Abstracts, Division of Biological Chemistry, 219th National Meeting of the American Chemical Society, March 26−30, 2000

H. M. Miziorko, Program Chair

Sunday Morning—Carbohydrate Enzymology (Cosponsored with Division of Carbohydrate Chemistry)—S. G. Withers, Organizer

1. Phosphoribulokinase: Structure/function correlations accounting for regulation & catalysis. Henry M. Miziorko, Jennifer A. Runquist, Guosheng Kung, and David H. T. Harrison. Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226-3548 (fax: 414-456-6510, miziorko@mcw.edu)

R. sphaeroides phosphoribulokinase (PRK) is an octamer of 32 kDa subunits which exhibit a nucleotide monophosphate (NMP) kinase fold. The ATP analogue AMP-PCP binds not to the consensus ATP site but at the interface of three subunits in a region containing numerous arginines. Since we previously observed mononucleotide binding to the effector (NADH/AMP) site, we investigated whether these arginines support allosteric regulation. Characterization of R234A, R257A, and R31A mutants indicates that R234 and R257 directly support effector binding. R31 is crucial to transmission of the allosteric stimulus. Since the high-resolution PRK structure includes no bound substrate, mutagenesis and mechanistic studies have been used to elucidate the function of active site residues. A "Walker B" carboxyl (E131) is appropriately situated between a "P-loop" and the putative general base catalyst (D42). In this NMP kinase fold protein, we identified the binding site of the nonnucleotide phosphoryl acceptor, Ru5P. Binding residues H45 and R49 are situated near the catalytic base, but K165 is in the NMP kinase fold "lid" region, which is separated from the other active site components in "open" forms of NMP kinase fold enzymes. [Support: USDA NRICRG (H.M.M.); H. Frasch Fdn. (D.H.T.H.).]

2. Covalent catalysis with UDP-glucose dehydrogenase. Martin E. Tanner, Robert E. Campbell, Xue Ge, Steve Mossiman, and Natalie C. J. Strynadka. Departments of Chemistry and Biochemistry, University of British Columbia, Vancouver, BC V6T-1Z1 (mtanner@chem.ubc.ca)

UDP-glucose dehydrogenase catalyzes the 2-fold NAD^+-dependent oxidation of UDP-glucose to give UDP-glucuronic acid. Evidence will be presented in support of a mechanism proceeding via an aldehyde intermediate and involving covalent catalysis. An active site thiol adds to the aldehyde and is oxidized to a thioester intermediate. A final hydrolysis generates the free acid. Crystal structures of the substrate and product complexes will be presented.

3. 2 Å structure of malarial purine phosphoribosyltransferase in complex with a transition-state analogue inhibitor. Vern L. Schramm,[1] Wuxian Shi,[1] Caroline M. Li,[1] Peter C. Tyler,[2] Richard H. Furneaux,[2] Sean M. Cahill,[1] Mark E. Girvin,[1] Charles Grubmeyer,[3] and Steven C. Almo.[1] [1]Albert Einstein College of Medicine, Bronx, NY 10461 (fax: 718-430-8565, verb@aecom.yu.edu), [2]Carbohydrate Chemistry Team, Industrial Research Ltd., Lower Hutt, New Zealand, and [3]Temple University School of Medicine, Philadelphia, PA 19140

Malaria is the second leading cause of worldwide mortality from infectious disease. *Plasmodium falciparum* proliferation in human erythrocytes requires purine salvage by hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRTase). The enzyme is a target for the development of novel antimalarials. Design and synthesis of transition-state analogue inhibitors permitted cocrystallization with the malarial enzyme and refinement of the complex to 2.0 Å resolution. Catalytic site contacts in the malarial enzyme are similar to those of human hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) despite distinct substrate specificity. The crystal structure of malarial HGXPRTase with bound inhibitor, pyrophosphate, and two Mg^{2+} ions reveals features unique to the transition-state analogue complex. Substrate-assisted catalysis occurs by ribooxocarbenium stabilization from the O5′ lone pair and a pyrophosphate oxygen. A dissociative reaction coordinate path is implicated in which the primary reaction coordinate motion is the ribosyl C1′ in motion between relatively immobile purine base and $(Mg)_2$-pyrophosphate. Several short hydrogen bonds form in the complex of the enzyme and inhibitor. The proton NMR spectrum of the transition-state analogue complex of malarial HGXPRTase contains two downfield signals at 14.3 and 15.3 ppm. Despite the structural similarity to the human enzyme, the NMR spectra of the complexes reveal differences in hydrogen bonding between the transition-state analogue complexes of the human and malarial HG(X)PRTases. The X-ray crystal structures and NMR spectra reveal chemical and structural features that suggest a strategy for the design of malaria-specific transition-state inhibitors.

4. Three views of the glycosyltransfer of *N*-acetylneuraminic acid. B. Horenstein, J. Yang, H. Sun, M. Bruner, K. Millar, and S. Schenkman. Department of Chemistry, University of Florida, Box 117200, Gainesville, FL 32611

N-Acetylneuraminic acid (NeuAc) is a nine-carbon ketose often found at the terminus of cell surface oligosaccharides. These linkages are created by the action of sialyltransferase enzymes, that use the activated sugar nucleotide form of NeuAc, CMP-NeuAc, as the glycosyl donor. Interestingly, the trypanosome *T. cruzi* uses an enzyme called trans-

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sialidase to transfer host cell surface NeuAc to its own cell surface. In this paper, we present the results of mechanistic studies for solvolysis of CMP-NeuAc, sialyltransferase-catalyzed transfer of NeuAc, and the trans-sialidase-catalyzed transfer of NeuAc. Utilizing kinetic isotope effect experiments, we have found that these three reactions for glycosyltransfer of NeuAc proceed by different mechanistic pathways.

5. X-ray crystal structure of rabbit *N*-acetylglucosaminyl transferase 1, a key enzyme in the biosynthesis of N-linked glycans. James M. Rini,[1] Ulug M. Ünligil,[1] Sihong Zhou,[1] Sivashankary Yuwaraj,[1] Mohan Sarkar,[2] and Harry Schachter.[3] [1]Departments of Medical Genetics & Microbiology and Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada (james.rini@utoronto.ca), [2]Department of Biochemistry, Hospital for Sick Children, Toronto, ON M5G 1X8, Canada, and [3]Medical Genetics & Microbiology and Biochemistry/Biochemistry, University of Toronto/Hospital for Sick Children

The medial-golgi enzyme UDP-*N*-acetylglucosamine:α-3-D-mannoside β-1,2-*N*-acetylglucosaminyl transferase I (GnT I, EC 2.4.1.101) is a key enzyme in the asparagine-linked glycosylation pathway; its action serves as the gateway from oligomannose to complex and hybrid glycans. We have solved the X-ray crystal structure of a soluble fragment containing the catalytic domain of rabbit (*Oryctolagus cuniculus*) GnT I in both the presence and absence of UDP-GlcNAc. The structure is formed by two mixed β-sheets, each of which is flanked by α-helices. The UDP-GlcNAc coordinates a manganese ion and is found in a pocket large enough to also contain the Man_5Gn_2 acceptor. The structure provides insight into the inverting catalytic mechanism, as well as an explanation for the observation that the reaction proceeds in an ordered sequential fashion.

6. Structural enzymology of enzymatic glycosyl transfer: Glycoside hydrolases, polysaccharide lyases, and glycosyltransferases. Gideon J. Davies,[1] Simon J. Charnock,[1] Annabelle Varrot,[1] and Martin Schülein.[2] [1]Department of Chemistry, Structural Biology Laboratory, Heslington, York, Y010 5DD, United Kingdom (fax: 00 44 1904 410519, davies@yorvic.york.ac.uk), and [2]Novo-Nordisk A/S, Novo AllÉ, DK 2880, Bagsavæ×f1rd, Denmark

Enzymatic glycosyl group transfer is performed by a diverse array of enzymes. Significant progress has been made in our understanding of glycoside hydrolase structure and function, in particular, the role of the numerous different enzyme families and their respective mechanisms. Features such as substrate distortion have now been viewed in many retaining β-glycoside hydrolases. Recent structure determinations have also revealed new structural families of polysaccharide lyases, which are presumed to utilize β-elimination chemistry for catalysis. The first NDP-dependent glycosyltransferases structures are also beginning to emerge. The first structure of a member of sequence family 2, SpsA from *Bacillus subtilis*, whose function is unknown, reveals significant sequence similarity with enzymes such as cellulose and chitin synthases, dolicyl phosphomannose transferases, and numerous lipopolysaccharide synthases. These enzymes all act with inversion of anomeric configuration using α-linked NCP sugars to generate β-linked products. The 3-D structure of SpsA begins to shed some light onto the mode of action of this enzyme family.

Sunday Afternoon—General Papers/Poster Presentations—H. Miziorko, Organizer, Presiding

7. *Escherichia coli* dimethylallyl diphosphate:tRNA dimethylallyltransferase: Site-directed mutagenesis of highly conserved residues. Timothy J. Soderberg and C. Dale Poulter. Department of Chemistry, University of Utah, 315 South 1400 East, RM Dock, Salt Lake City, UT 84112 (fax: 801-581-4391, soderber@chemistry.utah.edu)

Dimethylallyl diphosphate:tRNA dimethylallyltransferase (DMAPP-tRNA transferase) catalyzes the transfer of the hydrocarbon moiety in dimethylallyl diphosphate (DMAPP) to the exocyclic amine of adenosine at position 37 in some tRNA molecules. An alignment of the 24 known sequences for this enzyme reveals considerable homology, with 27 charged, polar, or aromatic residues either conserved or conservatively substituted. In this study, site-directed mutants of the recombinant enzyme from *E. coli* were constructed in which all of the highly conserved nonaliphatic residues were singly altered. Values of k_{cat}, K_m^{DMAPP}, and K_m^{RNA} were determined and compared to those of the wild-type enzyme. Mutants in which threonine, histidine, lysine, arginine, aspartate, glutamate, and tyrosine residues were changed displayed greater than 10-fold alterations in one or more of the kinetic parameters, suggesting possible roles for these residues in substrate binding or catalysis.

8. Purification and characterization of a recombinant non-head-to-tail monoterpene synthase that catalyzes the formation of chrysanthemyl diphosphate. Susan B. Rivera and C. Dale Poulter. Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112 (fax: 801-581-4391, baughman@chemistry.utah.edu)

Chrysanthemyl diphosphate synthase (CDS) is the first example of a monoterpene synthase catalyzing the condensation of two molecules of dimethylallyl diphosphate (DMAPP) to produce chrysanthemyl diphosphate (CPP), a c(1′-2-3) irregularly linked isoprenoid. CPP is found in *Chrysanthemum cinerariaefolium* and related species of the Asteraceae family and is a precursor of the insecticidal pyrethrins. When a gene complementary to CDS was cloned from a *C. cinerariaefolium* cDNA library at Agridyne technologies, sequence alignments showed that CDS exhibits homology to farnesyl diphosphate synthase, but the product of CDS is similar to the c(1′-2-3) isoprenoid intermediates formed by squalene synthase and phytoene synthase in the sterol and carotenoid biosynthetic pathways, respectively. Recombinant CDS containing an N-terminal polyhistidine affinity tag was purified to homogeneity from an overproducing *E. coli* strain by Ni^{2+} chromatography. Incubation of recombinant CDS with DMAPP produced CPP, as confirmed by GC/MS. A TLC assay was developed and kinetic constants obtained.

9. Key role in catalysis for histidine-89 of adenylosuccinate lyase of *Bacillus subtilis.* Jennifer L. Brosius and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 (fax: 302-831-6335, jbrosius@udel.edu)

Adenylosuccinate lyase of *Bacillus subtilis* is a tetrameric enzyme which catalyzes the cleavage of adenylosuccinate to AMP and fumarate. We have mutated histidine-89, one of three conserved histidines, to Gln, Ala, Glu, and Arg. The enzymes were expressed in *E. coli* and purified to homogeneity. As compared to a specific activity of 2.0 units/mg for wild-type enzyme, the mutant enzymes exhibit specific activities of 0.0225, 0.0036, 0.0036, and 0.0009 for H89Q, H89A, H89E, and H89R, respectively. Circular dichroism and FPLC gel filtration reveal that mutant enzymes have a similar conformation and oligomeric state to the wild-type enzyme. There are relatively small increases in the K_m values of H89Q: 2.5-fold for adenylosuccinate and fumarate, and 10-fold for AMP, indicating that the major effect of the mutation is on V_{max}. Incubation of H89Q with inactive H141Q enzyme [Lee, T. T., Worby, C., Bao, Z.-Q., Dixon, J. E., and Colman R. F. (1999) *Biochemistry 38*, 22−32] leads to a 30-fold increase in activity. This intersubunit complementation indicates that His-89 and His-141 from different subunits participate in the active site and that both are required for catalysis. (Supported by NSF MCB-97-28202 and NIH T32 GM-08550.)

10. Affinity labeling of rat liver glutathione-*S*-transferase by 17β-iodoacetoxy-estradiol-3-sulfate. Melissa A. Vargo and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 (fax: 302-831-6335, mvargo@udel.edu)

Rat liver glutathione-*S*-transferase (GST), isozyme 1-1, catalyzes the glutathione-dependent isomerization of Δ^5-androstene-3,17-dione and also binds steroid sulfates at a nonsubstrate steroid site. 17β-Iodoacetoxy-estradiol-3-sulfate (17β-IES), a reactive steroid analogue, produces a time-dependent inactivation of GST to a limit of 60% residual activity. The rate constant for inactivation (k_{obs}) exhibits a nonlinear dependence on [17β-IES] with $K_I = 71\ \mu$M and $k_{max} = 0.0133\ min^{-1}$. Complete protection against inactivation is provided by 17β-estradiol-3,17-disulfate, while Δ^5-androstene-3,17-dione and *S*-methylglutathione have little effect on k_{obs}. These results indicate that 17β-IES reacts as an affinity label of the nonsubstrate steroid site, rather than of the substrate sites occupied by Δ^5-androstene-3,17-dione or glutathione. Loss of activity occurs concomitant with incorporation of about 1 mol of [^{14}C]17β-IES/mol of enzyme dimer when the enzyme is maximally inactivated. Isolation of labeled peptide from the chymotryptic digest shows that Cys^{17} is the only enzymic amino acid modified. Covalent modification of Cys^{17} by 17β-IES on one subunit apparently prevents reaction of the steroid analogue with the second subunit, suggesting that interaction between the two subunits of GST 1-1 is important in binding steroid at the nonsubstrate site. (Supported by NIH CA-66561 and T32 GM-08550.)

11. Nonredox role for copper in a copper amine oxidase from yeast. Stephen A. Mills and Judith P. Klinman. Department of Chemistry/Klinman, University of California, 301 Hildebrand, Berkeley, CA 94720 (samills@eve.cchem.berkeley.edu)

The role of metal in copper amine oxidases was studied by removal and replacement of the copper in the enzyme from *Hansenula polymorpha*. Metal-free protein was found to have no activity in oxidizing methylamine and was unreactive toward phenylhydrazine. Addition of cobaltous ion to the metal-free protein restored 19% of the native activity and 75% of the native reactivity toward phenylhydrazine. The cobalt-substituted enzyme has a k_{cat} which is 73% of the native k_{cat} and a $K_{m(app)}$ 45-fold higher than the native enzyme. This study with the cobalt-substituted amine oxidase confirms the mechanism proposed by Su and Klinman in which a change in the valence state of the metal is not required during turnover, with electrons being passed directly from the reduced TPQ cofactor to prebound oxygen. Thus, copper is proposed to provide electrostatic stabilization of the superoxide anion formed during turnover.

12. Mechanism of TOPA quinone biogenesis. Benjamin Schwartz, Joanne E. Dove, and Judith P. Klinman. Chemistry Department, University of California, c/o Klinman Group, Berkeley, CA 94720 (bnsch@eve.cchem.berkeley.edu)

TOPA quinone is the novel redox cofactor found in all known copper amine oxidases. The quinocofactor is derived posttranslationally from a specific tyrosine residue within the protein itself. No other enzymes or cofactors are needed to process the precursor tyrosine residue to TOPA quinone, raising the interesting question of how this enzyme can carry out both its oxidative catalytic function and the oxygenation reaction necessary to produce the cofactor. Based on recent spectroscopic and kinetic results, a new mechanism is proposed for TOPA quinone formation, in which the precursor tyrosine, rather than oxygen, is activated by the mononuclear, active site copper. This mechanism, as well as the roles played by various active site residues, will be discussed.

13. Mechanism of the oxidative half-reaction in *Hansenula polymorpha* amine oxidase (HPAO): A nonmetal binding site? Yoshio Goto, Qiaojuan Su, and Judith P. Klinman. Department of Chemistry, University of California, Berkeley, CA 94720 (fax: 510-643-6232, yoshio@eve.cchem.berkeley.edu)

Trihydroxyphenylalanine quinone (TPQ)-dependent copper amine oxidases have been found in a wide variety of organisms. These enzymes catalyze the deamination of primary amines, concomitant with the reduction of molecular oxygen to hydrogen peroxide ($RCH_2NH_3^+ + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_4^+$). Su et al. have recently investigated the mechanism of the oxidative half-reaction of bovine serum amine oxidase (BSAO) by various steady-state kinetic analyses. They concluded that the first electron transfer from reduced cofactor to dioxygen is the rate-determining step and that O_2 prebinds to the enzyme [(1998) *Biochemistry 37*, 12513]. In this report, we present the mechanism of the oxidative half-reaction in HPAO, of which an X-ray structure is available [Li et al. (1998) *Structure 6*, 293]. The relevancy of an O_2 binding site in amine oxidases is investigated by a comparison of kinetics between BSAO, HPAO, and various HPAO active site mutants.

14. Novel inhibitor reveals an allosteric binding site for both soybean lipoxygenase-1 and human 15-lipoxygenase. Rakesh Mogul,[1] Eric Johansen,[1] and Theodore R. Holman.[2] [1]University of California, Santa Cruz, CA 95064 (rmogul@hydrogen.ucsc.edu), and [2]Chemistry and Biochemistry, University of California, 1156 High Street, Santa Cruz, CA 95064

Lipoxygenases (LO) are thought to proceed through simple competitive inhibition pathways. Using oleic sulfate (OS), a novel analogue of oleic acid, we have revealed a regulatory site, for SLO-1 and 15-HLO, that modulates the microscopic rate constants of catalysis via inhibitor binding. The kinetic isotope effect exhibits a striking hyperbolic response to increasing amounts of OS, supporting the formation of a catalytically active ternary complex. The steady-state kinetics show that SLO-1 proceeds through a hyperbolic partial inhibition pathway; where OS binding ($K_I = 0.4 \pm 0.1$ μM) causes an increase in the K_M(app) ($\alpha = 4$) and a decrease in the k_{cat} ($\beta = 0.7$). Inhibition of 15-HLO proceeds through a similar yet more complicated kinetic model involving two inhibitor binding sites. Taken together, these findings present broad implications regarding the biological regulation of LO and the treatment of LO-related diseases.

15. Marine natural products as lipoxygenase inhibitors. Erika Noel Jonsson and Theodore R. Holman. Department of Chemistry, University of California, 1156 High Street, Santa Cruz, CA 94064 (@hydrogen.ucsc.edu)

Lipoxygenase (LO) catalyzes the addition of dioxygen to 1,4-*cis*,*cis*-pentadiene-containing fatty acid, producing a chiral (*E*,*Z*)-congugated hydroperoxy fatty acid. Lipoxygenases are non-heme iron enzymes and serve integral functions in both the plant and animal kingdoms. The three major human lipoxygenases (HLO), 5-, 12-, and 15-HLO, have been implicated in both cancer growth and regulation. Thus, the need for potent, new inhibitors as therapeutic agents is indicated. In the current study, we have screened a marine natural products library with soybean lipoxygenase (SLO-1) and 15-HLO and found several potential inhibitors. One inhibitor, isolated from the dictyoceratid sponge *Coscinoderma sp*., has been structurally identified by mass spectrometry, H-NMR, and C-NMR. In addition, kinetic data of 15-HLO and 12-HLO were studied along with EPR data of the iron active site, and an inhibition mechanism is presented.

16. Oleic sulfate: A novel allosteric lipoxygenase inhibitor. Theodore R. Holman, Rakesh Mogul, and Eric Johansen. Department of Chemistry, University of California, 1156 High Street, Santa Cruz, CA 94064 (fax: 831-459-2935, tholman@chemistry.ucsc.edu)

Lipoxygenases are implicated in a variety of cancers and catalyze the oxidation of unsaturated fatty acids. A unique aspect of the lipoxygenase reaction, which has helped us investigate the mechanism in more detail, is the extremely large isotope effect. At 5 μM LA, the KIE for SLO-1 is temperature-dependent below 32 °C, but at 100 μM LA, the KIE is temperature-independent at all temperatures. This suggests a change in the microscopic rate constants and was postulated to be due to an allosteric site since addition of oleic acid (OA) also increased the KIE. This conclusion, however, is complicated by the fact that LA and OA form micelles. To address this issue, we have synthesized oleic sulfate (OS), which is over 3 times more soluble than OA and is 50 times more potent an inhibitor. In addition, OS exhibits hyperbolic inhibition which strongly indicates the presence of an allosteric site in both soybean and 15-human lipoxygenases.

17. Active site mapping and mutagenesis of squalene: Hopene cyclase. Tongyun Dang and Glenn D. Prestwich. Department of Medicinal Chemistry, University of Utah, 30 South 2000 East, Room 201, Salt Lake City, UT 84112 (fax: 801-585-9053, tdang@hnu.pharm.utah.edu)

Oxidosqualene cyclase (OSC) and squalene:hopene cyclase (SHC) are pivotal enzymes in triterpenoid biosynthesis that convert 2,3-oxidosqualene and squalene to tetra- and pentacyclic products, respectively. Selective inhibitors for OSC would uniquely suppress the committed step in sterol biosynthesis, thus acting as cholesterol-lowering drugs with minimal side effects. A bioavailable drug candidate, Ro48-8071, is a very potent inhibitor for both enzymes. By photoaffinity labeling and peptide mapping, we determined that Ala-44 of SHC was specifically modified. Molecular modeling suggests that Ro48-8071 may bind at the junction between the central cavity and substrate entry channel, thus inhibiting access of the substrate to the active site. Subsequent mutagenesis studies provide evidence that the nucleophilicity and positioning of Glu-45 are crucial for its stabilization of the carbocation intermediates. An E45Q mutant shows 118% of wild-type activity, and E45D produces a lower hopene:hopenol ratio. Replacement of the conserved "DDTAVV" motif in SHC with the "DCTAEA" motif from OSC changes the substrate specificity. This mutant cyclizes 2,3-oxidosqualene but cannot process squalene. Mono- and pentacyclic 3-hydroxy triterpenes were isolated and characterized from the cyclization mixture for this mutant.

18. Amino acid substrate specificity of phenylalanine hydroxylase and tyrosine hydroxylase. S. Colette Daubner, Julie Melendez, and Paul F. Fitzpatrick. Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128 (fax: 409-845-9274, colette@bioch.tamu.edu)

Tyrosine hydroxylase (TyrH) and phenylalanine hydroxylase (PheH) show 75% sequence identity in their catalytic domains. TyrH is able to hydroxylate phenylalanine to a lesser extent than tyrosine; PheH and the isolated catalytic domain of PheH are unable to hydroxylate tyrosine. Residues which differ greatly between the two enzymes and also showed proximity to the active site were mutated. Aspartate 425 of TyrH was found critical to the enzyme's ability to hydroxylate tyrosine. The corresponding residue in rat or human PheH is valine or isoleucine. D425ITyrH and V379DPheH were overexpressed and purified. D425ITyrH was unable to hydroxylate tyrosine, but was able to hydroxylate phenylalanine to a greater extent than TyrH. High levels of tyrosine and enzyme enabled the detection of low levels of DOPA production. Substitution of valine 379 with an aspartate did result in a slight increase in the ability of PheH to hydroxylate tyrosine but was detrimental to phenylalanine hydroxylation. Aspartate 425 is necessary for the hydroxylation of tyrosine by TyrH, but the inclusion of an

aspartate at the same position is not sufficient to enable PheH to hydroxylate tyrosine.

enzyme	V_m for DOPA formation	V_m for tyrosine formation
WT TyrH	150 ± 14	96 ± 12
WT PheH	0.13 ± 0.05	373 ± 21
D425I TyrH	1.6 ± 0.1	135 ± 8
V379D PheH	0.8 ± 0.04	38 ± 2

19. Anomeric sulfur analogue of CMP-sialic acid as a mechanistic probe for α-2,3-sialic acid transferase. Scott B. Cohen and Randall L. Halcomb. Department of Chemistry & Biochemistry, University of Colorado, Boulder, CO 80309-0215 (scott.cohen@colorado.edu)

α-2,3-Sialic acid transferase catalyzes the transfer of sialic acid from CMP-sialic acid (**1**) to a lactose acceptor. An analogue of CMP-sialic acid which has the anomeric oxygen atom replaced by a sulfur atom was synthesized (**1*s***). Solvolysis of CMP-sialic acid, yielding CMP and sialic acid, proceeded at a rate 50-fold faster than for **1*s***. Analogue **1*s*** was found to be a substrate for α-2,3-sialic transferase, affording the same trisaccharide product as obtained from reaction with **1**. The Michaelis−Menten constant (K_m) for **1*s*** was just 3-fold lower than for **1**, indicating competent substrate binding. A much greater affect was observed for the catalytic constant of the enzyme−substrate complex. The k_{cat} for **1*s*** was 2 orders of magnitude lower than for **1**. The data suggest a mechanism in which the transferase executes a rate-limiting protonation of the anomeric oxygen atom of CMP-sialic acid.

20. Base unstacking during promoter melting, and kinetics of promoter binding by bacteriophage T7 RNA polymerase. Rajiv P. Bandwar and Smita S. Patel. Department of Biochemistry, UMDNJ−Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854 (fax: 732-235-4783, bandwarp@umdnj.edu)

The promoter binding to T7 RNA polymerase during transcription initiation is a multistep process leading to the formation of open binary complex. The binding of a strong T7 promoter, ϕ10, to T7 RNA polymerase was measured by stopped-flow kinetic measurements, using the fluorescence of the adenine analogue 2-aminopurine (2-AP) singly incorporated in place of each of the TATA adenines in the −1 to −4 region of the duplex DNA promoter. The time-dependent fluorescence changes upon binding of singly 2-AP incorporated ϕ10 promoters were characterized in terms of observed rates and amplitudes. All the DNAs exhibited similar rates of open complex formation; however, the amplitude of the fluorescence change with 2-AP at −4 was found to be 5−15 times higher than at other positions. Based on a recently reported crystal structure of the T7 RNAP−promoter complex [Cheetham et al. (1999) *Nature 399*, 80], this large fluorescence change with 2-AP at −4 is explained on the basis of unstacking of 2-AP with the neighboring G at −5, which quenches the fluorescence of 2-AP when stacked. The ϕ10 promoter with 2-AP at −4 on the template strand was used for detailed kinetic studies of the promoter binding mechanism. It is proposed that two forms of the polymerase exist and only one form is capable of productive binding to the promoter. Computer simulation of the minimum kinetic pathway model predicts 35% of the competent form that binds the promoter. The following binding mechanism has been proposed, where E′ and E represent two forms of the polymerase. E binds promoter (D) to form a closed complex (ED) followed by promoter melting conformation change to form open complexes (ED_1 and ED_2).

21. Catalytic mechanism of L-ribulose-5-phosphate 4-epimerase as determined by kinetic isotope effects and spectroscopy. Lac V Lee, Maria V Vu, Russell R Poyner, and W Wallace Cleland. Institute for Enzyme Research, University of Wisconsin, Department of Biochemistry, 1710 University Avenue, Madison, WI 53705 (fax: 608-265-2904, lac@enzyme.wisc.edu)

On the basis of ^{13}C and deuterium isotope effects, L-ribulose-5-phosphate 4-epimerase catalyzes the epimerization of L-ribulose-5-phosphate to D-xylulose-5-phosphate by the aldol cleavage/condensation mechanism. ^{13}C isotope effects were measured at C-2, C-3, C-4, and C-5, and deuterium isotope effects were measured at C-3 and C-4 of the substrate. The 4-epimerase shows sequence similarity with L-fuculose-1-phosphate and L-rhamnulose-1-phosphate aldolases for residues in the active site of L-fuculose-1-phosphate aldolase, suggesting that Asp76, His95, His97, and His171 of the 4-epimerase may be metal ion ligands. H97N, H95N, and Y229F mutants had 10%, 1%, and 0.1%, respectively, of the activity of WT enzyme when activated by Zn(II), the physiological activator. Co(II) and Mn(II) replaced Zn(II) with Y229F and WT enzymes, although less effectively with the His mutants, while Mg(II) was a poorly bound, weak activator. Upon binding of the substrate to the WT and mutant enzymes, a marked change in the absorption/CD spectra was observed for the Co(II)-substituted enzymes and the EPR spectrum of the Mn(II)-substituted WT enzyme. The data suggest that substrate binding to the divalent metal ion is required for catalysis. (This work is supported by NIH Grant GM 18938.)

22. Determination of the O-18 fractionation factor of formate in LBHBs. Laurie A. Reinhardt,[1] Heather M. Christensen,[1] and W. Wallace Cleland.[2] [1]Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (fax: 608-265-2904, Reinhardt@enzyme.wisc.edu), and [2]Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin

The degree of covalency in strong hydrogen bonds is not fully known. Although some believe hydrogen bonding is solely electrostatic in nature, it has been proposed that low barrier hydrogen bonds (LBHBs) are largely covalent. While strong hydrogen bond strengths have previously been assessed by deuterium fractionation factors, it is the O-18 fractionation factor which is affected by covalent bonding. Electrostatic interactions have little effect on the O-18 fractionation factor. Therefore, we are measuring O-18 fractionation factors for a series of hydrogen bonded species. The O-18 fractionation of formate equilibrated between ion-exchange resin containing nitrogen bases of differing pK_a values and solution containing tetrabutylammonium ion should indicate the degree of covalency of the hydrogen

bonded species. Isotope ratio mass spectroscopy is used to determine the O-18 fractionation factor of formate, after its conversion to carbon dioxide. (Supported by NIH Grants GM18938 and GM51806.)

