansion of short repetitive DNA duplexes.

185. An investigation of the dynamic process of bacteriophage T4 DNA polymerase holoenzyme assembly through the eyes of the processivity factor. Michael Trakselis. Pennsylvania State University, Dr. Stephen Benkovic's Laboratory, 152 Davey Laboratory #87, University Park, PA 16827

The coordinated assembly of the DNA polymerase (gp43), the sliding clamp (gp45), and the clamp loader (gp44/62) to form the bacteriophage T4 DNA polymerase holoenzyme is a multistep process. Changes to the structure of gp45 were evaluated during this process, and a final solution structure for the holoenzyme is proposed. Stopped-flow fluorescence resonance energy transfer (FRET) was used to investigate the opening and closing of the gp45 ring during holoenzyme assembly. By utilizing three site-specific mutants of gp45, we tracked changes in distances across the gp45 subunit interface through seven conformational states associated with holoenzyme assembly. In the final gp45 state of holoenzyme assembly, one subunit interface is open about 11 Å. This open interface of gp45 is proposed to interact with the C-terminal tail of gp43, providing a point of contact between gp45 and gp43. These experiments further define the dynamic process of bacteriophage T4 polymerase holoenzyme assembly.

186. Structural nucleic acids microarray. Claude E. Gagna, Ph.D. New York Institute of Technology, NYCOM #2, Room #362, Department of Life Sciences, Old Westbury, NY 11568-8000

A novel technique has been developed which should accelerate basic biomedical research. This microarray system uses different double-stranded (ds-) (and single-stranded) DNA and RNA molecules of various base pairs. The technology can be applied to 96-, 384-, or 1536-well microplates. The molecules, low or high MW, are either in the right-handed (e.g., A-DNA, B-DNA, A-RNA) or in the left-handed (e.g., Z-DNA, Z-RNA) ds conformations. Other nucleic acids are also used: genomic or viral DNA, DNA−RNA hybrids, methylated nucleic acids, modified and specialty nucleosides (nucleotides), bases, T-DNA, C-DNA, t-RNA, r-RNA, m-RNA, snRNP, snRNA, hnRNA, catalytic RNA, kinked DNA, bent DNA, nucleic acid junctions, plasmid DNA, positive and negatively supercoiled DNA, DNA knots, catenanes, PCR and restriction fragments, and cruciform, slipped, mispaired, nodule, parallel-stranded, and anisomorphic DNAs. Normal and diseased genes (adult and embryonic), introns, exons, operons, TATA boxes, solenoids, Okazaki fragments, promoters, replicons, replication forks, oligodeoxynucleotides, oligoribonucleotides, polynucleotides, polyribonucleotides, triplex DNA, quadruplex DNA, and pentaplex DNA, along with many others, can be examined. Additionally, nucleic acid−protein complexes, drug intercalations, and cross-linking are also used. This sensitive microarray will allow for analysis of nucleic acid geometry and dynamics.

187. Conformational changes of ADAR2·RNA complex. Hye Young Yi-Brunozzi and Peter A. Beal. Department of Chemistry, University of Utah, 315 S. 1400 East, Room 2020, Salt Lake City, UT 84112 (fax: 801-581-8433, yi_brunozzi@hotmail.com)

ADAR2 is an RNA-specific adenosine deaminase, but little is known about how the enzyme interacts with the substrate or the catalytic mechanism. Using tryptophan and 2-aminopurine (2-AP) fluorescence, the conformational changes of the protein and the substrate were investigated. We have previously reported ADAR2-induced changes in the 2-AP fluorescence of modified substrates, indicating a possible base-flipping mechanism. Additional data have been obtained using full-length ADAR2 and the RNA binding domain (RBD) which illustrate that the 2-AP fluorescence is specific to the editing site and is dependent on the presence of the catalytic domain. Tryptophan fluorescence and acrylamide quenching show that conformational changes take place within ADAR2 in the presence of duplex RNA. Fe·EDTA footprinting indicates that the RBD is located around the editing site, suggesting that the RBD is primarily responsible for identifying potential ADAR2 editing sites, but further conformational changes induced by the catalytic domain must take place for deamination to occur.

188. Novel synthesis and RNA-binding properties of aminoglycoside dimers conjugated via a naphthalene diimide-based intercalator. Jeffrey B.-H. Tok and Jason Fenker. Department of Chemistry, York College and Graduate Center of the City University of New York (CUNY), 94−20 Guy R. Brewer Blvd., Jamaica, NY 11415

The utilization of small molecules to control cell expression is recently of immense interest. Binding of small molecular ligands to DNA to control the transcription process has been well demonstrated. The interaction processes between small molecules and nucleic acids have been primarily through mechanisms such as groove binding and

intercalation. For example, planar naphthalene diimide-based intercalators have recently been extensively reported to be an effective intercalator, even as a polyintercalator, for DNA. Herein, the synthesis and RNA-binding properties of naphthalene-based diimide-conjugated bis-aminoglycoside antibiotics was presented. Compared to the monomeric aminoglycoside, the conjugated ligands were observed to attain up to an approximately 30-fold enhancement in binding affinity toward a novel RNA construct that contained two 16S rRNA A-sites.

189. Does a stacked DNA base pair hydrate better than a hydrogen bonded one? An ab initio modeling study. Sivanesan Dakshanamurthy and William J. Welsh. Department of Chemistry, University of Missouri, 315, Benton Hall, St. Louis, MO 63121 (fax: 314-516-5342, siva@ozone.umsl.edu)

It is well-known that the two main factors responsible for the stabilization of DNA double helix are stacking and hydrogen bonding (H-bonding) interactions between the nucleic acid bases. It has been observed experimentally and theoretically that the structure of DNA is highly sensitive to humidity and adopts various types of conformations, depending upon the relative humidity. There are very few experimental studies on stacking interactions; comparative studies of specific solvation effects on nucleic acid base pair and stacked pair have not yet been carried out. This study is aimed at answering which hydrates better: H-bonded pairs or stacked pairs? To this end, a systematic comparative study has been made on the explicit hydration of the guanine-cytosine hydrogen-bonded base pair (GC) and guanine-cytosine stacked pair (G/C). An electrostatic-based approach has been used to identify the potential binding sites for water molecules around GC and G/C dimers. In particular, the Molecular Electrostatic Potential (MESP) surface for GC and G/C base pairs was explored. Several geometries of the complexes, GC...$(H_2O)_n$ and G/C...$(H_2O)_n$ ($n = 1-6$), were investigated using HF/6-31G**/6-31G+** calculations. Further minimization calculations were performed at both DFT/6-31G** and MP2/6-31G** levels to assess the role of electron correlation contribution in the hydration process. Energetics of these systems show a clear-cut additional stability for the G/C pair over the GC pair of about 1−6.1 kcal/mol at the HF/6-31G** level, 1.5−7.5 kcal/mol at the DFT level, and 1.5−9.5 kcal/mol at the MP2/6-31G** level of theories. The present study thus confirms that the stacked base pair hydrates better than the corresponding H-bonded base pair, which confirms the experimental evidence. It can be concluded from the present findings that DNA base pairs can accommodate up to 6 water molecules (shown in Figures a and b) in accordance with the experimental results.

190. Robustness of the dexamethasone−methotrexate yeast three-hybrid system. Wassim M. Abida, **Brian T. Carter**, Eric A. Althoff, Hening Lin, and Virginia W. Cornish. Department of Chemistry, Columbia University, 3000 Broadway, MC 3153, Havemeyer Hall, New York, NY 10027 (phone: 212-854-8616, fax: 212-932-1289)

A new chemical inducer of dimerization (CID) was recently developed in Professor Cornish's lab, which uses a heterodimer of methotrexate (MTX) and dexamethasone (DEX) which, when placed in the yeast three-hybrid system, reconstitutes transcription of the *lacZ* gene. The effect of altering the structure of the DEX−MTX CID and the protein chimeras in the three-hybrid assay was investigated. It was observed that all DEX−MTX CIDs, except the DEX−MTX CID with the shortest chemical linker, showed the ability to induce β-galactosidase levels at levels 400% above strains possessing no CID. The DEX−MTX CIDs showed little or no increase in β-galactosidase levels above background levels in strains where dihydrofolate reductase (DHFR) from *E. coli* was replaced by DHFR from murine. The three-hybrid system did show some directional preference to the way in which the receptors were fused to the DNA binding domain and the activation domain. These studies have led to a better understanding of the factors that are important in activating transcription in the DEX−MTX yeast three-hybrid system.

191. Solution structure of (1*S*,2*R*,3*S*,4*R*)-benz[*a*]anthracene guanine N^2 adduct in an oligonucleotide containing codon 12 of the N-*ras* protooncogene. Hye-Young H. Kim, Amanda S. Wilkinson, Constance M. Harris, Thomas M. Harris, and Michael P. Stone. Department of Chemistry, Center in Molecular Toxicology, and Vanderbilt−Ingram Cancer Center, Vanderbilt University, Nashville, TN 37235

The structure of the bay region (1*S*,2*R*,3*S*,4*R*)-N^2-[1-(1,2,3,4-tetrahydro-2,3,4-trihydroxybenz[*a*]anthracenyl)]-2′-deoxyguanosyl adduct in d(GGCAG$\underline{X^6}$TGGTG):d(CAC-CACCTGCC), bearing codon 12 of the human N-*ras* protooncogene (underlined), was determined. This adduct results from the trans opening of (1*R*,2*S*,3*S*,4*R*)-1,2-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracenyl-3,4-diol by the exocyclic N^2 of guanine. The benz[*a*]anthracene moiety is located in the minor groove toward the 5′-direction of the modified strand. X^6 H1′ shifted downfield, and C^{18} H1′ and T^{19} H1′, complementary to the adducted site, shifted upfield significantly. All base pairs, including modified base pair, are aligned through Watson−Crick pairing as in B-type DNA. Molecular dynamics simulations were restrained by 437 inter- and intrastrand NOEs, and 98 sugar puckering and phosphate backbone torsion angles. The restrained molecular dynamics calculations converged with maximum pairwise root-mean-square deviation 0.3 Å. Complete relaxation matrix analyses of the 11 base pairs yielded sixth root residual indices between calculated and experimental NOE intensities of 8.8×10^{-2} for the refined structure.

192. Oxidation of deoxyribose in DNA produces 3′-phosphoglycolaldehyde residues that give rise to glyoxal by a novel phosphonate rearrangement. Mohamad Awada and Peter C. Dedon. Division of BEH, MIT, 56-787, Cambridge, MA 02139

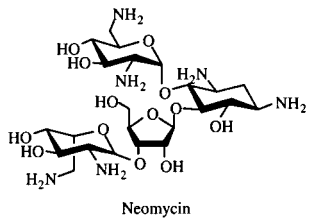
Oxidative damage to the sugar−phosphate backbone of DNA produces a variety of electrophilic residues that can react with neighboring bases to form mutagenic adducts. In this work, we have investigated one of these residues, phosphoglycolaldehyde, that arises from 3′-hydrogen abstraction from deoxyribose. We previously demonstrated that synthetic phosphoglycolaldehyde reacts with dG and DNA to form the mutagenic 1,*N*2-glyoxal adducts, consistent with published observations of glyoxal formation in oxidized

DNA. Using GC/MS, we have now established that 3′-phosphoglycolaldehyde residues form in DNA exposed to Fe(II)EDTA and other oxidizing agents. The results of ^{31}P NMR and GC/MS studies revealed that phosphoglycolaldehyde reacts to form a pentacoordinated phosphorane intermediate that rearranges to glyoxal and phosphonate. In addition to a demonstration of the biological relevance of phosphonate chemistry, these results illustrate the potential for amplification of DNA damage by formation of base adducts from the electrophilic products of strand breaks.

193. Stabilization of triple helical nucleic acid structures. R. Lane Coffee, Jr., and Dev P. Arya. Department of Chemistry, Clemson University, Clemson, SC 29634

Triple helical nucleic acids have the potential to serve as part of an antigene and antisense strategy, targeting DNA and RNA, respectively, to suppress the transcription and/or translation of specific genes. Several obstacles, which include low stability, must be overcome before triplex-forming oligonucleotides can be used for therapeutic purposes. Our goals aim at stabilizing triple helical nucleic acid structures by using aminoglycoside antibiotics. Aminoglycoside antibiotics are bactericidal agents that are comprised of two or more amino sugars joined in glycosidic linkage to a hexose nucleus. We have shown that neomycin (six amino groups) is the most active and triplex-selective stabilization agent among all aminoglycosides, previously studied minor groove binders, and polycations. Neomycin stabilizes DNA triple helices while the double helical structures composed of poly(dA)·poly(dT) are virtually unaffected. Neomycin has also been shown to selectively stabilize a mixed 22-base DNA triplex containing C and T bases in the pyrimidine strand. Recent studies are directed toward hybrid nucleic acid structures consisting of both DNA and RNA strands. Ligand-induced formation of new classes of hybrid triplexes has potential implications in the recognition of RNA·DNA hybrid duplexes by cellular and viral proteins. Recent results in the stabilization of such hybrid structures will be presented. Our efforts in the development of synthetic neomycin analogues and their triplex stabilization properties will also be discussed.

