

2007

South West
Macromolecular
Symposium

Session Abstracts

Table of Contents

Structural basis for conserved complement factor-like function in the antimalarial protein TEP1.....	2
Biophysical Studies on the Tp34 Lipoprotein of <i>Treponema pallidum</i>	3
Crystal Structures of Multidrug Resistance Regulator EbrR.....	4
Crystal Structure of the Novel “Open” Conformation of Copper-Zinc Superoxide Dismutase: Pseudosymmetry, Merohedral Twinning, and Implications for Copper Chaperone (CCS) Function and SOD1-linked Familial ALS.....	5
Crystal Structure of <i>Medicago truncatula</i> UGT85H2 - Insights into the Structural Basis of a Multifunctional (Iso)flavonoid Glycosyltransferase.....	6
Allosteric Motions in Structures of Yeast NAD ⁺ -Specific Isocitrate Dehydrogenase ...	7
An Excursion from Ser/Thr to Tyrosine Kinase Activity Underlies the Threshold Behavior of MAPK Modules.....	8
Crystal Structures of GSI-? Glutamine Synthetase from <i>Bacillus subtilis</i> bound to ATP with glutamate and the ATP analogue, AMP-PCP.....	9
Structural Biology of the <i>E. coli</i> stress response proteins: YedU and YciF.....	10
Structural Mechanism of Organic Hydroperoxide Induction of the Transcription Repressor OhrR.....	11
Segrosome structure revealed by ParR-centromere complex.....	12
Epigenetic control of transcription through histone demethylation by LSD1.....	13
Domain-domain flexibility leads to allostery within the cAMP Receptor Protein (CRP).....	14
Improving protein crystal quality by selective removal of a Ca ²⁺ -dependent membrane insertion loop.....	15
Trash to Treasure: Changing a Poorly Diffracting Frozen Crystal to a High Resolution Data Set.....	16
HomeLabs Vs. Synchrotrons: The Facts Will Surprise You.....	17

Structural basis for conserved complement factor-like function in the antimalarial protein TEP1.

Authors: Richard H. G. Baxter[1], Chung-I Chang[1], Yogarany Chelliah[1], Stéphanie Blandin[2], Elena A. Levashina[2], and Johann Deisenhofer[1]

[1] Howard Hughes Medical Institute and Department of Biochemistry, University of Texas Southwestern Medical Center, 6001 Forest Park Road, Dallas, TX 75390-9050.

[2] Institut de Biologie Moléculaire et Cellulaire, UPR9022 du Centre National de la Recherche Scientifique, Équipe "Avenir" Institut National de la Santé et de la Recherche Médicale, 67084 Strasbourg, France.

Abstract: Thioester-containing proteins (TEPs) are a major component of the innate immune response of insects to invasion by bacteria and protozoa. TEPs form a distinct clade of a superfamily that includes the pan-protease inhibitors alpha2-macroglobulins and vertebrate complement factors. The essential feature of these proteins is a sequestered thioester bond that, after cleavage in a protease-sensitive region of the protein, is activated and covalently binds to its target. Recently, TEP1 from the malarial vector *Anopheles gambiae* was shown to mediate recognition and killing of ookinetes from the malarial parasite *Plasmodium berghei*, a model for the human malarial parasite *Plasmodium falciparum*. Here, we present the crystal structure of the TEP1 isoform TEP1r. Although the overall protein fold of TEP1r resembles that of complement factor C3, the TEP1r domains are repositioned to stabilize the inactive conformation of the molecule (containing an intact thioester) in the absence of the anaphylotoxin domain, a central component of complement factors. The structure of TEP1r provides a molecular basis for the differences between TEP1 alleles TEP1r and TEP1s, which correlate with resistance of *A. gambiae* to infection by *P. berghei*.

Biophysical Studies on the Tp34 Lipoprotein of *Treponema pallidum*

Chad A. Brautigam¹, Ranjit Deka², Diana R. Tomchick¹, Mischa Machius¹, Farol L. Tomson², Sarah B. Lumpkins¹, and Michael V. Norgard².

Departments of ¹Biochemistry and ²Microbiology, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, 75390.