23. Determining the role of substituent effects in the binding of fluorinated benzenesulfonamides to carbonic anhydrase using microemulsion electrokinetic chromatography. Ryan Madder, A. Jain, and J. G. Voet. Department of Chemistry, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081 (fax: 610-328-7355, rmadder1@swarthmore.edu)

In an attempt to determine the role of substituent effects in the binding of several fluorinated benzenesulfonamides to the enzyme carbonic anhydrase, the hydrophobicities of the various inhibitors were sought using a branch of capillary electrophoresis known as microemulsion electrokinetic chromatography (MEEKC). Unexpectedly, the log *P* values of the fluorinated carbonic anhydrase inhibitors were not found to increase with the number of fluorines present in the molecule. Furthermore, when the log *P* values of the inhibitors were plotted against the dissociation constants of the inhibitors to the enzyme, no correlation was found, thereby dispelling the previous notion that the binding of the inhibitors increases with hydrophobicity. The log *P* values were then evaluated by examining the contributions substituent effects and positioning of fluorination make to the overall hydrophobicities of the inhibitors. The roles that fluorine substituent effects and positioning play in binding of the inhibitors to the enzyme will be discussed.

24. *Torpedo* AChE L282A mutant (destabilized) becomes PMSF sensitive. Daniel Kraut,[1] N. Morel,[2] S. Bon,[2] J. Massoulié,[2] I. Silman,[3] J. L. Sussman,[3] and J. G. Voet.[1] [1]Department of Chemistry, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081 (fax: 610-328-7355, dkraut1@swarthmore.edu), [2]Ecole Normale SupÉrieure, and [3]Weizmann Institute of Science

Acetylcholinesterase (AChE) is potentially susceptible to inactivation by phenylmethanesulfonyl fluoride (PMSF) and benzenesulfonyl fluoride (BSF). While BSF inhibits both mouse and *Torpedo* AChE, PMSF does not react measurably with the *Torpedo* enzyme. However, it does inactivate the *Torpedo* acyl pocket double mutant F288L/F290V, which has specificity properties similar to butyrylcholinesterase (BChE). To provide support for the idea that increased breathing motions and decreased stability cause mouse susceptibility to PMSF, we examined the *Torpedo* mutant L282A, which has decreased thermal stability. *Torpedo* L282A AChE is inactivated by PMSF at a rate similar to PMSF inactivation of the F288L/F290V double mutant, but is not inactivated by iso-OMPA, a BChE inhibitor. We suggest that the increased breathing of this destabilized mutant accounts for PMSF inactivation capability and that the increased stability and decreased breathing motions of the *Torpedo* wild-type enzyme relative to the mouse enzyme account for its resistance to PMSF inactivation.

25. Characterization of an L-amino acid oxidase isolated from southern copperhead snake (*Agkistrodon contortrix contortrix*) venom. Michael L. Merchant,[1] Stephanie L. Wegehaupt,[1] Robyn L. Thomas,[1] and Jing Lu.[2] [1]Chemistry and Physics, Texas Woman's University, P.O. Box 425859, Denton, TX 76204 (fax: 940-898-2548, f_merchant@twu.edu), and [2]Nutrition and Food Sciences, Texas Woman's University

We have isolated an acidic (p*I* 5.9) L-amino acid oxidase from the venom of the southern copperhead snake (*Agkistrodon contortrix contortrix*). The enzyme is a homodimer (MW ~133 000 Da) based on SDS−PAGE analysis and gel filtration chromatography results. The holoenzyme is composed of the homodimer and 2 equiv of FAD. Further, we show that the enzymatic oxidation of L-amino acids yields usual and unusual results. The specificity of the enzyme favors oxidation of hydrophobic amino acids (i.e., L-leucine K_m 0.2−0.5 mM). Unexpectedly, the oxidation of nitro-L-arginine occurs with a greater V_{max} than the oxidation of L-arginine. This suggests a potential role for the dissociation and diffusion of the product being included in the rate-determining step.

26. Cysteine 282 mutants of human cytosolic creatine kinases. Michael J. McLeish, **Pan-Fen Wang**, Malea M. Kneen, and George L. Kenyon. College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109 (fax: 734-615 3079, mcleish@umich.edu)

Creatine kinase (CK, EC 2.7.3.2) catalyzes the reversible transfer of the γ-phosphoryl group of MgATP to creatine, resulting in the formation of phosphocreatine and MgADP. The enzyme plays a key role in cell energy metabolism as well as in the transport of 'high energy phosphates'. There are two cytosolic creatine kinase isozymes, the muscle (CK-MM) and brain (CK-BB) isozymes, which exist as dimers. In addition, there are two mitochondrial isozymes which exist as octamers. There is significant sequence homology between the isozymes, and, in all CKs examined to date, there is at least one reactive cysteine residue per monomer. Recent mutagenesis experiments showed that, in chicken Mi_b-CK, the reactive cysteine was essential for synergism but not for catalysis. We now report the results of mutagenesis studies carried out on cysteine 282, the reactive cysteine of both human muscle and human brain CK. As with the mitochondrial CK mutants, although there was significant reduction in activity, the cysteine mutants of both cytosolic isozymes were still active. However, when compared to the Mi-CK mutants, there were distinct differences in pH−rate profiles as well as activation/inhibition by chloride ions.

27. Demethylation of *p*-nitroanisole by rabbit liver P450 1A2 involves multiple substrate-binding sites. Grover Paul Miller[1] and F. Peter Guengerich.[2] [1]Department of Biochemistry, Vanderbilt University, 642 Medical Research Building I, Nashville, TN 37232 (fax: 615-322-3141, grover@toxicology.mc.vanderbilt.edu), and [2]Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University

The P450 1A2-catalyzed demethylation of *p*-nitroanisole (pNA) was monitored by pNA depletion and *p*-nitrophenol (pNP) production simultaneaously. The rates of pNP formation could not be appropriately fit to a simple Michaelis−Menten scheme when including data at high [pNA]. Moreover, pNA depletion rates mirrored the trend observed for

the pNP data. A solvent effect or novel product could not explain the data. Titrating pNA at high enzyme concentration (10 μM) yielded a second titration curve ($K_{D1} = 1.7$ μM, $K_{D2} = 39$ μM). The pNP data were fit to two schemes involving multiliganded complexes. First, two sets of Michaelis−Menten parameters indicated similar k_{cat} ($\sim$1 s^{-1}), but K_M values differed 40-fold, 6 μM versus 260 μM. Second, the Hill equation demonstrated an unprecedented negative cooperativity ($n = 0.55$, $k_{cat} = 2.2$ s^{-1}, $K_{0.5} = 34$ μM). These findings have important implications for other P450 studies involving the turnover of small substrates.

28. Disparate apparent strengths for structurally equivalent hydrogen bonds in a peptide−MHC class II complex. **Benjamin J. McFarland**,[1] Andrea J. Sant,[2] and Craig Beeson.[1] [1]Department of Chemistry, University of Washington, P.O. Box 351700, Seattle, WA 98112 (benmc@u.washington.edu), and [2]Department of Pathology, University of Chicago, Chicago, IL 60637

Major histocompatibility complex (MHC) class II proteins and antigenic peptides form unusually long-lived complexes stabilized in part by hydrogen bonds to the peptide backbone. MHC residues Hisβ81 and Hisα68 are located pseudosymmetrically at each end of the peptide-binding groove and form hydrogen bonds that appear crystallographically equivalent. We have mutated each of these residues to Asn in the I-A^d-MHC. Although peptide dissociation rates are accelerated 20−200-fold by the N-terminal Hisβ81Asn mutation, the rates are accelerated less than 14-fold by the C-terminal Hisα68Asn mutation. We propose that disruption of the Hisβ81 hydrogen bond, and not Hisα68, is an obligate step in the dissociation transition state for all peptides. This is consistent with Arrhenius data and with an inverse correlation of mutation effect vs peptide stability. These results suggest that an antigen-processing molecule like HLA-DM could remove peptides most effectively by facilitating the loss of N-terminal contacts.

29. Grignard-mediated synthesis and biological evaluation of various 3-substituted diphosphate analogues. Todd J. Zahn,[1] Hale Wills,[1] Carolyn Weinbaum,[2] and Richard A. Gibbs.[1] [1]Pharmaceutical Sciences, Wayne State University, College of Pharmacy, and AHP, 613 Shapero, Detroit, MI 48202 (tzahn@Wizard.pharm.wayne.edu), and [2]Pharmacology and Cancer Biology, Duke University, Duke Medical Center, Durham, NC 27710

The fact that the key oncoprotein Ras is farnesylated has led to intense interest in protein farnesyl transferase (FTase) inhibitors as potential cancer chemotherapeutic agents. We have synthesized and evaluated several 3-substituted FPP analogues, and found that 3-tbFPP is a potent inhibitor of both yeast FTase [(1996) *J. Org. Chem.*, 8010] and mammalian FTase [(1999) *J. Med. Chem.* (in press)]. In fact, 3-tbFPP is one of the most potent FPP-based inhibitors of mammalian FTase, and is thus a promising lead compound for further inhibitor development. Therefore, we have adapted our synthetic route to incorporate a novel copper-mediated coupling of various Grignard reagents with an isoprenoid triflate. This new method has allowed us to generate a wide variety of 3-alkyl-substituted FPP analogues, including the three new derivatives shown below. These compounds have now undergone preliminary biological evaluation, utilizing a continuous spectrofluorimetric mammalian FTase assay. This procedure allowed us to determine both the ability of these compounds to act as mFTase inhibitors, and their potential to act as alternative substrates for this enzyme. (This study was supported by NIH Grant CA78819.)

30. Further characterization of a dissimilatory iron(III) reductase from *Enterococcus faecalis*. Leslie E. Sommerville, **Brook Simon**, and **Jennifer Richardson**. Chemistry Department, Fort Lewis College, 1000 Rim Drive, Durango, CO 81301 (sommervil_l@fortlewis.edu)

A dissimilatory iron(III) reductase activity has been partially characterized in whole cells and crude membrane fractions of *Enterococcus faecalis*. Previous studies showed that the enzyme can utilize ferric citrate as a soluble iron(III) substrate, and that its activity is optimal at a pH between 6 and 7, but no dependence on NADH was observed. Using a different protocol, preliminary experiments with the crude membrane fractions show that the reductase is dependent on NADH but no kinetic parameters could be determined due to a contaminating NADH oxidase activity in the membrane prep. In an attempt to further purify the iron(III) reductase activity, several detergents were used to extract the reductase from the membrane. Extractions using Tween 80, Brij, and Triton-X-100 resulted in loss of reductase activity. After further study, the activity was found to be solubilized and stabilized when extracted with deoxycholate. These experiments suggest a route for purification of the reductase that will allow for further characterization of this enzyme in the future.

31. Evidence for an ene mechanism in a "hydride equivalent" transfer with a nicotinamide coenzyme model system. **R. Daniel Libby** and Ryan A. Mehl. Chemistry Department, Moravian College, 1200 Main Street, Bethlehem, PA 18018 (fax: 610-861-3979, rdlibby@cs.moravian.edu)

Reactions of bis-1,4-di(trimethylsilyl)-1,4-dihydropyridine, **1**, were studied as a model system for NADH dehydrogenases. The reaction of **1** with acrylonitrile, **2**, initially produces an Ene adduct, bis-1,4-di(trimethylsilyl)-2-(1-cyanoethyl)-1,2-dihydropyridine, **3**, which is a stable liquid and was isolated and characterized. Heating **3** with a mixture of methanol and water at 50 °C for 3 days completes the reduction of **2** to propanenitrile, **4**. Reaction of **1** with **2** at 50 °C in methanol and water yields **4** with transient formation of **3**. The results are consistent with the mechanism originally proposed by Hamilton [(1971) *Prog. Biochem. 1*, 83−157] which describes the nicotinamide "hydride equivalent" transfer as a two-step process involving the initial formation of an Ene adduct which then decomposes by an ionic process. The demonstration that **3** is an obligatory intermediate in the reduction of **2** by **1** gives the first direct evidence for a covalent intermediate in a 1,4-dihydropyridine reduction reaction.

32. Mechanism of bromide activation of chloroperoxidase-catalyzed peroxidation of catechol: Comparison with the chloride activation mechanism. R. Daniel Libby, Jeremiah Eckhaus, and Daniel A. Moniot. Chemistry Depart-

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Bromide ion (Br^-) is a competitive activator of the chloroperoxidase (CPO)-catalyzed catechol peroxidation. The mechanism of activation is compared with that previously found with chloride (Cl^-). Both Br^- and Cl^- are competitive activators vs catechol. All data for both activation processes are consistent with a mechanism in which halide competes with catechol for binding to CPO Compound I. Catechol binding initiates the halide-independent path, in which Compound I acts as the oxidizing agent. Halide binding to Compound I initiates the halide-dependent reaction, where the enzymatic halogenating intermediate (EOX) is the oxidizing agent. As with Cl^- activation, Br^- activation is due to a shift from the Br^--independent pathway to the Br^--dependent process; however, substrate inhibition is an important factor in Br^- activation. This report presents the second example where exclusion of the substrate from its primary binding site activates an enzymatic reaction.

33. GDP-mannose mannosyl hydrolase (GDPMH) catalyzes nucleophilic substitution at carbon, unlike its homologues. Patricia M. Legler,[1] Michael A. Massiah,[1] Maurice J. Bessman,[2] and Albert S. Mildvan.[1] [1]Biological Chemistry, Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205 (fax: 410-955 5759, plegler@welchlink.welch.jhu.edu), and [2]Biology, Johns Hopkins University

GDPMH is a 36.8 kDa homodimer which catalyzes the hydrolysis of GDP-α-mannose or GDP-α-glucose to sugar and GDP in the presence of Mg^{2+}. GDPMH is a member of the Nudix family of enzymes which catalyze the hydrolysis of nucleoside diphosphate derivatives by nucleophilic substitution at phosphorus. However, GDPMH has a sequence inversion in the conserved Nudix motif (-REL- in MutT; -ERL- in GDPMH). By ^1H NMR, the initial hydrolysis product of GDP-α-glucose is β-glucose, indicating nucleophilic attack and inversion at the C1 of glucose. Attack at carbon was confirmed by 2D $^1H-^{13}C$-HSQC spectra of the products of hydrolysis in 49% ^{18}O-labeled water which showed an upfield shift of 18 ppb at C1 of β-glucose. No ^{18}O isotope shifts were found in the ^{31}P NMR spectrum of the GDP product. Thus, enzymes showing conservation of active site sequences do not necessarily catalyze the same reactions.

34. Structural basis for the low pK_a of the general base in 4-oxalocrotonate tautomerase (4-OT). Christian P. Whitman,[1] Robert M. Czerwinski,[1] Thomas K. Harris,[2] and Albert S. Mildvan.[2] [1]College of Pharmacy, University of Texas, Austin, TX 78712 (fax: 512-232-2606, cwhitman@uts.cc.utexas.edu), and [2]Department of Biological Chemistry, Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

4-OT catalyzes the isomerization of β,γ-unsaturated enones to their α,β-isomers through a dienol intermediate. Pro-1, the catalytic base in the reaction, has a pK_a value of 6.4, which is 3 units lower than that of the model compound, proline amide. Recent studies show that the abnormally low pK_a is not due to the electrostatic effects of nearby cationic residues but is likely the result of a low local dielectric constant of 15.3 ± 1.0 [(1999) *Biochemistry 38*, 12358]. This mechanism is supported by studies of two mutants (F50A and F50Y) of the active site Phe-50. By ^{15}N NMR titration, the pK_a of Pro-1 in F50A is 7.4 ± 0.2, comparable to the pK_a of 7.6 ± 0.2 found in the pH vs k_{cat}/K_m rate profile. $^1H-^{15}N$ HSQC spectra of F50A show diffuse structural changes. Replacing Phe-50 with Tyr results in a pK_a of 6.3 ± 0.1 for Pro-1 in the pH vs k_{cat}/K_m rate profile, comparable to that observed for wild type. In addition, the pK_a of Tyr-50 in this mutant is 11.7 ± 0.1 by UV titration. This unusually high pK_a value indicates the local dielectric constant at the active site to be 16.6 ± 0.9.

35. How do serine proteases recognize their substrates? Robert C Bergstrom and David R. Corey. Department of Pharmacology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75205 (fax: 214-648-5095, bergstro@utsw.swmed.edu)

We have characterized the substrate specificity of the serine proteases prostate-specific antigen, plasmin, trypsin, urokinase-type plasminogen activator (u-PA), and tissue-type plasminogen activator (t-PA) and have found the K_M for hydrolysis of protein substrates is 10−1000-fold reduced in comparison to analogous peptides. We have tested whether K_M is equivalent to K_D, the true dissociation constant. The K_M, K_I, and K_D values of trypsin and u-PA for peptides and analogous protein substrates were measured. Kinetic analysis demonstrates that for both u-PA and trypsin that K_M is equivalent to K_D. The finding that K_M is a true dissociation constant indicates that there is a structural basis for the large change in binding affinity that is seen between protein and peptide substrates. In attempt to determine this structural basis, we have recently collected crystallographic data at 1.6 Å of a complex between trypsin and a peptide substrate.

36. Inhibition of *Leishmania donovani* 3′-nucleotidase by 9-substituted adenine derivatives. Maciej B. Szczepanik,[1] Praveen Pande,[1] Wolfgang G. Laux,[1] Alain Debrabant,[2] Denis Dwyer,[2] and Roger A. Johnson.[1] [1]Physiology and Biophysics, SUNY, Health Science Center, Stony Brook, NY 11794-8661 (fax: 516-444-3432, mszczepa@physiology.pnb.sunysb.edu), and [2]Cell Biology, NIAID, NIH, Bethesda, MD 20892

Several species of the protozoan family Trypanosomatidae contain exocellular membrane-bound 3′-nucleotidase (EC 3.1.3.6), an enzyme for which there is no evidence in mammalian cells. The protozoans include Leishmania and Trypanosomas, which are pathogenic parasites of man, responsible for sleeping sickness, leishmaniasis, and Chagas disease. The structure−activity relationship for inhibition of 3′-nucleotidase from *Leishmania donovani* was evaluated. The enzyme was inhibited by a variety of 5′- and 3′-substituted adenosine derivatives in a competitive manner. The most potent inhibitors were 9-substituted acyclic adenine derivatives [e.g., 9-(phosphonylmethoxyethyl)adenine] with IC_{50} values in the low micromolar range. Consequently, a series of dialdehyde and pyridoxal phosphate covalent affinity probes were synthesized and tested. These compounds caused irreversible inactivation of 3′-nucleotidase, suggesting their usefulness for labeling the enzyme to elucidate structures in and around the enzyme catalytic active

site. These studies form a basis for rational drug design for inhibition of an important purine salvage enzyme of these parasitic organisms.

37. Insight into the partial reactions of HMG-CoA synthase. Dmitriy A. Vinarov, Kelly Y. Chun, and Henry M. Miziorko. Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 (fax: 414-456-6570)

HMG-CoA synthase reacts with acetyl-CoA to form an acetyl-*S*-enzyme reaction intermediate which slowly hydrolyzes in the absence of the second substrate, acetoacetyl-CoA. Several mutant HMG-CoA synthases (D99A, D159A, and D203A) exhibit a 10^2-10^4-fold decrease in the overall k_{cat} which is largely attributable to retarded kinetics of acetyl-*S*-enzyme formation (10^2-10^3-fold decrease). Single turnover experiments indicate that these enzymes are not significantly impaired in condensation or hydrolysis to release HMG-CoA. Differential isotope shift ^{13}C NMR has been used to investigate the reaction intermediate. When [^{13}C]-acetyl-*S*-enzyme is incubated with $H_2{}^{18}O$ vs $H_2{}^{16}O$, an ^{18}O-dependent upfield shift for the C1 resonance is detected. This observation of ^{18}O exchange indicates transient formation of a tetrahedral species as a prelude to slow hydrolysis and release of acetate. The time required for the maximal development of the ^{18}O-induced isotope shift for D159A's and D203A's acetyl-*S*-enzyme intermediate is markedly increased in comparison with wild-type enzyme. These observations (1) suggest that enzyme-catalyzed hydrolysis of acetyl-CoA in the absence of second substrate requires prior formation of the acetyl-*S*-enzyme reaction intermediate; (2) confirm the hypothesis that D159 and D203 participate in acetyl-*S*-enzyme formation. In contrast, at times sufficient for complete formation of the acetyl-*S*-enzyme reaction intermediate, D99A exhibits no ^{18}O-induced shift.This result suggests that D99 may support either attack of solvent-derived OH^- or protonation of the C1 carbonyl oxygen in the formation of the *gem*-diol-containing tetrahedral adduct. If acetyl-*S*-enzyme hydrolysis mimics the productive hydrolysis of HMG-CoA from enzyme, release of the condensation product may also proceed through a tetrahedral adduct. [Supported by NIH Grant DK21491 (H.M.M.); D.A.V. is an American Heart Association postdoctoral fellow; K.Y.C. is an American Heart Association predoctoral fellow.]

38. Phosphoribulokinase: Evaluation of potential cation ligands in the "P-loop". **Jennifer A. Runquist** and Henry M. Miziorko. Biochemistry Department, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 (fax: 414-456-6570)

Phosphotranferases that utilize a divalent cation−nucleotide triphosphate substrate typically provide two amino acid side chain ligands to the cation. For phosphotransferase sequences that exhibit "P-loop" (or Walker A) motifs, ligands to cation are commonly supplied by serine/threonine residues. In proteins with "Walker B" motifs, a carboxyl group commonly supplies a second ligand to cation. In *R. sphaeroides* phosphoribulokinase (PRK), we have proposed that E131 represents a "Walker B" carboxyl. Elimination of the carboxyl side chain decreases V_m by 200-fold and inflates the K_m for substrate ribulose 5-phosphate (Ru5P) by 6-fold. The N-termini of PRKs contain consensus "P-loops", but no serine or threonine is absolutely invariant. In *R. sphaeroides* PRK, three alcohol side chains, contributed by T18, S19, and T20, map within the P loop and represent potential cation ligands. Each of these has been individually replaced with an alanine. The PRK mutants have been expressed, isolated, and partially characterized. Each mutant retains the ability to tightly bind effector (0.7−1.0/site) and exhibits normal allosteric activation (40-fold) by NADH, suggesting that the proteins retain a high degree of structural integrity. However, the mutants contrast sharply with respect to catalytic efficiency. T20A exhibits V_m comparable to wild-type PRK. T18A's V_m is diminished by 8-fold, and S19A's V_m is diminished by 500-fold. S19A and T18A exhibit only modest (6−7-fold) increases in K_m for ATP but larger (30−45-fold) increases in K_m for Ru5P. These observations underscore the importance of T18 and S19 to P-loop function and support the hypothesis that PRK's S19 provides a ligand to cation of the Mg-ATP substrate. (Supported by USDA NRICRG−Photosynthesis.)

39. Inhibitor candidates for tyrosyl protein sulfotransferases. John W. Kehoe and Carolyn R. Bertozzi. Chemistry Department/Bertozzi Group, University of California, B87 Latimer Hall, Berkeley, CA 94720 (jkehoe@uclink4.berkeley.edu)

Tyrosyl protein sulfotransferases (TPSTs) are a recently discovered class of enzymes which play important roles in inflammation and HIV infection. These enzymes transfer a sulfate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to tyrosine residues in various proteins. It is thought that the sulfates then mediate protein−protein interactions. The TPSTs' entire role in living systems is not yet understood, and may extend far beyond these two instances. We have begun screening inhibitor candidates for the two known human TPSTs. A small molecule inhibitor of the TPSTs would prove useful in the investigation of these enzymes' role in vivo, and may eventually lead to a new class of pharmaceuticals. PAPS is quite similar to adenosine triphosphate, so we have begun our screening program with known tyrosine kinase inhibitors. We are also screening small molecule libraries synthesized in our laboratory.

40. Investigation of cooperation between active-site bases in the reaction catalyzed by cholesterol oxidase. Ye Yin and Nicole Sampson. Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400 (fax: 631-632-5731, nicole.sampson@sunysb.edu)

Cholesterol oxidase is a monomeric flavoenzyme. It catalyzes the oxidation of steroids containing a 3β-hydroxyl group followed by isomerization of the Δ^5-enone into a Δ^4-3-ketosteroid. Previous studies suggest that His447 is the base for oxidation and Glu361 might assist the oxidation by a hydrogen-bonding network mediated by Asn485 and Wat541. We propose that the catalytic tetrad involving His447, Glu361, Asn485, and Wat541 helps to position the substrate and coordinates catalysis for oxidation. A single mutant, N485L, was prepared. Steady-state kinetic analysis shows that k_{cat} for N485L is 1000-fold slower than wild type in oxidation and for isomerization is only 20-fold slower. In pH-dependence kinetic studies, the oxidation activity of

N485L increased with increasing pH [V_{max}/K_m: $pK_{a(app)} = 10.33$, V_{max}: $pK_{a(app)} = 8.68$]. The isomerization activity did not. This suggests that the hydrogen-bonding network is the structural basis of cooperation of these active-site residues. Two double-mutants, H447E/E361Q and H447Q/E361Q, were prepared to examine the cooperation between His447 and Glu361. H447Q/E361Q has a lower oxidation activity than H447Q and E361Q individually. H447E/E361Q has no oxidation activity.

41. Kinetic isotope effects of thymidine phosphorylase. Mansoureh Rezaei, Micheal Kearny, and Paul C. Kline. Department of Chemistry, Middle Tennessee State University, 1301 East Main Street, Murfreesboro, TN 37132 (fax: 615-898-5182, Pkline@mtsu.edu)

Thymidine phosphorylase is one of two pyrimidine phosphorylases that operates in the salvage pathway. It catalyzes the irreversible phosphorolysis of uridine to yield uracil and ribose-1-phosphate. In addition, thymidine phosphorylase has been shown to be identical to platelet-derived endothelial cell growth factor. Recent studies have shown that platelet-derived endothelial cell growth factor (PD-ECGF) is associated with the development of new blood vessels necessary for tumor growth and metastasis in a variety of malignant tumors including breast, stomach, colon, and head and neck tumors. A family of kinetic isotope effects (KIEs) was determined for *E. coli* thymidine phosphorylase using stable isotopes. The experimentally determined KIEs were obtained for [1′-^{13}C]-, [2′-^{13}C]-, [1′-^{2}H]-, [2′-^{2}H]-, [5′-^{2}H]-, and [1-^{15}N]-uridine. The KIEs obtained for the arsenolysis reaction were matched to a vibrational transition state using bond energy bond order vibrational analysis. In this transition state, substantial oxocarbenium ion character has been developed, the C−N glycosidic linkage is largely cleaved, and an enzyme-directed attacking oxygen nucleophile lies just within bonding distance to its target carbon.

42. Relationship of spectroscopic changes and deuterium isotope effects to hydrogen bond strength. Marily H. Lantz and Vernon E. Anderson. Department of Chemistry, Case Western Reserve University, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106 (fax: 216-368-3419, mhl7@po.cwru.edu)

C_1-^2H stretching frequencies of hydrogen-bonded secondary alcohols have been studied by IR, NMR, and quantum chemical calculations. In particular, the C_1-^2H stretch observed by IR is red-shifted 104 cm^{-1} when dideuteriotrifluoroethanol is ionized, paralleling the frequencies calculated. Calculations suggest that the effect is largest on the α C−D bond anti to the O−H bond donor, and that the magnitude of the decreased frequency is related to the enthalpy of bond formation. We relate the changes in frequency to increased overlap of the oxygen lone pair orbital with the C_1-^2H antibonding orbital. The findings suggest this will give rise to an equilibrium isotope effect (EIE), confirmed experimentally and by quantum chemical calculations. An NMR titration experiment yields information relating differences in the ^{13}C chemical shifts to an estimated deuterium isotope effect of 1.13 for the ionization of the two molecules. Calculations of the stretching frequencies of 2-propanol complexed with a series of increasingly stronger bases suggest the expected EIE is directly related to C_1-^2H bond lengths and hydrogen bond strength. Using a model nucleoside/formate complex, our results offer an explanation for the four-bond kinetic isotope effect reported for the hydrolysis of [5′-^{3}H]inosine by nucleoside hydrolase [Horenstein, B. A., et al. (1991) *Biochemistry 30*, 10788−10796].

43. Orientation of CoA substrates, nicotinamide and active site functional groups in (di)enoyl-CoA reductases. Kerry L. Fillgrove and Vernon E. Anderson. Department of Biochemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4935 (fax: 216-368-3419, kerry@biochemistry.cwru.edu)

The stereochemical course of reduction of dienoyl-CoA thiolesters catalyzed by the 2,4-dienoyl-CoA reductase from rat liver mitochondria was investigated. The configuration of the double bond in the 3-enoyl-CoA products was determined by ^{1}H NMR, and studies to determine the absolute stereochemistry of reduction at Cα and C*δ* using [4-^{2}H]NADPH were conducted in H_2O and D_2O. The results indicate that the configuration of the double bond of the 3-enoyl-CoA product is trans and that a general acid-catalyzed addition of a solvent-derived proton/deuteron occurs on the *si* face at Cα of the dienoyl-CoA. The addition of a hydride equivalent from NADPH occurs on the *si* face at C*δ* of *trans*-2,*cis*-4-dienoyl-CoA and on the *re* face at C*δ* of *trans*-2,*trans*-4-dienoyl-CoA. The stereochemistry of reduction of the InhA enoyl-thiolester reductase from *Mycobacterium tuberculosis* was also determined using [4-^{2}H]-NADH in D_2O. The reduction of *trans*-2-octenoyl-CoA by InhA resulted in the syn addition of $^2H_2$ across the double bond, yielding (2*R*,3*S*)-[2,3-$^{2}H_2$]octanoyl-CoA. The mechanisitic implications will be discussed.