Scheme 1.



Neomycin

194. Spectroscopic & electrochemical studies between DNA and [(η^5-C_5H_5)$_2$Mo((−)-2-amine-6-mercaptopurine ribose)]Cl$_2$ antitumor systems. Túlio E. Chávez-Gil, Carmen A. Vega, Vilmary López, and Enrique Meléndez. Department of Chemistry, University of Puerto Rico, PO Box 9019, Mayagüez, PR 00681 (tulgil@yahoo.com, e_Melendez@rumac.uprm.edu)

A new antitumor [(η^5-C_5H_5)$_2$Mo(L)]Cl$_2$ (L = (−)-2-amine-6-mercaptopurine ribose) complex has been synthesized in order to investigate its relationship and stability constants (K_b) by its interaction with calf thymus DNA as well as its electrochemical behavior in DMSO and buffer solutions. The compound has been characterized by elemental analysis, cyclic voltammetry, and spectroscopic (UV−visible, infrared, and ^1H, ^{13}C NMR) methods. Stoichiometric amounts of Cp_2MoCl_2 and the ligand L lead to cytostatic adduct complexes. The ^1H NMR spectrum shows the down- and upfield shift signals of adduct when compared with the former Cp_2MoCl_2 and the purine ribose ligand. The H8 is downfield-shifted to $\delta = 8.338$ ppm instead of 8.165 ppm for the former, whereas the NH_2 is upfield-shifted to 6.653 ppm instead of the initial 6.812 ppm. The UV/VIS spectra showed three sets of absorption bands with maxima at 248 (LCMT), 312, and 343 (MCLT) nm, which suffer hypochromism, as well as about a 10 nm shift of the maximum to a longer wavelength under DNA titration depending on the ionic strength of the buffer. Intrinsic binding constants, K_b, ranging from 10^3 to 10^5 M^{-1} cm^{-1} have been determined, suggesting DNA-complex interaction in agreement with similar systems [Melendez, E., Marrero, M., Rivera, C., Hernandez, E., and Segel, A. (2000) *Inorg. Chim. Acta 298*, 178]. Cyclic voltammetry showed a reversible wave at +600 and 513 mV vs Ag/AgCl, consistent with a single one-electron transition for a Mo^V/Mo^{IV} coupling. However, in the presence of an equivalent of 100 μL of DNA, the redox potential changed to more negative and reversible values at 0.585 and *V* versus SHE and also voltammogram signals decayed as a consequence of DNA-complex binding. We have reported recently that similar complexes exhibit considerable potential in medicinal chemistry [Melendez, E., Marrero, M., Rivera, C., Hernandez, E., and Segel, A. (2000) *Inorg. Chim. Acta 298*, 178], and the aim of this work is investigate biophysical aspects related with DNA and metallocene interactions, in an attempt to solve the question of whether DNA and Cp_2MCl_2 interaction is truly weak or not [Chavez-Gil, T. E., Perez, Y., Rodriguez, M., and Melendez, E., manuscript in preparation].

195. Synthesis, spectroscopy, electrochemistry and kinetics interactions of titanocene(amino acids)−DNA antitumor systems. Yajaira Pérez, Tulio E. Chávez-Gil, and Enrique Meléndez. Department of Chemistry, University of Puerto Rico, PO Box 9019, Mayagüez, PR 00681

Titanocene dichloride (Cp_2TiCl_2) is the first metallocene known to exhibit high and more effective antitumor activity. This complex showed the greatest activity against colorectal, lung, and breast carcinomas in comparison to other metallocene complexes. However, its antitumor activity could be improved by incorporating more biocompatible ligands such as amino acids and nucleobases, making this complex more accessible to the target places in the cell. The major goal of our research is to prepare thiolated-amino acid complexes with high water solubility and study their interactions with the DNA structure. Two new complexes, with the general formula [Cp_2TiL_2]Cl$_2$ [L = L-cysteine (**1**) and L = D-penicillamine (**2**)], were synthesized and characterized via UV−Vis, ^1H NMR, and IR spectroscopies and elemental analysis. Kinetics of ligand hydrolysis have been monitored by UV−Vis and ^1H NMR spectroscopies. Observed rate constants for the amino acid loss are $k_{obs} = 1.12 \times 10^{-2}$ min^{-1} (**1**) and $k_{obs} = 1.47 \times 10^{-3}$ min^{-1} (**2**). Metal−DNA interactions have been pursued by UV−Vis spectroscopy and

electrochemical methods. The binding constants for Ti-amino acids are on the order of 10^3. Mechanism of action at the molecular level and binding parameters will be discussed.

196. Development of synthetic viroids. Demin Zhou and **Peter G. Schultz**. Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037

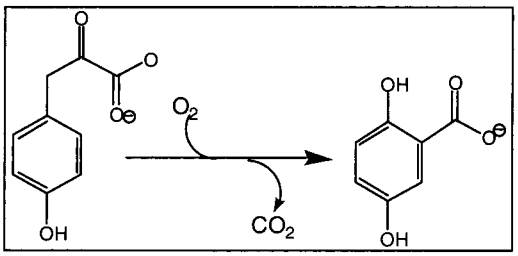
Natural viroids are the smallest pathogenic agents yet described, which are single-stranded circular RNA molecules varying in length from 246 to 463 nucleotides. The aims of this project are to develop artificial viroids that can be amplified by exponential rolling-circle replication and to apply the synthetic viroid as a novel system for RNA selection and evolution. The first generation artificial viroid with the characteristics of rolling-circle replication has been successfully developed, and its potential application as a novel RNA vector is now developing.

197. A novel motif for the design of transition state analogues for phosphatases. Alvan C. Hengge and **Przemyslaw G. Czyryca**. Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300

Kinetic isotope effect studies of several phosphatases carried out by our laboratory indicate that the transition state for enzymatic phosphoryl transfer is loose in nature, characterized by extensive bond cleavage to the leaving group. We have designed molecules to mimic certain aspects of such a transition state geometry. The first simple prototypes of these compounds have been synthesized and tested as inhibitors of alkaline phosphatase from *E. coli* and for the protein tyrosine phosphatase YOP from *Yersinia.* These compounds are competitive inhibitors of both phosphatases, with better inhibition toward alkaline phosphatase, with K_i values in the micromolar range.

198. A prelude to kinetic and structural characterization of 4-hydroxyphenylpyruvate dioxygenase from *Streptomyces avermitilis*. Kayunta Johnson-Winters, Tamara N. Nelson, and Graham R. Moran. Department of Chemistry, University of Wisconsin−Milwaukee, Milwaukee, WI 53211

The second step of the tyrosine catabolism pathway is the quite extraordinary conversion of 4-hydroxyphenylpyruvate to homogensitate by the FeII-dependent enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27). This enzyme is the target of specific inhibitors that are used in the treatment of Type 1 Tyrosinemia. Although several plausible mechanisms have been proposed for this enzyme, no direct evidence has yet been presented for any of the reaction coordinate intermediates. The initial goal of our research is to study HPPD using pre-steady-state methods to observe oxygenated intermediates as they accumulate in turnover. However, since the absorption spectra of the substrate and product overlap that of HPPD, stopped-flow spectral observation under pseudo-first-order conditions is hindered by the relatively high molar extinction coefficient of the protein (40 465 $M^{-1}cm^{-1}$). We propose to reduce this number by mutating nonconserved tryptophans to phenylalanine to create a form of the enzyme more amenable to UV−Vis studies. HPPD from *S. avermitillis* has only three tryptophans, all of which are nonconserved (W85, -200, -385). We have subcloned the gene for HPPD from *S. avermitillus* into the pET expression system and expressed the enzyme in *E. coli* to 35% of total cell protein. Methods for purification of the holo- and apo-forms of HPPD have been developed, yielding, in each case, pure enzyme of high activity (TN 4 s^{-1}). As isolated, the holo-(Fe^{III}) form has a purple color due to a weak absorbance band at 600 nm which is lost upon reduction. A concurrent goal is to determine the structure of HPPD in complex with its substrate, 4-hydroxyphenylpyruvate. To this end, we initially hope to crystallize the apo-enzyme equilibrated with a divalent metal ion that is stable to oxidation in an atmosphere such as Mn^{II} or Cu^{II} so that 4-hydroxyphenylpyruvate can be soaked into these crystals without turnover.



199. Acyl-CoA hydrolase assay by capillary electrophoresis. Kathleen Healy Moore, Anagha B. Dandekar, and **Sree D. Panuganti**. Department of Chemistry, Oakland University, Rochester, MI 48309

Acyl-CoA hydrolases (ACH) hydrolyze acyl-CoAs of various chain lengths to free fatty acids and CoASH. These enzymes have the ability to regulate the physiological acyl-CoA concentrations as well as to detoxify nonphysiological acyl-CoA metabolites. In this study, substrate (acyl-CoA) and product (CoASH) of ACH are separated and quantified using capillary electrophoresis (CE). The retention times of standard CoASH and *n*-decanoyl-CoA ranged from 4.43 to 21.88 min and from 4.58 to 14.83 min, respectively, depending on pH (1.8−9.5), voltage (−20 to +30 kV), and type and concentration of buffer. The peak areas for standards increased with concentration over a range of 0.01−0.3 mM. Based on these findings, CE is being used to monitor hydrolysis of physiological acyl-CoAs (decanoyl-CoA, myristoyl-CoA) by rat liver enzymes. Enzyme activity results correlate directly with spectrophotometric analyses. With respect to xenobiotic acyl-CoAs, analysis of a series of ethylene glycol-derived oxaacyl-CoAs indicates decreased hydrolysis compared with physiological acyl-CoAs. (Supported by The Research Excellence Program in Biotechnology, Oakland University.)

200. An LC-MS based kinetic assay to explore the amine selectivity of NAD-synthetase. B. S. Pybus,[1] S. R. Harville,[1] Raj K. Singh,[2] Lawrence J. DeLucas,[2] and D. D. Muccio.[1] [1]Department of Chemistry and [2]Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham, Birmingham, AL

NAD-synthetase catalyzes the last step in the biosynthesis of NAD using the chemical energy of ATP in the following reaction: MgATP + NaAD + NH_3 → AMP + NAD + $MgPP_i$. Previous kinetic studies on this system employed a

coupled spectrophotometric assay in which the alcohol dehydrogenase reaction: NAD + ETOH → NADH + acetaldehyde, was used to report NAD production. This assay does not allow for the exploration of the selectivity of the NaAD or NH_3 enzyme binding sites, due to its dependence on NAD formation. Using LC-MS with single ion detection, a method was developed for the separation, identification, and quantification of products and reactants in the NAD-synthetase-mediated reaction in a time-dependent manner. Several alternative amines were evaluated in the NAD-synthetase reaction. Of the amines evaluated, only NH_2NH_2, NH_2OH, and NH_2CH_3 reacted to form product. K_m and k_{cat} for each amine were determined by the LC-MS method.

201. Avoidance of aluminum by mycorrhizal fungus, *Wilcoxina mikolae*. Guozhang Zou and Gregory L. Boyer. Department of Chemistry, State University of New York, College of Environmental Science and Forestry, 1 Forest Dr., Syracuse, NY 13210

Wilcoxina mikolae is of environmental importance in the forest due to its symbiosis with many forest trees. *Wilcoxina mikolae*, an ectendo-mycorrhizal fungus, secretes the siderophore deferriferricrocin. This paper was initiated by the strong binding ability of deferriferricrocin with aluminum, which will potentially affect the bioavailability of aluminum in the environment. *Wilcoxina mikolae* was grown in iron-deficient and iron-replete culture with different levels of aluminum(III) added. The growth of this mycorrhizal fungus was monitored by dry weight. The concentration of extracellular deferriferricrocin, alumicrocin [Al(III) complex], and ferricrocin [Fe(III) complex] was measured by LC/MS. The chelation directly decreased the concentration of free cationic aluminum, and the complexed aluminum lost its toxicity toward *Wilcoxina*. However, high concentration of aluminum was able to compete with relatively low concentration of iron to form a complex with deferriferricrocin. This reduced the siderophore-mediated iron uptake by *Wilcoxina*, which resulted in decreased growth under iron-deficient conditions.