44. Kinetic analysis of ligand interactions with rho, *E. coli* transcription termination factor. Dong-Eun Kim and Smita S. Patel. Department of Biochemistry, UMDNJ−Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854 (fax: 732-235-4783, kimdo@umdnj.edu)

Escherichia coli transcription termination factor rho is an ATPase-dependent helicase that can unwind RNA−DNA duplex. The active form of rho is a hexamer with three catalytic subunits that turn over ATP at a fast rate and three noncatalytic subunits that turn over ATP at a relatively slow rate. In the presence of RNA cofactor, the rho-catalyzed ATPase rate is fast, close to 30 s^{-1}. However, under these conditions, the three tightly bound nucleotides dissociate from the hexamer at a slow rate of 0.02 s^{-1}, indicating that the three tight nucleotide binding sites of rho do not participate in the fast ATPase turnover. The slowly exchanging nucleotide binding sites of rho are capable of hydrolyzing ATP, but the resulting products bind tightly and dissociate from rho slower than the fast ATPase turnover. Using the pre-steady-state pulse−chase experiments, the hydrolysis rate of three ATPs bound to the noncatalytic sites and the apparent bimolecular rate constant for ATP binding were determined in the presence of RNA. The mechanism of the ATPase reaction at the noncatalytic sites was proposed and compared with the ATPase mechanism at the catalytic sites that exhibit a sequential mechanism of ATP binding and

hydrolysis like the binding change mechanism of the F_1-ATPase protein. To investigate how the ring-shaped hexameric rho protein interacts with an RNA strand, we have used transient-state kinetic approaches. The transient protein fluorescence changes upon RNA binding were monitored in real time using the stopped-flow method. Multiple phases of protein fluorescence changes were observed, suggesting multiple steps are present in the RNA interactions to rho protein. Chemical-quench-flow rho ATPase assay supports the mechanism in which rho:RNA assembly needs to undergo multiple conformational changes prior to stimulating the steady-state catalytic ATP turnover. The analysis of the kinetic data by global fitting provided the intrinsic rate constants for each kinetic step in the proposed mechanism.

45. Kinetic study of cis-trans isomerization of proline peptide bonds in model compounds for oxidoreductase enzymes. Dallas R. Rabenstein, **Tiesheng Shi**, and Stephen M. Spain. Department of Chemistry, University of California, Riverside, CA 92521 (dallas.rabenstein@ucr.edu)

Oxidoreductase enzymes have in common the Cys-Xaa-Xaa-Cys active site motif. Model peptides for thioltransferase and thioredoxin, two members of the family of oxidoreductase enzymes, have been prepared. The active site of thioltransferase has the sequence Cys-Pro-Phe-Cys, while that of thioredoxin is Cys-Gly-Pro-Cys. Results of kinetic studies of cis-trans isomerization for both dithiol and intramolecular disulfide forms of the model peptides will be presented. Both the cis and trans isomers with respect to the conformation across the Xaa−Pro peptide bond are observed in NMR spectra. Rate constants and activation parameters were determined for cis-trans isomerization by the inversion-magnetization transfer NMR method. The results highlight that the dithiol and the corresponding cyclic disulfide forms of the model peptides have significant differences in conformational flexibility.

46. Mechanistic studies of microsomal epoxide hydrolase. Constance S. Cassidy and Richard N. Armstrong. Department of Biochemistry, Vanderbilt University, 842 MRBI, 23rd and Pierce, Nashville, TN 37232 (connie@toxicology.mc.vanderbilt.edu)

Microsomal epoxide hydrolase (MEH) catalyzes the addition of water to epoxides and arene oxides to give vicinal diol products. MEH is a member of the α/β-hydrolase fold family of enzymes. The catalytic triad consists of a nucleophile (D226), a general base (H431), and a charge relay residue (E404). The reaction occurs in two steps, involving nucleophilic attack of D226 on the oxirane carbon to give a covalent ester intermediate followed by attack of water on the carbonyl carbon of D226. Previous studies support a simplified kinetic mechanism in which rapid equilibrium binding of the substrate is followed by reversible formation and irreversible hydrolysis of the alkyl-enzyme intermediate. The crystal structures of several related epoxide hydrolase proteins have been solved and suggest that two tyrosine residues may be involved in the stabilization of the oxirane intermediate within the alkylation step. We present kinetic and spectroscopic studies of MEH and several mutants (Y299F, Y374F, and W332F) with the substrates phenanthrene 9,10-oxide, epichlorohydrin, and glycidyl 4-nitrobenzoate.

47. Metabolic oxidation of carcinogenic arylamines by P-450 monooxygenases: Theoretical support for the one-electron-transfer mechanism. **R. S. Fellers**,[1] J. Sasaki,[2] and M. Colvin.[2] [1]Center for Applied Scientific Computing, Lawrence Livermore National Laboratory, P.O. Box 808, L-551, Livermore, CA 94550 (fellers1@llnl.gov), and [2]Computational Biology Group, Lawrence Livermore National Laboratory, P.O. Box 808, L452, Livermore, CA 94550

N-Oxidation is an initial step in carcinogenic arylamine activation. The precise mechanism of oxidation is not completely understood. However, current opinion favors two likely metabolic pathways that are characterized by either one- or two-electron-transfer from the substrate to the heme moiety. Based upon the observed kinetics and product distributions observed experimentally, and charge distributions calculated by Huckel theory, Hammons et al. (1985) concluded that the two-electron-transfer mechanism was the preferred model. In this work, we have used a more sophisticated level of theory, DFT, to better calculate charge distributions and energies of the reactants, products, and proposed intermediates. Our data indicate that the one-electron model is more consistent with previous experimental findings.

48. NSAIDs and cyclooxygenase: A study of protein−small molecule interactions. G. Phillip Hochgesang, Scott W. Rowlinson, and Lawrence J. Marnett. Department of Biochemistry and Chemistry, Center in Molecular Toxicology, School of Medicine, Vanderbilt University, Nashville, TN 37232 (fax: 615-343-7534, gerald.p.hochgesang@vanderbilt.edu)

NSAIDs exert their beneficial effects through the inhibition of cyclooxygenase-2 (COX-2). Our group recently developed a COX-2 selective inhibitor, 2-acetoxyphenylhept-2-ynyl sulfide (APHS), based on aspirin as a template. Both aspirin and APHS inhibit COX-2 through covalent modification of serine 516. Although the region of COX-1 that interacts with aspirin has been determined by X-ray crystallography, the equivalent region of COX-2 that interacts with APHS is unknown. We have used site-directed mutagenesis coupled with polyacrylamide gel electrophoresis to probe the binding site of APHS within COX-2 as well as to determine the molecular requirements for acetylation of the enzyme. Our data show that tyrosines 334 and 371 participate in a general acid catalysis mechanism which allows aspirin to specifically modify serine 516. Our data also suggest that APHS binds in the so-called "top channel" region of the arachidonate binding site of the protein above serine 516. This would represent a novel NSAID binding site.

49. Pre-steady-state kinetics of carbamoyl phosphate synthetase of *E. coli.* B. W. Miles and F. M. Raushel. Department of Chemistry, Texas A&M University, College Station, TX 77843 (bwmiles@MSN.com)

Carbamoyl phosphate synthetase of *E. coli* catalyzes the synthesis of carbamoyl phosphate through a series of four reactions generating three intermediates occurring at three active sites connected together by a 100 Å molecular tunnel. To understand the mechanism, the pre-steady-state time courses for product formation were determined. When

bicarbonate and ATP are mixed with CPS, a stoichiometric burst of phosphate and ADP is observed followed by the steady-state rate. The addition of glutamine or ammonia does not affect the burst kinetics but increases the steady-state rate. The initial phosphorylation event is independent of the binding or hydrolysis of glutamine. The glutamate production time courses also demonstrated burst kinetics. The addition of ATP affects both burst formation kinetics and the steady-state rate. Thus, phosphorylation of bicarbonate synchronously controls glutamine hydrolysis. The overall rate-determining step is the formation or release of carbamoyl phosphate. (Supported by NIH Grant DK30343.)

50. Roles of hinge loop linking N- and C-terminal domains of small subunit of carbamoyl phosphate synthetase from *Escherichia coli.* Xinyi Huang and Frank M. Raushel. Department of Chemistry, Texas A&M University, College Station, TX 77843 (fax: 409-845-9452, huang@mail.chem.tamu.edu)

The heterodimeric carbamoyl phosphate synthetase from *Escherichia coli* synthesizes carbamoyl phosphate from bicarbonate, glutamine, and two molecules of ATP. The N- and C-terminal domains of the small subunit are linked by a type-II turn loop. The roles of this hinge-like loop in the formation of carbamoyl phosphate were determined by mutagenesis. Mutations G152I, G155I, and Δ155 were intended to disrupt the turn conformation. However, they only had minor effects on kinetic properties of the enzyme. L153W, L153G/N154G, and the ternary complex of the large subunit and two domains of the small subunit retained ability to synthesize carbamoyl-P, but partially uncoupled glutamine hydrolysis from the synthetase reaction. We conclude that the hinge loop, but not the type-II turn structure of the loop per se, is important for proper interface interactions between the two subunits and consequently coupling of the partial reactions. (Supported by NIH Grant DK30343.)

51. Triosephosphate isomerase: New tricks from an old dog. John P. Richard, **AnnMarie C. O'Donoghue**, and Tina L. Amyes. Department of Chemistry, SUNY, Buffalo, NY 14260-3000 (fax: 716-645-6963, jrichard@chem.buffalo.edu)

Values of k_{cat}/K_m for the isomerization of D-glyceraldehyde 3-phosphate (DGAP) and D-glyceraldehyde (DGA) catalyzed by triosephosphate isomerase (TIM) in D_2O at pD = 7.5 and 25 °C have been determined as 2×10^8 and 0.23 M^{-1} s^{-1}, respectively. The rate constant for nonenzymatic isomerization of DGA catalyzed by 3-quinuclidinone ($pK_{BD} = 8.3$) is $k_B = 5.4 \times 10^{-3}$ M^{-1} s^{-1}. The utilization of the binding energy of the phosphate group results in a 12 kcal/mol transition state stabilization and may account for all but 50-fold of the enzymatic rate acceleration. ^1H NMR analysis shows that 60% of [^1H] derived from deprotonation of DGAP at C-2 undergoes intramolecular transfer to product, so that isotope exchange with solvent D_2O at the enediol(ate) intermediate is *slower* (!) than proton transfer to give dihydroxyacetone phosphate. The observation that only 20% of [^1H] derived from deprotonation of DGA undergoes intramolecular transfer to give product provides evidence for an interaction between TIM and the phosphate group of the substrate which results in "shielding" of the transferred hydrogen from exchange with solvent.

52. Nuclear magnetic resonance and mass spectroscopic evidence for the flavin-1-phenylcyclopropylamine inactivator adduct of monoamine oxidase. Deanna J. Mitchell,[1] Richard B. Silverman,[1] Thomas P. Singer,[2] Sergey O. Sablin,[3] Richard B. van Breemen,[4] Dejan Nikolic,[4] and Edwin Rivera.[5] [1]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113 (fax: 847-491-7713, djmitchell@nwu.edu), [2]Molecular Biology Division, Veterans Affairs Medical Center, San Francisco, CA 94121, [3]Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94121, [4]Department of Medicinal Chemistry & Pharmacognosy, University of Illinois, Chicago, IL 60612-7231, and [5]Department of Medicinal Chemistry & Molecular Pharmacology, Purdue University, West Lafayette, IN 47907-1333

Flavoenzymes catalyze a variety of redox and monooxygenation reactions important in medicinal chemistry. The flavin cofactor, in either the flavin adenine dinucleotide (FAD) or the flavin mononucleotide (FMN) form, plays an essential role in the mechanism of action of many enzymes, including monoamine oxidase (MAO; EC 1.4.3.4). The MAO inactivator 1-phenylcyclopropylamine has been found to inactivate by forming a covalent attachment with an active site cysteine residue (reversible) and the flavin cofactor (irreversible). Use of a mutant form of this enzyme, which lacks the covalent attachment to the flavin cofactor, has allowed for isolation of the flavin−inactivator adduct. Analysis by on-line liquid chromatography−electrospray mass spectrometry (LC-ESMS) and nuclear magnetic resonance (NMR) spectroscopy has provided an avenue to explore the structure of the flavin−inactivator adduct of MAO.

53. Mechanistic comparisons of KDO 8-P synthase and DAH 7-P synthase. R. W. Woodard, W. P. Taylor, A. K. Sundaram, G. Ya. Sheflyan, D. L. Howe, H. S. Duewel, and M. R. Birck. Department of Medicinal Chemistry, University of Michigan, College of Pharmacy, 428 Church Street, Ann Arbor, MI 48109-1065 (fax: 734-763-2022, rww@umich.edu)

The enzyme 3-deoxy-D-*manno*-octulosonic acid 8-phosphate (KDO 8-P) synthase (EC 4.1.2.16), an enzyme in LPS biosynthesis, catalyzes the condensation of D-arabinose 5-phosphate (A 5-P) with phosphoenolpyruvate (PEP) to give KDO 8-P and inorganic phosphate (P_i) while 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAH 7-P) synthase (phe), an enzyme involved in the biosynthesis of the aromatic amino acid phenylalanine, catalyzes the condensation of D-erythrose 4-phosphate (E 4-P) with PEP to give DAH 7-P and P_i. Protein sequence alignments of the two enzymes reveal only a few regions of either identity or homology; however, both catalyze an aldol-type condensation in which a "carbanion" generated on the *si* face of PEP attacks the *re* face of the aldehyde portion of the monosaccharide (E 4-P or A 5-P). DAH 7-P synthase is a homotetramer requiring a divalent metal for activity while KDO 8-P synthase is a homotrimer and does not require a metal. Results from mechanistic and mutational studies comparing these two enzymes as well as studies of both an unusual DAH 7-P synthase from *Bacillus subtilis* 168 and a metallo-KDO 8-P synthase from *Aquifex aeolicus* will be presented.

54. Mutation at non-cysteine positions eliminates the oxidation/reduction effect on the activity of phenol sulfotransferase. Yuh-Shyong Yang. Department of Biological Science and Technology, National Chiao Tung University, 75 Po-Ai Street, Hsinchu, Taiwan (fax: 886-3-5729288, ysyang@cc.nctu.edu.tw)

Phenol sulfotransferases catalyze sulfuryl group transfer between nucleotide and a variety of nucleophiles that may be sugar, protein, small molecules, and xenobiotics. Binding of nucleotides strongly affects the activity and stability of phenol sulfotransferase at various oxidation/reduction states. Phenol sulfotransferase (PST VI) contains five cysteines, and C66 was found to be important for the catalysis of physiological reaction. The presence of C66 and oxidation are required for PST VI to reach maximum activity. We are characterizing a mutant (K65E,R68G) that contains the entire cysteines, but is insensitive to the effect of oxidation/reduction. Oxidation of this mutant is not required for maximum activity. K_m and V_{max} of both mutant and wild-type enzymes at various oxidation/reduction states were determined and compared. Mutant enzyme exhibits much better activity than that of wild-type enzyme. A model based on the kinetic data, mutagenesis, and molecular modeling is proposed.

55. Progress toward the expansion of the genetic alphabet. Yiqin Wu, Anthony Ogawa, Dustin McMinn, Markus Berger, Floyd Romesberg, and Peter Schultz. Department of Chemistry, CVN 22, Scripps Research Institute, La Jolla, CA 92037 (yiqin@scripps.edu)

Hydrophobic bases that can be incorporated into DNA enzymatically have been designed and synthesized. Enzymatic incorporation of unnatural nucleotide into DNA was demonstrated by Klenow fragment (KF) of DNA polymerase I from *E. coli*. In general, hydrophobic nucleobase in template would prefer to pair with other hydrophobic bases including itself. We have synthesized homopair nucleobases which can be incorporated into DNA by KF with nativelike activity and selectivity. Very selective heteropair has not been achieved because of the competition with the homopair. Possible homopair candidates that could be used to expand the genetic alphabet will be presented. Although we have achieved very selective single incorporation of unnatural nucleotide into DNA, further extension after the incorporation of the unnatural nucleotide is generally not very efficient, and it is sequence-dependent. Possible explanation about this observation will be discussed. We have initiated structural studies (NMR and X-ray) of these unnatural nucleobases in duplex DNA. New strategies to replicate full-length DNA after the initial incorporation of hydrophobic nucleotides are in progress.

56. Mechanisms of retaining β-hexosaminidases: functionally related enzymes, differing catalytic mechanisms. David J. Vocadlo, Christoph Mayer, and Stephen G. Withers. Protein Engineering Network of Centres of Excellence of Canada and Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada

Owing to their potential uses in biotechnology, there has been increasing interest in the mechanism of action of retaining β-hexosaminidases. There are two possible catalytic mechanisms these enzymes may employ: the double displacement mechanism, which is found for most retaining glycosidases (path A, scheme); and another involving anchimeric assistance from the 2-acetamido group of the substrate (path B, scheme). Recently, retaining β-hexosaminidases have been classified into families 3 and 20 of glycosyl hydrolases. We have undertaken a comparative study of the *Vibrio furnisii* ExoII and *Streptomycetes plicatus* β-hexosaminidases as representatives from families 3 and 20, respectively. Using a number of mechanistic probes including Bronsted and Taft-like analysis, secondary kinetic isotope effects, site-directed mutagenesis, and novel inhibitors, we have developed a detailed concept of the mechanism of action of these enzymes. We have clear evidence that these two β-hexosaminidases employ different catalytic mechanisms to accomplish the same goal. These results and their implication on the catalytic mechanism of chicken egg white lysozyme will be presented.

57. Mechanistic studies of α-L-iduronidase. Catharine E. Nieman,[1] **Alexander W. Wong**,[1] Lorne A. Clarke,[2] John J. Hopwood,[3] and Stephen G. Withers.[1] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (fax: 604-822-2847), [2]British Columbia Research Institute for Children's and Women's Health, 950 West 28th Avenue, Vancouver, BC V5Z 4H4, Canada, and [3]Department of Chemical Pathology, Adelaide Children's Hospital, 72 King William Road, North Adelaide, South Australia, 5006, Australia

α-L-Iduronidase (IDUA) is a Family 39 lysosomal glycosidase whose deficiency in humans leads to mucopolysaccharidosis type I (MPS I). This enzyme catalyzes the hydrolysis of iduronic acid residues from the nonreducing end of heparan sulfate and dermatan sulfate. The deficiency of IDUA causes a buildup of these polysaccharides within lysosomes, causing such abnormalities as mild skeletal deformities and corneal clouding, as seen in Scheie syndrome, and hepatosplenomegaly, mental retardation, and early death, as seen in the more severe Hurler syndrome. Through the use of nuclear magnetic resonance spectroscopy, we found that the hydrolysis reaction proceeds with net retention of anomeric configuration; thus, a double displacement mechanism involving a covalent glycosyl-enzyme intermediate is presumably involved. Labeling of the enzyme's catalytic nucleophile was accomplished using 5-fluoro-α-L-idopyranosiduronic acid fluoride, a novel mechanism-based inactivator. The labeled peptide present in a peptic digest of this trapped glycosyl-enzyme intermediate was identified and isolated using HPLC/electrospray ionization mass spectrometry. Its sequence, as determined by MS/MS analysis, is $_{291}$ADTPIYNDEADPLVG$_{305}$. The label was localized to either Asp298 or Glu299. Based on sequence alignments of Family 39 glycosidases, the predicted nucleophile is Glu299. Additional mechanistic studies were also performed and will be presented.

58. Mechanistic studies of chondroitinase AC from *Flavobacterium heparinum*. Carl S. Rye and Stephen G. Withers. Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (fax: 604-822-2847, crye@chem.ubc.ca)

Chondroitinase AC from *Flavobacterium heparinum* cleaves the GalNAc-β(1,4)-GlcA linkage in chondroitin sulfate glycosaminoglycans using an elimination mechanism. The presence of a double bond in the product provides a chromophore for assaying the enzymatic activity ($\lambda_{max} = 232$ nm). However, the UV region of the spectrum is often masked by strong absorptions from protein and other components. Novel chromogenic substrates have been synthesized which provide a facile assay of enzymatic activity. Substrates bearing a fluoride leaving group have also provided a convenient assay through the use of a fluoride ion selective electrode. Mechanistic insight into the elimination reaction has been provided by a primary deuterium kinetic isotope effect combined with a linear free energy relationship. A potential transition state analogue has been synthesized for use in X-ray crystallographic studies which would allow the location of the enzyme active site and critical catalytic residues to be identified.

59. Strategies for trapping the covalent glycosyl-enzyme intermediate of a retaining α-galactosyltransferase from *Neisseria meningitidis.* Hoa D. Ly and Stephen G. Withers. Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (fax: 604-822-2847, hoaly@chem.ubc.ca)

α-Galactosyltransferase from *Neisseria meningitidis* catalyzes the transfer of galactose from uridine diphosphogalactose (UDP-Gal) to glycoconjugates bearing a galactose residue at the nonreducing terminus. The anomeric configuration of the product relative to that of the donor, UDP-Gal, is retained in the reaction. By analogy with the mechanism of retaining glycosidases, α-galactosyltransferase is believed to proceed via a double displacement mechanism involving the formation of a covalent glycosyl-enzyme intermediate. A number of strategies to trap this intermediate and to identify the catalytic nucleophile involved have been tried. These have included the use of incompetent acceptor substrates and also various fluorinated analogues of the donor substrate. The synthesis, kinetic evaluation, and results of trapping experiments using these compounds will be discussed.

60. Studies on the role of a conserved chloride in human pancreatic α-amylase. Shin Numao,[1] Yili Wang,[2] Christopher M. Overall,[3] Gary D. Brayer,[2] and Stephen G Withers.[1] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T1Z1 (numao@chem.ubc.ca), [2]Biochemistry and Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada, and [3]Department of Oral Biological and Medical Sciences, University of British Columbia, 2199 Wesbrook Mall, Vancouver, BC V6T 1Z3, Canada

α-Amylases, found throughout Nature, are enzymes that catalyze the digestion of starch. Although the primary structure of this group of enzymes is only conserved in four short regions, the tertiary structure is fairly well conserved. Interestingly, within this group of enzymes there exist two subgroups—the chloride-dependent and chloride-independent α-amylases. The chloride-dependent amylases contain several conserved arginines that in the crystal structure are seen to coordinate the chloride. We have mutated these residues in the chloride-dependent human pancreatic α-amylase to remove the chloride-binding site of this enzyme. Results of kinetic and crystallographic studies on these mutants aimed at elucidating the role of the chloride ion in this enzyme will be presented.

61. Turbo-Glycosynthases: Enhanced glycosylation activity obtained by substituting serine at the nucleophile position in retaining glycosidases. David L. Zechel,[1] Christoph Mayer,[1] Oyekanmi Nashiru,[2] Stephen P. Reid,[1] R. Antony J. Warren.[2] and Stephen G. Withers.[1] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (fax: 604-822-2847, dlzechel@chem.ubc.ca), and [2]Department of Microbiology, University of British Columbia

Glycosynthases are nucleophile mutants of retaining glycosidases that catalyze the glycosylation of sugar acceptors using α-glycosyl fluoride donors, thereby synthesizing oligosaccharides. The 'original' glycosynthase, derived from *Agrobacterium* sp. β-glucosidase (Abg) by mutating the nucleophile glutamate to alanine (E358A), synthesizes oligosaccharides in yields exceeding 90% [Mackenzie, L. F., Wang, Q., Warren, R. A. J., and Withers, S. G. (1998) *J. Am. Chem. Soc. 120*, 5583−5584]. A dramatic, 24-fold, improvement in synthetic rates has been achieved by substituting the nucleophile with serine, resulting in improved product yields, reduced reaction times, and an enhanced synthetic repertoire. Thus, poor acceptors for Abg E358A, such as PNP-GlcNAc, are successfully glycosylated by E358S, allowing the synthesis of PNP-β-LacNAc. Likewise, the analogous substitution of alanine for serine in *Cellulomonas fimi* β-mannosidase converted a weak glycosynthase to one with nearly quantitative glycosylation power. The increased glycosylation activity likely originates from a stabilizing interaction between the serine hydroxyl group and the departing anomeric fluorine of the α-glycosyl fluoride.

62. Glycosynthases: Enzymes for oligosaccharide synthesis. David L. Jakeman,[1] Stephen G. Withers,[1] Melanie Mah,[1] Christoph A. Mayer,[1] R. Antony J. Warren,[2] and Oyekanmi Nashito.[2] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (djakeman@chem.ubc.ca), and [2]Department of Microbiology, University of British Columbia, BC, Canada

Glycosynthases are a new and exciting class of functional enzymes which synthesize oligosaccharides [(1998) *J. Am. Chem. Soc. 120*, 5583]. The chemical synthesis of oligosaccharides is nontrivial, and, similarly, the biological synthesis remains problematic for large-scale production of oligosaccharides. This synthetic difficulty has limited the utility of oligosaccharides as therapeutic agents. Transferase enzymes, which readily synthesize oligosaccharides in vivo, remain limited in their synthetic capacity in vitro for two reasons: poor enzyme availability, and high cost of uridine diphosphate (UDP)-sugars. The advent of glycosynthase enzymes (developed through mechanism-guided evolution) allows the use of readily prepared α-fluoro sugars as activated donors to join the oligosaccharide chain. We shall present research directed toward broadening the range of acceptor and donor sugars employed as substrates for glycosynthases and, to

increase the rate of reaction, the diversity and the specificity of the glycosidic linkages synthesized.

63. New, simple inhibitor of a retaining xylanase: Use in probing the roles of active site residues. Spencer J. Williams,[1] Valerie Notenboom,[2] Jacqueline Wicki,[1] David R. Rose,[2] and Stephen G. Withers.[1] [1]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z1, Canada (spencerw@chem.ubc.ca), and [2]Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, Toronto, M5G 2M9, Canada

Xylanases are glycosyl hydrolases that cleave polymeric chains of β-1,4-linked xylose, present in Nature as part of many hemicellulose complexes. These enzymes have been widely used in biotechnological applications, e.g., to facilitate chemical bleaching in the processing of pulp fiber, and in the treatment of animal feeds to improve digestibility. Mechanistic studies of these enzymes are lacking owing to the difficulty in preparing substrates and inhibitors in the xylobiose series, typically the minimal unit recognized by xylanases. Herein, we describe the synthesis and kinetic analysis of **1**, a simple, nonbasic, and extremely potent inhibitor of the retaining xylanase, Cex, from *Cellulomonas fimi*. The remarkable inhibition shown by this compound prompted further investigations into the mode of interaction of **1** with Cex, including the determination of the 3-dimensional X-ray structure of the complex and kinetic studies with mutants.

64. Probing the ser-pinch mechanism in base-flipping and catalysis by uracil DNA glycosylase (UDG) using directed mutagenesis and phosphorothioate (Ps) substitutions. R. M. Werner, R. Gordley, and **J. T. Stivers**. Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, MD 20850 (fax: 301-738-6255, stivers@carb.nist.gov)

The DNA repair enzyme UDG from *E. coli* (eUDG) catalyzes the hydrolysis of premutagenic uracil residues from DNA by flipping the uracil base from the DNA helix. On the basis of crystallographic studies, a conserved trio of serine residues (S88, S189, and S192 of eUDG) has been proposed to pinch the phosphodiester backbone of the DNA and facilitate flipping of dU from the DNA helix. We have investigated the energetic role of the hydrogen bonds provided by these Ser side chains to the nonbridging phosphodiester oxygens using the complementary approaches of directed mutagenesis and stereospecific Ps substitutions. The S88A mutation shows significant damaging effects of 94-fold and 21-fold on chemistry and binding, respectively, while the much larger damaging effect of the S189A mutation is mostly on the chemical step (1360-fold). In contrast, the S192G mutation shows only a small 1.5-fold effect on the chemical step, but a significant effect on binding (21-fold). Substitution of sulfur for the nonbridging oxygens that interact with Ser-88 or Ser-189 results in large thio effects of 74- and 63-fold, respectively. A free energy analysis of the combined effects of Ps substitution and mutation at these individual positions indicates nonadditivity, with interaction energies of Ser-88 and Ser-189 with the phosphodiester oxygens of -1.5 and -1.15 kcal/mol, respectively. In addition, Ser-189 shows a significant interaction energy of -0.78 kcal/mol with the other nonbridging oxygen with which it does not directly interact. Since the product of the R_p and S_p thio effects at this position (820-fold) is similar to the effect of removing Ser-189, then removal of this group may disrupt enzyme binding energy with both nonbridging oxygens. Overall, these results suggest a role for "serine-pinching" in binding and probably uracil flipping, but also in catalysis, perhaps by orientation or substrate strain.

65. Role of hydrogen bonding in transition-state stabilization by uracil DNA glycosylase. Alexander C. Drohat and James T. Stivers. Center for Advanced Research in Biotechnology of the NIST and University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850 (fax: 301-738-6255, drohat@carb.nist.gov)

An important question regarding the mechanism of *N*-glycosidic bond cleavage by pyrimidine-specific DNA glycosylases is how the enzyme activates the pyrimidine leaving group for expulsion. Recent studies in our lab indicate that *Escherichia coli* uracil DNA glycosylase (UDG) provides a hydrogen bond from a neutral His187 to stabilize the developing negative charge on uracil O2 in the transition state by 20 kJ/mol [Drohat, A. C., et al. (1999) *Biochemistry 38*, 11876−11886]. We show here that uracil bound to the product complex at neutral pH is in the N1−O2 imidate form and has an N1 p$K_a = 6.4 \pm 0.1$. This pK_a is a surprising 3.4 units lower than for free uracil, corresponding to 20 kJ/mol of stabilization energy by the enzyme. Thus, the negative charge that develops on the uracil base during glycosidic bond cleavage resonates to O2 and not O4. This is consistent with the observation of a highly deshielded ^{1}H NMR resonance ($\delta = 15.6$ ppm) that is assigned to a hydrogen bond from His187-Nϵ^2 to uracil O2. The D/H fractionation factor ($\phi = 1.0 \pm 0.1$), solvent exchange rate and protection factor ($k_{ex} = 7$ s^{-1} and PF $= 500$), and change in ^{15}N chemical shift upon hydrogen bond formation ($\Delta\delta = 10$ ppm) indicate that this is a fairly strong, short hydrogen bond. These findings suggest a general mechanism for activation of pyrimidine leaving groups by DNA glycosylases involving a preorganized active site that has been highly evolved to solvate the developing negative charge in a concerted transition state as nucleophilic attack at C1′ proceeds.