202. Binding interactions of vancomycin tracers with a bacterial cell wall peptidoglycan analogue. Zhiguang Yu, Maciej Adamczyk, Jonathan Grote, Jeffrey A. Moore, and Sushil D. Rege. Department of Chemistry, Diagnostics Division, Abbott Laboratories, 100 Abbott Park Rd., Department 09NM, Building AP-20, Abbott Park, IL 60064-6016

Binding interactions between several vancomycin tracers and (N,N'-diacetyl)K_DA_DA in solution were evaluated in a competition format using a surface plasmon resonance instrument. Tracers derivatized from the carboxy terminus or the *N*-vancosaminyl sugar moiety of vancomycin bind the peptide with an affinity similar to that of underivatized vancomycin. In contrast, *N*-methylleucyl-derivatized vancomycin tracers bind the peptide with a reduced affinity relative to vancomycin.

203. Binding of antibodies to α-galactosyl derivatives incorporated into liposomes. Patricia Mowery,[1] Yi He,[2] and Laura L. Kiessling.[3] [1]Department of Biochemistry, [2]Department of Chemistry, and [3]Department of Biochemistry and Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (pmowery@biochem.wisc.edu)

The number of people needing organ transplants outweighs the number of donors. A major obstacle to developing a viable solution, pig-to-human xenotransplantation, is the carbohydrate epitope Galα(1−3)Galβ(1−4)GlcNAc (α-galactosyl), which is displayed on pig but not human cells. Humans have developed large titers of anti-α-galactosyl antibodies (anti-Gal), presumably due to constant exposure to bacteria that display α-galactosyl residues. The binding properties of anti-Gal to α-galactosyl derivatives are an active area of interest. Anti-Gal antibodies bind weakly (μM) to a single α-galactosyl epitope but tightly (pM) to multiple epitopes. The optimal concentration of α-galactosyl needed for tight binding is unknown. To explore the densities required for optimal binding, we have synthesized α-galactosyl derivatives and incorporated them into liposomes. By changing the density of the α-galactosyl derivatives, we can probe how anti-Gal binding is influenced by carbohydrate presentation.

204. Biological and chemical properties of lipid/polydiacetylene colorimetric vesicle biosensors. Marina Katz,[1] Sofiya Kolushev,[1] Haim Tsubery,[2] Mati Fridkin,[2] and Raz Jelinek.[1] [1]Department of Chemistry, Ben-Gurion University, Ben-Gurion St., 84105 Beer-Sheva, Israel (fax: 972-8-6472943, marinaka@bgumail.bgu.ac.il), and [2]Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel 76100

Supramolecular chemical assemblies, composed of polydiacetylene, exhibit rapid colorimetric transitions upon specific interactions with a variety of biological analytes in aqueous solutions. Among the analytes that give rise to the unique blue−red color changes are lipophilic enzymes, antibacterial peptides, ions, antibodies, and membrane penetration enhancers. The chemical assemblies include conjugated polydiacetylene, responsible for the chromatic transitions, and the molecular recognition elements, which are either chemically or physically associated with the polydiacetylene. Thus, by incorporation of specific recognition elements, the system can be designed in ways allowing for highly selective identification of analytes. In particular, receptors and epitopes can be incorporated within the sensor assembly, which then determine the specificity of the colorimetric transitions. The polydiacetylene-based molecular assemblies are robust, and can be readily applied to the diagnosis of physiological molecules, and for rapid screening of chemical and biological libraries.

205. Calcium-binding properties of an important calcite growth inhibitor protein, lithostathine. D. Mustafi,[1] B.-I. Lee,[2] A. Sosa-Peinado,[1] Y. Nakagawa,[1] and W. Cho.[2] [1]Department of Biochemistry & Molecular Biology, The University of Chicago, Chicago, IL 60637, and [2]Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60607

The pancreas excretes two types of metabolically important proteins: digestive enzymes and hormones. Lithostathine is the only protein excreted from the pancreas that has no digestive or hormonal activities. Since it is found in pancreatic stone, it is believed that this protein inhibits pancreatic stone (calcium carbonate crystals) formation. Human lithostathine (HLIT) is a 144-amino acid protein

synthesized by the exocrine pancreas. It has been shown that the 166-amino acid sequence deduced from the human *reg* cDNA contained the 144-amino acid sequence of HLIT. We have cloned this *reg* protein cDNA in *E. coli* strain BL21 and purified HLIT after culturing the cloned cells. Calcium-binding studies were carried out using equilibrium dialysis, circular dichroism (CD), and electron paramagnetic resonance (EPR) spectroscopic methods. Equilibrium dialysis with $^{45}Ca^{2+}$ showed that HLIT binds Ca^{2+} in a 1:1 molar ratio. For EPR studies, we have used the divalent vanadyl (VO^{2+}) ion as a suitable paramagnetic substitute for Ca^{2+}. We have demonstrated that VO^{2+} binds to HLIT with a metal:protein binding stoichiometry of 1:1 and that VO^{2+} competes with Ca^{2+} in binding to HLIT. Based on the crystal structure of HLIT [Bertrand et al. (1996) *EMBO J. 15*, 2678], we have carried out modeling studies and identified a core of acidic residues that would be important for metal binding. We have prepared six mutants, E30A, D31A, E33A, D37A, D72A, and D73A, and carried out CD and EPR studies with mutant forms of the protein. Neither Ca^{2+} nor VO^{2+} binds to any of these six mutant forms of HLIT. Results from spectroscopic and molecular modeling studies will be discussed. (Supported by NSF Grant MCB-0092524.)

206. Characterization of PC12 cell proliferation and differentiation stimulated by ECM proteins collagen, laminin and by growth factors NGF, b-FGF. Darlene G. Attiah, Ross Kopher, and Tejal A. Desai. Department of Bioengineering, University of Illinois at Chicago, Chicago, IL 60607

Nerve cell transplantation is a common approach for the treatment of neurodegenerative diseases where it is desired to restore functionality in the host system by substituting for the damaged tissue. It is proposed that neuronal outgrowth and differentiation is controlled by the presence of extracellular matrix adhesion molecules, growth factors, and other environmental cues. Pheochromoytoma (PC12) cells were used as a model for investigating the influence of the cell adhesion molecules collagen and laminin and of growth factors NGF and b-FGF on the in vitro maintenance of nerve cell proliferation and differentiation. Characterization was achieved by the assessment of different parameters including: the percentage of PC12 neurite bearing cells, neurite stability, density, and mean length for an experimental duration of 7−14 days.

207. Characterization of the reverse reaction of (*S*)-mandelate dehydrogenase from *Pseudomonas putida.* Asteriani R. Dewanti and Bharati Mitra. Department of Biochemistry and Molecular Biology, Wayne State University, 4374 Scott Hall, 540 E. Canfield, Detroit, MI 48201 (fax: 313-577-2765, adewanti@med.wayne.edu)

(*S*)-Mandelate dehydrogenase (MDH), an FMN-containing hydroxy acid oxidizing enzyme, oxidizes (*S*)-mandelate, a bulky aromatic α-hydroxy acid. In contrast, other enzymes in this family oxidize small, saturated aliphatic substrates. It has been shown that two homologous enzymes, yeast flavocytochrome b_2 (FCB2) and long-chain hydroxy acid oxidase (HAO) from rat kidney, can catalyze the reverse reaction, the conversion of keto acid to hydroxy acid. In this study, we show that MDH is also able to catalyze the reverse reaction. For FCB2, the large difference in the k_{cat} values between the forward and the reverse reactions is utilized to discriminate between substrate and product. Unlike FCB2, for MDH the discrimination between mandelate and benzoylformate is determined by the large difference in the K_d values. This may be due to the unique nature of its constrained, aromatic substrate for MDH. However, slow substrates and products have similar affinities for MDH. (Supported by NIH Grant GM-54102.)

208. Design of novel matrix metalloproteinase inhibitors based on three-dimensional quantitative structure−activity relationship (3D-QSAR) models. William J. Welsh and **Elizabeth A. Amin**. Department of Chemistry and Center for Molecular Electronics, University of Missouri−St. Louis, 8001 Natural Bridge Rd., St. Louis, MO 63121 (fax: 314-516-5342, wwelsh@umsl.edu, ea@ozone.umsl.edu)

Matrix metalloproteinases (MMPs) constitute a class of structurally related, zinc-binding enzymes which mediate the breakdown of extracellular matrix proteins such as collagen, gelatin, and proteoglycan. MMPs have been identified in tissue surrounding invasive carcinoma, and directly enable tumor metastasis through proteolysis and blood vessel formation (angiogenesis). Degenerative and inflammatory diseases such as osteoarthritis also depend on MMPs to spread to unaffected tissue. These enzymes are therefore attractive targets for small-molecule synthetic inhibitors (MMPIs) which would serve as adjuncts to traditional treatments such as radiation and chemotherapy. Comparative molecular field analysis (CoMFA), a widely used 3D paradigm of quantitative structure−activity relationship (QSAR) models, is useful when the binding area is unknown or difficult to model, and is therefore well suited to MMPI design. Three highly predictive CoMFA models have been derived and applied to the design of a new series of nonpeptidic MMPIs which demonstrate high predicted biological activity against stromelysin-1 (MMP-3).

209. Active intermediates of the thermophilic cytochrome P450 (CYP119) from *Sulfolobus solfataricus*. Shao-Ching Hung, Ilia G. Denisov, Kara E. Weiss, and Stephen G. Sligar. Departments of Biochemistry and Chemistry and College of Medicine, University of Illinois, Urbana, IL 61801

CYP119 is a thermophilic cytochrome P450 from *Sulfolobus solfataricus* which was found in a high-temperature and sulfur-rich environment. So far, the substrates and redox partner that perform its enzymatic function are still unknown. To gain insights into the possible intermediates in the reaction mechanism, the ferric, ferrous, oxygenated, and reduced hydroperoxy forms of CYP119 are formed as generated from chemical and radiolytic reduction methods. UV−vis and EPR spectroscopic studies have been performed for detailed characterization of these intermediates. Furthermore, the low-spin and fast autoxidation properties of this enzyme can be explained based on the three-dimensional crystal structure. Finally, the reaction of organic peroxides with CYP119 is presented. (Supported by NIH Grants GM31756 and GM33775.)

210. Chemical precedent for early events in coenzyme B_{12}-dependent enzyme reactions: evidence for quantum

mechanical tunneling in hydrogen abstraction reactions even without the enzyme. Kenneth M. Doll and Richard G. Finke. Department of Chemistry, Colorado State University, 200 W. Lake St., Fort Collins, CO 80523 (fax: 970-491-1801, kdoll@lamar.colostate.edu)

Recently, there have been numerous reports regarding the possible involvement of quantum mechanical tunneling in enzyme reactions that involve the transfer of hydrogen. The enzyme's involvement in tunneling can only be studied with a comparison of the same reaction both inside and outside of the enzyme. The solution thermolysis reaction of coenzyme B_{12} has been shown to cleanly generate adenosyl radicals that can abstract hydrogen from ethylene glycol, the same reaction that is observed in coenzyme B_{12}-dependent diol dehydratase. We have compared our solution data to the literature enzyme data, and also to another well-studied enzyme, methylmalonyl-CoA mutase. We have also synthesized 8-methoxyadenosylcobalamin, a methoxy analogue of coenzyme B_{12}, in high purity. This methoxy analogue has demonstrated similar abstraction reactivity to coenzyme B_{12}, but with simpler reaction kinetics. These systems have allowed us to study questions about the ability of enzymes to exploit or enhance quantum mechanical tunneling in their hydrogen abstraction reactions.

211. Biochemical and spectroscopic characterization of human heart L-3-hydroxyacyl-CoA dehydrogenase. Pravin Nair,[1] Alasdair F. Bell,[1] Joseph J. Barycki,[2] Leonard J. Banaszak,[2] and Peter J. Tonge.[1] [1]Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794, and [2]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455

Human heart L-3-hydroxyacyl-CoA dehydrogenase (HAD) is the penultimate enzyme in the β-oxidation pathway and catalyzes the NAD-dependent oxidation of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. We are using site-directed mutagenesis (SDM), kinetics, and Raman spectroscopy to elucidate the mechanism of the enzyme. X-ray crystallographic studies suggested key residues that hydrogen bond to the substrate and cofactor, namely, histidine 158, glutamate 170, serine 137, and asparagine 208. Histidine 158 has been proposed to be the key active site residue, acting as a catalytic base and abstracting from the 3-OH group of the substrate; the glutamate residue is proposed to stabilize the positive charge that results from the proton abstraction. Raman spectroscopy has been used to characterize the structure of the complex of wild-type HAD + NAD^+ + AcAc-CoA which was discovered to form a charge-transfer complex. Labeled compounds will be used to assign the Raman bands from the 3-ketoacyl-CoA and find structure−reactivity correlation.