66. Proton movements control the reduction of FAD in *p*-hydroxybenzoate hydroxylase. Bruce A. Palfey,[1] Kendra King Frederick,[1] Graham R. Moran,[2] Barrie Entsch,[2] David P. Ballou,[1] and Vincent Massey.[1] [1]Department of Biological Chemistry, University of Michigan, 1301 Catherine Street, Ann Arbor, MI 48109-0606 (fax: 734-763-0289, brupalf@umich.edu), and [2]Molecular and Cellular Biology, University of New England, School of Biological Sciences, Armidale, NSW 2351, Australia

The FAD of *p*-hydroxybenzoate hydroxylase adopts two conformations—the "in" conformation, where the flavin is buried; and the solvent-exposed "out" conformation, where it is reduced by NADPH. This movement is controlled by an internal hydrogen bond network involving *p*-hydroxybenzoate and several protein residues (Tyr 201, Tyr 385, and His 72). Mutations in this network result in the existence of

two distinct enzyme populations: rapidly reducing enzyme, which has the phenolate form of pOHB bound; and slowly reducing enzyme, which has the phenolic form of pOHB bound. These two forms equilibrate slowly on the time scale of reduction (<0.1 s), presumably because an ionizable group responsible for this process is buried. The hydrogen bond network of PHBH provides a solution to the problem of discriminating between pOHB and the analogue *p*-aminobenzoate, a vital metabolite; it links a deprotonation not involved in the chemical reaction to the required conformational change.

67. Purification and characterization of copper chaperone for copper−zinc superoxide dismutase. Haining Zhu,[1] Eric Shipp,[1] P. John Hart,[2] Aram M. Nersissian,[1] and Joan S. Valentine.[1] [1]Department of Chemistry and Biochemistry, Universitiy of California, 405 Hilgard Avenue, Los Angeles, CA 90095-1569 (haining@chem.ucla.edu), and [2]Department of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7760

Mutations in the gene encoding copper−zinc superoxide dismutase (CuZnSOD) have been linked to the familial form of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder. Most of these mutations are point mutations scattered throughout the protein. A gain of function(s), rather than the loss of SOD activity, has been postulated to be responsible for the disease development. In addition, altered metal binding properties could significantly change the biochemical and biophysical properties of the protein, which may convert CuZnSOD into an enzyme capable of generating toxic species by aberrant chemistry. Therefore, it appears very plausible to direct our studies toward examination of metal binding properties of mutant CuZnSOD and elucidation of pathway(s) by which metal ions are delivered to CuZnSOD. The mechanisms by which metal ions are imported into cells and subsequently incorporated into specific metalloproteins have been extensively studied during the past few years. Several genes encoding putative metal transporter proteins or chaperones have been identified. One of them, human ccs (copper chaperone for superoxide dismutase), is thought to encode a protein that delivers copper specifically to CuZnSOD. The ccs gene product is predicted to be a 274 amino acid polypeptide consisting of three distinct sequence domains. The N-terminal domain carries a consensus sequence, MXCXXC, which is known to be involved in heavy-metal binding. The second domain displays a high degree of sequence identity to human CuZnSOD. The C-terminal domain is relatively short and contains two conserved cysteine residues. We have successfully expressed CCS in *E. coli* and purified the protein that is currently under examination for its metal binding properties and its ability to reconstitute apo-CuZnSOD. The preliminary results of these studies will be discussed.

68. Rational design of selective submicromolar inhibitors of *Tritrichomonas foetus* hypoxanthine-guanine-xanthine phosphoribosyltransferase. Alex M. Aronov, Narsimha R. Munagala, Paul R. Ortiz de Montellano, Irwin D. Kuntz, and Ching C. Wang. Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446 (aaronov@cgl.ucsf.edu)

All parasitic protozoa lack the ability to synthesize purine nucleotides de novo, relying instead on purine salvage enzymes for their survival. Hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT) from the protozoan parasite *Tritrichomonas foetus* is a rational target for antiparasitic drug design because it is the primary enzyme the parasite uses to salvage purine bases from the host. The present study is a continuation of our efforts to use the X-ray structure of *T. foetus* HGXPRT−GMP complex to design compounds that bind tightly to the purine pocket of HGXPRT. The goal of the current project was to improve the affinity and selectivity of previously identified HGXPRT inhibitor TF1. A virtual library of substituted 4-phthalimidocarboxanilides was constructed using methods of structure-based drug design, and was implemented synthetically on solid support. Compound 20 was then used as a secondary lead for the second round of combinatorial chemistry, producing a number of low micromolar inhibitors of HGXPRT. One of these compounds, TF2, was further characterized as a competitive inhibitor of *T. foetus* HGXPRT with respect to guanine with $K_i = 0.49\ \mu M$ and a 30-fold selectivity over the human HGPRT. TF2 inhibited the growth of cultured *T. foetus* cells in a concentration-dependent manner with $ED_{50} = 2.8\ \mu M$, and this inhibitory effect could be reversed by addition of exogenous hypoxanthine. These studies underscore the efficiency of combining structure-based drug design with combinatorial chemistry to produce effective species-specific enzyme inhibitors of medicinal importance.

69. Residues important for substrate recognition of *E. coli* MutY identified by cross-linking and site-directed mutagenesis. Cindy Lou Chepanoske, Marie-Pierre Golinelli, and Sheila S. David. Department of Chemistry, University of Utah, 315 South 1400 East, RM Dock, Salt Lake City, UT 84112 (fax: 801-581-8433, cindylou@chemistry.chem.utah.edu)

E. coli MutY is a DNA repair glycosylase that removes an adenine base when mispaired with 7,8-dihydro-8-oxo-2′-deoxyguanosine (OG), and G. (1) MutY contains an $[Fe_4S_4]^{+2}$ cluster and is highly homologous to *E. coli* endo III, although these two enzymes possess very different substrate specificity. Insight into potential active site residues has been provided by the X-ray structure of a truncated form of the protein. (2) However, biochemical evidence is needed to support a mechanism of specific damage recognition. Our approach to providing insight into this mechanism has previously focused on preparing noncleavable substrate analogues to probe the DNA−protein complex. (3) To identify important amino acids of MutY involved in specific DNA recognition, we have utilized DNA−protein cross-linking and site-directed mutagenesis. Specifically, cross-linking occurred between Lys142 and OG using $Na_2Ir\ Cl6$ oxidation of OG. (4) To further probe the active site, photo-cross-linking using 4-thiothymidine incorporated into substrate DNA adjacent to the OG:A mismatch was employed. The cross-linking conditions have been optimized to obtain a 16% reaction yield, and the covalent complex was isolated and digested for mass spectrometry experiments to obtain the modified amino acid. Initial results from MALDI-TOF spectroscopy and negative ion ESI-MS show that the covalent modification can be isolated, and the progress toward

identifying this residue will be discussed. Additionally, the role of the iron−sulfur cluster loop (FCL) domain, a positively charged region in MutY between the first two ligating cysteines of the iron−sulfur cluster, was investigated. In endo III, the FCL domain has been identified to be important in DNA binding; however, the role of the FCL in MutY remains to be delineated. Three positively charged residues in the FCL domain, Arg194, Lys196, and Lys198, have been replaced with alanine, and each mutated form retains high but reduced affinity binding to the substrate relative to WT. Kinetic analysis and DNA binding properties of these mutated forms of MutY will be reported. Combining the information obtained from cross-linking and site-directed mutagenesis is providing insight into the properties of MutY's recognition and repair of mismatched DNA.

70. *S*-2-Hydroxyglutarate: An alternate substrate provides mechanistic insights into the glutamate mutase reaction. E. Neil, G. Marsh, and **Ipsita Roymoulik**. Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, MI 48105 (nmarsh@umich.edu)

Adenosylcobalamin-dependent glutamate mutase catalyzes the reversible rearrangement of L-glutamate to L-*threo*-3-methylaspartate, through a mechanism involving free-radical intermediates. The enzyme exhibits a very high substrate specificity, and so far, no other molecules have been found to be substrates. We report studies on the reaction of glutamate mutase with the substrate analogue *S*-2-hydroxyglutarate and the corresponding structural isomer *RS-threo*-3-methylmalate. In the presence of the coenzyme, both analogues give rise to UV−visible spectral changes which are consistent with the formation of the cob(II)alamin species on the enzyme. Exchange of tritium between the 5′-position of 5′-deoxyadenosine on the coenzyme and the analogues also has been demonstrated, suggesting the formation of a substrate-based radical. The interconversion of *S*-2-hydroxyglutarate and *RS-threo*-2-hydroxy-3-methylsuccinate by the holo-enzyme has been confirmed by NMR spectroscopy. $K_M = 1.13 \pm 0.35$ mM and $k_{cat} = 0.056 \pm 0.0034$ s^{-1} have been obtained for *S*-2-hydroxyglutarate. Furthermore, the effect of deuterium on the EPR spectrum of Cob(II) when *S*-[4,4-$^2H_2$]-2-hydroxyglutarate is used as a substrate provides indirect evidence for the mechanism to proceed via the formation of a substrate-based radical at C-4. *S*-2-Hydroxyglutarate is the first example of a nonnatural substrate to be described for this enzyme; it is also turning out to be a very useful mechanistic probe.

71. Spectroscopic characterization of a cytochrome *c* variant with a single thioether bond. Federico I. Rosell and A. Grant Mauk. Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC V6T 1Z3, Canada (mauk@interchg.ubc.ca)

A yeast cytochrome *c* variant (Cys14Ser) lacking one thioether bond that anchors the heme to the protein has been expressed in *E. coli*. This variant adopts a native conformation with His/Met coordination (Met80 ϵ-CH 23.6 ppm; principal *g*-values: 3.01, 2.29, $\gg$1.3), but it does not exhibit the characteristic 695 nm CT band. CD spectroscopy results suggest that the structure of the variant is disordered compared to wild-type cytochrome *c*. The electronic spectrum of the ferrous form of the variant exhibits a symmetrically broadened α-band $\gg$4 nm to lower energy. In the MCD spectrum, a band appears above 390 nm that is more intense than the Soret *A*-term which is also shifted to lower energy. The complementary variant, Cys17Ser, is expressed poorly, suggesting that Cys17 plays a more significant role in the incorporation of heme into cytochrome *c*. (Supported by MRC Grant TM-7182.)

72. Studies on the reaction mechanism of catalase-mediated oxidation of cyanamide. John Bumpus, Darren Bates, Amber Russell, Mohamed Abouleish, Douglas Baker, and Jordan Frost. Department of Chemistry, University of Northern Iowa, McCollum Science Hall, Cedar Falls, IA 50614 (fax: 319-273-7127, john.bumpus@uni.edu)

Cyanamide, an alcohol deterrent drug, is known to be oxidized by bovine hepatic catalase to nitroxyl ion, hydrogen cyanide, and nitrite. Carbon dioxide has also been reported to be an oxidation product. Cyanamide, however, is a poor substrate. The enzyme must be present in large amounts, and, in vitro, only a relatively small amount of the cyanamide originally present is oxidized. There are several reasons for this: First of all, two of the oxidation products, nitrite and cyanide, are catalase inhibitors which would be expected to effectively compete with the parent compound for the active site; second, catalase and cyanide form a relatively stable complex that is, apparently, catalytically inactive. No evidence for a ferrous−NO complex (which might be expected to form by reduction of ferric catalase by nitroxyl ion) was observed. Third, catalase was partially inactivated during cyanamide oxidation. Previous investigations by others have postulated that *N*-hydroxycyanamide, nitrosyl cyanide, and other cyanamide derivatives are intermediates in this oxidation. The involvement of some of these intermediates was postulated in order to account for production of nitrite and carbon dioxide. In the present study, we provide an alternative reaction mechanism/pathway that does not invoke the intermediacy of these compounds but is, nevertheless, consistent with the accepted chemistry of catalases and their interactions with cyanamide and nitrogen oxides. (Supported by NIH Grant R15 GM54326-01.)

73. Study of the mechanistic roles of the Thr134, Tyr160, and Lys164 in dTDP-glucose-4,6-dehydratase. Barbara Gerratana, Adrian D. Hegeman, and Perry A. Frey. Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (gerratana@biochem.wisc.edu)

dTDP-D-glucose-4,6-dehydratase and UDP-galactose 4-epimerase are members of a subfamily included in the short-chain dehydrogenase/reductase (SDR) family. The enzymes in this family are able to catalyze a wide number of different reactions such as alcohol dehydrogenation, carbonyl reduction, and ketoacyl reduction. Even though these enzymes do not have similar activities or a high sequence homology, they do share amino acid primary sequence motifs such as YxxxK and a highly conserved Ser shown to be critical for enzyme activity. Tyr149, Lys153, and Ser124 have been shown to be important for the activity of the UDP-galactose 4-epime-

rase. In dTDP-D-glucose-4,6-dehydratase, the conserved Tyr160 and Lys164 are present, while the Ser is replaced with Thr134. These residues are collinear with the epimerase conserved residues when the active sites of these two enzymes are superimposed. In light of these similarities and considering that both enzymatic mechanisms involved as the first step the formation of a 4-ketopyranose intermediate with concomitant reduction of the cofactor NAD^+, we are investigating the roles of the Tyr, Lys, and Thr residues in the reaction of dTDP-D-glucose-4,6-dehydratase by studying the catalytic properties of Y160A, Y160F, T134A, T134V, T134S, K164A, and K164M variants.

74. Walking the fine line between two enzyme activities: UDP-galactose-4-epimerase and dTDP-glucose-4,6-dehydratase. Adrian D. Hegeman, Jeffrey W. Gross, and Perry A. Frey. Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (hegeman@biochem.wisc.edu)

UDP-galactose-4-epimerase [uridine 5′-diphosphate (α)-D-galactoside-4-epimerase, EC 5.1.3.2] and dTDP-glucose-4,6-dehydratase [2′-deoxythymidine 5′-diphosphate (α)-D-glucoside-4,6-hydro-lyase, EC 4.2.1.46] are closely related enzymes (25% amino acid sequence identity) which share common catalytic machinery. As shown schematically below, both enzymes employ tightly bound NAD^+ to oxidize nucleotide−sugar substrates (1.) to nucleotide−4-keto-sugar intermediates (2.). After the 4-keto intermediate is formed, the mechanisms diverge. Epimerase reduces the 4-keto intermediate on either face of the ketone to give either UDP-glucose or UDP-galactose (3.). Dehydratase, in contrast, directs the elimination of water from the 4-keto intermediate, generating a 4-keto-5,6-glucosene intermediate (4.) which is then reduced to yield dTDP-4-keto-6-deoxyglucose product (5.). This study seeks to elucidate the differences between epimerase and dehydratase that control the contrasting chemistries following the generation of the keto-sugar intermediate. Alignment of epimerase and dehydratase amino acid sequences and comparison of available 3-dimensional structures have been used to identify residues that may be involved in the water elimination portion of the dehydratase mechanism. Site-directed mutagenesis has been used to modify key dehydratase active-site residues (D135, E136, E198, K199, and Y301) to probe their roles in catalysis. Steady-state kinetic analysis of each mutant shows a modest (∼100-fold) drop in V_{max}, over WT, with marginal affects in *V/K*. Rapid mixing techniques coupled to mass spectral analysis have been used to attribute loss of activity to changes in catalysis for specific chemical steps.

75. Pre-steady-state kinetics of deoxythymidine diphosphate glucose 4,6-dehydratase: Detection and quantification of reaction intermediates by mass spectroscopy. Jeffrey W. Gross,[1] Adrian D. Hegeman,[1] Martha M. Vestling,[2] and Perry A. Frey.[1] [1]Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (fax: 608-265-2904, gross@enzyme.wisc.edu), and [2]Department of Chemistry, University of Wisconsin

Deoxythymidine diphosphate glucose 4,6-dehydratase (dehydratase; EC 4.2.1.46) catalyzes the formation of dTDP-4-keto-6-deoxyglucose (4.) from dTDP-glucose (1.). The dehydratase mechanism has been proposed to proceed sequentially through two intermediates. Formation of the first intermediate, dTDP-4-ketoglucose (2.), is associated with the reduction of an enzyme-bound NAD^+; the second intermediate, dTDP-4-keto-5,6-glucosene (3.), is reduced by the NADH. Here, we have used rapid chemical quench techniques, followed by either matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy or high-pressure liquid chromatography analysis to identify and characterize the reaction intermediates. For the first time, the dTDP-4-keto-5,6-glucosene intermediate has been detected. This reaction intermediate must be stabilized by chemical reduction with $NaBH_4$, resulting in detection at +2 mass units above the predicted value for the unmodified intermediate. Reduction with $NaBD_4$ results in a further +1 mass unit increase. These observed mass shifts, along with those detected using deuterated substrates, confirm the authenticity of the intermediate and demonstrate that reduction is occurring at the ketone functionality. Mass spectroscopy will be used to quantify the dehydratase reaction intermediates at times preceding the steady state, thus allowing the determination of the microscopic rate constants.

76. Synthesis, characterization, and enzymatic analysis of 3′,4′-anhydroadenosylcobalamin: A coenzyme B_{12} analogue with unusual properties. Olafur Th. Magnusson and Perry A. Frey. Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (omagnusson@biochem.wisc.edu)

Our laboratory is interested in how coenzyme B_{12}-dependent enzymes facilitate the cleavage of the Co−C bond of the cofactor. We have synthesized an analogue of adenosylcobalamin (AdoCbl) designed to stabilize the 5′-deoxyadenosyl radical that is produced upon homolytic cleavage of the Co−C bond. Upon replacement of the upper axial ligand of the coenzyme by a 3′,4′-anhydroadenosyl moiety, the radical formed on the nucleoside analogue is stabilized by allylic delocalization. The compound 3′,4′-anhydroadenosylcobalamin (3′,4′-anAdoCbl) was synthesized by chemical and enzymatic methods where the final step was the coupling of cob(I)alamin and 3′,4′-anhydroATP via CobA, an ATP:corrinoid adenosyltransferase. The compound was characterized by UV−Vis spectoscopy, by ESI-MS, and by examination of the aromatic region of the NMR spectra. 3′,4′-anAdoCbl displays some very interesting properties. It is both thermally labile and sensitive to oxygen. Photolysis experiments conducted under anaereobic conditions reveal no appreciable formation of cob(II)alamin whereas the compound breaks down rapidly under aerobic conditions as measured by cob(III)alamin formation. The bond dissociation energy of the compound was estimated using radical trapping techniques. The results show that the Co−C bond of the analogue is considerably weaker than in adenosylcobalamin and that it is rapidly cleaved at ambient temperatures. The surprising resistance of 3′,4′-anAdoCbl to anaerobic photolysis is discussed in the context of these results. The cofactor analogue is able to activate the AdoCbl-dependent enzymes diol dehydrase and ethanolamine ammonia-lyase, although the observed activity is very low. These results along with further enzymatic analysis on the properties of this coenzyme B_{12} analogue are discussed.

77. Thiamin diphoshate-dependent decarboxylase active centers: similarities and differences. Frank Jordan. Department of Chemistry, Rutgers University, 73 Warren Street, Newark, NJ 07102 (fax: 973-353-1264, frjordan@newark.rutgers.edu)

With the publication of high-resolution structures of three enzymes which carry out similar thiamin diphosphate-dependent decarboxylations of 2-oxo acids (pyruvate decarboxylase from yeast and *Zymomonas mobiliz* and benzoylformate decarboxylase), it is becoming possible to start evaluating how closely related function can be accomplished by rather unrelated active centers. Site-directed mutagenesis studies have been carried out for several active center residues of the yeast enzyme in the author's lab. Structure−function correlations of this enzyme are made more challenging since superimposed on the complicated mechanism there is also substrate activation in evidence (hysteretic kinetic behavior). Several active center mutants also exhibit pronounced inhibition at high substrate concentration (more so than in the wild-type enzyme), an apparent inhibition that is also dependent on pH. The studies identified several key residues in the mechanism (E51, D28, H114, H115, I415, and E477 in catalysis and C221, H92, E91, and W412 in regulation), and a comparison of residues at the same locus in the different enzymes has made it possible to identify states of ionization of residues near the coenzyme. The extent to which 'solvent effects' participate in rate acceleration is being evaluated on several such enzymes. (Supported by NIH Grant GM-503980.)

78. Substrate selectivity of unnatural tyrosine derivatives for protein tyrosine kinase csk. Kyonghee Kim, **Keykavous Parang**, Ontario D. Lau, and Philip A. Cole. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Hunterian 317, Baltimore, MD 21205 (fax: 410-614-7717)

Protein tyrosine kinases are critical enzymes in cell signal transduction. Details of tyrosine substrate specificity were investigated including features of charge, steric bulk, stereochemistry, aryl displacement from peptide backbone, and hydrogen bonding. For example, a negative charge ortho to the phenol hydroxyl was incompatible with substrate reactivity, consistent with previous pH−rate profiles which indicated the importance of the neutral phenol. Overall, these studies confirm the interpretation of a previous linear free energy relationship analysis which suggested that the enzyme follows a dissociative transition state mechanism.

79. Synthesis and biological evaluation of ω-modified farnesyl pyrophosphate analogues. Kareem A. H. Chehade, Douglas A. Andres, and H. Peter Spielmann. Department of Biochemistry and Kentucky Center for Structural Biology, University of Kentucky, Lexington, KY 40536 (fax: 606-323-1037, kacheh0@pop.uky.edu)

While farnesylation is essential for Ras to become membrane-associated and induce cellular transformation, it is unknown whether the prenyl group functions as a hydrophobic membrane anchor or as a target for protein−protein recognition. Studies aimed at understanding the biological role of protein isoprenylation are limited by the lack of farnesyl pyrophosphate (FPP) analogues containing heteroatoms and more widely varied structures that are transferable to Ras by protein farnesyltransferase (FTase). We have previously designed and synthesized a new class of transferable FPP analogues where the ω-isoprene unit of FPP was replaced by an aniline functionality (8-anilinogeranyl pyrophosphate, AGPP). We continue our studies by replacing the aniline ring of AGPP with a wide variety of substituted anilines. Two of these analogues, ETAZ-AGPP and TAZ-AGPP, are heterobifunctional photoaffinity FPP analogues. Preliminary analysis of NAGPP, PFAGPP, and IAGPP with mammalian FTase using dansyl-GCVLS as a cosubstrate followed by fluorescence detection indicates that these FPP analogues serve as substrates of FTase. The design, synthesis, and biological evaluation of all FPP analogues will be discussed.

80. Anatomy of a proficient enzyme: The structure of OMP decarboxylase in the absence and presence of a postulated transition state analogue. Brian Miller,[1] Anne Hassell,[2] Richard Wolfenden,[1] Michael Milburn,[2] and Steven Short.[2] [1]Department of Biochemistry and Biophysics, University of North Carolina, CB# 7260, Chapel Hill, NC 27599, and [2]Glaxo Wellcome Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709

The crystal structure of orotidine 5′-phosphate decarboxylase, determined in the absence and presence of the proposed transition state analogue 6-hydroxyuridine 5′-phosphate (BMP), shows that ODCase folds as a TIM barrel with the ligand binding site near the open end of the barrel. Binding of BMP is accompanied by protein loop movements that envelop the ligand almost completely, forming critical interactions with the phosphate, the ribose, and with the pyrimidine ring. Interactions between enzyme and the phosphoryl group help to explain this group's remarkable contribution of substrate susceptibility to enzymatic decarboxylation. Lysine-93 appears to be anchored in such a way as to optimize interactions with C-6 of the pyrimidine ring, and to donate the proton that appears at C-6 of the product. In addition, H-bonds from the active site to O-2 and O-4 may help to delocalize negative charge that develops on the pyrimidine ring in the transition state.

81. Temperature dependence of the transition state affinity of cytidine deaminase. Mark Snider, Stefan Gaunitz, Caroline Ridgway, and Richard Wolfenden. Department of Biochemistry and Biophysics, Program in Molecular and Cellular Biophysics, University of North Carolina at Chapel Hill, CB# 7260, Chapel Hill, NC 27599 (msnider@med.unc.edu)

The catalytic proficiency of an enzyme can be expressed by dividing k_{cat}/K_m, the apparent second-order rate constant at low substrate concentration, by k_{non}, the rate constant for the uncatalyzed reaction in water. For *E. coli* cytidine deaminase, k_{cat}, K_m, and k_{non} are insensitive to changes in pH near neutrality, and viscosity studies indicate that neither substate binding nor product release is rate-determining. Surprisingly, we find that k_{cat}/K_m for cytidine deaminase is almost invariant with temperature ($E_{act.} = 2.5$ kcal), whereas the rate of the nonenzymatic reaction increases steeply with increasing temperature ($E_{act.} = 22.7$ kcal). As a result,

catalytic proficiency and transition state affinity increase steeply with decreasing temperature ($\Delta H_{binding} = -20.2$ kcal), furnishing a new test of potential transition state analogues. Interestingly, the second-order rate constant, k_{cat}/K_m, exhibits a more favorable entropy of activation (−22 eu) than does the first-order rate constant, k_{non} (−28 eu).

Monday Afternoon—Eli Lilly Award Symposium—X. Wang, Organizer

82. Biochemical studies of apoptosis. Xiaodong Wang. Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235 (xwang@biochem.swmed.edu)

Apoptosis is a form of cell death that is essential for development, maintenance of tissue homeostasis, and elimination of harmful cells in multicellular organisms. The execution of apoptosis is carried out by a group of intracellular cysteine proteases, namely, caspases, that cleave their substrates after aspartic acid residues. Caspases normally exist as inactive zymogens that only become activated during apoptosis. Using classical biochemical fractionation and reconstitution experiments, we have been studying the biochemical mechanism of caspase activation in human cells. Using caspase-3 activation as an assay, we isolated several protein factors that are necessary for this reaction. The first factor identified is cytochrome *c*, a mitochondrial protein that is released from mitochondria to cytosol during apoptosis. Once in the cytosol, cytochrome *c* binds to another protein, Apaf-1, and triggers the oligomerization of Apaf-1/cytochrome *c* complex in the presence of ATP, or dATP. Oligomerized Apaf-1/cytochrome *c* complex is sufficient to recruit procaspase-9, which is subsequently activated through auto-catalysis. In this meeting, I will discuss the detailed biochemical mechanism of how these proteins interact with each other to initiate the activation of apoptotic protease.

83. Nucleotide requirements for the in vitro activation of the APAF-1-mediated caspase pathway. Lorenzo M Leoni,[1] Davide Genini,[1] Imawati Budihardjo,[2] William Plunkett,[3] Xiaodong Wang,[2] Carlos J. Carrera,[1] Howard B. Cottam,[1] and Dennis Carson.[1] [1]Department of Medicine and Sam and Rose Stein Institute for Research on Aging, University of California, 9500 Gilman Drive, La Jolla, CA 92093 (fax: 858-534-5399, lleoni@ucsd.edu), [2]Department of Biochemistry, University of Texas Southwestern Medical Center, and [3]University of Texas M. D. Anderson Cancer Center

Adenine deoxynucleosides, such as 2-chlorodeoxyadenosine (2CdA), fludarabine, induce apoptosis in quiescent lymphocytes. We used a fluorometric-based assay of caspase activation to extend the analysis to several other clinically relevant adenine deoxynucleotides in B-chronic lymphocytic leukemia extracts. As estimated by the V_{max}/K_m ratios, the relative efficiencies of different nucleotides were Ara-ATP > F-Ara-ATP > dATP > 2CdATP > AraGTP > dADP > ATP. In contrast to dADP, both ADP and its nonhydrolyzable α,β-methylphosphonate analogue were strong inhibitors of APAF-1-dependent caspase activation. The hierarchy of nucleotide activation was confirmed in a fully reconstituted system using recombinant APAF-1 and recombinant procaspase-9. These results suggest that the potency of adenine deoxynucleotides as cofactors for APAF-1-dependent caspase activation is due both to stimulation by the 5′-triphosphates and to lack of inhibition by the 5′-diphosphates. The capacity of adenine deoxynucleoside metabolites to activate the apoptosome pathway may be an additional biochemical mechanism that plays a role in the chemotherapy of indolent leukemia.

84. Oncogenes as a Trojan horse. Y. Lazebnik. Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724 (fax: 516 367 8461, lazebnik@cshl.org)

No abstract available.

85. Structural studies of APAF-1 Mediated Apoptosis. Y. Shi. Molecular Biology, Princeton University, Washington Road, Princeton, NJ 08544 (yshi@phoenix.princeton.edu)

No abstract available.

Tuesday Morning—Hydrogen Bonding in Macromolecules: Methodology and Applications—A. S. Mildvan, Organizer

86. NMR and quantum chemical approaches to the evaluation of hydrogen bond strengths in proteins. John L. Markley and William M. Westler. National Magnetic Resonance Facility, University of Wisconsin, Department of Biochemistry, 433 Babcock Drive, Madison, WI 53706 (fax: 608-262-3759, markley@nmrfam.wisc.edu)

Information about H-bonding can be derived from chemical shifts, differences between ^{1}H and ^{3}H chemical shifts, ^{2}H/^{1}H fractionation factors, couplings transmitted through H-bonds, and activation enthalpies and rates for hydrogen exchange. The first four of these quantities can be evaluated from high-level quantum mechanical calculations based on structural models. We have used a tandem approach of experiment and theory to investigate three protein systems. Studies of serine proteases focused on the strengths of active-site H-bonds in the zymogen, active enzyme, and first tetrahedral transition state in the catalytic mechanism. Results from these studies support the hypothesis that stabilization of the transition state by generation of a strong H-bond between His57 and Asp102 contributes to catalytic efficiency. Investigations of rubredoxin reveal strong H-bonds between peptide backbone amides and sulfur atoms of the cluster. *J*-coupling through H-bonds in brazzein is providing additional constraints for refinement of the solution structure of this protein. (Supported by NIH Grants GM58667 and RR02301.)

87. NMR studies of hydrogen bonding: Chemical shift databases. Ann McDermott, X. Song, and Y. Wei. Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027 (fax: 212-932-1289, mcdermot@chem.columbia.edu)

Chemical shifts for the carboxylate—imidazole motif, with N—H bond lengths, were systematically studied in several crystalline characterized systems, using solid-state NMR methods. We will discuss several enzyme active sites in light of the knowledge of these model systems.

88. NMR studies of strong hydrogen bonds on enzymes. Albert S. Mildvan,[1] Thomas K. Harris,[1] and Qinjian Zhao.[2] [1]Department of Biological Chemistry, Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21210 (fax: 410-955-5759), and [2]Departments of Biological Chemistry and Pharmacology, Johns Hopkins School of Medicine

H-bond lengths on enzymes have been determined with high precision (±0.05 Å) from both the proton chemical shifts and the H/D fractionation factors of the protons involved. H-bond lengths from chemical shifts were based on an empirical correlation of 59 O−H···O H-bond lengths measured by small molecule X-ray crystallography, with chemical shifts measured in the same crystals by solid-state NMR [McDermott and Ridenour (1996) *Encycl. NMR*]. H-bond lengths were independently measured by fractionation factors which yield distances between the two proton wells in quartic double minimum potential functions [Kreevoy and Liang (1980) *J. Am. Chem. Soc. 102*, 3315] by adding two covalent bond lengths. These H-bond lengths agree well with each other (±0.05 Å) and with those found in protein X-ray structures within the larger errors in distances obtained by protein crystallography (±0.2−0.8 Å). From such measurements, short strong H-bonds have been detected in several enzymes including ketosteroid isomerase, triose phosphate isomerase, and serine proteases, and in a dihydroxynaphthalene analogue of proton sponge.

89. Scalar couplings across hydrogen bonds in nucleic acids and proteins. Stephan Grzesiek,[1] Michael Barfield,[2] Florence Cordier,[1] Andrew Dingley,[3] Juli Feigon,[4] and Linda K Nicholson.[5] [1]Biozentrum/Structural Biology, University of Basel, Klingelbergstrasse 70, Basel, CH-4056, Switzerland (fax: ++41 61 267 2109, stephan.grzesiek@unibas.ch), [2]University of Arizona, [3]University of Duesseldorf, Germany, [4]University of California, and [5]Cornell University, Ithaca, NY

Direct evidence for the existence of individual hydrogen bonds in biomacromolecules has been provided by the detection of trans-hydrogen bond scalar couplings in RNA, DNA, and proteins. These scalar couplings can be used to identify the three partners involved in the hydrogen bond, i.e., the donor, the acceptor, and the proton. The size of the trans-hydrogen bond scalar couplings correlates with the strength of the hydrogen bond and with the chemical shift of the proton involved in the H-bond. The results of density functional theory quantitatively reproduce the trans-hydrogen bond coupling effect and the experimental correlations and show that the NMR parameters can be used to gain important insights into the nature of the hydrogen bond. The very existence of the couplings indicates an overlap of donor, hydrogen, and acceptor electronic wave functions and suggests a limited covalent character of the hydrogen bond. Basic features of the trans-hydrogen scalar coupling are qualitatively interpretable in terms of a simple sum-over-states molecular orbital description of a three-orbital fragment.We will show applications to a number of different RNA, DNA, and protein molecules and explore the possibility to obtain further insights into macromolecular structures from the quantitation of the H-bond couplings.

Tuesday Afternoon—Pfizer Award Symposium—E. T. Kool, Organizer

90. Catalytic strategies of ribozymes revealed by chemically modified nucleic acids. Joseph Piccirilli. Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL 60637 (jpicciri@midway.uchicago.edu)

Divalent metal ions are critical for the folding and function of most catalytic RNA molecules (ribozymes). However, understanding the roles of specific metal ions during RNA catalysis is a formidable challenge because the metal ions that are important for catalytic function are bound within a sea of metal ions that coat the charged phosphodiester backbone. Chemically modified nucleic acids have provided some of the most intricate features of catalysis by RNA and have been especially powerful in assessing the presence and roles of metal ions at RNA active sites. This talk describes how chemically modified nucleic acids and quantitative analysis can be used to elucidate catalytic strategies used by the *Tetrahymena* ribozyme. These approaches reveal that the transition state for the reaction catalyzed by this ribozyme contains four ligands which interact with at least three metal ions bound at the active site. In addition to stabilizing the transition state, one of these metal ions serves to bind, position and activate the hydroxyl nucleophile for splicing. Understanding the catalytic strategies used by this ribozyme reveals how an active site composed of RNA can achieve significant catalytic rate accelerations, and the approaches and principles derived are also relevant for understanding catalysis by protein enzymes.

91. Unnatural ligands for engineered proteins: New tools for chemical genetics. Kevan M. Shokat. Department of Cellular and Molecular Pharmacology, University of California, 513 Parnasus Avenue, Box 0450, San Francisco, CA 94143 (shokat@cmp.ucsf.edu)

Research in our laboratory is focused on the development of novel chemically based tools to decipher signal transduction pathways on a genome-wide scale. Our lab has developed a method for producing these valuable reagents using an approach combining protein design and chemical synthesis. We use protein design to engineer a functionally silent yet structurally significant mutation into the active site of the protein of interest. Next, a nonspecific inhibitor of the wild-type enzyme is synthesized which contains substituents which specifically complement the mutation introduced into the active site of the protein of interest. Importantly, the new substituent is designed to preclude binding of the inhibitor to any wild-type enzymes because they would "bump" into the large residue in the wild-type enzyme. This makes choosing a residue which is conserved in the entire protein family critical for the success of the method.

92. Engineering RNA and DNA biocatalysts. Ronald R. Breaker. Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520 (fax: 203-432-5713, ronald.breaker@yale.edu)

Biocatalysis is primarily driven by polypeptides whose specific three-dimensional shapes define the extraordinary substrate recognition and catalytic characteristics that are the hallmark of enzymes. Nucleic acids also exhibit immense structural diversity, suggesting that RNA and DNA, like their polypeptide counterparts, might have significant potential for molecular recognition and chemical catalysis. It is speculated

that sophisticated nucleic acid enzymes might have played a major role early in the chemical evolution of living systems. If true, then new enzymes made from RNA and DNA that reflect the catalytic activity of these long-extinct ribozymes could be applied to modern challenges in chemical and biological catalysis. We have employed a variety of rational design and combinatorial engineering strategies to probe the catalytic repertoire of nucleic acids. Recently, we have created a series of precision RNA switches that specifically recognize and report the presence of various organic compounds. Similarly, we have created numerous catalytic DNAs or "deoxyribozymes" that exhibit robust enzyme-like function, despite the long-standing view that DNA is catalytically inert. These findings indicate that the true catalytic potential of RNA and DNA has yet to be fully explored.

93. Synthetic mimics of DNA base pairs: Probing replication mechanisms. Eric T. Kool. Department of Chemistry, Stanford University, Palo Alto, CA 94305

The ongoing study of modified DNAs has been useful in lending insight into the physical properties of natural DNA and the proteins that interact with it. In this talk are described studies of the replication and repair processes mediated by DNA polymerase enzymes. We have studied these processes with new analogues of DNA in which Watson−Crick hydrogen bonds are missing. Despite this important chemical difference, many of these compounds retain the ability to function like natural DNA bases. For example, some are replicated by polymerase enzymes very efficiently, and some bind proteins as well as, or better than, natural DNAs. The importance of specific polar and nonpolar interactions between proteins and the DNA is discussed in some detail. Understanding the chemical details of these processes is important both for the basic information gained about gene expression and repair as well as for applications in medical diagnostics and therapeutics.

Wednesday Morning−General Papers; Session "B"/ Continuation of Poster Presentations−H. M. Miziorko, Presiding

94. Rapid and direct sequencing of double-stranded DNA using exonuclease III and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Uraiwan Puapaiboon,[1] Jaran Jai-nhuknan,[2] and James A. Cowan.[1] [1]Evans Laboratory of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210 (upuapaib@ chemistry.ohio-state.edu), and [2]Bruker Daltonics, Inc., 47697 Westinghouse Drive, Fremont, CA 94539

The use of MALDI-TOF MS in concert with exonuclease III is demonstrated to be a rapid and sensitive approach for direct sequencing of double-stranded DNA without the need to prepare ssDNA from dsDNA samples. This technique avoids the use of radioactive labeling and time-consuming gel electrophoresis, and is particularly suited to sequence characterization of short oligonucleotides that are not accessible by current methods. Major obstacles attributed to interference by salts in mass spectrometric techniques have been overcome. Two different kinds of dsDNA substrate were employed, and the advantages of each approach are described. This technique is likely to be applicable to the diagnosis of genetic diseases and human genome sequencing.

95. Simple method to identify cysteine-containing peptides by isotopic labeling and ion trap mass spectrometry. Jiang Wu, Maciej Adamczyk, and John C Gebler. Department of Chemistry (9NM), Abbott Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6016 (fax: 847-938-5188, jiang.wu@ add.ssw.abbott.com)

A simple method was developed to facilitate the identification of cysteine-containing peptides based on *S*-carboxymethylation of cysteine residues with a 50:50 mixture of natural and $^{13}C_2$-labeled bromoacetic acid. An ion trap mass spectrometer with high-resolution scan functionality showed a unique doublet pattern specific to the modified peptides, with $\Delta m/z$ values of 2 and 1 for $[M+H]^+$ and $[M+2H]^{2+}$ ions, respectively. The method has been applied to the identification of active cysteine residue(s) in creatine kinase, as well as the recognition of cysteine-containing segments in an IgG antibody of unknown sequence.

96. Charge derivatization of peptides to simplify their sequencing with an ion trap mass spectrometer. Jiang Wu, Maciej Adamczyk, and John C Gebler. Department of Chemistry (9NM), Abbott Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6016 (fax: 847-938-5188, jiang.wu@add.ssw.abbott.com)

The low-energy collision-induced dissociation (CID) of N-terminal fixed-charge derivatives [tris(2,4,6-trimethoxyphenyl)phosphonium] of peptides was investigated using an electrospray ion trap mass spectrometer. The fixed-charge directed the fragmentation pattern and generated solely N-terminal fragments with minimal internal rearrangement, regardless of the presence and position of basic amino acids in the peptide chain. Generally only b-type ions, accompanied by less intensive a-type ions, were observed depending on the collision energy. The fixed charge controlled fragmentation beyond typical MS/MS; thus, the capacity of ion trap to perform multiple stage fragmentation (MS^n) was found particularly useful for obtaining the complete sequence information of the peptides. The application of the charge derivatization to peptide sequencing will be presented.

97. Small peptide in the α subunit of clusterin is responsible for its complement inhibitory activity. Tuajuanda C. Jordan-Starck, Joy L. Marshall, James C. Dirden, and **Alvin C. Collins, III**. Department of Chemistry, Xavier University of Louisiana, 7325 Palmetto Street, Box 48A, New Orleans, LA 70125-1098 (fax: 504-485-7942, tjordans@xula.edu)

Clusterin is an inhibitor of the complement system. Clusterin's effect(s) on the assembly of the membrane attack complex (MAC) was (were) determined. In guinea pig serum, clusterin holoprotein inhibits terminal complement complex (TCC)-mediated hemolysis in a time- and concentration-dependent manner. The subunits of this dimeric protein also reduce the TCC activity. To understand the inhibition

mechanism, clusterin was presented at different points in human MAC assembly. Clusterin inhibits complement at C8 and C9, having an IC_{50} of 0.005 and 0.006 μM, respectively. The α and β subunits have IC_{50} values of 0.07 and 0.25 μM, respectively. To further define the inhibitory domain, synthetic peptides derived from both subunits were tested: CL18 and CL150 of the α subunit inhibited 20% and 80% of TCC-mediated hemolysis, respectively; CL221 and CL407 of the β subunit inhibited 40% and <10%, respectively. In the future, the essential functional residues will be determined using site-directed mutagenesis.

98. β-1,3-Glucanase and chitinase from *Trichoderma harzianum* rifai. Momein H. El-Katatny, Walter Somitsch, Karl-Heinz Robra, and Georg M. Gubitz. Microbiology Graz University of Technology, Petersgasse 12, Graz, 8010, Austria (fax: ++433168738815, elkatatny@ima.tu-graz.ac.at)

Twenty-four isolates of *Trichoderma* were screened for β-1,3-glucanase and chitinase activity. Out of these organisms, a strain identified as *T. harzianum* rifai secreted the highest activities. In vitro production of chitinases and β-1,3-glucanases by *T. harzianum* was examined under various culture conditions. Enzyme production was significantly influenced by the carbon source incorporated into the medium and was stimulated by acidic pH from 5.5 to 6.0. Addition of glucose or GlcNAc along with chitin and laminarin repressed production of chitinase and β-1,3-glucanase, respectively, while the polysaccharides as sole carbon source enhanced production of the respective enzymes. Production of both enzymes was also enhanced by polysaccharides contained in the mycelium of *Sclerotium rolfsii*. Both enzymes were purified, and their molecular biochemical parameters were determined. Chitinase and β-1,3-glucanase from *T. harzianum* were capable of hydrolyzing dried or fresh mycelium of *S. rolfsii*, and growth of this plant pathogen fungus was significantly inhibited (up to 33.7%) by enzyme preparations from *T. harzianum*. The importance of β-1,3-glucanase and chitinase as key enzymes responsible for fungal cell and sclerotial wall lysis and degradation should be considered. These polysaccharide degrading enzymes from *T. harzianum* may be an important factor in biological control.

99. Calcium ion down-regulates soluble guanylyl cyclase activity: Evidence for a second metal ion binding site. Heather S. Carr, Lucile Serfass, Laura M. Aschenbrenner, and Judith N Burstyn. Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, WI 53706 (fax: 608-262-6143)

Ca^{2+} plays a vital role in the NO/cGMP signaling pathway by interacting with calmodulin and NO synthase to stimulate NO production; it has also been speculated that Ca^{2+} plays a more direct role in cGMP production by regulation of soluble guanylyl cyclase (sGC), the NO receptor. We demonstrate that Ca^{2+} reversibly inhibits both the basal and NO-stimulated forms of sGC. Inhibition of sGC by Ca^{2+} is noncompetitive with respect to MgGTP and competitive with respect to Mg^{2+} in excess of substrate. Furthermore, inhibition of activated sGC by Ca^{2+} is independent of activator identity or concentration. The catalytic sites of sGC and adenylyl cyclase (AC) are highly homologous, and recent structural and biochemical studies of AC suggest that two metal ions are required for cyclization of ATP to cAMP. The mode of inhibition of sGC by Ca^{2+} supports a mechanism in which two divalent ions participate in the catalysis. We suggest that Ca^{2+} down-regulates cGMP production by coordination to a secon metal ion binding site in place of Mg^{2+}.

100. Role of calcium and calmodulin on ethylene production in apple fruit. Keiichi Tanaka, Toshikazu Asakura, and Noboru Muramatsu. Department of Breeding, National Institute of Fruit Tree Science, 2-1 Fujimoto, Tsukuba, Ibaraki, 305-0852, Japan (fax: +81−298−38−6437)

The ACC oxidase converted 1-amino-cyclopropane-1-carboxylic acid (ACC) into ethylene. Whole fruits supplied with calcium delayed the rate of senescence. Calmodulin mediated the biochemical action of calcium in plants. Tanaka et al. (1991) reported that calcium and calmodulin regulated the conversion of ACC to ethylene in Japanese pear disks. However, there was no test of stereospecific conversion of 1-amino-2-ethylcyclopropane-1-carboxylic acid (AEC) by ACC oxidase. We found that the ACC oxidase, which required calcium and calmodulin, converted mixtures of (1*R*,2*S*)- and (1*S*,2*R*)-AEC into 1-butene but did not convert mixtures of (1*R*,2*R*)- and (1*S*,2*S*)-AEC into 1-butene. The ACC oxidase activity depended on pH and calcium concentrations. The ACC oxidase did not require iron and ascorbate as cofactor. Calmodulin promoted the ethylene production from ACC. This study suggested that the ACC oxidase of apple fruit was controlled by calcium and calmodulin in vivo.

101. Calcium release from sarcoplasmic reticulum is both stimulated and inhibited by anthracyclines and their metabolites. Susan E. Shadle,[1] **Sean S. Frink, Jr.**,[1] Xuande Li,[2] Barry J. Cusack,[2] Phillip S. Mushlin,[3] Hervé A. Gambliel,[2] and Richard D. Olson.[2] [1]Department of Chemistry, Boise State University, 1910 University Drive, Boise, ID 83725 (fax: 208-426-3027, sshadle@chem.boisestate.edu), [2]Research Service (151), VA Medical Center, Boise, ID 83702, and [3]Department of Anesthesia, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115

Daunorubicin belongs to a family of widely used chemotherapy agents known as anthracyclines, whose use is limited by a well-documented, yet poorly understood cardiotoxicity. Experiments aimed at understanding this cardiotoxicity have pointed to the ability of these drugs to interfere with sarcoplasmic reticulum (SR) calcium channel function. At micromolar concentrations, daunorubicin (Daun) and its primary C-13 metabolite, daunorubicinol (Daunol), have been shown to stimulate SR calcium release. Experiments presented here measured calcium release from canine SR vesicles spectroscopically using the metallochromatic indicator antipyrylazo III. At nanomolar concentrations, preincubation with Daun and Daunol inhibits caffeine-induced SR calcium release. This effect is independent of drug exposure time but dependent on the extent of SR calcium loading. Results of these studies and others examining the dependence of this effect on a variety of variables will be presented. Implications for the mechanism of drug cardiotoxicity will be discussed.

102. Comparative studies of bile pigment complexation by dihydroxy and trihydroxy bile salts. William E. Kurtin. Department of Chemistry, Trinity University, 715 Stadium Drive, San Antonio, TX 78212 (fax: 210-736-7569, wkurtin@trinity.edu)

Bilirubin, the metabolic product of heme degradation in humans, is insoluble in water, but is soluble in aqueous solutions of bile salts such as sodium taurocholate. Knowledge of the chemistry of these complexes is required for the understanding of the biophysical chemistry of bile. We have used UV−Vis, fluorescence, light scattering, and NMR spectroscopy in comparative studies of the complexation of bilirubin by dihydroxy and trihydroxy bile salts. These studies have revealed that the microenvironments for bilirubin in micelles of the two types of bile salts are very similar, but that the degree of complexation is higher with dihydroxy bile salts. The implications of these studies for the biophysical chemistry of bile will be discussed.

103. Crystal structure of the Msx-1 homeodomain/DNA complex. Stacy L. DeWees,[1] Cory Abate-Shen,[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 Nueroscience and Cell Biology, University of Medicine and Dentistry, Piscataway, NJ 08854

The Msx-1 homeodomain protein is a developmental regulator that is important in craniofacial development. Homeodomains are DNA-binding motifs that have been studied to determine protein−DNA interactions in great detail. The structure of the Msx-1 homeodomain complexed to DNA has been solved to 2.2 Å resolution. The protein−DNA contacts are very similar to other homeodomain/DNA structures, with the closest match being the HoxB1 homeodomain/DNA complex. The HoxB1 homeodomain induces a 10° bend in the DNA while Msx-1 seems to induce a more severe bend at 28°. Comparisons with other homeodomain structures and models lead to identification of conserved waters and interactions. Previous mutational analysis and transcription assays can be explained by the structure. The interaction of Msx-1 with another homeodomain, Dlx-2, and TBP will be discussed in the context of the structural data.

104. Dynamic modeling of EDG1 receptor structural changes induced by site-directed mutations. Abby L. Parrill,[1] **Debra L. Bautista**,[1] Daniel L. Baker,[2] De-an Wang,[2] David J. Fischer,[2] Gabor Tigyi,[2] James van Brocklyn,[3] and Sarah Spiegel.[3] [1]Department of Chemistry, University of Memphis, Memphis, TN 38152 (fax: 901-678-3447, aparrill@memphis.edu), [2]Department of Physiology, University of Tennessee, Memphis, TN 38163, and [3]Department of Biochemistry and Molecular Biology, Georgetown University, Washington, DC 20007

EDG1 is a member of the G protein coupled receptor family and serves as a cellular receptor responsive to phospholipids. EDG1 binds sphingosine-1-phosphate (SPP) with high affinity and lysophosphatidic acid (LPA) with low affinity. A model has been developed, based on an experimentally based model of the structure of rhodopsin, to evaluate the features that contribute to the different binding affinities of phospholipids for EDG1. Hydrogen bonding in the EDG1 model was optimized to improve contacts between polar conserved positions in the EDG family. This model was then used for docking studies with SPP and LPA. The docking studies predict three residues (R90, E91, and R291) make ion pairs with charged functional groups in SPP as shown below. Twenty-one mutations expressed transiently in HEK293 cells were evaluated by radioligand binding assays. Binding results for 17 of these mutations, including decreased binding to mutations at positions 90, 91, and 291, are well explained by the current model. The remaining four mutations and five additional control mutations are being modeled using molecular dynamics. Polar and nonpolar residue surface areas in the binding pocket reflect differences in the binding pocket that develop during the simulations. These results correlate well with the experimentally observed binding affinities.

105. Electron-transfer steps within redox enzymes: Using thermodynamics as a guide. Todd P. Silverstein. Chemistry Department, Willamette University, Olin Building, 900 State Street, Salem, OR 97301 (fax: 503-375-5425, tsilvers@willamette.edu)

Electron-transfer reactions catalyzed by oxidoreductases are often probed kinetically, but rarely thermodynamically. Thermodynamic analysis can offer insight into the energetics and mechanism of the catalytic cycle. In this paper, individual electron-transfer steps in the catalytic cycles of four redox enzymes (cytochrome *c* oxidase, cytochrome *c* reductase, NO synthase, and NADH dehydrogenase) are examined thermodynamically. Cytochrome *c* oxidase features steps that are all exergonic, particularly the steps believed to be responsible for driving proton pumping. Cytochrome *c* reductase and NO synthase each feature crucial endergonic steps that may be run in concert with proximal spontaneous steps. Two recent models describing the catalytic cycle of NADH dehydrogenase are compared from thermodynamic and topological perspectives. The model of Dutton et al. [(1998) *Biochim. Biophys. Acta 1364*, 245−257] is thermodynaically superior to that of Brandt [(1997) *Biochim. Biophys. Acta 1318*, 79−91]. Nevertheless, a key aspect of Brandt's model (redox gating of the proton channel) could be incorporated into Dutton's model to provide a simpler catalytic cycle.

106. Genomic analysis of the bacterial large conductance mechanosensitive channel. Donald E. Elmore,[1] **Joshua A. Maurer**,[1] Henry A. Lester,[2] and Dennis A. Dougherty.[1] [1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Mail Code 164-30 Cr, Pasadena, CA 91125 (fax: 626-564-9297, elmore@its.caltech.edu), and [2]Division of Biology, Caltech, 156-29 Caltech, Pasadena, CA 91125

Mechanosensitive channels play a central role in biological systems from bacterial osmoregulation to human circulation and touch. Although very few mechanosensitive channels have been cloned, extensive biochemical and electrophysiological data have been obtained for the bacterial large-conductance channel (MscL). Electrophysiological data have been obtained for MscL in nine bacterial species; however, most experimental studies have focused on the *E. coli* form

of this protein. Thirty-one putative MscL homologues have been identified from extensive database searching. Several sequence analysis techniques have been applied to compare these protein sequences. These methods included global and segmented sequence alignments, pattern identification, and statistical comparisons. These sequence comparisons can be combined with experimental data to highlight functionally important regions in the homologues. Also, this sequence information has been combined with the recently determined crystal structure of *M. tuberculosis* MscL through homology modeling to probe the connection between sequence and structure. Results from genomic analyses allow for better understanding of channel structure−function relationships and provide insight into future biochemical and electrophysiological experiments.

107. Inhibition of human immunodeficiency virus type 1 reverse transcriptase by a novel imidazole nucleotide derivative, 2′-deoxyimidine-5′- triphosphate (dImdTP). **Stephen G. Kerr**,[1] **Sundeep S. Dhareshwar**,[1] and Thomas I. Kalman.[2] [1]Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, 179 Longwood Avenue, Boston, MA 02115 (fax: 617-732-2737, skerr@mcp.edu), and [2]Department of Medicinal Chemistry, State University of New York, Buffalo, NY 14260

A study of the inhibition of human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) by a novel five-membered imidazole nucleotide derivative was undertaken. 2′-Deoxyimidine (dImd) is a structural analogue of thymidine (dThd). In contrast to the chain-terminating RT inhibitors AZT, DDC, D4T, and 3TC, dImd permits DNA extension. In assays of single-nucleotide extension by HIV-1 RT, using a homoduplex of DNA/DNA (45/22-mer template/primer), dImdTP inhibited competitively the incorporation of dTTP (0.5 μM), with an IC_{50} value of 1.0 ($\pm$0.2) μM. In multiple extension assays, using a pre-designed homoduplex DNA/DNA (32/22-mer template/primer), which allows for incorporation of two and four consecutive dThd (or dImd) molecules, dImdTP could not support the extension to full-length product, and caused the enzyme to stall after the incorporation of three consecutive dImd bases, indicating that the cumulative effects of the slightly distorted base-pairing with adenine by the analogue are not tolerated by HIV-1 RT.

108. Mechanical unfolding and refolding of carbonic anhydrase B in buffer measured by atomic force microscope. Tong Wang and Atsushi Ikai. Department of Bioscience, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama, Japan (fax: 81-045-924-5806, twang@bio.titech.ac.jp)

Atomic force microscopy (AFM) was used to mechanically unfold and refold the globular protein carbonic anhydrase B (CAB), which had -SH groups at its C- and N-termini and fixed to a silanized surface of silicon wafer. The AFM tip was modified with a reactive end to -SH and brought into contact with the protein on the surface. The native CAB resisted a tensile force of 1.6 $\pm$ 0.3 nN before its breakdown, while in 6 M GdmCl solution CAB was extended close to its full length of 100 nm. A dimer CAB was extended to 100 nm, showing that one of the units was not folded tightly compared with the native monomer. When piezo movement was limited to 150−180 nm, dimer CAB that was unfolded gave reversible extension−contraction curves, representing a more compact conformation than random coil; occasionally, we observed curves with middle peaks, which may represent the formation of some intermediate states while the chain was contracted.

109. Modulation of enzyme activity by membrane torque tension. Jason Beard and George S. Attard. Department of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, United Kingdom (fax: 1703 593781, jbeard@soton.ac.uk)

Anti-tumor lipids prevent cell growth, inhibiting lipid production by blocking CT, a key enzyme in the synthesis of phosphatidylcholine. It has been suggested that the regulatory effect for CT is due to the ratio of bilayer- to nonbilayer-forming lipids in the cell membrane. We postulate that this ratio is important because it controls the membrane torque tension (MTT). We have constructed a computer simulation of the membrane lipid metabolic pathway to test these ideas. The model correctly predicts changes in the relative composition of lipid species. It shows that CT exerts a large influence on the MTT and predicts the difference in behavior of CT and ET. Predictions of the lipid dependence of other enzymes are consistent with literature studies. Our postulate for feedback leads to an increase in robustness while facilitating lipid accumulation. The model provides an explanation for the action of ATLs and can be used to model the effect of ATLs on membrane lipid composition.

110. Photomodification of antibodies for western blot analysis and radioimmunotherapy. Manjula Nakka and Boyd Haley. Department of Chemistry, University of Kentucky, Rose Street, Lexington, KY 40506 (fax: 606-257-3040, manju@engr.uky.edu)

Antitumor antibodies such as CC49 have been proposed to be ideal vehicles for carrying radioactive compounds, drugs, toxins, genes, and anti-sense oligonucleotides to the tumor site, in vivo. Until now these compounds have been attached to the antibody by classical chemical conjugation methods which have disadvantages such as nonspecific attachment and inactivation of the antibodies to various extents. In the photolabeling technique, we use modified nucleotides, such as [γ-^{32}P]-2-azidoadenosine triphosphate ([γ-^{32}P]2N_3ATP), which are activated by brief photolysis with short-wavelength UV light and, in the presence of protein, covalently modify the protein at the binding site and hence are site-specific. Antibodies have been shown to have a high-affinity site for ATP and can be photolabeled with this technique. We photolabeled CC49 antibody with 2N_3-ATP, and preliminary results show that the photolabeling efficiency of heavy versus light chain was better in Tris-buffered systems than in phosphate-buffered systems. To prevent phosphate hydrolysis, we synthesized γ-PO_4-blocked compounds of the nucleotide ([γ-^{32}P]2N_3ATP) and photolabeled the CC49 antibody. Preliminary results show that such γ-PO_4-blocked compounds are more stable in serum than the unblocked compounds. Potentially, this technique can produce photomodified antibodies useful for detection and radioimmunotherapy of tumors.

111. Protein structure modeling of an aminoglycoside 6′-*N*-acetyltransferase involved in antibiotic resistance. Goragot Wisedchaisri,[1] Marcelo E. Tolmasky,[2] and Katherine A. Kantardjieff.[1] [1]Department of Chemistry and Biochemistry and W. M. Keck Foundation Center for Molecular Structure, California State University, Fullerton, CA 92834, and [2]Department of Biological Science, California State University

Inactivation of aminoglycoside antibiotics due to enzymatic modification is a common process in establishing drug resistance in bacteria. The *Klebsiella pneumoniae* aminoglycoside 6′-*N*-acetyltransferase [AAC(6′)-Ib] inactivates amikacin by acetylation using acetyl-CoA. Obtaining the structure of AAC(6′)-Ib would help interpret the existing functional data and assist in constructing the mutants for testing new functional hypotheses. The AAC(6′)-Ib possibly belongs to the GCN5-related *N*-acetyltransferase (GNAT) superfamily, even though global sequence alignment shows less than 20% identity with sequences of known structures of acetyltransferases in the superfamily. Structure-based sequence alignment of these structures shows similar secondary structure and folding patterns. Crystal structures of two GNAT superfamily members, *Serratia marcescens* aminoglycoside 3-*N*-acetyltransferase and *Saccharomyces cerevisiae* histone acetyltransferase, were used as templates for modeling the tertiary structure of AAC(6′)-Ib. The resulting model might not be very accurate since it was based on only two templates but it was adequate enough to give rational ideas on amino acid selection for mutation.