212. Chromophoric spin-labeled β-lactam antibiotics for ENDOR structural characterization of reaction intermediates of class A and class C β-lactamases. D. Mustafi, J. E. Hofer, and M. W. Makinen. Department of Biochemistry & Molecular Biology, The University of Chicago, Chicago, IL 60637

The chromophoric spin-label substrates 6-*N*-[3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-propen-2-oyl]-penicillanic acid (SLPPEN) and 7-*N*-[3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-propen-2-oyl]-cephalosporanic acid (SLPCEP) were synthesized by acylation of the amino-β-lactam antibiotic with 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-propen-2-oic acid and characterized by physical methods. Both substrates exhibited UV absorption properties that allowed more accurate monitoring of hydrolysis than the spin-labeled β-lactam antibiotics synthesized earlier in this laboratory without the bridging olefinic group, in addition to "burst kinetics" with TEM-1 β-lactamase of *E. coli* and the β-lactamase of *Enterobacter cloacae* P99, indicative of formation of an acylenzyme reaction intermediate. The maximum value of $\Delta\epsilon$ for substrate-to-product hydrolysis was 2500 M^{-1} cm^{-1} at 277 nm compared to 670 M^{-1} cm^{-1} at 232 nm for SLPPEN and SLPEN [Mustafi, D., and Makinen, M. W. (1995) *J. Am. Chem. Soc*. *117*, 6739], respectively. For SLPPEN, steady-state kinetic parameters determined under initial velocity conditions were a k_{cat} of 640 ± 35 s^{-1} and a k_{cat}/K_M of (13.8 ± 0.8) × 10^6 M^{-1} s^{-1} for TEM-1 enzyme and a k_{cat} of 0.5 ± 0.02 s^{-1} and a k_{cat}/K_M of (5.3 ± 0.3) × 10^4 M^{-1} s^{-1} for P99 enzyme. These results follow the catalytic reactivity pattern of TEM-1 β-lactamase as a good penicillinase and poor cephalosporinase and P99 β-lactamase as a good cephalosporinase but poor penicillinase, as has been demonstrated with other classical β-lactam antibiotics. In contrast to the TEM-1 enzyme for which MeOH/water mixtures are optimal for cryokinetic studies, DMSO/water mixtures were optimal for the P99 β-lactamase, with DMSO exhibiting only noncompetitive inhibition up to concentrations of 40% (v/v) at pH* 7 and 25 °C. The $t_{1/2}$ of the acylenzyme intermediates formed upon reaction of the P99 enzyme with SLPPEN or SLPCEP at −30 °C was ≥3 min, allowing optical characterization. Electron nuclear double resonance (ENDOR) studies of the acylenzyme reaction intermediates of the P99 enzyme formed with SLPPEN and SLPCEP under cryokinetic conditions are presently in progress. (Supported by NIH Grant GM21900.)

213. Cloning, overexpression, purification and characterization of catalase/peroxidase from enterohemorrhagic *E. coli* O157:H7. Douglas C. Goodwin. Department of Chemistry, Auburn University, Auburn, AL 36849-5312

Enterohemorrhagic *Escherichia coli* O157:H7 is a highly virulent foodborne pathogen. Although mechanisms of virulence have not been fully elucidated, several proteins have been identified in *E. coli* O157:H7 that are absent from nonpathogenic strains, including an extracellular, heme-containing catalase/peroxidase, KatP. As a first step toward understanding the potential role that this enzyme may have in the virulence of *E. coli* O157:H7, we have cloned, overexpressed, and purified KatP from *E. coli* O157:H7. Visible absorption spectra recorded for purified KatP [Fe(III), Fe(III)-CN, and Fe(II)] were consistent with similar spectra recorded for other bacterial catalase/peroxidases. Likewise, the k_{cat} for the catalase activity of KatP (7500/s) 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. The significance of the reactivity of KatP with H_2O_2 and other hydroperoxides in the context of *E. coli* O157:H7 virulence will be discussed.

214. Crystal structure of the rabbit phosphoglucose isomerase complexed with D-arabinonhydroxamic acid 5-phosphate at 1.9 Å resolution. Diana Arsenieva,[1] Renaud Hardré,[2] Laurent Salmon,[2] and Constance Jeffery.[1] [1]Laboratory for Molecular Biology, MC567, Department of Biology, University of Illinois, 900 S. Ashland, Chicago, IL 60607, and [2]Laboratoire de Chimie Bioorganique et Bioinorganique, CNRS−FRE 2127, Institut de Chimie Moléculaire d'Orsay, Bât. 420, Université Paris-XI, 91405 Orsay, France

Phosphoglucose isomerase (PGI; EC 5.3.1.9) catalyzes the second step in glycolysis: the interconversion of D-glucose 6-phosphate and D-fructose 6-phosphate. Mutations in the gene encoding PGI cause the disease nonspherocytic hemolytic anemia. The competitive inhibitor D-arabinonhydroxamic acid 5-phosphate (5PAH) has a high constant of enzyme inhibition, $K_D = 2 \times 10^{-7}$ M, and resembles the intermediate of the reaction catalyzed by PGI. The structure of the complex was solved to 1.9 Å resolution with a final *R*-factor of 21.2% and an *R*-free of 24.7%. The positioning of the inhibitor in the active site supports the model that amino acid residues Glu357 and Arg272 participate in the isomerization step of catalysis. The comparison of our new structure to the structure of PGI without bound ligands shows that the active site adopts a more closed conformation upon inhibitor binding. The helix containing residues 513−520 shifts, bringing Lys518 closer to the bound ligand.

215. Effect of guanidinium display on the translocation of molecules through membranes. Stephen M. Fuchs[1] and Ronald T. Raines.[2] [1]Department of Biochemistry and [2]Departments of Biochemistry and Chemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (sfuchs@biochem.wisc.edu)

Thirty years ago, it was recognized that cationic polymers enhance the uptake of proteins and drugs and proteins by cells. More recently, conjugation to certain peptides has been shown to facilitate membrane translocation. The mechanism of this translocation is unclear but often depends on the presence of the guanidinium group of arginine residues. Little is known about the spatial requirements of the guanidinium functionalities. To address these issues, fluorescently labeled scaffolds have been synthesized displaying guanidinium groups in a variety of configurations. The ability of these scaffolds, as well as other polycations, to enter cells was assessed by fluorescence-activated cell sorting. The data suggest factors critical for the efficient translocation of proteins and small molecules through lipid bilayers.

216. A conformationally restricted analogue of vigabatrin as an inactivator of gamma-aminobutyric acid aminotransferase. Kristi Calvert and Richard B. Silverman. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113 (calvert@chem.nwu.edu)

A major inhibitory neurotransmitter in the central nervous system is γ-aminobutyric acid (GABA). Low levels of GABA have been associated with disorders such as epilepsy, Parkinson's disease, and Alzheimer's disease. The major pathway for degradation of GABA is via transamination with α-ketoglutarate, catalyzed by the enzyme GABA aminotransferase (GABA-AT). Inhibition of this enzyme results in an increased availability of GABA and could have therapeutic applications in treating neurological disorders such as those aforementioned. Vigabatrin, a mechanism-based inactivator of GABA-AT used to treat epilepsy in Europe, inactivates the enzyme through two pathways: a Michael addition or an enamine mechanism. A previous conformationally restricted analogue of vigabatrin, 3-amino-4-cyclohexenecarboxylic acid, inactivated GABA-AT solely through the enamine mechanism. An exocyclic, conformationally rigid analogue will be synthesized and assayed in an attempt to inactivate GABA-AT solely through the Michael addition pathway.

217. Electrochemical properties of the proline dehydrogenase domain of PutA from *Escherichia coli*. M. P. Vinod and D. F. Becker. Department of Chemistry, University of Missouri−St. Louis, St. Louis, MO 63121-4499

Electrochemical analysis of redox active proteins is of much current research interest especially since such an understanding of the inherent redox activity of proteins will help in designing novel biosensors and biochemical fuel cells. PutA is a multifunctional flavoprotein from *Escherichia coli* that associates peripherally with the membrane and catabolizes proline to glutamate. PutA is also a DNA-binding protein that regulates the transcription of the proline utilization regulon. In the present study, we report the isolation and electrochemistry of the proline dehydrogenase domain of PutA (PutA669), which is comprised of the first 669 amino acids of the PutA polypeptide (1320 total amino acids). Potentiometric titrations of $PutA_{669}$ by spectroelectrochemical methods using redox mediator dyes demonstrated a two-electron reduction of the flavin and an $E°$ of −0.085 V (pH 7.5) for $FAD/FADH_2$. Our current research is also directed toward immobilizing $PutA_{669}$ on the electrode surface using lipid bilayer membranes, self-assembled monolayers, and LB film techniques.

218. Enantiomeric characterization of citrus peel oils. Solomon Mitiku Bassore, Masayoshi Sawamura, and Hiroyuki Ukeda. Bioresources Science, Kochi University, B-200 Monobe, Nankoku, Kochi, 783-8502, Japan (fax: 81-88-864-5200, sawamura@cc.kochi-u.ac.jp)

Citrus peel oils were classified and characterized based on the enantiomeric distribution of limonene, alpha-pinene, beta-pinene, sabinene, and linalol. A total of 80 different self-prepared citrus oils, which belong to 37 varieties of 12 citrus species cultivated in Ethiopia, Kenya, Italy, and Japan, were investigated. Most of the citrus oils contained excess (+)-isomer of all the components analyzed. Only lemon, bergamot, Thahiti lime, and tachibana oils were identified to contain (−)-enantiomers of alpha-pinene, beta-pinene, and sabinene. Ujukitsu, ichang lemon, or kiyookadaidai was identified to contain the least enantiomeric purity of alpha-pinene, beta-pinene, or sabinene. Determination of the enantiomeric distribution of linalol was identified as an important tool to discriminate Satsuma mandarin oil from the rest of mandarin and its hybrid oils. It was also important to discriminate yuzu (C. junos Seib. ex Tanaka) from other citrus oils. For some of the citrus oils, enantiomeric distributions of the chiral components at different growth stages were investigated.

219. Investigation of the role of active site loops determining the substrate specificity of cholesterol oxidase. Jingyi Xiang and Nicole S. Sampson. Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400

Cholesterol oxidase catalyzes the oxidation and isomerization of cholesterol to cholest-4-en-3-one and uses different sterols as substrates, such as cholesterol, β-sitosterol, stigmasterol, and campesterol. The difference between these sterols is their C17 carbon tail structures. We used a combination of computer modeling and library screening to explore which residues are important for substrate specificity because an X-ray crystal structure of cholesterol oxidase with one of the above substrates has not been obtained. Using molecular dynamics, we identified Met58, Leu82, Val85, Met365, and Phe433 as most likely to be responsible for binding the C17 carbon tail of sterols. To test our prediction, we prepared mutant libraries in which each of these residues was substituted with the aliphatic amino acids plus phenylalanine and serine by cassette mutagenesis. We screened for mutants that were still active using an agar plate assay. These mutants were further characterized using in vitro kinetic assays with detergent micelles and small unilamellar vesicles. Our results thus far suggest that the wild-type and mutant enzymes do not have a preference for one sterol tail structure over another.

220. Kinetic and structural analysis of the role of asparagine 485 in the reaction catalyzed by cholesterol oxidase. Ye Yin,[1] Paula Lario,[2] Alice Vrielink,[3] and Nicole S. Sampson.[1] [1]Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400, [2]McIntyre Medical Sciences Building, McGill University, 3655 Drummond St., Montréal, Québec, H3G 1Y6, Canada, and [3]Department of Biochemistry, University of California, Santa Cruz, CA

Cholesterol oxidase catalyzes the oxidation and isomerization of cholesterol. We proposed that the His447, Asn485, Wat541 hydrogen-bonding network helps to position the substrate and coordinates general base and eletrophilic catalysis for oxidation. A single mutant, N485L, was prepared. Kinetic analysis shows that k_{cat} for N485L is 1000-fold slower than wild type in oxidation and for isomerization is 20-fold slower. Clearly, residue Asn485 plays a key role in the catalysis of oxidation.