112. Regulation of plant growth regulators on cotton somatic embryogenesis and plant regeneration. Bao-Hong Zhang. Department of Biotechnology, Cotton Research Institute, Chinese Academy of Agricultural Sciences, Baibi, Anyang 455112 China (zbh68@hotmail.com)

Effects of 11 exogenous hormones on the proliferation of cotton embryogenic calli, and the differentiation and development of cotton somatic embryos were studied. The results indicated that most of hormones inhibited the growth and the proliferation of cotton embryogenic callus except 2,4-D and BA, and it was influenced by culture time and genotypes. The formation and development of cotton somatic embryos were greatly influenced by exogenous hormones; 2,4-D inhibited either formation or development of cotton somatic embryos, the embryos which were obtained on the medium only with 2,4-D arrested at globular or heart stages. The role of TDZ was similar to 2,4-D; all the somatic embryos obtained on the medium only with TDZ were arrested at globular stage. GA inhibited the formation of somatic embryos, and was a disadvantage to the maturation and germination of cotton somatic embryos. 4BU-30 had little effect on formation and development of cotton somatic embryos. IBA, ABA, IAA, BA, KT, ZT, and 2iP promoted the differentiation of cotton somatic embryos, and the role was increased progressively by the above-mentioned sequence; the total number of somatic embryos induced by those seven plant growth regulators ranged from 1.193- to 3.852-fold of that of control without any plant growth regulators, but they were not greatly influenced by the development of embryo. 2iP was the best hormone for the formation and the development of cotton somatic embryo; the increase in the number of embryos per 1 g of callus was 2.852-fold.

113. Soluble and active N-terminal-truncated casbene and taxadiene synthases overexpressed in *Escherichia coli*. Qiulong Huang, Charles A. Roessner, and A. Ian Scott. Department of Chemistry, Texas A&M University, College Station, TX 77843 (Qhuang@mail.chem.tamu.edu)

Casbene synthase (CS) from castor bean seedlings and taxadiene synthase (TS) from the Pacific yew catalyze the formation of casbene and taxadiene, respectively. These typical diterpene skeletons are derived from the universal diterpene precursor geranylgeranyl pyrophosphate (GGPP). Sufficient amounts of these enzymes for detailed mechanistic and structural studies are difficult to obtain because of low natural levels in plants. Previous attempts to overexpress the full-length enzymes in *E. coli* resulted in either low levels or in formation of intractable inclusion bodies. We have found that high levels of soluble, active casbene and taxadiene synthases can be produced in *E. coli* by removing the N-terminal amino acids constituting a signal peptide, predicted by homology and secondary structure. The truncated casbene synthase and taxadiene synthase are respectively 66 and 78 residues shorter than the corresponding full-length proteins.

114. Spectroelectrochemical study of green fluorescent protein: Implications for the redox mechanism and interactions with dioxygen. Kimberley A. Waldron and David E. Malerba. Department of Chemistry, Regis University, 3333 Regis Boulevard, Denver, CO 80221 (fax: 303-964-5480, kwaldron@regis.edu)

This paper presents the first formal electrochemical study of green fluorescent protein (GFP) to our knowledge; it employs an indirect spectroelectrochemical method based on fluorescence. Indirect chronoamperometry with an inorganic mediator is performed on S65T-GFP mutant in the 600−950 mV potential range. A reduction potential for GFP is determined from the results of a Nernst plot of the data. This value contradicts earlier rough predictions based on the reactivity of GFP with various redox reagents. The biological implications of the observed reduction potential are discussed, along with possible consequences for the GFP mechanism of fluorescence, including the role of dioxygen.

115. Spectral variants of green fluorescent protein using DNA shuffling. A. M. Nolan, R. R. Naik, and M. O. Stone. AFRL/MLPJ, Air Force Research Laboratory Materials & Manufacturing Directorate, 3005 P Street, Suite 1, Wright-Patterson Air Force Base, Dayton, OH 45433 (fax: 937-255-1128, alexandra.nolan@ml.afrl.af.mil)

Green fluorescent protein (GFP) is a bioluminescent protein isolated from the jellyfish *Aeqorea victoria*. GFP has been widely used as a labeling tool in the imaging of cells. It is composed of 238 amino acids and produces a greenish fluorescence when irradiated with a long ultraviolet light (395 nm). The fluorescent signal is due to the presence of a chromophore containing an imidazolone ring structure which is formed by the posttranslational modification of the tripeptide Ser65-Tyr66-Gly67. Crystallographic studies of GFP reveal a cylindrical β-barrel structure protecting its

buried chromophore. Several mutant forms of GFP have already been obtained through a genetic engineering process. These variants emit at wavelengths ranging from 440 to 530 nm. Our goal is to obtain spectral variants of GFP using error-prone polymerase chain reaction (PCR) followed by an evolutionary method known as "DNA shuffling" to obtain a greater variety of mutants. Clones are selected using ultraviolet or visible light lamps. The goal of this work is to obtain a variety of red-shifted variants of GFP that can be used in energy transfer and amplification in polymeric films.

116. Nonlinear fluorescent behavior of green fluorescent protein. Rajesh R. Naik, Sean M. Kirkpatrick, and Morley O. Stone. Biotechnology Group, Air Force Research Labs, AFRL/MLPJ, Building 651, 3005 P Street, Suite 1, Dayton, OH 45433-7701 (rajesh.naik@afrl.af.mil)

Green fluorescent protein (GFP) has become an important tool in cell biology and has been extensively used in monitoring gene expression and intracellular protein localization. The autocatalytic, posttranslational cyclization and oxidation of the tripeptide Ser65-Tyr66-Gly67 generate the greenish-yellow fluorescence of GFP. The wild-type GFP has two absorption maxima: a strong peak at 395 nm and a weak one at 476 nm, but one emission peak at 509 nm. The absorption peaks correspond to the neutral and anionic chromophore states, respectively, that relax to a common emission state. Crystallographic studies of the wild-type GFP have revealed a cylindrical barrel protecting its buried chromophore; therefore, GFP is stable in a variety of harsh conditions. These properties give GFP broad utility in various applications. Two-photon absorption (degenerate) is a nonlinear phenomenon that is observed in materials when irradiated with two photons at half the energy of the difference between ground-state and excited-state levels. Molecules exhibiting strong two-photon absorption have great potential in a wide range of applications such as three-dimensional fluorescence imaging, optical data storage. and microfabrication. Here we present recent results on the nonlinear behavior of GFP by two-photon absorption, including nonlinear fluorescence.

117. Subunit-specific molecular code of hemoglobin cooperativity. Gary K. Ackers, **Jo M. Holt**, and **Alexandra L. Klinger**. Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, Box 8231, St. Louis, MO 63110 (fax: 314-747-3467, ackers@biochem.wustl.edu)

Extensive research on the mechanism of cooperative O_2 binding to human hemoglobin (Hb) has focused on finding the contributions to cooperativity by the eight combinatorial forms of partially ligated tetramers, and on relating these contributions to the molecule's well-known quaternary and tertiary structural transitions. Thermodynamic linkage analysis led to the Symmetry Rule model, which employs features of both traditional models (MWC and KNF): (a) the concerted quaternary transition is triggered by binding O_2 on both dimeric halves of the Hb tetramer; and (b) sequential cooperativity accompanies binding of ligands on the same dimer prior to the quaternary transition. These findings have been challenged on grounds that the observed energy distribution of the intermediates (a) was created by undetected ligand rearrangement among the heme sites, and (b) is incompatible with the traditional MWC model which is accepted as correct. (Supported by NIH and NSF.)

118. Theoretical study on the electronic and molecular structres of peptide nanotubes. Maki Kasahara,[1] Hajime Okamoto,[1] Kyozaburo Takeda,[1] and Kenji Shiraishi.[2] [1]Department of Materials Science and Engineering, School of Science and Engineering,Waseda University, 169-8555, Tokyo, Japan, and [2]NTT Basic Reserch Laboratories, 243-0198, Atsugi, Japan

Peptide nanotubes (PNTs) are synthesized by self-assembling polypeptide rings (PPRs). Based on the ab initio HF calculations, we investigate the electronic and molecular structures of PPRs and PNTs, focusing on the effect by substituting amino acid residues (Gly and Cys). Ring formation of peptide skeleton generates two new intraring hydrogen bonds. One produces a novel peptide plane of $\psi = \pi$, which differs from the normal peptide plane of $\omega = \pi$ found in the secondary structure of β-sheets. The other plays an important role in forming the most stable peptide ring skeleton, and gives its maximum value at the eight-residue ring. Thus, the total energy shows the minimum value at the eight-residue ring for both Gly- and Cys-PPRs. We also investigate the electronic function of the interring hydrogen bonds in the PNT. In addition to aggregation of PPRs to form the tertiary structure of PNTs, these interring hydrogen bonds cause a possibility of the electronic delocalization toward the tube direction.

119. Insertion-competent coat protein conformation of the filamentous phage fd: Fusion of I-forms with lipid vesicles. Scott A. Robertson and Linda M. Roberts. Department of Chemistry, California State University, Sacramento, 6000 J Street, Sacramento, CA 95819 (lroberts@saclink.csus.edu)

The filamentous phage fd deposits its coat protein in the inner membrane of host *E. coli* cells during infection by a complex process involving both phage and bacterial proteins. We have been using an in vitro system to study the initial steps of infection. Modified phage forms, produced by exposure of the phage to a chloroform/water interface, have previously been shown to have properties, such as detergent susceptibility, that are compatible with insertion into membranes. The coat protein conformational changes that occur in these modified phage (termed I-forms when produced at low temperatures or S-forms when produced at high temperatures) may represent conformational changes necessary for insertion of the coat protein into membranes. Here, we show that both I-forms and S-forms fuse with preformed lipid vesicles. In both cases, the protein is incorporated into the bilayer, as determined by quenching of the intrinsic fluorescence by brominated lipids. I-forms fuse to a much greater extent than S-forms, suggesting that the I-form coat protein conformation is competent for membrane insertion. The significance of these results with respect to infection mechanisms will be discussed.

120. Biochemical efficiency: A metrics to quantitate the impact of the biochemical environment on the outcome of a drug's interaction with its target. David C.

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One major hurdle in drug discovery is the identification of molecules that are efficacious. The absence of in vivo efficacy is generally attributed to poor pharmacokinetics. However, poor in vivo activity can also result when the mechanism of action does not synergize with the biochemistry or the target is not quantitatively important to the phenotype. Consequently, the intrinsic affinity of the inhibitor (KI) may not be realized in the in vivo outcome. Biochemical efficiency is introduced as a method to quantitate the influence of the biochemical environment on the biological outcome (phenotype) and to formalize a common practice in biomedical research. It is determined by comparing the inhibitory binding constant for the interaction (IC_{50} or KI) to the effect on a targeted outcome (ED_{50}). The ED_{50} for the most efficient drugs and targets will approach the IC_{50} (KI). Conceptually, biochemical efficiency helps to identify and focus attention to the factors that are quantitatively important to an outcome related to clinical efficacy. It can be used as a pharmaceutical metrics to help quantitate the quality of potential new drugs and targets. In this work, I will show the biochemical efficiency of some marketed drugs and of some targets and compounds that are still under evaluation, and discuss biochemical factors that can influence the biochemical efficiency.

121. Calcium regulation of synaptotagmin function via C2 domains. Ricardo A. Garcia,[1] Benjamin Staehlin,[1] and Hilary Arnold Godwin.[2] [1]Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, 2153 North Campus Drive, Evanston, IL 60208 (fax: 847-491-7713, r-garcia1@nwu.edu), [2]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113

The C2 domain is a calcium-binding motif that is thought to play a ubiquitous role in membrane trafficking and exocytosis. The exact role of C2 domains in signaling is a matter of debate, and some fundamental questions remain unanswered. For instance, how does calcium binding to C2 domains regulate protein activity? How are these C2 domains involved in calcium-dependent protein−protein interactions? The answers to these questions are central to defining a mechanistic role for C2 domains in calcium-regulated protein function. An attractive model system for investigating how calcium binding to C2 domains regulates these activities is the synaptic vesicle protein synaptotagmin (Syt). Syt is a well-studied example of a calcium-responsive, C2 domain-containing protein. In the present study, the effects of calcium binding to the C2 domains of Syt were investigated by fluorescence resonance energy transfer using the intrinsic fluorescence of tryptophan as the energy donor and a coumarin derivative conjugated to a single cysteine residue as the energy acceptor. Our results show that calcium binding to the C2 domains of Syt increases the efficiency of intramolecular energy transfer. These results indicate that calcium induces a conformational change within the protein. The global conformational changes observed with Syt are shown to be important for its fusogenic activity.

122. Characterization of reconstituted high-density lipoprotein containing apolipoprotein A-I deletion mutants. Nathan Smyth,[1] Ruben Almarez,[1] Flora Galvan-Rueckert,[1] Christie G. Brouillette,[2] Jeffrey A. Engler,[3] and Linda M. Roberts.[1] [1]Department of Chemistry, California State University, 6000 J Street, Sacramento, CA 95819 (lroberts@saclink.csus.edu), [2]Center for Macromolecular Crystallography, University of Alabama, and [3]Department of Biochemistry and Molecular Genetics, University of Alabama

The properties of reconstituted high-density lipoproteins (rHDL) containing apolipoprotein A-I (apo A-I) deletion mutants were examined. The apo A-I proteins used in the study are human apo A-I from serum; Δ1−65, an amino-terminal deletion mutant; Δ88−98, an internal deletion mutant; and Δ187−243, a carboxy-terminal deletion mutant. The rHDL prepared with these proteins were characterized by size, protein−lipid composition, and protein stoichiometry. Our results indicate that the predominant particle formed with all of the proteins except Δ187−243 was between 9 and 10 nm in diameter and contained two apo A-I molecules. The rHDL formed with either Δ1−65 or Δ88−98 were stable for at least 2 weeks whereas rHDL formed with Δ187−243 were significantly less stable. Initially, association of Δ187−243 with lipid produced two major particles with diameters of 12.1 and 8.7 nm. The larger rHDL were converted within 3 days to smaller rHDL with diameters of 9.8 nm. After 6 days, all of the original rHDL were gone, and small rHDL with diameters of 7 nm or less appeared along with substantial amounts of lipid-free protein. The instability of rHDL produced with a carboxy-terminal deletion mutant supports previous work indicating the importance of the carboxy terminus of apo A-I in lipid binding. In other experiments, rHDL prepared in the presence of two proteins of different length (e.g., human apo A-I and a deletion mutant) yielded only complexes containing one kind of protein. These results support the belt, rather than the picket fence, model of apo A-I binding to lipid.

123. Constitutive expression of highly active barley α-amylase 1 in *Saccharomyces cerevisiae* and characterization of its properties. Dominic W. S. Wong, Sarah B. Batt, and George H. Robertson. Western Regional Research Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710 (fax: 510-559-5777, dwsw@pw.usda.gov)

The barley α-amylase 1 gene was isolated from the barley cDNA library, and the gene construct was cloned into a yeast expression vector. The recombinant enzyme was constitutively secreted by *Saccharomyces cerevisiae* at a concentration of ∼0.2−0.3 μg/L of culture When propagated in YEPD/2 mM $CaCl_2$ medium, the α-amylase activity increased with cell density, and reached a maximum after 24 h. The enzyme was purified to homogeneity by affinity chromatography. The protein had an N-terminal sequence of His-Gln-Val-Leu-Phe-Gln-Gly-Phe-Asn-Trp, indicating that the signal peptide was correctly processed. The activity of the purified α-amylase was 1.9 mmol of maltose (mg of protein)$^{-1}$ min^{-1}, equivalent to that observed for the native seed enzyme. The kinetic parameters of the recombinant enzyme were measured using the substrate ET-G7PNP. A K_m of 0.45 mM and a k_{cat} of 1.2×10^2 s^{-1} were obtained for

the recombinant α-amylase, with a k_{cat}/K_m of 2.7×10^2 $mM^{-1} \cdot s^{-1}$, consistent with those of α-amylases from plants and other sources.

124. Dexamethasone−methotrexate: An efficient chemical inducer of protein dimerization in vivo. Virginia W. Cornish,[1] **Hening Lin**,[1] Wassim M. Abida,[1] and Robert T. Sauer.[2] [1]Chemistry Department, Columbia University, 3000 Broadway, MC 3111, Havemeyer Hall, New York, NY 10027 (fax: 212-932-1289, virginia@chem.columbia.edu), and [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

"Chemical Inducers of Dimerization" (CIDs) that can manipulate protein−protein interactions in vivo are a powerful tool in studying protein function and controlling cellular processes. Based on the well-characterized ligand−receptor pairs dexamethasone (Dex)−glucocorticoid receptor (GR) and methotrexate (Mtx)−dihydrofolate reductase (DHFR), we designed a new CID, a dexamethasone−,ethotrexate (Dex−Mtx) small molecule. It was synthesized from commercially available starting materials, and the yeast two-hybrid system was used to verify that it could dimerize GR and DHFR protein chimeras. We constructed plasmids encoding LexA-DHFR and B42-GR protein chimeras, and transformed these plasmids into *S. cerevisiae* strain FY250 containing a lacZ reporter gene. LacZ transcription assays showed that Dex−Mtx can dimerize proteins efficiently in vivo, increasing the level of lacZ transcription about 150-fold. Control experiments showed that activation of lacZ transcription was Dex−Mtx-dependent. Given the ease with which Dex−Mtx can be synthesized and the efficeincy with which it can induce protein dimerization, the Dex−Mtx system should find widespread utility in biological research.

125. Effect of amino acid mutations and pressure on the stability and flexibility of hyperthermophilic glutamate dehydrogenase. Michael M. Sun,[1] **Gary S. Mak**,[1] Dennis Maeder,[2] Mikyung Lee,[2] Costantino Vetriani,[2] Frank T. Robb,[2] and Douglas S. Clark.[1] [1]Department of Chemical Engineering, University of California, 473 Tan Hall, Berkeley, CA 94720 (fax: 510-643-1228, mikesun@socrates.berkeley.edu), and [2]Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202

We have investigated the effect of amino acid mutations on the stability and activity of hexameric glutamate dehydrogenase (GDH) from the hyperthermophile *Thermococcus litoralis*. Thermostability studies with charged mutants in the presence of KCl revealed that electrostatic repulsion between subunits is an important factor in the stability of GDH. Computer modeling was performed to elucidate the effect of these charge mutations on the structure and stability of GDH. The effect of pressure on GDH stability was also investigated. Moderately high pressure was found to stabilize all GDHs against thermoinactivation. A mechanism for pressure stabilization is proposed based on the kinetics of thermoinactivation, the crystal structure of the wild-type GDH, and the different degrees of pressure stabilization. Hydrogen/deuterium exchange experiments at both ambient and high pressure were also conducted to further probe GDH flexibility.

126. Enhanced minding of a "dimerized" A-site 16S rRNA construct with dimeric aminoglycosides. Jeffrey B.-H. Tok and Gordon R. Huffman. Department of Chemistry, Indiana University−Purdue University, 2101 East Coliseum Boulevard, Fort Wayne, IN 46803 (fax: 219-481-6070, tokj@ipfw.edu)

Aminoglycosides are a class of antibiotics known to function in large part by binding to the A-site decoding region of bacterial 16S rRNA, causing premature termination and mistranslation of proteins. Understanding the rules that govern the recognition of RNA by the aminoglycoside antibiotics has been of prime importance. In this work, we examined and compared the binding properties of a series of dimeric aminoglycoside molecules to the monomeric aminoglycoside molecule with a 56 nucleotide "dimerized" A-site 16S rRNA construct. It was observed that the dissociation constants of the dimeric aminoglycosides to a dimerized A-site 16S rRNA construct are approximately 8−25-fold tighter than the monomeric aminoglycoside molecule.

127. Hazelnut (Filbert), a new source for taxanes. Angela M. Hoffman, Maggie Ross, Katie Weigandt, Harnik Gulati, and Waseem Khan. Chemistry and Physics Departments, University of Portland, 5000 North Willamette Boulevard, Portland, OR 97203 (fax: 503-943-7399, hoffman@up.edu)

Paclitaxel (Taxol), an anticancer drug, and several related diterpenoid taxanes were originally isolated from the Pacific yew. Taxanes have also been isolated from fungi associated with yew. We have recently recovered these compounds from branches of several hazelnut (Corylus) cultivars and from a variety of fungal endophytes associated with Corylus. Paclitaxel has also been isolated from sterile cells cultured from hazelnut. A similar mixture of taxanes was obtained from both yew and hazelnut, suggesting that the same pathway is operating in both organisms. Concentrations of taxanes from hazelnut and associated fungi were generally less than from yew. Taxanes from both fungal isolates and cell cultures have been verified by LC/mass spectroscopy. (Financial support from the University of Portland and the Oregon Hazelnut Commission is gratefully acknowledged.)

128. Intrinsic bending in oligonucleotides as probed by fluorescence resonance energy transfer (FRET). Catherine J. Murphy and **Jessi M. Wildeson**. Department of Chemistry and Biochemistry, University of South Carolina, Jones Physical Science Center, Columbia, SC 29208 (fax: 803-777-9521, murphy@psc.sc.edu)

We have conducted studies utilizing fluorescence resonance energy transfer (FRET), a spectroscopic process by which energy is passed nonradiatively between molecules over distances of 10−100 Å, as a probe to characterize the sequence-specific structure of bent or kinked oligodeoxynucleotides in solution. This method entails labeling the 5′ ends of complementary hybridized oligodeoxynucleotides with "donor" and corresponding "acceptor" fluorophores. Based on Förster's theories, the efficiency of energy transfer between these chromophores is proportional to the inverse sixth power of the distance separating them. From this calculated dye to dye distance, the angle of duplex curvature

can be evaluated using geometric models. This presentation will focus on the analysis of sequence-specific conformations of short (16−20 bp) oligodeoxynucleotides containing GGCC and other sequence tracts thought to induce conformations on DNA duplexes. We have observed greater angles of sequence-specific bending in solution than previously reported by studies of DNA constrained in the crystal state.

129. Mechanistic studies of cyclopamine, an inhibitor of the hedgehog signaling pathway. James K. Chen, Jussi Taipale, Michael K. Cooper, and Philip A. Beachy. Department of Molecular Biology and Genetics, PCTB 701, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205 (fax: 410-955-9124, jkchen@mail.jhmi.edu)

Perturbations of developmental pathways can cause human diseases and disorders. In particular, activating mutations within the hedgehog (Hh) signaling cascade have been linked to several cancers, whereas genetic alterations that inactivate Hh signaling can produce developmental defects. Our ability to prevent or remedy these abnormalities, however, is limited by our incomplete understanding of Hh signal transduction. The recent finding that plant-derived steroid alkaloids, jervine and cyclopamine (11-deoxojervine), can abrogate Hh signaling provides a unique vantage point for dissecting this developmental pathway. In this context, cyclopamine derivatives have been synthesized and evaluated using a cell-based assay for Hh signaling, leading to the discovery of more potent inhibitors and the generation of cyclopamine-based photoaffinity reagents and fluorophores. Coupled with genetic analyses, studies with these synthetic probes have provided insights into Hh signaling at the molecular level and may foster new strategies for the chemical regulation of this important biochemical pathway.

130. Molecular cloning of an olfactory-specific gene from the olfactory epithelium in Atlantic salmon, *Salmo salar*. Jiongdong Pang,[1] Karl A. Hartman,[2] Joel M. Chandlee,[2] Dennis E. Rhoads,[3] Jia Yu,[4] and Joan Lloyd.[1] [1]Chemistry Department, Southern Connecticut State University, 501 Crescent Street, New Haven, CT 06514 (fax: 203-392-6396, pang@scsu.ctstateu.edu), [2]Department of Biochemistry, Microbiology and Molecular Genetics, University of Rhode Island, Kingston, RI 02881, [3]Biology Department, Monmouth University, West Long Branch, NJ 07764, and [4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215

Olfaction drives many vital behaviors of salmon. Pang et al. have previously demonstrated that salmon olfactory glutamate receptor (oGluR) is likely a subtype of the metabotropic glutamate receptor (mGluR) family in mammalian brain [(1994) *FEBS Lett. 354*, 301−304]. We therefore attempted to isolate the gene for oGluR using probes and PCR primers for mGluR subtypes. *Materials and Methods:* A salmon olfactory cDNA library was constructed and screened using cDNA of mGluR1 as probe. Putative positive clones were picked, verified, and characterized. *Results:* (1) Northern blot analysis showed that one clone, designated 24h, is only expressed in the olfactory tissue. (2) Clone 24h is currently being sequenced. Although the anticipated regions of homology to mGluR1 have not yet been established, two other short regions of interest have been identified. The aa sequences deduced from the nt sequences suggest that clone 24h protein has significant regions of homology to the yeast mating pheromone receptor protein (Mid2). (a) The N-terminal end of clone 24h possesses a long Ser/Thr-rich domain. It is possible that this region of clone 24h protein is extracellular and O-glycosylated. (b) The C-terminal region of clone 24h shows high homology with the Ca^{2+} binding site of human α-lactalbumin, which is also a feature of Mid2. Ca^{2+} plays an important role in olfactory signal transduction. Ca^{2+} achieves some of its many biological effects through highly specific interactions with signal transduction enzymes such as PKC and PDE, both known to operate in olfactory reception. The fact that clone 24h contains a Ca^{2+} binding site suggests that it would respond to varying concentrations of Ca^{2+} inside of the cell. This sensitivity might be used to modulate the activity of oGluR so as to reduce the noise in olfactory neuronal circuits by turning off transduction when Ca^{2+} levels are high. *Conclusions:* An olfactory-specific gene was cloned from a salmon olfactory cDNA library. The deduced amino acid sequence shows a Ser-rich region and a high-affinity Ca^{2+} binding site. Many more questions will be answered when the entire sequence data are evaluated.

131. NMR studies of horse cytochrome *c* in the mixed solvents. Sivashankar Sivakolundu,[1] Susan Pochapsky,[2] and Patricia Ann Mabrouk.[1] [1]Department of Chemistry, Northeastern University, 112 Hurtig Hall, Boston, MA 02115 (ssivakol@lynx.neu.edu), and [2]Center for Magnetic Resonance, 170 Albany Street, MIT, Cambridge, MA 02139

Horse cytochrome *c* (cyt *c*) undergoes structural changes in the mixed solvent media (0−40% organic solvent). Resonance Raman spectroscopic studies [Sivakolundu, S., and Mabrouk, P. (1999) *J. Am. Chem. Soc.* (submitted for publication)] show a change in the ligation of the heme active site. A mixture of cyt *c* conformers is observed in mixed solvent media, which is similar to cyt *c* in the alkaline media. Met80, which is the axial ligand in native cyt *c*, is replaced by Lys73, Lys79, or OH- in the alkaline and the mixed solvent media, resulting in different conformers of cyt *c*. The percentage of each conformer is found to be dependent on the solvent dielectric constant in the mixed solvents and pH in the alkaline media. NMR studies (500 MHz ^1H) are being done to study the nature of the changes undergone by cyt *c* in the mixed solvent media. The results of NMR studies will be discussed.

132. NMR studies of the dynamics of DNA oligonucleotides using heteronuclear labeling and relaxation measurements. Ryszard Michalczyk,[1] Charles C. Orji,[2] and Louis A. Silks, III.[1] [1]Bioscience Division, Los Alamos National Laboratory, B-2, MS E-529, Los Alamos, NM 87545 (fax: 505-665-5052, rmichalczyk@lanl.gov), and [2]Paradigm Organics Inc., 840 Main Campus Drive, Suite 3900, Raleigh, NC 27606

Our ongoing efforts to develop methods for DNA labeling have resulted in a number of singly and multiply labeled DNAs. One of our major goals has been to develop a common synthetic pathway to both deoxyadenosine (dA) and deoxyguanosine (dG). We have devised a synthetic strategy

in which 2,4,5-triamino-6-chloropyrimidine serves as a pivotal intermediate. Annulation of this intermediate gives 2-amino-6-chloropurine, which is subsequently converted to either deoxyguanosine (via 2,6-diaminopurine) or deoxyadenosine (via 6-chloropurine). This route allows for straightforward and efficient labeling at any desired position of dA or dG. We have applied this new synthetic scheme to the synthesis of [8-^{13}C, 9-*amino*-$^{15}N_2$]-2′-deoxyadenosine on a multigram scale. The labeled deoxynucleoside was incorporated at all positions of the DNA 16-mer d(CGACCGAAAACGGTGC)·d(GCACCGTTTTCGGTCG), containing the binding site for human papillomavirus E2 protein. Assignments of proton and carbon resonances using ^{1}H−^{1}H NOESY and ^{1}H−^{13}C HMQC NMR experiments have demonstrated the usefulness of this labeling. In addition, measurements of ^{13}C, ^{15}N, and ^{1}H relaxation lead to the characterization of the dynamics of adenine residues in this DNA. The measurements in the temperature range between 5 and 45 °C are in progress, and the results of the analysis of relaxation data will be presented in detail.