The most significant change observed in the mutant X-ray crystal structure is a repositioning of Met122 away from O_4 of the FAD. This Met122 also positions Asn485 over the pyrimidine ring of the cofactor in the wild-type structure. Redox potential measurements for the FAD of wild type and N485L showed that the E_m for wild type is 87 mV more positive than that of N485L. This reveals that the reason for the reduced catalytic activity of the N485L is the reduced reduction potential of the FAD. This reduction is consistent with the loss of the stabilizing interaction between Asn485, Met122, and the cofactor.

221. Crystal structure of rabbit phosphoglucose isomerase complexed with its substrate, D-fructose-6-phosphate. Ji Hyun Lee, Kathy Z. Chang, Vishal Patel, and Constance J. Jeffery. Department of Biology, University of Illinois at Chicago, 900 S. Ashland Ave., Room 4260, Chicago, IL 60607-7173 (jlee66@uic.edu)

Phosphoglucose isomerase catalyzes the interconversion of D-glucose-6-phosphate (G6P) and D-fructose-6-phosphate (F6P) and plays important roles in glycolysis and gluconeogenesis. Catalysis proceeds in both the G6P to F6P and F6P to G6P directions, so both G6P and F6P are substrates. X-ray crystal structure analysis of rabbit and bacterial PGI has previously identified the location of the enzyme active site, and a recent crystal structure of rabbit PGI identified Glu357 as a candidate functional group for transferring the proton. However, it was not clear which active site amino acid residues catalyze the ring-opening step. We report the X-ray crystal structure of rabbit PGI complexed with the cyclic form of its substrate, D-fructose-6-phosphate, at 2.1 Å resolution. The location of the substrate relative to the side chains of His388 and Lys518 suggests that His388 protonates the ring oxygen and Lys518 and Thr214 position a water molecule that accepts a proton from the hydroxyl group at C2 to provide the open-chain form of the substrate.

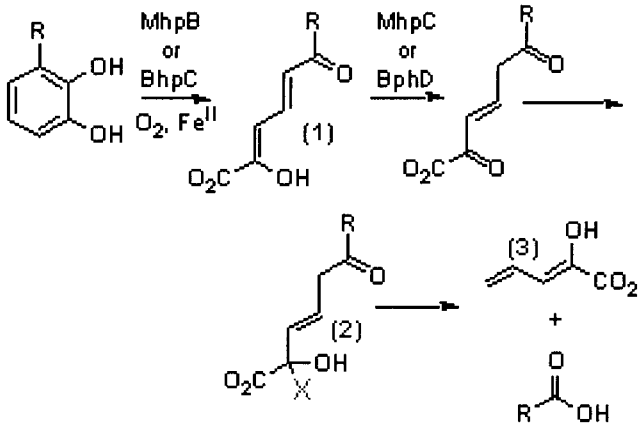
222. Kinetic investigation of the reaction catalyzed by yeast cystathionine beta-synthase. Shinichi Taoka and Ruma Banerjee. Department of Biochemistry, University of Nebraska−Lincoln, 1901 Vine St., Lincoln, NE 68588-0664

Elevated levels of homocysteine are correlated with cardiovascular diseases and neural tube defects. Human cystathionine beta-synthase is one of two key enzymes that removes homocysteine from mammalian cells. It catalyzes the condensation of serine and homocysteine to produce cystathionine. Yeast cystathionine beta-synthase is a pydoxial 5-phosphate dependent enzyme. The kinetic parameters of the enzyme, k_{cat} and K_m, obtained when the enzyme is preincubated with homocysteine are 2−3 times higher than those seen with serine-preincubated enzyme. We are investigating kinetic characteristic by stopped flow spectroscopy. Global analysis indicates that at least three species are formed when the enzyme is preincubated with 30 mM serine and shot against with 10−25 mM homocysteine. The species with peaks at 413, 425, and 460 nm are assigned as the internal aldimine, the external aldimine, and the aminoacrylate, respectively. A fourth unassigned species with a peak at 430−450 nm is observed; this is presumably a product−enzyme species. The detailed pre-steady-state analysis of the reaction will be described.

223. Mechanistic studies of hydrolase enzymes on the aromatic degradation pathways of *Escherichia coli*. Damian M. Speare and Timothy D. H. Bugg. Department of Chemistry, University of Warwick, Coventry, United Kingdom (fax: +44 (024) 765 24112, d.m.speare@warwick.ac.uk)

The persistence in the environment of man-made chemicals, particularly chlorinated aromatics and polychlorinated biphenyls (PCB's), in the form of pesticides and industrial chemicals is an issue of increasing public concern. By studying the bacterial pathways involved in biodegradation of aromatic compounds, we seek to understand the enzymatic processes involved in their breakdown. We are currently studying two catabolic pathways found in *Escherichia coli* responsible for the degradation of phenylpropionic acid and

biphenyl. In both degradation pathways, there is an oxidative ring cleavage catalyzed by a dioxygenase enzyme to give dienoic acid products (**1**). These then undergo tautomerism followed by cleavage to give 2-hydroxypentadienoic acid (**3**) and either succinic or benzoic acid. The cleavage involves attack of a nucleophile onto the carbonyl to give a quaternary center (**2**) and subsequent fragmentation, but the nature of the nucleophile remains unknown. We have developed a general synthetic route to these dienoic acids allowing further research to be carried out. Results will be shown of investigations into the stereospecific enzyme mechanism by spectroscopy and isotope labeling experiments.



224. Poly(ethylene glycol) as a means to identify the site of enzyme inactivation. Christine A. Schering and Richard B. Silverman. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208 (schering@chem.nwu.edu)

Poly(ethylene glycol) is used to develop a new methodology to determine the site of enzyme inactivation. The unique solubility properties of PEG (it is soluble in most solvents except ether and hexanes) allow for homogeneous reaction conditions while retaining simple isolation and purification via precipitation and filtration. To develop this new method, TPCK, an inactivator of α-chymotrypsin, was linked to PEG (MW 5000). Incubation of the PEG-bound TPCK with α-chymotrypsin (MW 25 000) led to 92% inactivation of the enzyme. MALDI-TOF mass spectroscopy showed a new peak at 30 000 Da, supporting the covalent attachment of PEG-TPCK to α-chymotrypsin. Following enzymatic digestion, the MALDI-TOF spectrum indicates a peptide adduct to the PEG. The sequence and site of inactivation are being determined via analytical techniques.

225. Regulation of the constitutive nitric oxide synthases through their reductase domains. Dawn E. Harris, Linda J. Roman, Pavel Martasek, Thomas M. Shea, and Bettie Sue S. Masters. Department of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900 (fax: 210-567-6984, harrisd@uthscsa.edu)

The nitric oxide synthases (NOS) are composed of a heme and a reductase domain, with a calmodulin (CaM) binding site connecting the two. The reductase domain is highly homologous to NADPH−cytochrome P450 reductase (CPR), with the exception of an insert in the FMN domain in the constitutive NOSs and an elongated tail in all the NOSs. We have investigated the role of CaM, the autoinhibitory insert, and the C-terminal tail in controlling electron transfer in the reductase domain. An autoinhibitory peptide from the insert in the FMN domain inhibits activity by displacing CaM, while the C-terminal tail represses activity in the absence of CaM. We propose that the reductase domain contains all the elements for controlling activity in the constitutive NOSs. (Supported by NIH Grants GM52419 and HL30050 and Robert A. Welch Foundation Grant AQ-1192 to B.S.S.M.)

226. Effect of fluoroproline in the X-position on the triple-helical stability of collagen. Jonathan A. Hodges and Ronald T. Raines. Department of Biochemistry and Department of Chemistry, University of Wisconsin−Madison, Madison, WI

The polypeptide strands of natural collagen consist of tripeptide repeats of the sequence Gly-X-Y. The Y-position is often occupied by 4(*R*)-hydroxyproline (Hyp), which stabilizes the collagen triple helix. Hydroxyproline in the X-position has long been considered destabilizing, but recent work has provided an example to the contrary. In the Y-position, 4(*R*)-fluoroproline (Flp) further enhances triple helix stability due to the increased electron-withdrawing ability of −F relative to −OH. The effect of Flp in the X-position on triple-helix stability is examined by the synthesis of $(FlpProGly)_7$ and comparison of the conformation stability of its triple helices to that of other collagen mimics.

227. Study of lipid exchange in children with rachit. Shoira M. Ibatova,[1] **Robiya A. Nasimova**,[2] Shuhrat M. Sayitkulov,[2] Nurali Q. Mukhamadiev,[2] and Gulzoda F. Fayziyeva.[2] [1]Samarkand Medical Institute (fax: 998-662 333487, info@chem.samuni.silk.org) and [2]Physical Chemistry Department, Samarkand State University, University Blvd., 15, A. Temur, 148, India

The blood serum of healthy and hospitalized children up to the age of 3 has been investigated. The group composition of blood serum has been carried out by the thin-layer chromatography method and by the individual composition of fatty acids, by the method of gas−liquid chromatography. Lipase activity was estimated as the fraction of free fatty acids. Primary attention is given to such fractions of lipids found as free fatty acids and glycerides. As the obtained data show, children who are ill with rachit are observed to have a reduction in the contents of free fatty acids and glycerides in comparison to the control group. There is a reduction in the contents of unsaturated fatty acids and the lipase activity with a constant content of saturated lipid. Probably, it is connected with low assimilation of lipids by the pathological organism. Thus, study of the group composition of lipids of individual composition and contents of fatty acids allows estimation of the status of lipid disbalance in the organism.

228. Effects of multivalent, chemotactically active, saccharide polymers on bacterial swarmer cell dedifferentiation. Allison C. Lamanna,[1] Jason E. Gestwicki,[1] Julius Adler,[1] and Laura L. Kiessling.[2] [1]Department of Biochemistry and [2]Departments of Chemistry and Biochemistry, University of Wisconsin−Madison, 433 Babcock Dr., Madison, WI 53706 (alamanna@biochem.wisc.edu)

Swarmer cells are multinucleoid, hyperflagellated, elongated, differentiated bacteria that tend to grow on surfaces. They have been shown to be important in pathogenicity and have been implicated in biofilm formation because they secrete exopolysaccharides similar to those that encase biofilm colonies. Many different species of bacteria, including *E. coli*, *S. typhimurium*, *P. mirabilis*, and *B. subtilis*, are able to differentiate into swarmer cells. The mechanisms underlying swarmer cell growth, differentiation, and especially dedifferentiation are largely unexplored. It is known that the chemotaxis proteins are essential for swarmer cell differentiation. We have synthesized multivalent compounds that alter bacterial chemotaxis. We have developed a new assay that can be used to distinguish between swarmer cells and undifferentiated bacteria. The effects of our synthetic ligands on swarmer cell dedifferentiation in various bacterial species will be presented. Our results suggest new approaches to alter microbial pathogenicity.

229. Substrate recognition and activation in β-oxidation. Peter J. Tonge,[1] Alasdair F. Bell,[1] Yuguo Feng,[1] Jiaquan Wu,[1] Hilary Hofstein,[1] Polina Novichenok,[1] Marian T. Stankovich,[2] and Avery W. Stephens.[2] [1]Department of Chemistry, SUNY Stony Brook, Stony Brook, NY 11794-3400, and [2]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

We are studying the mechanism of action of enzymes involved in fatty acid oxidation. Using site-directed mutagenesis coupled with spectroscopic methods such as Raman and NMR spectroscopy, we are exploring how enoyl-CoA hydratase and acyl-CoA dehydrogenase bind and activate their fatty acid substrates. The observation using Raman spectroscopy that enoyl-CoA hydratase binds two conformers of the substrate analogue hexadienoyl-CoA strongly suggests that the enzyme's stereochemistry results from preferential hydration of one bound substrate conformer rather than preferential binding of the correct conformer. In addition, the effect of modifying hydrogen bonding interactions between the enzyme and substrate carbonyl in enoyl-CoA hydratase (G141P) and acyl-CoA dehydrogenase (E376Q, 2-deoxyFAD) is being explored using Raman spectroscopy. Finally, we have synthesized a range of acyl-oxyCoA esters to probe the importance of the CoA thioester in the reaction catalyzed by each enzyme.

230. Tale of two C2s: Distinct roles of a common Ca^{2+}-binding domain. Bernd U. Sehgal,[1] Ricardo A. Garcia,[2] Jovana Grbic,[1] and Hilary Arnold Godwin.[1,2] [1]Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3113, and [2]Department of Biochemistry, Molecular Biology, and Cell Biology, 2153 N. Campus Dr., Northwestern University, Evanston, IL 60208-3300

C2 domains are found in a large number of eukaryotic proteins in a variety of signaling pathways and often serve as protein−protein or protein−lipid interaction modules. Although C2 domains were originally identified as the calcium-binding motif in Ca^{2+}-dependent isoforms of protein kinase C, not all C2 domains are Ca^{2+}-dependent. Here, the C2 domains of two proteins with very different functions are investigated and compared: the C2 domain of the yeast ubiquitin ligase Rsp5 (Rsp5-C2) and the C2 domains (C2A and C2B) of the neurological calcium sensor synaptotagmin II. Because C2 domains have widely been considered to act as an oligomerization motif, we determined the association state of Rsp5-C2, C2A, C2B, and full-length synaptotagmin II by conducting sedimentation equilibrium experiments using analytical ultracentrifugation, and of full-length Rsp5 by native PAGE. Both full-length proteins self-associate, but whereas synaptotagmin II dimerizes in a Ca^{2+}-dependent manner, Rsp5 is constitutively self-associated. The association states of the individual C2 domains were also investigated, to determine their role in the association of the proteins. Although both full-length proteins self-associate, neither Rsp5-C2 nor C2A does, regardless of whether Ca^{2+} is present. By contrast, C2B oligomerizes in a Ca^{2+}-independent fashion; presumably Ca^{2+} binding to C2A is required for the oligomerization to be Ca^{2+}-dependent. These studies suggest that the C2B domain of synaptotagmin II behaves as an oligomerization motif, but that the C2 domain from Rsp5 is not responsible for self-association.

231. Exploring the active site hydrogen bonding network of a protein tyrosine phosphatase. Christin L. Thomas, Kristin A. White, and Robert L. Van Etten. Chemistry Department, Purdue University, West Lafayette, IN 47907

An extensive hydrogen bonding network can be found in the active site of low molecular weight protein tyrosine phosphatases. The interaction is between several residues in the phosphate binding loop and nearby histidine and serine residues. This network appears to be important for maintaining the optimal active site configuration for substrate recognition and catalysis. We are exploring the roles of highly conserved histidine and serine residues in the hydrogen bonding network. Site-directed mutagensis is being used to systematically alter the protein sequence. Substrate binding and catalysis by mutant enzymes is being characterized by Michaelis−Menten kinetics. Proton nuclear magnetic resonance spectroscopy is being used to explore structural changes.

232. Factors affecting the conformational stability of collagen. Cara L. Jenkins,[1] Lynn E. Bretscher,[2] and Ronald T. Raines.[1,2] [1]Department of Chemistry and [2]Department of Biochemistry, University of Wisconsin−Madison, Madison, WI 53706

Collagen is the major structural protein in animals, and is made up of three polypeptide strands with the repeating sequence $(Xaa\text{-}Yaa\text{-}Gly)_n$. These strands adopt a polyproline II-type conformation and wind around a common axis to form the collagen triple helix. The Xaa and Yaa positions of the repeating sequence are often occupied by proline (Pro) and 4(*R*)-hydroxyproline (Hyp) residues, respectively. These heterocyclic amino acids, particularly Hyp, have been shown to be critical for collagen stability, but the molecular basis of the contribution of Hyp to collagen stability has not been thoroughly elucidated. We have made several model peptides based on the sequences $(Pro\text{-}Hyp\text{-}Gly)_n$ and $(Pro\text{-}Pro\text{-}Gly)_n$ (where $n = 7$ or 10) to explore the factors that contribute to collagen stability. The syntheses of these peptides and the results of their study will be presented.

233. Gum polysaccharide of two Venezuelan specimens from *Albizia niopoides* var. colombiana. Olga Beltrán,[1]

Gladys León de Pinto,[1] Maritza Martínez,[1] José Manuel Igartuburu,[2] Fernando Rincón,[1] and Lilian Sanabria.[1] [1]Centro de Investigaciones en Química de los Productos Naturales, Facultad de Humanidades y Educación, La Universidad del Zulia, Apartado 526, Maracaibo, Venezuela, and [2]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510, Puerto Real, Cádiz, Spain

The genus *Albizia durazzini* comprises 150 species; some of these species are disseminated in Venezuela. *Albizia niopoides* var. colombiana exudes a clear gum, very soluble in cold water. Analytical data of the gum exudates from two specimens of that species showed little variation. The gums studied have interesting properties which support the complexity of the *Albizia* genus. The polysaccharide isolated from the gums contains galactose, arabinose, rhamnose, and glucuronic acid and its 4-*O*-methyl derivative. The high content of rhamnose is comparable to that reported for *A. forbessi*. Mannose was not detected, although the presence of this sugar in most *Albizia* gums has been taken as a feature of great diagnostic significance. The analytical data reported in this work are a contribution to augment the data existing for the gums from *Albizia* spp. On the other hand, it was shown that the analytical differentiation between *Acacia* and *Albizia* gums may be achieved by NMR spectra.

234. Importance of a buried polar d−g′ interaction in a model antiparallel coiled coil. Diana L. McClain, Howard L. Woods, III, and Martha G. Oakley. Department of Chemistry, Indiana University, 800 East Kirkwood Ave., Bloomington, IN 47405-7102 (fax: 812-855-8300)

Coiled coils are formed by two or more α-helices that align in a parallel or an antiparallel relative orientation. Residues at the **a**, **d**, **e**, and **g** positions define the hydrophobic interface between the helices and are important for determining coiled-coil specificity. In Acid-a1-Base-a1, a buried **a**−**d′** Asn−Asn interaction results in a 50-fold preference for the antiparallel orientation. Although this type of interaction is found frequently in parallel coiled coils, there are no examples of an **a**−**d′** Asn−Asn interaction in naturally occurring antiparallel coiled coils. There are, however, numerous examples of **d**−**g′** and **a**−**e′** salt bridges. We have designed peptides that explore the role of a potential interhelical interaction between an Arg at an interior **d** position and a Glu at the appropriate **g′** position. We have determined the effect of this potential **d**−**g′** interaction on helix orientation preference, oligomerization state, and stability of this model coiled coil.

235. Inhibition of the enoyl-acyl carrier protein reductase from *E. coli* by triclosan. Sharada Sivaraman and Peter J. Tonge. Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794

The enoyl-acyl carrier protein reductase (ENR) is involved in bacterial fatty acid biosynthesis and is the target for the antibacterial diazaborine compounds and the frontline antituberculosis drug isoniazid. Recent reports have implicated triclosan (TCN) as a third structurally distinct class of ENR inhibitors based on resistant strains of *Escherichia coli* and *Mycobacterium smegmatis*. The widespread use of triclosan in several personal care products such as toothpaste, mouthwashes, and soaps can be questioned in light of these reports. For this reason, we have chosen to investigate the molecular mechanism of inhibition of the *E. coli* ENR by triclosan. Our data suggest that triclosan is a tight-binding inhibitor of the *E. coli* ENR with subnanomolar K_i. The dissociation constant, K_d, obtained from fluorescence for the enzyme·NADH·I complex is 1 μM. Based on the crystal structure of this ternary complex, we have made mutations to investigate the role of Tyr 156 in the binding of triclosan. The triclosan-resistant mutants identified by Levy and coworkers, G93V, F203L, and M159T, have been overexpressed, and the data show that triclosan binding is weaker although the k_{cat} for the substrate is unaffected. MIC experiments have been carried out with triclosan and other dihydroxydiphenyl ether derivatives that corroborate the reactivity experiments.

236. Interaction of *S*-adenosylmethionine with the iron−sulfur cluster of pyruvate formate-lyase activating enzyme. Wei Hong,[1] Charles Walsby,[2] William E. Broderick,[1] Brian M. Hoffman,[2] and Joan B. Broderick.[1] [1]Department of Chemistry, Michigan State University, East Lansing, MI 48824 (broderij@cem.msu.edu), and [2]Department of Chemistry, Northwestern University, Evanston, IL 60208

Pyruvate formate-lyase activating enzyme (PFL-AE) is a member of a growing family of enzymes that utilize [4Fe-4S] clusters and *S*-adenosylmethionine (AdoMet) to generate catalytically essential radicals. PFL-AE generates a glycyl radical on pyruvate formate-lyase (PFL), and converts AdoMet stoichiometrically to methionine and 5′-deoxyadenosine. An AdoMet-derived adenosyl radical has been implicated as the intermediate responsible for abstraction of the *pro-S* hydrogen atom of PFL Gly734. To probe the mechanism of radical generation, and the role of AdoMet, we have undertaken an investigation of the interaction of PFL-AE with a variety of isotopically labeled AdoMets and AdoMet analogues. The syntheses and characterization of the labeled AdoMets and AdoMet analogues will be presented, along with the results of kinetic and spectroscopic studies aimed at probing the interaction of AdoMet with the [4Fe-4S] cluster of PFL-AE.

237. Enzymatic enantioselective esterification of ketoprofen in organic solvent. Du Wei, **Zong Min-hua**, and Guo Yong. College of Food and Biotechnology, South China University of Technology, Guangzhou 510640, PR, China

Since the mid-1980s there has been an increasing demand on enantiopure chiral drugs rather than racemates which contain enantiomers with different bioactivity. The enzymatic enantioselective resolution of 2-substituted acids (naproxen, ibuprofen, and ketoprofen) by lipases has been the subject of intense investigation because their *S*-isomers often have higher activities than their *R*-isomers. However, for the resolution of racemic ketoprofen [1-(3-benzoyl)phenylpropionic acid], few convincing results have been reported. The effect of reaction media, cosolvent, and additives on *Candida antarctica* lipase B (Novozym435)-catalyzed enantioselective esterification of ketoprofen was systematically explored. Novozym435 showed high catalytic activity and enantioselectivity in cyclohexane; cosolvent and additives have profound effects on Novozym435-catalyzed enantioselective

esterification of ketoprofen: the polar cosolvents benzene and toluene can improve enzymatic enantioselectivity; a small amount of 18-Crown-6 could increase both the esterification rate and the enantioselectivity. DMF, however, could not improve the enantioselective esterification within the scope studied. Through the above-mentioned studies, a ee% of 97% of the remaining substrate was achieved at the conversion of 60% after 28 h reaction. To the best of our knowledge, the result reported here is the most satisfactory one.

238. Mechanism of reaction of phosphate esters coordinated to a model of dinuclear metallophosphatases. Alvan C. Hengge,[1] **Tim Humphry**,[1] Marcello Forconi,[2] and Nicholas H. Williams.[2] [1]Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300, and [2]Department of Chemistry, University of Sheffield, Sheffield, U.K. S3 7HF

Kinetic isotope effects have been used to probe the mechanism of reaction of *p*-nitrophenyl phosphate monoester, and methyl *p*-nitrophenyl phosphate diester coordinated to a dinuclear Co(III) complex, $Co_2(tacn)_2(OH)_2$ (tacn = 1,4,7-triazacyclononane). Comparisons are made between the reactions of uncomplexed phosphate monoester and diesters with the reactions of the metal−phosphate ester complexes. Analyses reveal that both the mechanism and the transition state of the rate-limiting steps are very different for the complexed phosphate esters than for reactions of free phosphate esters in solution. For example, diesters of *p*-nitrophenol undergo phosphoryl transfer reactions by a concerted mechanism with no intermediate, with a fairly tight transition state. In contrast, the complexed diester undergoes reaction by a two-step reaction in which departure of the leaving group occurs in the rate-limiting step. Kinetic isotope effects have been used to characterize the nature of the transition state.

239. Mechanistic studies of phosphite dehydrogenase: An unusual phosphoryl transfer reaction. Stacey Rimkus,[1] Jennifer M. Vrtis,[1] Joshua Wheatley,[2] Andrea K. White,[3] William W. Metcalf,[3] and Wilfred A. van der Donk.[1] [1]Department of Chemistry, [2]Department of Biochemistry, and [3]Department of Microbiology, University of Illinois at Urbana−Champaign, 600 S. Mathews, Urbana, IL 61801 (srimkus@uiuc.edu)

Phosphite dehydrogenase (PtxD) represents the first protein that catalyzes redox chemistry on an inorganic phosphorus compound. PtxD oxidizes phosphite to phosphate in an NAD-dependent reaction. Three putative mechanisms for the reaction catalyzed by PtxD include a dissociative pathway, an associative mechanism, and a concerted mechanism. We have previously established the stereoselectivity of the hydride transfer and the kinetic isotope effect on V_{max}. To elucidate the mechanism, we have synthesized the substrate analogue thiophosphite for kinetic assays. Kinetic parameters obtained from assays with thiophosphite as compared to phosphite may distinguish between mechanisms. Solvent isotope effect assays currently underway in our laboratory will also further probe the mechanistic details of this reaction. We also investigated the catalytic roles of the proposed active site residues using site-directed mutagenesis.