133. Poly γ-D-glutamic acid as a template for functionalized water-soluble biomaterials. Emmanuel J.-P. F. Prodhomme and T. D. H Bugg. Chemistry Department, Warwick University, Coventry, CV4 7AL, United Kingdom (msrpx@warwick.ac.uk)

Polymeric materials can provide a very useful template for the delivery of biologically active small molecules, provided that the polymer can be efficiently functionalized, giving a high local concentration of attached ligands. Poly γ-D-glutamic acid is an unusual γ-linked polypeptide of high molecular weight (150−200 kDa) isolated from *Bacillus licheniformis* and offers several advantages for such studies: it is a high molecular weight, water-soluble polymer; its α-carboxylate side chains can be covalently modified in aqueous solution; its γ-linked D-amino acid backbone is resistant to mammalian proteases. The immobilization of protein ligands on the polymer can lead to several applications including the immobilization of tumor-specific antibodies which bind to multimeric receptors on the tumor cell surface and the coimmobilization of tumor-specific antibodies with anticancer agents. Some linkers were developed to be attached via their free amino group to the polymer followed by an addition of the given protein on the conjugate via a free thiol.

134. Purification and partial characterization of 2-(2′-hydroxyphenyl)benzenesulfinate desulfinase. Rene C. Rodriguez,[1] Dawn S. Schneider,[1] and Linette M. Watkins.[2] [1]Department of Chemistry, Southwest Texas State University, 601 University Drive, San Marcos, TX 78666 (rr43448@swt.edu), and [2]Department of Chemistry and Waste Minimization and Management Research Center, Southwest Texas State University, 601 University Drive, San Marcos, TX 78666

Dibenzothiophene is the model organosulfur compound used to study the biocatalytic removal of sulfur from petroleum and coal products. The enzyme 2-(2′-hydroxyphenyl)benzenesulfinate desulfinase (HPBS desulfinase) catalyzes the final step in the desulfurization pathway, the cleavage of the carbon−sulfur bond of 2-(2′-hydroxyphenyl)benzenesulfinate (HPBS) to form 2-hydroxybiphenyl and sulfite. HPBS desulfinase was purified from *Rhodococcus* strain sp. IGTS8. Efficient cell lysis was obtained using a French press. Purification was achieved using ion-exchange chromatography and hydrophobic interaction chromatography. The purification was monitored using spectrofluorometric and colorimetric assays and further assessed by SDS−polyacrylamide gel electrophoresis. An overall 20-fold increase in purification was obtained for the enzyme that was determined by SDS−PAGE to be greater than 90% pure. The K_m and V_{max} of the enzyme were measured using the substrate HPBS. Inhibition was observed in the presence of the product 2-hydroxybiphenyl.

135. Selective transport of Pb(II) by the polyether antibiotic ionomycin. Douglas R. Pfeiffer,[1] **Richard W. Taylor**,[2] Clifford J. Chapman,[1] and Warren L. Erdahl.[1] [1]Department of Medical Biochemistry, The Ohio State University, Columbus, OH 43210, and [2]Department of Chemistry & Biochemistry, University of Oklahoma, Norman, OK 73019

Studies utilizing phospholipid vesicles show that ionomycin transports divalent cations with the following selectivity sequence: $Pb^{2+} > Cd^{2+}$, $Zn^{2+} > Mn^{2+} > Ca^{2+} > Cu^{2+} > Co^{2+} > Ni^{2+}$, Sr^{2+}. Using individual transport rates for Pb^{2+} and Ca^{2+}, a selectivity factor, $S_{Pb/Ca}$, of 450 is calculated. This rises to ∼3200 when both cations are present and transported simultaneously. 1 μM Pb^{2+} inhibits the transport of 1 mM Ca^{2+} by ∼50%, whereas the rate of Pb^{2+} transport approaches a maximum at a concentration of 10 μM Pb^{2+}, when 1 mM Ca^{2+} is also present. The concentration dependence of Pb^{2+} transport indicates that the primary transporting species has 1:1 Pb^{2+}/ionophore stoichiometry. The species transporting Pb^{2+} may include HI·Pb·OH, where HI represents singly ionized ionomycin and the coordinated OH^- maintains charge neutrality. Studies using A20 B lymphoma cells show that ionomycin catalyzes both Pb^{2+} influx and efflux, with concentration behavior similar to that found for the phospholipid vesicles.

136. Structure−activity relationships in anti-neoplastic type I amphiphiles. M. K. Dymond and G. S. Attard. Department of Chemistry, Southampton University, University Road, Highfield, Southampton, SO17 1BJ, England (mkd1@soton.ac.uk)

HDPC and ET-18-OMe, both type I amphiphiles and analogues of the naturally occurring biomembrane component lysophosphatidylcholine, are potent anti-neoplastic agents. A series of novel, type I amphiphiles have been synthesized, and their cytostatic activity against cancer cells in culture was determined. The data suggest that cytostatic activity is a generic property of type I amphiphiles. Furthermore, the primary target for these amphiphiles appears to be the inhibition of phosphatidylcholine synthesis via perturbation in the elastic properties of intracellular bilayer membranes.

137. Sulfate release from 2-diisopropylaminoethylsulfonic acid, a derivative of the nerve agent VX, by taurine-utilizing bacteria. Amber Elise Schrank and Michael P. Labare. Department of Chemistry, United States Military

Academy, P.O. Box 1759, West Point, NY 10997 (x02074@exmail.usma.edu)

The United States is searching for an environmentally safe way to dispose of the nerve agent VX, *O*-ethyl-*S*-(2-diisopropylaminoethyl)methylphosphonothiolate. VX can be transformed to 2-diisopropylaminoethylsulfonic acid (pSA) by sequential treatment with water and performic acid. Previously our laboratory has shown that pSA was biodegradable. However, the consortium proved to be phenotypically unstable. A phenotypically stable bacterial isolate capable of growing on 2-aminoethylsulfonic acid has been isolated from Hudson River sediment. A resting cell assay was developed to quantify the release of sulfate from bacterial growth on taurine. When cells [$OD_{(600\ nm)}$] were exposed to 5 mM taurine for 15 min, 1 h, and 24 h, 0.00246, 0.00442, and 0.0413 mg of SO_4^{2-} were released, respectively. This assay will be used to determine if the isolate can remove the sulfate group from pSA. In addition, diethylaminoethylsulfunic acid, derived from Russian VX, will also be tested for the release of sulfate.

138. Threading intercalators: Effects of side chain structure on DNA interaction for anthraquinones and naphthalene diimides. Dabney W. Dixon, Vera Steullet, and Sophia Edwards-Bennett. Department of Chemistry, Georgia State University, University Plaza, Atlanta, GA 30303 (fax: 404-651-1416, ddixon@gsu.edu)

Intercalators with one side chain in the minor groove and the other in the major groove are termed "threading intercalators". Topological constraints require that one chain pass through an opening in the DNA as the molecule intercalates. To investigate the effect of size and structure of the intercalator on the threading process, we have synthesized a series of anthraquinones and naphthalene diimides. The association rates are a function of the size of the molecule, but the dissociation rates are not. Examples with side chains ending in aromatic moieties show less dependence on size than those ending in aliphatic moieties. The molecular basis of these observations will be discussed.

139. Transient outward K^+ current inhibition by homocysteine in rat ventricular myocytes. Robert Dean Shontz,[1] George J. Rozanski,[2] and Zhi Xu.[2] [1]Department of Chemistry, University of Northern Iowa, 412 West 18th Street, Apartment 2, Cedar Falls, IA 50613 (shontzr7548@uni.edu), and [2]Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, NE

Clinical studies of atherosclerosis, coronary heart disease, and congestive heart failure find patients have high levels of homocysteine in their blood. The action potential of ventricular cardiomyocytes in congestive heart failure is lengthened. Past research has determined this to be due to the reduction of a specific voltage-dependent, Ca^{2+}-independent, transient outward K^+ current (I_{TO}). The aim of the present study was to examine the electrophysiological effects of homocysteine on the I_{TO} using rat ventricular myocytes. Heart cells were obtained via a surgical procedure followed by enzymatic treatment. Whole-cell patch clamp techniques were performed to study changes in current density, voltage-dependent activation and inactivation, and time-dependent inactivation kinetics and recovery from inactivation. Intracellular and extracellular drug applications were performed. Voltage-clamp experiments revealed that the maximum I_{TO} density, measured at +60 mV, was reduced 34% when homocysteine was added extracellularly to the myocytes ($P < 0.01$). Intracellular application of homocysteine resulted in a 17% decrease in maximum I_{TO} density at +60 mV ($P < 0.02$). Homocysteine did not affect the voltage-dependent activation or inactivation. This suggests the voltage-sensitive region of the K^+ channel is not affected. Similarly, the time-dependent activation kinetics and recovery from inactivation were not altered; thus, the ball and chain region of the K^+ channel was presumably not affected. These findings indicate that homocysteine may bind and/or cause a conformational change in the pore of the K^+ channel. Thus, homocysteine decreases myocyte I_{TO}, which may relate to impaired contractility and arrhythmogenesis, both of which are characteristic of a failing heart.

140. Xylose and carbocyclic adenine analogues as potent inhibitors of adenylyl cyclase. Praveen Pande,[1] Fritz Theil,[2] and Roger A. Johnson.[1] [1]Physiology and Biophysics, SUNY, Health Science Center, Stony Brook, NY 11794-8661 (fax: 516-444-3432, praveen@physiology.pnb.sunysb.edu), and [2]Chemistry Department, University of Liverpool, Liverpool, United Kingdom

Adenylyl cyclases are a family of enzymes that catalyze the conversion of adenosine 5′-triphosphate to adenosine 3′,5′-cyclic monophosphate (cAMP), a second messenger that is involved in diverse cellular responses. Adenylyl cyclase is potently inhibited by derivatives of adenosine via a domain referred to as the P-site, and this inactivation occurs through a post-transition configuration of the catalytic active site. To further our studies on the structure−activity relationship of inhibitors of adenylyl cyclase, additional ribose-modified derivatives of adenosine were synthesized and tested for inhibition; IC_{50} values were as follows: -D-(xylose)adenine, 3.2 μM; -D-(2′-deoxyxylose)adenine, 15.5 μM; and -D-(2′,5′-dideoxyxylose)adenine, 16.4 μM. These compare with inhibition by the diasteromers -D-adenosine ($IC_{50} = 82\ \mu$M) and -D-(arabinose)adenine ($IC_{50} = 30\ \mu$M). The addition of a triphosphate group to a 9-substituted carbocyclic adenine derivative containing a cyclopentane ring instead of ribose showed strong potency ($IC_{50} = 0.45\ \mu$M). However, the hydroxyl derivative of this compound was a poor inhibitor ($IC_{50} > 1$ mM). This suggests that the conformation of the sugar/nonsugar five-membered ring of these inhibitors plays a key role in orienting the molecule to fit into the catalytic site of the enzyme. These studies form a basis for rational drug design for inhibition of one of the most important transmembrane signal transduction enzymes in biology.

141. Practical serum test that predicts/assesses the effectiveness of cancer therapy. E. T. Bucovaz and W. D. Whybrew. Biochemistry Department, University of Tennessee, 800 Madison Avenue, Memphis, TN 38163 (fax: 901-448-7126, EBUCOVAZ@UTMEM1.UTMEM.EDU)

A simple quantitative assay procedure for measuring the serum titer of cancer-induced B-Protein, which is directly related to the degree of proliferation of cancer cells,was described in Cancer Research Therapy and Control [(1992) *Cancer Res. Ther. Control 3*, 57−74]. Previous studies

revealed that approximately 13% of the 2700 cancer patients tested did not exhibit an elevated level of cancer-induced B-Protein. Moreover, it was observed that patients who either initially did not have a measurable level of cancer-induced B-Protein or who did not have a measurable level of cancer-induced B-Protein following treatment responded favorably to therapy. In contrast, patients who initially had an elevated serum titer of cancer-induced B-Protein that remained elevated during and after treatment did not respond favorably to therapy. In a focused study involving 135 cancer patients, it was concluded that the measurement of cancer-induced B-Protein can serve as a prognostic indicator to predict and to assess the response of a patient's malignancy to a particular therapy principally at a time when a more aggressive therapy might have an opportunity for success. In addition, it was concluded that cancer-induced B-Protein is functioning in an anti-apoptotic(-like) capacity to inhibit the body's mechanism, which is designed to destroy cancer cells, thus permitting all biological functions related to tumor growth and development to proceed, and, as a consequence, rendering any form of treatment less effective.

142. Molecular pathogenesis of Parkinson's disease: results from atomic force microscopy of nonfibrillar α-synuclein assemblies. Tomas T. Ding,[1] Jean-Christophe Rochet,[1] Kelly A. Conway,[1] Jason H. Hafner,[2] Seung-Jae Lee,[1] Charles M. Lieber,[2] and Peter T. Lansbury, Jr.[1] [1]Brigham and Women's Hospital/Department of Neurology, Harvard Medical School, Center for Neurologic Diseases, 77 Avenue Louis Pasteur, Boston, MA 02115 (fax: 617-525-5305, ding@cnd.bwh.harvard.edu), and [2]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Parkinson's disease (PD) brain is characterized by Lewy bodies, i.e., inclusion bodies containing fibrillar α-synuclein (αS), in some surviving neurons of the substantia nigra. In addition, two point mutations in the gene encoding αS (A53T and A30P) are associated with early-onset familial forms of PD. In vitro studies of aggregating αS revealed that one of the mutant proteins (A53T) forms amyloid fibrils more rapidly than both the wild-type (wt) protein and the second mutant (A30P), while wt fibrillizes faster than A30P. However, the rate of disappearance of monomeric A30P from aggregation solutions is comparable or slightly faster than wt, while the turnover of monomeric A53T is much faster than that of wt. This suggests that nonfibrillar (oligomeric) forms of αS are involved in the aggregation pathway, presumably at early time points. Here we introduce data rendered by atomic force microscopy (AFM) of nonfibrillar assemblies formed by αS (wt as well as mutant proteins). All three proteins formed spherical assemblies of various heights (e.g., 3 and 5 nm). Large ring-like morphologies (diameter >40 nm) were detected in a 1:1 mixture of A53T and wt, and in a solution of wt alone. The temporal relationships between the various species will be discussed. The question of whether one or more of these assemblies or other nonfibrillar forms of αS might be suitable targets for therapeutic intervention will also be addressed.

143. Role of proteins containing open-chain tetrapyrroles in the photoresponses of the cyanobacterium *Fremyella diplosiphon* PCC7601. Christine Ramos-Boudreau,[1] John Cobley,[1] and Nicole Tandreau de Marsac.[2] [1]Chemistry Department, University of San Francisco, 2130 Fulton Street, San Francisco, CA 94117, and [2]Physiologie Microbienne, Institut Pasteur, 28 Rue du Dr. Roux, Paris, France

In the cyanobacterium *Fremyella diplosiphon* (*Calothrix* sp. PCC7601), green light causes the expression of genes encoding the light harvesting protein, phycoerythrin (PE), and prevents the expression of some of the genes which encode phycocyanin (PC2). These actions of green light can be antagonized by red light. In higher plants, when the actions of red light are antagonized by far red light, the photoreceptor is the photochromic protein phytochrome. In the cyanobacterium, three genes have been identified (rcaE, cphA1, and cphA2) which encode proteins related to higher plant phytochrome. The protein RcaE has been demonstrated to play an essential role in the adaption of the cyanobacterium to green and red light. The roles of the proteins CphA1 and CphA2 in the cyanobacterium are unknown. In CphA1, it is expected that an open-chain tetrapyrrole chromophore will be covalently bound to a conserved cysteine. However, in CphA2 this particular cysteine is not conserved. To understand the roles of CphA1 and CphA2, we have created, by reverse genetics, strains of the cyanobacterium in which the genes for these proteins are mutated by frameshifts. Investigation of these strains should enable us to determine which of the cyanobacterial photoresponses require CphA1 and/or CphA2.

144. Novel dichotomy in the redox chemistry of HNO. Surendra N. Mahapatro,[1] Christina L. Closken,[1] Rhiannon C. ReVello,[1] and Markandeswar P. Panda.[2] [1]Chemistry Department, Regis University, 3333 Regis Boulevard, Denver, CO 80221 (smahapat@regis.edu), and [2]Biochemistry Department, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284

Nitrite and N_2O are the only two known reported products of the spontaneous self-decomposition of trioxodinitrate(II), $HN_2O_3^-$, in aqueous medium in the pH range 4−10. The intermediacy of HNO in trioxodinitrate(II) decomposition has been unequivocally demonstrated by kinetic, ^{15}N NMR, and isotope studies. We report for the first time that hydroxylamine is a significant product of trioxodinitrate(II) decomposition at concentrations of $HN_2O_3^-$ of 10 mM, in aqueous phosphate buffer (pH 7.0−7.4). Hydroxylamine is most probably formed via an intermediate "NHOH" species, which results from the self-oxidation and reduction of HNO. This novel dichotomy in the redox behavior of HNO is evident in the reactions of copper-containing proteins such as cytochrome *c* oxidase. Reduced cytochrome *c* oxidase reacted with HNO to give ferronitrosyl cytochrome a_3.

145. Use of experimental data to calculate negative ion Morse potential surfaces for cytosine, thymine, and uracil. Edward C. M. Chen[1] and Edward S. Chen.[2] [1]School of Natural and Applied Sciences, University of Houston, CLC, 2700 Bay Area Boulevard, Houston, TX 77058 (ecmc@hia.net), and [2]CRPC, Rice University, MS-41, 6100 South Main, Houston, TX 77005

Recently published experimental and theoretical data for the electron affinities, gas-phase acidities, photoelectron

spectra, electron impact, and transmission spectra for adenine, guanine, cytosine, uracil, and thymine are reviewed. Negative ion mass spectrometry data for thermal electron attachment to these molecules will be presented which show they are very strong gas-phase acids. Morse potentials for dipole-bound and valence-state negative ions are calculated which consolidate all of these data. These curves illustrate the change in geometry predicted in semiempirical multiconfiguration configuration interaction calculations. For example, the NH_2 group in the negative ion of cytosine is twisted out of the plane of the ring as shown in the accompanying figures.

146. Folding pathway of some small proteins: β-Hairpins and zinc fingers. Vijay S. Pande. Department of Chemistry, Stanford University, Stanford, CA 94305-5080 (fax: 650-725-0259, pande@stanford.edu)

Is there a universal mechanism by which small proteins fold? One means to answer this question is to examine simulations of folding trajectories. Small proteins fold in tens of microseconds to milliseconds, beyond the reach of current atomistic simulations. However, the time required to traverse the free energy barrier in these systems is only a small fraction of the total folding time and is within the reach of detailed simulations. We discuss techniques for sampling these trajectories for small proteins (a β-hairpin and a zinc finger). Our results shed light on the folding mechanism of small proteins and in particular can discriminate between potentially universal and nonuniversal aspects of the kinetic mechanism.

147. Analysis of biothiols by HPLC using ThioGlo maleimide reagents. Nuran Ercal,[1] Nukhet Aykin,[1] and Ping Yang.[2] [1]Chemistry Department, University of Missouri, 142 Schrenk Hall, Rolla, MO 65409 (fax: 573-341-6033, nercal@umr.edu), and [2]Washington University, St. Louis, MO

The importance of thiols has stimulated the development of a number of methods for determining glutathione and other biologically important thiols. However, currently available methods have some limitations such as lengthiness and complexity. In the present study, a new high-performance liquid chromatography (HPLC) method for determining biological thiols was developed by using 9-acetoxy-2-[4-(2,5-dihydro-2,5-dioxo-1*H*-pyrrol-1-yl)phenyl]-3-oxo-3*H*-naphtho-[2,1-*b*]pyran (ThioGlo) as a derivatizing agent. ThioGlo reacts selectively and rapidly with the thiols to yield fluorescent adducts which can be detected fluorometrically (λ_{ex} = 365 nm, λ_{em} = 445 nm). The within-run coefficient of variation for glutathione (GSH) by this method ranges from 1.08% to 2.94%, while the between-run coefficient of variation for GSH is from 4.31% to 8.61%. The detection limit is around 50 fmol for GSH; the GSH derivatives remain stable for a month if kept at 4 °C. Results for oxidized glutathione (GSSG), homocysteine, and cysteine are also included. Moreover, captopril (a well-known angiotensin converting enzyme inhibitor), widely used for treatment of hypertension, has recently been under extensive investigation for its possible role as a free radical scavenger. The present method was applied to determine captopril levels in biological tissues. The ThioGlo method was compared to our previous method, in which *N*-(1-pyrenyl)maleimide (NPM) is used to derivatize thiol-containing compounds. The present method offers various advantages over currently accepted techniques, including speed and sensitivity.

148. Bradykinin-like immunoreactivity and pharmacological action in the sea cucumber *Holothuria glaberrima*. Mark Anthony Vergara-Gómez,[1] Gladys Escalona-de Motta,[2] Marña Rubio-Dàvila,[2] and Jose E. Garcña-Arraràs.[3] [1]Biology Department, University of Puerto Rico, Rño Piedras, P.O. Box 23360, UPR Station, San Juan, PR 00931-3360 (fax: 787-764-3360, markvergara@hotmail.com), [2]-Institute of Neurobiology, San Juan, PR, and [3]University of Puerto Rico

Bradykinin (BK), a peptide with a wide range of effects, has been commonly found associated with vertebrate nervous systems. Little information is available on the presence of BK in invertebrate systems. We have been interested in studying the presence and function of neuropeptides in organisms of the phylum Echinodermata, particularly in the sea cucumber *Holothuria glaberrima*. Using single-labeling immunohistochemistry, we were able to localize BK-like immunoreactivity in nerve fibers associated with muscle layers within the digestive tract as well as in the radial nerve, dermis wall, ambulacral feet, and tentacles. Double-labeling immunohistochemistry using anti-BK and a muscle-specific monoclonal antibody suggests that the immunoreactive fibers are innervating the muscular tissues. Force test were performed to determine a possible action of BK on the muscle systems. Pharmacological assays showed no response upon BK application. However, a negative modulatory response to the ACh-induced contraction was observed in body wall preparations at doses ranging from 10^{-6} to 10^{-9} M. Our results suggest that a BK-like peptide occurs in holothurians and is responsible for some type of modulation of the neuromuscular system. (Funded by EPSCoR, AMP, FIPI, and Howard Hughes programs of the University of Puerto Rico.)

149. Control of phosphorylation state of an ion channel containing caged tyrosine. Gabriel S. Brandt,[1] Yanhe Tong,[2] Ming Li,[2] Henry A. Lester,[2] and Dennis A. Dougherty.[1] [1]Division of Chemistry and Chemical Engineering, California Institute of Technology, 164-30, Pasadena, CA 91125 (fax: 626-564-9297, gabrielb@its.caltech.edu), and [2]Division of Biology, California Institute of Technology

The use of caged compounds or phototriggers (compounds derivatized with protecting groups which can be removed in situ) is a well-established technique for controlling the kinetics of biological events. The advent of unnatural amino acid mutagenesis has allowed this technique to be extended to caging proteins as well as their ligands. Here, a tyrosine analogue with its phenolic oxygen protected by a nitrobenzyl group, Tyr(ONB), is site-specifically-introduced into an ion channel. Photolysis of cells expressing this protein restores the wild-type tyrosine residue at the position of incorporation. The potassium channel Kir2.1 is modulated by tyrosine phosphorylation of a single tyrosine residue, Y242. The introduction of Tyr(ONB) at this site eliminated kinase-mediated reduction of the whole-cell current. However, UV irradiation of cells containing Tyr(ONB) at position 242

restored the ability of kinases to decrease potassium currents, consistent with the notion that uncaging the tyrosine permits kinase recognition. Thus, the use of caged tyrosine allows for experimental control of the initiation of tyrosine phosphorylation.

150. Cleavage of DNA abasic sites by amines and intercalator-amines. Dabney W. Dixon, Vera Steullet, Sophia Edwards-Bennett, and Josë L. Gonzalez-Romàn. Department of Chemistry, Georgia State University, University Plaza, Atlanta, GA 30303 (fax: 404-651-1416, ddixon@gsu.edu)

Amines are known to cleave plasmid DNA at abasic sites (apurinic or apyrimidinic, AP sites). We report a systematic study of abasic site cleavage by amines and intercalator-amines. Amines studied include piperidine, spermine, spermidine, and 12 other di-, tri-, and tetraamines. Triamines are more effective than diamines, which are substantially more effective than monoamines. Examples with three carbon atoms between neighboring nitrogens atoms cleave more efficiently than those with two carbons between adjacent nitrogens. This may reflect a particularly favorable geometry for proton abstraction for these species. The effect of nitrogen−nitrogen spacing on the pK_a values of the nitrogens may contribute as well. Anthraquinone and naphthalenediimide intercalators with amine-containing side chains generally cleave most of the abasic sites at micromolar concentration (30 min at 37 °C). Thus, cleavage of plasmid DNA at adventitious abasic sites by intercalator-amines bearing two nitrogens in a single side chain occurs readily.

151. Synthesis and biochemical characterization of DNA containing C-1′ oxidized abasic site lesions. Terry L. Sheppard, **Hamilton J. Lenox**, and Chris McCoy. Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113 (fax: 847-491-7713, sheppard@chem.nwu.edu)

Damage of DNA by reactive oxygen species produces nucleobase and sugar lesions that compromise the coding potential and strand integrity of DNA. Oxidation of C-1′ of the sugar portion of nucleotides produces 2-deoxyribonolactone (or oxidized abasic site) lesions in DNA. The biological effects of these lesions are unknown. To gain insight into the chemical biology of these lesions, an effective synthesis of DNA containing these labile sites is reported. A nucleoside phosphoramidite analogue has been synthesized and used to introduce the lesion in a photocaged form site-specifically into DNA by solid-phase DNA synthesis strategies. The stable analogue is incorporated into oligonucleotides with high efficiency and is subsequently decaged with light (355 nm) to reveal the oxidized abasic site lesion within the DNA strand. The chemical characterization and biochemical properties of the modified DNA, the lactone-containing DNA, and the DNA cleavage products will be reported.

152. DNA−protein cross-links generated by the flash−quench technique: Dependence upon DNA sequence. Suzie R. Ward, Alexis M. Lueras, and Eric D. A. Stemp. Department of Physical Sciences and Mathematics, Mount St. Mary's College, 12001 Chalon Road, Los Angeles, CA 90049 (suziward@msmc.la.edu)

DNA−protein cross-links are one consequence of oxidative damage to DNA. Guanine is the most easily oxidized DNA base, and its oxidation potential is known to depend on sequence. The flash−quench technique was used to produce the guanine radical, and the yield of DNA−protein cross-links was examined as a function of DNA sequence. Specifically, we compared cross-linking in the polynucleotides poly(dG-dC), poly(dG)-poly(dC), poly(dA-dT), poly(dA)-poly(dT), poly(dA-dC)·poly(dG-dT). As expected, the formation of cross-links requires guanine. Comparing was also examined for 20-mer DNA duplexes containing isolated G's and easily oxidized GG and GGG sequences.

153. Site-specific photo-cross-linking of the double-stranded RNA binding domain of PKR to a synthetic RNA ligand. Peter A. Beal and **Richard J. Spanggord**. Department of Chemistry, University of Utah, 315S 1400E RM DOCK, Salt Lake City, UT 84112 (fax: 801-581-8433, beal@chemistry.chem.utah.edu)

We are conducting site-specific modification of the PKR RNA binding domain with a photo-cross-linking reagent to study the interaction of this protein with a highly structured activating RNA. We initially replaced two cysteine residues that occur naturally in this protein with valine and showed that RNA binding and kinase activation were minimally affected. Cysteine was reintroduced at various amino acid positions, and the resulting mutant proteins were modified with the cysteine-specific photoactivable cross-linker TFPAM-3. TFPAM-3-modified proteins were characterized via electrospray mass spectrometry and bound RNA with comparable dissociation constants to wild-type protein. Primer extension mapping of the cross-linked RNA has identified modification sites within the 3′and 5′ loops of the RNA at high protein concentration. Further studies have resulted in similar cross-linking efficiencies at lower protein concentrations. These experimental results allow for the refinement of models for complexes formed between this RNA and PKR.

154. Effect of iron(III) chitosan as a phosphate binder on the reduction of serum phosphorus in rats, and phosphate binding ability of cross-linked iron chitosan. Joseph Baxter,[1] Shimizu Fuki,[1] Yasuyuki Takiguchi,[1] Masahiro Wada,[2] and Tatsuaki Yamaguchi.[1] [1]Department of Industrial Chemistry, Chiba Institute of Technology, 2-17-1, Narashino-shi, Chiba-ken, Tsudanuma, Japan (fax: (81)-476-47-0717, joseph@cba.att.ne.jp), and [2]Department of Nutrition, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Tokyo, Japan

An iron(III) chitosan complex and cross-linked iron chitosan were synthesized to study their phosphate binding capacity for control of hyperphosphatemia. Two *Wistar* strain rat groups ($n = 38$), a control and a treatment group, were studied for 30 days following administration of iron(III) chitosan. The serum phosphorus levels of the control group decreased slightly over the 30 day period whereas the treated group was significantly reduced after 15 days as compared to the respective control group ($P = 0.004$; control $= 5.7 \pm 0.9$ mg dL^{-1}, treatment $= 4.4 \pm 0.5$ mg dL^{-1}) and after 30 days ($P = 0.002$; control $= 5.5 \pm 0.9$ mg dL^{-1}, treatment $= 4.1 \pm 0.6$ mg dL^{-1}). Differences were also found in urine

phosphorus, feces phosphorus, feces iron, total cholesterol, HDL-C, and serum iron levels ($P < 0.05$). Other serum parameters were measured, but no significant differences were found.

155. Equilibrium constant measurements for the association of cyanine dyes with DNA. Jeffrey T. Petty, Jason A. Bordelon, and Mary E. Robertson. Chemistry Department, Furman University, Greenville, SC 29613 (jeff.petty@furman.edu)

The association of three monomeric cyanine dyes with double-stranded DNA was studied. The two thiazole orange derivatives and one oxazole yellow derivative are structurally similar, with a cationic aromatic system composed of a quinoline ring connected by a bridge to a benzothiazole or a benzoxazole ring. One significant difference is a positively charged alkylammonium substituent or an uncharged methyl substituent on the quinoline. Equilibrium constants were measured as a function of the ionic strength of the buffer to determine the number of condensed Na^+ that are released from the DNA backbone. In contrast to the other dyes, the benzothiazole dye with the alkylammonium side chain (TOPRO-1) releases fewer Na^+ than expected based on its net +2 charge. By comparison with the other cyanine dyes, the cationic side chain of TOPRO-1 is the source of the difference.