240. Peptidomimetic approach to potent and selective inhibitors of neuronal nitric oxide synthase. Richard B. Silverman and **Erik P. Erdal**. Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208

The dipeptide L-Arg^{NO2}-Dbu^{NH2} was found to be a potent, selective inhibitor of neuronal NOS; therefore, it was chosen as a lead molecule. Because amide bonds are hydrolyzable and highly polar, amide bond isosteres were synthesized to produce stable inhibitors. Several peptidomimetic isosteres of L-Arg^{NO2}-Dbu have been synthesized and are being tested for activity and potency.

241. Proposed commonality between cancer, pregnancy, and tissue repair. E. T. Bucovaz and W. D. Whybrew. Department of Molecular Sciences, University of Tennessee, Health Science Center, Memphis, TN 38163

There are two different B-proteins produced by the body: one is produced in response to the proliferation of aberrant cells, e.g., cancer; the other is produced in response to the proliferation of normal cells, e.g., pregnancy and tissue repair. Pregnancy cells differ significantly from cells involved in the tissue repair process in that half of the DNA of the fertilized ovum and fetus is contributed by the father. Nevertheless, they are normal cells. Both B-proteins appear to serve as antiapoptotic(-like) agents that inhibit apoptotic-(-like) mechanisms that prevent and destroy localized abnormal growth and development of cells whether these cells are aberrant or normal. In pregnancy, tissue repair, and cancer, an elevation in the serum titer of either B-protein is associated with an increase in localized cell growth and development. Although the biological role of the two B-proteins and their physical and chemical properties are similar, the effect of the two B-proteins on the body appears to be in direct contrast. An elevation of the cancer-induced B-protein in the serum of cancer patients is an undesirable condition, whereas an elevation of the pregnancy-tissue repair-induced B-protein in the serum of pregnant patients, or in patients undergoing some form of tissue repair, is a desirable condition.

242. Gankyrin counteracts against INK4 proteins in the regulation of CDK4-mediated phosphorylation of Rb in vitro. Junan Li and Ming-Daw Tsai. Departments of Biochemistry and Chemistry, The Ohio State University, Columbus, OH 43210

The newly discovered oncogenic protein gankryin, which contains six ankyrin repeats, has been reported to be involved in the phosphorylation and degradation of the retinoblsatoma gene product, Rb. Using in vitro systems, we have identified a peptide fragment of gankyrin, ^{176}LHLACDEERN^{185}, which is responsible for binding of gankyrin to Rb. We further demonstrated a different mechanism for gankyrin to facilitate the phosphorylation of Rb—by binding with cyclin-dependent kinase 4 (CDK4). This binding does not inhibit the Rb-phosphorylating kinase activity of CDK4, but it competes with p16 binding to CDK4 and counteracts the inhibitory function of p16. We then showed that binding of gankyrin to CDK4 and the consequent counteraction of p16 function were not affected by the Rb-binding peptide ^{176}LHLAC-DEERN^{185}, indicating that the two mechanisms are inde-

pendent. They also involve different structural regions of gankyrin. While the Rb-binding motif is located at the fifth ankyrin repeat, truncation mutants of gankyrin, with the first three or four ankyrin repeats remaining, are sufficient for binding to CDK4 and for counteracting the inhibitory function of p16. These results demonstrate the potential importance of gankyrin in cell cycle control and tumorigenesis, and expand the INK4-CDK4/6-Rb pathway.

243. Bridged bimetallic enzymes: Two heads are better than one. Dagmar Ringe,[1] Gregory A. Petsko,[1] William Desmarais,[1] and Richard C. Holz.[2] [1]Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02454, and [2]Department of Biochemistry, Utah State University, Logan, UT 84332

An unusually large number of enzymes have an active site that contains two transition metal ions connected by a bridging ligand. The metals may be identical (two zinc ions is a common motif) or different (iron and zinc in the case of calcineurin); the bridging ligand is usually the carboxylate side chain of glutamate or aspartate. Despite these common features, many different substrates are acted on by these enzymes, and they catalyze an astonishingly diverse range of chemical reactions: from sugar isomerization via hydride transfer (xylose isomerase) to nucleic acid synthesis (DNA polymerase). One such reaction is the cleavage of the N-terminal amino acid of many proteins, a hydrolytic reaction catalyzed by a family of aminopeptidases, all of which have a pair of carboxylate-bridged zinc or cobalt ions at their active site. We have been studying the aminopeptidase of *Aeromonas* as a model system for understanding how bridged bimetallic centers contribute to catalysis. *Aeromonas* aminopeptidase (AAP) prefers to cleave hydrophobic N-terminal amino acids, but is otherwise nonspecific. We have solved the crystal structures of AAP with a variety of inhibitors and substrate analogues, including butaneboronic acid and leucine phosphonate. These structures have led us to propose a detailed mechanism of AAP including binding of the carbonyl oxygen of the scissile peptide bond between the metal ions, plus binding of the terminal amino group to one of the metals followed by attachment of a metal-bound water or hydroxide on the amide linkage. A detailed understanding of an enzyme mechanism is impossible without knowing which residues are protonated and which are not. NMR can sometimes be used to determine protonation states of active site groups, but this is complicated, particularly for large proteins. We have been developing an approach to this problem that involves the use of ultra-high-resolution X-ray crystallography to observe the hydrogen atoms directly. AAP diffracts strongly at higher than 1 Å resolution, and electron density maps of this protein have revealed a number of surprising features, including several bound sodium ions, alternative positions for about 10% of the side chains, and an unsuspected bound inhibitor at the active site.

Inhibition of Enzymes Important in Medicine Symposium—Thursday Morning

244. Inactivation of γ-aminobutyric acid aminotransferase by conformationally-rigid analogues. Sun Choi,[1] Jian Qiu,[1] Paola Storici,[2] Tilman Schirmer,[2] and **Richard B. Silverman**.[1] [1]Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60208-3113, and [2]Division of Structural Biology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

When the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) diminishes, convulsions can be triggered. GABA does not cross the blood−brain barrier, so administration of GABA has no effect on convulsions. However, compounds that cross the blood−brain barrier and inhibit the pyridoxal 5′-phosphate-dependent enzyme GABA aminotransferase, the enzyme that degrades GABA, exhibit anticonvulsant activity. The epilepsy drug vigabatrin (Sabril®; γ-vinyl GABA) irreversibly inactivates GABA aminotransferase. Several aspects of our research involved with the design and mechanism of GABA aminotransferase inactivators will be presented. Our early work on the mechanism of inactivation of GABA aminotransferase by vigabatrin, leading to our current molecular modeling and X-ray crystallographic studies that confirm the hypotheses, will be presented. These molecular modeling studies led us to synthesize conformationally rigid analogues of vigabatrin, which will be described as an approach to control the mechanism of inactivation. Studies on halogenated analogues of substituted GABA and on conformationally rigid analogues of these inactivators also will be presented. Crystallography has been employed as a means to establish the inactivation mechanism of one of these compounds.

245. Matrix metalloproteinases: structures, function, and inhibition. S. Mobashery. Department of Chemistry, Wayne State University, Detroit, MI 48202

Matrix metalloproteinases (MMPs) are important hydrolytic enzymes with profound physiological and pathological functions in living organisms. MMPs are produced in their inactive zymogenic forms, which are subsequently proteolytically activated in an elaborate set of events. Recent availability of structures for a number of these enzymes has been instrumental, allowing evaluation of the incremental processes in the elaborate events that lead to enzyme activation. The structures were useful in the design and development of highly selective inhibitors for gelatinases (MMP-2 and -9), enzymes implicated in angiogenesis and cancer metastasis. These and other properties of these important enzymes will be discussed.

246. Studies on estrone sulfatase. Theodore S. Widlanski, Cheri L. Stowell, and Kevin K. Barvian. Department of Chemistry, Indiana University, Bloomington, IN 47405

Estrone sulfatase is highly overexpressed in breast tumor cells. A variety of metal-ligating phosphonates have been explored as inhibitors of this enzyme. However, in keeping with the presence of a unique electrophilic aldehyde in the enzyme active site, nucleophiles such as hydrazines provide an important entry into new types of inactivating agents of this enzyme.

247. Bifunctional enzymes in de novo purine metabolism. V. Jo Davisson. Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47906

One of the unifying principles in modern biology is that all organisms show marked similarities in their central metabolic pathways. This generalization is based upon a common evolutionary relationship among the constituent metabolic enzymes that control the chemistry associated with each of these pathways. Therefore, few examples of enzymes in primary metabolism represent opportunities for selective intervention using mechanism-based inhibition. However, there are distinctions emerging from the detailed comparative studies of de novo purine biosynthetic enzymes. The bifunctional ADE2 proteins in mammals and fungi represent a clear example. These proteins are functionally related by the 5′-aminoimidazole ribonucleotide (AIR) carboxylase activities, and the constituent PurE domains contained within these proteins are linked by their genetic ancestry and structure. In contrast, the substrate specificity of the AIR carboxylases coupled with our recent studies with azole ribonucleotide inhibitors has established that the PurE proteins from eubacteria, fungi, vertebrates, and archaea diverge in their active site chemistry.

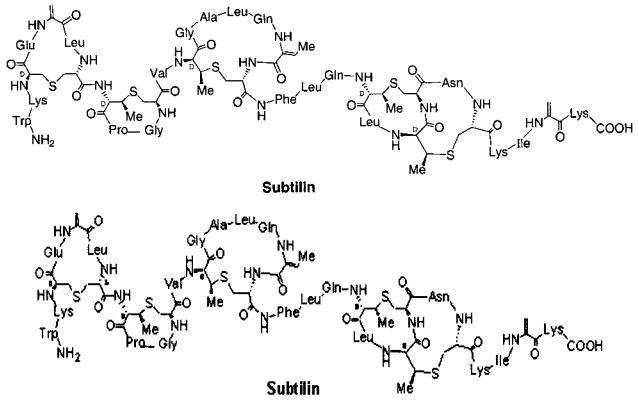
General Oral Session—Thursday Afternoon

248. Chloroperoxidase-catalyzed enantioselective oxidation of cyclopropanes. Shanghui Hu and Jonathan S. Dordick. Department of Chemical Engineering, Rensselaer Polytechnic Institute, 110 Eighth St., Troy, NY 12180

Chloroperoxidase (CPO), a versatile and efficient biocatalyst, catalyzes a variety of oxidative reactions. Particularly, it catalyzes asymmetric epoxidation of alkenes, hydroxylation of alkynes, and oxidation of sulfides. Recently, we have discovered CPO-catalyzed oxidation of cyclopropanes to aldehydes with excellent enantioselectivity and high turnover, using *tert*-butylhydroperoxide as the terminal oxidant. This is the first example for the heme protein family. This finding provides a novel route for the synthesis of optically active cyclopropanes, which occurs widely in natural products and compounds of pharmaceutical interests. In this presentation, the substrate specificity and the mechanisms of this oxidation will be addressed.

249. Study of SpaB dehydratase involved in subtilin biosynthesis. Lili Xie and Wilfred van der Donk. Department of Chemistry, University of Illinois at Urbana−Champaign, Urbana, IL 61801

Subtilin is a member of the so-called lantibiotics, which are ribosomally synthesized and posttranslationally modified antibiotics containing several unusual amino acids (see figure below). A biosynthetic pathway for subtilin has been proposed; however, little is known about the enzymology of the first modification step involving a dehydratase—SpaB. Our efforts toward studying the mechanism of dehydration focused on cloning the *spaB* gene into a high-level expression system in *E. coli*. Sequencing of the *spaB* gene revealed seven nucleotide changes compared with the published sequence. Although the SpaB protein (120.5 kDa) was reported to be membrane-associated, it has been overexpressed in soluble form and was purified by Ni^{2+}-chelated affinity chromatography. The proper assay conditions for SpaB activity are under investigation.



250. Matched enzyme−substrate mutagenesis and Brønsted LFER study show strong cooperative effects in catalytic mechanism of phospholipase C. Karol S. Bruzik,[1] Cornelia Mihai,[1] Robert J. Kubiak,[1] Alex V. Kravchuk,[2] and Ming-Daw Tsai.[2] [1]Department of Medicinal, Chemistry, and Pharmacognosy, University of Illinois, 833 S. Wood St., Chicago, IL 60612 (fax: 312-996-7107, kbruzik@uic.edu), and [2]Department of Chemistry, The Ohio State University, Columbus, OH

We have performed a comprehensive kinetic study of phospholipase C-catalyzed cleavage of phosphatidylinositol analogues with wild-type and active site mutant enzymes, where the mutation positions were in close proximity to the modified substrate residues. In addition, we have examined Brønsted relationships for the series of hydrophobic and nonhydrophobic alkyl and aryl phosphoinositols with varying pK_a of the leaving group. Our results indicate that the three common elements of phosphoryl transfer catalysis: general acid, general base, and phosphate protonation, are integrated into a highly cooperative catalytic entity. The assembly of catalytic residues is controlled by hydrophobic interactions of the leaving group at the remote positions.