156. Human anti-Gal antibody affinity chromatography with mass spectrometric detection: An efficient method for screening a-Gal derivatives. Jianqiang Wang,[1] Boyan Zhang,[1] Jianwen Fang,[2] Wei Zhang,[2] Keiko Sujino,[1] Adam Janczuk,[2] Xiangping Qian,[1] Xi Chen,[2] Ole Hindsgaul,[1] Monica Palcic,[1] and Peng George Wang.[2] [1]Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada (fax: 780-492-7705, jianqiang.wang@chem.ualberta.ca), and [2]Department of Chemistry, Wayne State University, Detroit, MI 48202

Xenotransplantation (*1*), which is considered a means of overcoming the shortage of human organs, tissues, and cells for transplantation, has been hampered because of severe immunological rejection by the human body (*2*). The hyperacute rejection of organs by the human anti-Gal antibody is particularly important. Inhibition of human anti-Gal antibody binding to xenograft cells by infusing soluble synthetic a-Gal oligosaccharides is a potential method to overcome rejection. This requires tight-binding a-Gal derivatives (*3*). In this report, a series of a-Gal derivatives were designed and synthesized, and a new efficient method for screening a-Gal derivatives using the FAC/MS binding assay (*4*) was developed. The FAC/MS assay was developed recently in this laboratory for high-throughput screening of compound libraries and extract proteins such as selectins and enzymes (*4*). We here applied this method for screening a-Gal derivative binding to immobilized anti-Gal antibodies on a micro-column. This proved to be an effective method in screening a-Gal derivatives, and the K_d values of a-Gal derivative binding to anti-Gal antibodies could be easily determined by this method. (*1*) (a) Platt, J. L. (1998) *Nature* (April, Supplement to *392*), 11. (b) (1998) *Nature* (Special Issue) (January) *391*, 309. (c) Rother, R. P., and Squinto, S. P. (1996) *Cell 36*, 185. (*2*) (a) Galili, U. (1998) *Sci. Med. 5* (5), 28. (b) Cooper, D. K. C. (1992) *Clin. Trans. 6*, 178. (c) Cooper, D. K. C., Good, A. H., Koren, E., Oriol, R., Malcolm, A. J., Ippolito, R. M., Neethling, F. A., Ye, Y., Romano, E., and Zuhdi, N. (1993) *Transplant. Immunol. 1* (3), 198. (*3*) Wang, J.-Q., Chen, X., Zhang, W., Zacharek, S., Chen, Y., and Wang, P. G. (1999) *J. Am. Chem. Soc. 121*, 8174, and references cited therein. (*4*) (a) Schriemer, D. C., Bundle, D. R., Li, L., and Hindsgaul, O. (1998) *Angew. Chem., Int. Ed. Engl. 37*, 3383. (b) Schriemer, D. C., and Hindsgaul, O. (1998) *Combinat. Chem. High Throughput Screening 1*, 155.

157. Lactate dehydrogenase activity in wild populations of Chesapeake Bay sports fish species: Striped bass (*Morone saxatilis*), summer flounder (*Paralichthys dentatus*), Atlantic croaker (*Micropogonias undulatus*), and bluefish (*Pomatomus saltatrix*). Amelia G. Potter,[1] Steven G. Hughes,[2] Reginal M. Harrell,[3] Eric B. May,[2] and Theodore A. Mollett.[1] [1]Natural Sciences/Chemistry, University of Maryland Eastern Shore, Carver Hall, Backbone Road, Princess Anne, MD 21853 (fax: 410-651-7739, agpotter@mail.umes.edu), [2]Maryland Fish and Wildlife COOP Unit, Trigg Hall, Princess Anne, MD 21853, and [3]Horn Point Environmental Laboratory, Cambridge, MD

Preliminary profiles of lactate dehydrogenase (LDH) activity in four Chesapeake Bay sport fish species were determined to identify possible relationships between fish muscle LDH activity and their ability to cope with metabolic acidosis. The species used in this study were striped bass (*Morone saxatilis*), summer flounder (*Paralichthys dentatus*), Atlantic croaker (*Micropogonias undulatus*), and bluefish (*Pomatomus saltatrix*), and their selection was based on their physiological differences and their relative importance to the Maryland sport fishing industry. The results of our study indicated a trend for decreasing LDH activity in white muscle when comparing either muscle composition (i.e., white vs red) or relative activity levels. These fish were ranked, in order of decreasing LDH activity, as bluefish, striped bass, Atlantic croaker, and summer flounder. From the data collected in this study, we conclude that differences are observed in LDH activity for muscles or tissues producing differing amounts of lactate. The results of these studies further indicate lower LDH activity for pyruvate in each species when comparing white muscle to other tissues (heart muscle, red muscle, or liver tissue). These findings agree with literature reports that lactate is removed at its site of origin (i.e., white muscle).

158. Metal uptake and phytochelatin production in *Schizosaccharomyces pombe.* Joyce P. Whitehead, Catherine A. Guerrein, Erin C. Gramm, Nicole M. Gourley, and Margaret V. Mintz. Department of Chemistry, Dickinson College, Carlisle, PA 17013 (whitehea@dickinson.edu)

Cellular resistance to heavy-metal toxicity in plants is mediated by the binding of metal ions to cysteine-rich peptides known as phytochelatins (PC). Phytochelatins are synthesized by PC synthase from glutathione and can vary in length from 5 to 23 amino acids, depending on the metal ion to which the plant is exposed. The yeast, *S. pombe*, has the capacity to produce phytochelatins, and thus enables the study of these peptides. Yeast cultures were inoculated with different transition metal salts, and metal uptake, culture

health, and length of the resulting phytochelatins were monitored. By doing a systematic study where only the size of the metal ion used is varied, more can be learned about the production of these short peptides. The results of the metal uptake and culture health studies as well as the purification and analysis of the phytochelatins will be presented.

159. Molecular studies on *N*-acetylmuramyl-L-alanine amidase. Ross Lyndon Harding and T. D. H Bugg. Department of Chemistry, Warwick University, Coventry, CV4 7AL, United Kingdom (r.l.harding@warwick.ac.uk)

N-Acetylmuramyl-L-alanine amidase is a hydrolase enzyme which degrades bacterial cell walls (peptidoglycan) in order for bacteria to grow and separate. The inhibition of the biosynthesis of peptidoglycan has been widely studied; however, its breakdown by peptidoglycan hydrolases as a target for antibacterial action is not well established. A synthetic route has been devised for the assembly of thioester analogues of MurNAc-dipeptide, for use as enzyme substrates in a DTNB-coupled assay. The synthesis, involving a novel route to (*S*)-thiolactic acid, couples protected muramic acid with a dipeptide thiol. A hydroxamic acid and an epoxide have also been prepared as potential inhibitors of the enzyme and novel antibiotics.

160. Partial purification and characterization of glycosidases from *Aspergillus oryzae* and *Penicillium canescens*. Nataliya M. Samoshina,[1] Lyubov V. Yugova,[2] Paul H. Gross,[1] Galina B. Bravova,[2] Amelia A. Shishkova,[2] and Vyacheslav V. Samoshin.[1] [1]Department of Chemistry, University of the Pacific, 3601 Pacific Avenue, Stockton, CA 95211 (fax: 209-946-2607, vsamoshi@vms1.cc.uop.edu), and [2]Research Institute "Biotekhnologiya", Nauchny proezd 8, Moscow, 117246, Russia

Multienzyme compositions were purified from *Aspergillus oryzae* and *Penicillium canescens* with α-D-galactosidase, *β*-D-galactosidase, α-D-glucosidase, *β*-D-glucosidase, *β*-D-mannosidase, *β*-D-xylosidase, α-L-fucosidase, *β*-D-fucosidase, α-L-arabinofuranosidase, *β*-L-arabinofuranosidase, and α-L-rhamnosidase activities that varied from 10 to 2000 units/g against *p*-nitrophenyl-glycopyranosides. Kinetic parameters (K_M, V_{max}) and basic enzymatic properties (pH_{opt}, T_{opt}) were determined. Interesting regularities observed for these parameters will be discussed. The multienzyme preparations were used for preliminary testing of C-glycosides as potential enzyme inhibitors.

161. Protein renaturation by the liquid organic salt ethylammonium nitrate (EAN). Catherine A. Summers and Robert A. Flowers, II. Department of Chemistry, University of Toledo, Toledo, OH 43606 (fax: 419-530-4033, csummer@uoft02.utoledo.edu)

The room temperature liquid salt ethylammonium nitrate (EAN) has been used to enhance the recovery of denatured−reduced proteins. Our results show that EAN has the ability to prevent aggregation of denatured proteins while simultaneously assisting in their refolding. The use of EAN as a refolding additive is advantageous because the renaturation is a one-step process. Of particular interest is the fact that the refolded active protein can be separated from the molten salt by simple desalting methods. Although the use of a low-temperature molten salt in protein renaturation is unconventional, the power of this approach lies in its simplicity and utility.

162. Reagentless optical detection of glucose using genetically engineered galactose/glucose binding protein. Lyndon L. E. Salins and Sylvia Daunert. Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055 (fax: 606-323-1069, llesal1@pop.uky.edu)

The galactose/glucose binding protein (GBP) is a periplasmic protein that binds galactose and glucose in a highly specific manner. The tertiary structure of GBP consists of two globular domains connected by three short peptide linkages that serve as a hinge. The binding site is located in the deep cleft between these domains. The ligand induces a hinge motion in GBP that is accompanied by a change in the conformation of the protein. This constitutes the basis of the sensing system development. Wild-type GBP lacks cysteine residues in its structure; therefore, the introduction of a unique cysteine residue allows for the labeling of the protein at a desired position. Six mutants of GBP, where native amino acids were converted into cysteines, were prepared by site-directed mutagenesis using PCR. Site-specific labeling was achieved by attaching environment-sensitive fluorophores to the protein through the sulfhydryl group of the cysteine residue. Calibration plots for glucose and galactose were constructed by relating the fluorescence intensity with the concentration of ligand present in the sample solution.

163. Studying the organization of the MerR−RNA polymerase transcription complex by FeBABE-mediated cleavage. Saul A. Datwyler,[1] Resham D. Kulkarni,[2] Anne O. Summers,[2] and Claude F. Meares.[1] [1]Department of Chemistry, University of California, One Shields Avenue, Davis, CA 95616 (fax: 530-752-3386, sadatwyler@ucdavis.edu), and [2]Department of Microbiology, University of Georgia, Athens, GA 30602

Gene transcription is regulated through the interactions of many different transcription regulators with RNA polymerase (RNAP). There is limited information regarding the positioning of the metalloregulator MerR relative to σ^{70} when complexed with RNAP. MerR binds as a dimer to the promoter of the mercury resistance operon (*mer*) between the −10 and −35 recognition elements and sequesters RNAP in a closed complex. The presence of Hg(II) induces open complex formation by binding to MerR and underwinding the promoter. We examined the interactions of σ^{70} with MerR using a set of single cysteine mutants of σ^{70} conjugated to FeBABE [iron (*S*)-1-(*p*-bromoacetamidobenzyl)ethylenediaminetetraacetate]. The cleavage patterns of DNA and MerR with the σ^{70} conjugates provide evidence for protein−protein interactions between σ^{70} and MerR as well as the positioning of MerR relative to σ^{70} on the promoter.

164. Development of mutant RecA proteins with unique fluorescent residues. Scott F. Singleton, Michael D. Berger, Jr., Rebecca A. Simonette, and Andrew M. Lee. Departments of Chemistry and Biochemistry & Cell Biology, Rice University, P.O. Box 1892, MS 60, Houston, TX 77251-1892 (fax: 713-285-5155, sfs@rice.edu)

RecA-mediated DNA strand exchange is recognized as the prototypic recombination system and has been studied over the past 30 years. Research is focused on the use of intrinsic protein fluorescence as a spectroscopic handle to allow a stepwise dissection of the strand exchange process. The two tryptophans present in the wild-type protein have been replaced to create a fully functional nonfluorescent RecA protein. The permissivities of W290 and W308 to replacement are not identical. Moreover, the A289S mutation mitigates the tolerance of RecA recombination activity toward W290 mutation. Our "dark" RecA protein (W290Y and W308F) exhibits wild-type activity in all in vivo and in vitro assays. Replacement of other aromatic residues with tryptophan (e.g., H97W, Y103W, H163W, and F203W) in the dark RecA background generates readily interpretable spectroscopic changes that can be used to develop a more precise kinetic scheme.

165. Understanding the specificity of desulfurization by *Rhodococcus erythropolis* sp. IGTS8. Todd R. Smith, **Philip J. Smith**, and Linette M Watkins. Department of Chemistry and Waste Minimization and Management Research Center, Southwest Texas State University, 601 University Drive, San Marcos, TX 78666 (ts35163@hotmail.com)

Rhodococcus erythropolis sp. IGTS8 (IGTS8) has been identified as a potential biocatalyst for the selective removal of sulfur from petroleum and coal. Four enzymes are used in the desulfurization pathway in which IGTS8 utilizes a wide range of organosulfur compounds as its sulfur source. Over 2 dozen organosulfur compounds were tested for their ability to act as the sole sulfur source for IGTS8. Analogues were tested in whole-cell and cell-free assays using HPLC and spectrofluorometric assays. The level of desulfurization activity induced in the presence of a wide variety of organosulfur compounds was measured. Synthetically oxidized organosulfur compounds were prepared and purified. These compounds were used as standards to monitor partial activity of the desulfurization pathway and to define the specificity of the different enzymes in the desulfurization pathway. Some inferences about the possible mechanism of the enzymes in the pathway can be drawn from these data.

166. Differential scanning calorimetric study of the isoforms of apolipoprotein E. Prathima Acharya,[1] Julian Snow,[1] Phillips Michael,[2] Sissel Lund-Katz,[2] Karl Weisgraber,[3] Julie Morrow,[3] and Muhammed Zaion.[2] [1]Department of Chemistry & Biochemistry, University of the Sciences, 600 South 43rd Street, Philadelphia, PA 19104-4495 (fax: 215-596-8543, prasud@aol.com), [2]Department of Biochemistry, Medical College of Pennsylvania−Hahnemann, 2900 Queen Lane, Philadelphia, PA 19129, and [3]Department of Pathology, Gladstone Institute for Cardiovascular Research, Universitiy of California, San Francisco, CA 94140

Apo E, first identified as a component of VLDL, is now known to be associated with other lipoproteins as well, such as HDL and chylomicron remnants. As a ligand for cell surface receptors of the LDL family, apo E directs the uptake of chylomicron remnants and of VLDL. Three allele products, designated apo E2, apo E3, and apo E4 have been identified. A differential scanning calorimetric analysis of the unfolding patterns of the three isoforms and their 22 kDa fragments reveals interesting differences related to differences in conformations. That the heat capacity profiles of the 22 kDa fragments are very similar, if not identical, to those of the intact proteins is consistent with the idea that the corresponding domains in the intact proteins have similar structures. The intact proteins exhibit unfolding at lower temperatures than the fragments, indicating that the N-terminal domains are more stable than the C-terminal domains. The conformational changes that occur during thermal unfolding appear to be similar to those that occur when the lipid-free protein interacts with phospholipid. Results also suggest that the C- and N-terminal domains of apo E4 exhibit a high degree of structural independence, whereas those of apo E3 interact.

167. Comparison of various fluorescent tags for oligosaccharide mapping and characterization. Shirish T. Dhume and Kalyan R. Anumula. Bioanalytical Science, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, Mailcode UW2960, King of Prussia, PA 19406 (fax: 610-270-6727, Shirish_T_Dhume@SBPHRD.com)

Anthranilic acid (AA, 2-aminobenzoic acid) was introduced as the most sensitive and versatile fluorescent label for the analysis of glycosylation in terms of both monosaccharides and oligosaccharides (*1*). Methods based on AA labeling offer a significant improvement over the HPAEC−PAD methods for further characterization (e.g., sensitivity, resolution, low pH, and isolation of peaks in the salt-free form), and AA is compatible with spectroscopic methods. In this study, we have evaluated other fluorescent tags for carbohydrate analysis and their comparison to AA. AA was found to be superior in terms of sensitivity in comparison to all the other fluorescent tags tested. In addition, all the other tags tested either were not suitable or had a poor resolution in the HPLC mapping method that is used for AA (*2*). MALDI-TOF MS can be obtained with all the tags tested, and the intensities were comparable within a factor of 2.

168. Purification and characterization of the ADP-D-glycero-D-mannoheptose synthetase from *Helicobactor pylori.* Xin Du,[1] Anthony R. Welch,[2] and William G. Coleman, Jr.[1] [1]Laboratory of Biochemistry and Genetics, NIDDK, National Institutes of Health, Room 2A03, Building 8, 9000 Rockville Pike, Bethesda, MD 20892 (fax: 301-402-0240, xind@intra.niddk.nih.gov), and [2]Department of Research and Development, Diagnon Corporation, Rockville, MD

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, which is widely recognized as an important virulence factor in infection caused by Gram-negative bacteria. ADP-D-glycero-D-mannoheptose is a key intermediate of LPS inner core biosynthesis. The product of the *H. pylori* rfaE gene, the ADP-D-glycero-D-mannoheptose synthetase, is proposed to catalyze the synthesis of ADP-D-glycero-D-mannoheptose from D-glycero-D-mannoheptose-1P. The rfaE gene from *H. pylori* was cloned into the expression vector pGEX-6p-1 for overexpression in *E. coli*. The enzyme was purified using the Glutathione Sepharose 4B affinity column (Pharmacia Biotech). NH_2-terminal sequencing confirms that the purified

enzyme is the rfaE gene product. The enzymatic activity is confirmed by using its native substrate, D-glycero-D-mannoheptose-1P, and the product ADP-D-glycero-D-mannoheptose is formed. An assay was developed to measure this activity. This is the first demonstration of such enzyme activity in any Gram-negative bacteria.

169. Surveying the secondary structures of RNA random library. Tianbing Xia, Shuwei Li, Jie Xu, and Richard W. Roberts. Division of Chemistry and Chemical Engineering, California Institute of Technology, MC 147-75, Pasadena, CA 91125 (fax: 626-568-9430, tbxia@its.caltech.edu)

There is enormous sequencing information for nucleic acids, but the sequence space of RNA secondary structures has not been systematically investigated. The fundamental question of the likelihood of foldedness for any particular sequence remains unanswered. We present a strategy to determine the amount of secondary structure in RNAs with unknown folds using ^{1}H NMR spectroscopy. This method involves comparison of exchangeable and nonexchangeable protons, including a chemically inserted internal reference. Study on small-model nucleic acid systems showed that base pairing information can be readily obtained. The method is simple, rapid, and suitable for surveying secondary structures of the RNA library of random sequences.

170. Electroactive substrates that modulate cell growth. Muhammad N. Yousaf and Milan Mrksich. Department of Chemistry, University of Chicago, 5735 South Ellis Avenue, Chicago, IL 60637 (fax: 773-702-0805, mnyousaf@rainbow.uchicago.edu)

Cells do not exist in a static environment. The behavior of cells is influenced by changes in the ligands presented in the surrounding extracellular matrix. The activity of ligands can be modulated by enzymes, conformational changes, association with proteins, or wounding. Model substrates that can mimic these important processes would be extremely valuable for fundamental studies in cell biology. We now report a method based on an electroactive substrate that can alter, in real-time, the presentation of ligands to an attached cell. We use an in-situ Diels−Alder reaction and illustrate this approach by turning on the growth of cells patterned to a substrate.

171. Role of PC1 subunits in the regulation of PBS composition in the cyanobacterium *Fremyella displosiphon*. Dana C. Ciobanu and John Cobley. Department of Chemistry, University of San Francisco, 2130 Fulton Avenue, San Francisco, CA 94117-1080 (ciobda00@usfca.edu)

Efficient light harvesting is possible in *Fremyella diplosiphon* because this filamentous photosynthetic bacterium can alter the composition of its phycobilisome (PBS), composed of colored phycobiliproteins, in response to light quality. Although many of the genes encoding the phycobiliproteins of PBS have already been identified, the actual mechanism that connects perception of light to regulation of PBS composition still remains to be solved. One could attempt to put together the pieces of this puzzle by exploring the regulation of PBS synthesis through the use of colored *F. diplosiphon* mutants. Therefore, I am investigating how *F. diplosiphon* will deal with a deletion in the two genes, cpcB1 and cpcA1, encoding for the PC1 (phycocyanin) subunits of PBS that are synthesized in both green and red light. Here it will be examined what happens when the bacterium cannot synthesize the PC1 components and the effects of green and red light on cells missing these components. The genes cpcB1 and cpcA1 were deleted from a genomic fragment of DNA by standard recombination. The mutant cells were grown in red and green light in order to observe the phenotypic effects of this deletion. Southern blotting, which is currently in progress, will confirm that the deletion in the bacteria investigated is in fact the one desired.

Wednesday Afternoon—Repligen Award Symposium—P. A. Frey, Organizer

172. Low barrier hydrogen bonds and enzymatic catalysis. W. Wallace Cleland. Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (fax: 608-265-2904, cleland@enzyme.wisc.edu)

Short, strong (low barrier) hydrogen bonds occur when the p*K*'s of the atoms sharing the proton are similar. The overall distance is 2.5 Å or less, the deuterium fractionation factor is less than 0.5, the proton NMR chemical shift can approach 20 ppm, and deuterium or tritium substitution causes an upfield change in the chemical shift. Such bonds can have **H* values of 25 kcal/mol in the gas phase, and at least half that in water or other high dielectric medium. The strength of the hydrogen bond in an active site drops by ∼1 kcal/mol for each pH unit mismatch in p*K*'s. When a weak hydrogen bond in the initial enzyme−substrate complex is converted into a low barrier one by alteration of the p*K* of the substrate or catalytic group so that the p*K*'s match, the increase in hydrogen bond strength can be used to help catalyze the reaction. A well-established example of this is the reaction catalyzed by serine proteases. The p*K* of neutral histidine is 14, while that of aspartate is ∼6. Proton transfer from serine to permit attack on bound substrate produces protonated histidine, with a p*K* now matching that of aspartate. Studies with trifluoromethyl ketone inhibitors that form tetrahedral adducts show up to 5 orders of magnitude in binding strength as the result of formation of a low barrier hydrogen bond between aspartate and histidine. Other enzymes whose mechanisms appear to involve low barrier hydrogen bonds include liver alcohol dehydrogenase, steroid isomerase, triose-P isomerase, aconitase, citrate synthase, and zinc proteases. It is likely that low barrier hydrogen bonds form at the transition state of any reaction involving general acid or base catalysis, as at that point the p*K*'s of catalytic group and reactant will be equal.

173. Organic free radicals as enzymatic intermediates. Perry A. Frey. Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705 (frey@enzyme.wisc.edu)

5′-Deoxyadenosin-5′-yl is postulated to initiate radical formation in adenosylcobalamin- and *S*-adenosylmethinonine-dependent enzymatic reactions. Although 5′-deoxyadenosin-5′-yl has never been observed spectroscopically, an allylic analogue has been observed and characterized as

an intermediate in the reaction of lysine 2,3-aminomutase (KAM). Upon activation of KAM with 3′,4′-anhydroadenosylmethionine in the presence of a substrate, followed by freezing in liquid nitrogen, the EPR spectrum of 5′-deoxy-3′,4′-anhydroadenosin-5′-yl is observed, presumably because it is the most stable free radical in the mechanism. Activation with *S*-adenosylmethionine followed by freezing leads to substrate- or product-based free radicals, which have been characterized as intermediates. Suicide inactivation of diol-dehydrase with glycolaldehyde is adenosylcobalamin-dependent and leads to the cleavage of the coenzyme to cob(II)alamin and 5′-deoxyadenosine. The glycolaldehyde product has been characterized as a semidione free radical, which is presumably too stable to react further. (Supported by Grant DK 28607 from the NIDDK.)

174. Quinocofactor biogenesis in the copper amine oxidases: How does a single active site perform multiple functions? Judith P. Klinman, Joanne P Dove, Neal K. Williams, and Benjamin Schwartz. Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley, CA 94720-1460 (fax: 510-643-6232, klinman@socrates.berkeley.edu)

A single active site within the copper amine oxidases performs multiple functions that include cofactor biogenesis, amine oxidation to an aldehyde, and dioxygen reduction to hydrogen peroxide. Although cofactor biogenesis involves uptake of molecular oxygen, analogous to the oxidative half-reaction of enzyme turnover, its chemical process of tyrosine hydroxylation is far more difficult. Through a combination of kinectic and spectroscopic probes, together with the use of a mutant protein designed to have an altered redox potential at the active site copper, evidence has been obtained for a novel mechanism of tyrosine functionalization. This involves (i) tyrosine, rather than dioxygen, activation at the active site copper and (ii) dioxygen binding at a nonmetal site. The latter is both the trigger for tyrosine interaction with metal and separate from the subsequent chemical reaction of dioxygen. This mechanism can be related to that for oxygen consumption during catalytic turnover. [Supported by a grant from the NIH (GM 39296).]

175. Two solutions to the same chemical problem: Transferring methyl groups to thiols. Rowena G. Matthews. Biophysics Research Division and Department of Biological Chemistry, University of Michigan, 4028 Chemistry, 930 North University, Ann Arbor, MI 48109-1055 (fax: 734-764-3323, rmatthew@umich.edu)

Two enzymes in *Escherichia coli* catalyze the transfer of a methyl group from methyltetrahydrofolate to homocysteine: cobalamin-dependent methionine synthase (MetH) and cobalamin-independent methionine synthase (MetE). These two enzymes lack homology in their primary sequences and appear to have evolved independently. We are interested in determining their detailed reaction mechanisms and in understanding the latitude that enzymes enjoy in catalyzing challenging chemical reactions. Is there really more than one way to skin a cat? We have recently shown that the two enzymes activate homcysteine for nucleophilic attack at neutral pH in a similar manner: by coordination of the thiolate to a catalytically essential zinc ion. However, the scaffolds provided by each protein for zinc are completely different, reflecting independent evolutionary origin. We are now seeking to elucidate the manner in which methyltetrahydrofolate is activated for nucleophilic displacement of the methyl group.

Thursday Morning—Protein Folding—G. Lorimer, Organizer

176. Genesis of Methuselah proteins: Roles for barriers, metastability, and pro regions. David A. Agard. Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of California, 513 Parnassus Avenue, Room S-1057, San Francisco, CA 94143-0448 (fax: 415-476-1902, agard@msg.ucsf.edu)

Abstract text not available.

177. Intramolecular chaperone mediated folding of subtilisin. Ujwal P. Shinde, Xuan Fu, Cynthia Marie-Claire, Yukihiro Yabuta, and Masayori Inouye. Department of Biochemistry, Robert Wood Johnson Medical School—UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854 (fax: 732-235-4559, shinde@rwja.umdnj.edu)

The subtilisin propeptide acts as an intramolecular chaperone (IMC) that imprints structural information onto its protease domain. This phenomenon was termed as 'protein memory'. IMC-mediated folding occurs in numerous protein families, and their sequence analysis suggests that IMC domains have higher mutational frequencies than their cognate enzymatic domains. Using a chimera, we show that IMC from aqualysin can modulate the enzymatic properties and stability of mature subtilisin. On the basis of the X-ray structure of the IMC—subtilisin complex, we introduced a mutation that destabilizes the interactions at the interface between the IMC and subtilisin. Our results demonstrate that folding is not obligatorily linked to the inhibitory potency of the IMC domain. Furthermore, we trapped a stable folding-competent intermediate by introducing a disulfide bridge between Met(−60) within the IMC and Ser(188) in the subtilisin domain. We will discuss how IMC facilitates folding and imprints structural information onto subtilisin.

178. Cotranslational folding of bacterial luciferase. Thomas O. Baldwin. Biochemistry Department, University of Arizona, Tucson, AZ 85721 (fax: 520-626-9204, tbaldwin@u.arizona.edu)

One function shared by all proteins, and encoded in their amino acid sequences, is folding of the growing nascent chain within the cellular milieu to yield the biologically active structure. We have found that the bacterial luciferase β subunit reaches its final native form, the $\alpha\beta$ heterodimer, much more rapidly during biosynthetic folding than during refolding from urea. The rate of formation of active enzyme is determined by an unstable short-lived folding intermediate, which is able to associate with the α subunit very rapidly following release from the ribosome. The short-lived intermediate is formed in part by interactions involving the C-terminal section of the β subunit, interactions that do not exist in the final, biologically active, heterodimer; this intermediate equilibrates with a more stable species which does not interact with α. This mechanism embodies a major strategy, the avoidance of slow-folding intermediates and

kinetic traps, that may be employed by many proteins for achieving fast and efficient biosynthetic folding.

179. Chaperonins: Nanomachines that facilitate protein folding. George H. Lorimer. Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742 (fax: 301-352-5539, GL48@umail.umd.edu)

The in vitro folding of some proteins under quasi-physiological conditions is facilitated by the chaperonin proteins, GroEL and GroES, driven by the hydrolysis of ATP. The chaperonins function as mechano-chemical nanomachines. The two heptameric rings of GroEL operate 180° out-of-phase with one another, in the manner of a 'two-stroke' reciprocating engine. The binding of ATP to one ring (ring A) of GroEL triggers the power stroke of the chaperonin cycle, resulting in huge, concerted domain movements in the GroEL ring. These are thought to be coupled to a mechanical, stretch-induced unfolding of the substrate protein and ultimately to the transient encapsulation of the substrate protein. Encapsulation involves the vectoral displacement of the substrate protein by the 'mobile loops' of GroES into the expanded central cavity of GroEL. Binding of ATP to the other GroEL ring (ring B) signals the 'exhaust stroke' in ring A, resulting in the dissociation of GroES and the expulsion of the 'exhaust products' of the chaperonin cycle, ADP and the substrate protein.

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