251. Intermediates of heme enzyme catalysis as studied by cryogenic radiolytic reduction with phosphorus-32. Ilia G. Denisov,[1] Thomas M. Makris,[1] Masao Ikeda-Saito,[2] and Stephen G. Sligar.[3] [1]Department of Biochemistry, University of Illinois, 405 N. Mathews Ave., Urbana, IL 61801 (fax: 217-244-7100, denisov@uiuc.edu), [2]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, and [3]Departments of Biochemistry, Chemistry, and College of Medicine, University of Illinois, Urbana, IL 61801

Recently, substantial progress was reached in mechanistic studies of metalloenzymes using radiolytic reduction via X-ray and γ-irradiation at cryogenic temperatures. We describe the application of a new method of preparation of unstable intermediates of cytochromes P450 and heme oxygenase. The radiolysis of glycerol/aqueous phosphate buffer matrix at 77 K is provided by b-decay of ^{32}P-enriched phosphate, used as an internal source of radioactivity. The progress of the reduction of oxygenated enzymes and UV−Vis spectra of highly reactive hydroperoxo−heme enzyme complexes are documented. These compounds are stable at 77 K, but undergo chemical relaxation and form product at 190−210 K. The cryoradiolytic reduction with ^{32}P makes

possible the generation and spectroscopic investigation of active intermediates of diverse biochemical reactions, without use of fast kinetic methods and large radioactive sources. (Supported by NIH Grants GM31756 and GM33775 to S.G.S., and GM57272 to M.I.-S.)

252. Mechanistic studies of the regulation of the multifunctional PutA flavoprotein from *Escherichia coli*. **Donald F. Becker**, Paul Johnson, Dan Gu, and M. P. Vinod. Department of Chemistry and Biochemistry, University of Missouri−St. Louis, St. Louis, MO 63121

The PutA flavoprotein from *Escherichia coli* associates with the membrane to catalyze the two-step oxidation of proline to glutamate. In addition, PutA also regulates the transcription of the *put* (proline utilization) regulon by binding to the *put* control DNA region (419 bp). We seek to understand the mechanism by which PutA switches between its two functions. Presently, we are assessing the effects of the flavin redox state on PutA−DNA interactions by evaluating PutA binding to different sites (20−30 bp) in the *put* control DNA. These experiments will identify specific redox-sensitive DNA-binding sites. We are also determining whether the transcription of the *put* regulon is redox-activated by performing in vitro transcription assays under different redox conditions. As a first step toward developing a structure−function model for the multifunctional PutA protein, we have genetically engineered fragments of the PutA polypeptide (1320 total amino acids) to identify functional domains. Thus far, the proline and aldehyde dehydrogenase domains have been located.

253. Oxidation of phenethylamine derivatives by cytochrome P450 2D6 reveals complex steady-state kinetics. **G. P. Miller**, I. H. Hanna, Y. Nishimura, C. J. Rizzo, and F. P. Guengerich. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN

To investigate P450 2D6 catalysis, we analyzed the binding and oxidation of phenethylamine substrates. Both 3-methoxyphenethylamine and 4-methoxyphenethylamine underwent sequential oxidations to form hydroxyphenethylamines by *O*-demethylation and then 3,4-dihydroxyphenethylamine by ring hydroxylation. Based on binding and catalytic studies, P450 2D6 discriminated between the substrates. Whereas the affinities for 3-methoxyphenethylamine and 4-methoxyphenethylamine were similar, the affinity for 4-hydroxyphenethylamine was 12-fold weaker than for 3-hydroxyphenethylamine, respectively. The observation of Type I and Type II binding spectra indicated both protonated and unprotonated forms of these compounds were present. Further analyses of 3-methoxyphenethylamine and 4-methoxyphenethylamine binding as a function of pH demonstrated an apparent pK_a for a P450 2D6 residue involved in binding and the lowering of the pK_a of the substrate amine group upon binding P450 2D6. The rates of substrate turnover were fit globally to a scheme incorporating both the multiple oxidations and the presence of protonated and unprotonated substrate.

254. Isoform-selective interaction of cyclooxygenase-2 with indomethacin amides studied by real-time fluorescence and inhibition kinetics. **S. L. Timofeevski** and L. J. Marnett. Department of Biochemistry, Vanderbilt University, Nashville, TN 37232

Cyclooxygenase-2 (COX-2) is an important target for pharmaceutical intervention in a variety of inflammatory conditions. Conversion of carboxylate-containing NSAIDs, such as indomethacin, to esters or amides results in potent and selective COX-2 inhibitors. We used fluorescent coumarinyl and cinnamyl groups, coupled with the indomethacin carboxyl group via an amide bond, to monitor the interaction of indomethacin derivatives with COX-2 and COX-1 in real time. Following a rapid association of the inhibitors with apoenzyme, a time-dependent fluorescence enhancement of the inhibitors was observed with COX-2, but not with COX-1. This slow, COX-2-specific phase was prevented or reversed by (*S*)-flurbiprofen, and corresponded to a unimolecular event. Site-specific substitutions of Tyr-355, Glu-524, and Arg-120, that comprise the constriction in the substrate access channel, resulted in a marked decrease in the fluorescence enhancement and faster onset of the equilibrium. The heme prosthetic group quenched the fluorescence of the enzyme-bound inhibitors, allowing us to monitor the binding of heme to apoenzyme. The steady-state inhibition studies revealed that the potency of indomethacin amides for COX-2 could be attributed to a time-dependent transition near the constriction of the active site. The inhibition mechanism, consistent with the binding and inhibition kinetics, is proposed to include two or more time-dependent, reversible transitions in the COX-2 active site, with the constriction site residues critically involved in retaining the indomethacin moiety in the upper cyclooxygenase active site.

255. Cyclic acyl phosph(on)ates: "Penicillin-like" inhibitors of the class C β-lactamase of *Enterobacter cloacae* P99. **Kamaljit Kaur**, Martin J. K. Lan, and Rex F. Pratt. Department of Chemistry, Wesleyan University, Hall-Atwater Laboratories, Lawn Ave., Middletown, CT 06459 (fax: 860-685-2211, kkaur@wesleyan.edu)

Class C β-lactamases have recently gained heightened clinical significance to the still increasing bacterial resistance to current commercial β-lactam antibiotics. Salicyloyl cyclic phosphate, **1**, inactivates the class C β-lactamase of *Enterobacter cloacae* P99 in a covalent fashion [(1998) *J. Am. Chem. Soc. 120*, 3004]. As with penicillins, in the covalent enzyme complex the leaving group does not leave and thereby obstructs the incoming nucleophile. Nonetheless, the inactivated enzyme slowly reverts to the active form. Now we show that reactivation involves a recyclization reaction that regenerates **1** rather than hydrolysis of the covalent intermediate, i.e., that the inactivation is a slowly reversible covalent modification of the active site. The thermodynamic dissociation constant of the inhibitor is 160 nM. Also we show that **1** inactivates the enzyme by phosphorylation rather than acylation of the active site serine residue. The phosphonate analogue of **1** did not appear to inactivate the P99 β-lactamase in a time-dependent fashion. It was found, however, to act as a fast reversible inhibitor ($K_i = 10\ \mu$M).

256. Computer-aided identification of kinetically significant anion-binding sites in human serum transferrin.

William J. Welsh, **Elizabeth A. Amin**, and Wesley R. Harris. Department of Chemistry and Center for Molecular Electronics, University of Missouri−St. Louis, 8001 Natural Bridge Rd., St. Louis, MO 63121 (fax: 314-516-5342, wwelsh@umsl.edu, ea@ozone.umsl.edu)

Human serum transferrin is an approximately 80-kDa iron-binding glycoprotein responsible for transporting iron(III) cations from erythrocyte processing locations to tissues that require iron. HST is normally ∼30% saturated with Fe, but frequent blood transfusions can result in toxic iron overload, where HST becomes totally saturated and unable to remove excess iron from the bloodstream. The immediate therapeutic goal is to facilitate iron removal from HST in order to safely chelate and excrete it; this is complicated by the extremely slow iron release process. Certain anions selectively affect Fe release from HST and are believed to bind at one or more allosteric "KISAB" (kinetically significant anion-binding) sites. Probable KISAB sites have been identified and rank-ordered using molecular dynamics, molecular mechanics, and energy grid calculations.

257. β_2-Microglobulin mutant: A novel selective immune-suppressor. Meir Glick and Graham Richards. Physical and Theoretical Chemistry Laboratory, University of Oxford, S. Parks Rd., Oxford, OX1 3QZ, United Kingdom (glick@bellatrix.pcl.ox.ac.uk)

The peptide−MHC class I complex (pMHC) on a target cell is recognized by a specific T cell receptor on the surface of $CD8^+$ cytotoxic T lymphocytes (CTL). The pMHC consists of a heavy chain, which is attached to the cell membrane and contains the peptide binding site, and a light chain, β_2-microglobulin (β2m). The CD8 molecule is a 'co-receptor' and is not peptide-specific, but binds to a conserved site on the pMHC molecule which comprises several regions on the heavy chain and the small DE loop of β2m consisting of residues 58−60. Exogenous soluble β2m can exchange with cell-surface-associated β2m complexed to pMHC. Therefore, by mutating the CD8 contact site on β2m, and exchanging the mutant β2m into the native MHC, it should be possible to inhibit CTL activation. We employed molecular dynamic simulations to study the interactions between the CD8αα, HLA-A2, and β2m molecules, to design and predict the effects of novel β2m mutants on the interaction between CD8αα and pMHC. The K58→E mutant was found to inhibit fresh peripheral blood mononuclear cells lytic response to endogenously presented pMHC class I antigen in comparison to equimolar levels of wild-type β2m. These data suggest that mutant forms of β2m could be used to modulate the cellular immune response therapeutically, especially if delivered from within targeted cells.

258. Probing enzyme−ligand interactions in crystal and solution states. Jian Dong and Paul Carey. Department of Biochemistry, Case Western Reserve University, Cleveland, OH

Demonstrating the protein function in the crystalline and solution states provides a criteria that the crystal structure is biologically relevant, thereby allowing meaningful structure−function relationships to be studied. Raman spectroscopy is best suited to study both the solution and the crystalline states of enzyme−ligand interactions. Here three examples are presented: uracil DNA glycosylase, 4-chlorobenzoyl-CoA dehalogenase, and urokinase plasminogen activator (a serine protease). In UDG, the enzyme activates the extrahelical uracil base in the ground state mainly through H bonds to its C═O groups, without destroying its quasi-aromaticity. This result is at variance with the conclusion from a recent crystal structure in which a change in hybridization at C1 occurs. In dehalogenase, although the structure of the W137F mutant is unknown, we can identify two protonation states of the product bound to the mutant but only one protonation state in the wt. In urokinase, the use of protein crystals permits competitive binding experiments that cannot be undertaken in solution. The Raman spectrum for a urokinase single crystal that had been exposed to equimolar amounts of a number of inhibitors showed only the Raman signature of the compound with the lowest K_i, suggesting that the Raman approach may offer a novel route in the screening of compounds in drug design applications.

259. Detecting protein−protein interactions involving transcriptional activators by in vivo cross-linking. Daniel B. Hall and Kevin Struhl. Department of Biological Chemistry, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115 (fax: 617-432-2529, daniel_hall@hms.harvard.edu)

Although transcriptional activator proteins are critical components for the regulation of gene expression, their mechanism of action is not well understood. Presumably they function by recruiting general transcription factors, RNA polymerase, and/or chromatin-modifying factors. We wished to develop a new technique to detect direct protein−protein interactions utilizing in vivo cross-linking. Formaldehyde is an ideal cross-linking agent since it is readily cell-permeable, and the cross-link can be reversed to facilitate identification of the cross-linked proteins. Initially, we decided to determine whether transcriptional activators contact TBP or any of the TBP-associated factors (TAFs). Indeed, TAF68 and TAF90 were cross-linked to the VP16 activation domain while TBP and the other 12 TAFs were not. These cross-links were not formed in the absence of an activation domain or with a truncated, inactive construct. Currently we are determining whether both cross-links indeed represent direct protein−protein contacts and searching for other potential targets of transcriptional activators.

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