Treponema pallidum, an obligate human pathogen and the causative agent of syphilis, is not cultivatable *in vitro*, making it intractable to conventional genetic approaches to discovering the functions of gene products. This difficulty has prompted our labs to pursue a structure-to-function approach to study the lipoproteins of *T. pallidum*. One target is the ~20 kDa protein called Tp34 (TP0971). The crystal structure of a nonacylated, recombinant form of Tp34 (rTp34), determined at 1.9Å resolution, revealed two metal-occupied binding sites within a dimer; the identity of the ion most likely was zinc. Residues from both of the monomers contributed to the interfacial metal-binding sites. Previous reports had implied that Tp34 could bind human lactoferrin (hLF). Analytical ultracentrifugation showed that, in solution, rTp34 formed a metal-stabilized dimer and that rTp34 bound hLF with a stoichiometry of 2:1. Isothermal titration calorimetry further revealed that rTp34 bound hLF at high (submicromolar) affinity. Finally, membrane topology studies revealed that native Tp34 is not located on the outer surface (outer membrane) of *T. pallidum* but, rather, is periplasmic. How the propensity of Tp34 to bind zinc and the iron-sequestering hLF may relate overall to the biology of *T. pallidum* infection in humans is discussed.

Crystal Structures of Multidrug Resistance Regulator EbrR

Jinhui Dong, Lisheng Ni, and Richard G. Brennan

*Department of Biochemistry and Molecular Biology, MD Anderson Cancer Center,
Houston, TX*

In bacteria, Multidrug resistance transporters are tightly controlled by transcription regulators. EbrR is a putative DNA binding protein of the AcrR/TetR family. It is suggested that EbrR function as the repressor of its upstream *ebrA* gene, a putative multidrug resistance transporter of small multidrug resistance (SMR) family. Preliminary data indicate that EbrR can bind multiple structurally dissimilar chemicals. We use EbrR as a model protein to study the structural mechanisms of multidrug recognition.

Crystal structures of EbrR in complex with three different chemicals have been solved with MAD data or molecular replacement. Overall fold of EbrR structure is similar to those of other TetR family members. Like another multidrug resistance regulator of TetR family QacR, EbrR also has a large flexible substrate-binding pocket, which utilizes aromatic and negative-charged residues to form specific interactions with substrates. Substrate binding causes large conformational changes. Moreover, the structures might also explain the interactions between “drugs” observed in drug binding.

Crystal Structure of the Novel “Open” Conformation of Copper-Zinc Superoxide Dismutase: Pseudosymmetry, Merohedral Twinning, and Implications for Copper Chaperone (CCS) Function and SOD1-linked Familial ALS.

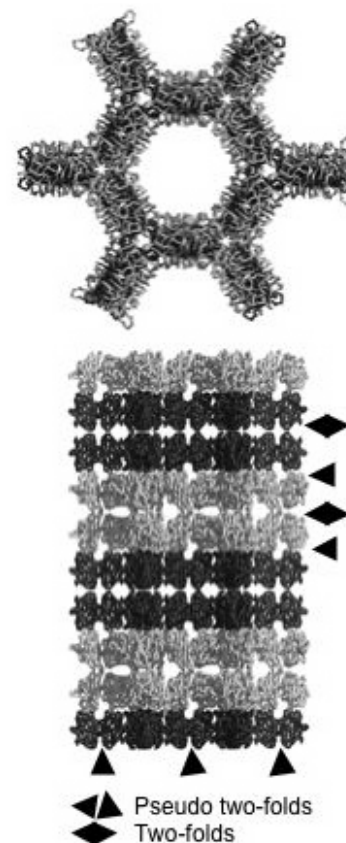
Jonathan P. Schuermann, Angela J. Rodriguez, Xiaohang Cao, Alexander B. Taylor, and P. John Hart

Department of Biochemistry and the X-ray Crystallography Core Laboratory, The University of Texas Health Science Center at San Antonio, San Antonio, TX USA

Approximately 115 distinct mutations in human copper-zinc superoxide dismutase (SOD1) cause an inherited form of amyotrophic lateral sclerosis (ALS, motor neuron disease). A helper protein, the copper chaperone for SOD (CCS), modifies nascent SOD1 by inserting the catalytic copper and oxidizing the disulfide bond in each subunit. These posttranslational alterations impart enormous stability to the enzyme and are therefore critical for the understanding of SOD1-linked fALS, which is now accepted to be a protein misfolding and aggregation disease.

One emphasis of this talk will be the significance of the crystal structure of an immature, pathogenic human SOD1 variant that is recognized, but cannot be acted upon by CCS. The asymmetric unit contains one canonical Zn-bound SOD1 homodimer and one completely novel, metal-free, disulfide reduced homodimer. The unanticipated “open” conformation appears only to newly synthesized SOD1 polypeptides. Gel shift and solution biophysical data strongly support the notion that the open conformation of SOD1 is bound tightly by CCS. Modeling studies using existing CCS structures suggest a mechanism of copper delivery different from that currently accepted in the field. The enhanced understanding of SOD1-CCS interactions that arises from the structure suggests, for the first time, a unifying characteristic shared by each of the ~115 pathogenic SOD1 mutations identified to date.

The other emphasis of this talk will be on complications caused by crystals with perfect hemihedral twinning combined with pseudosymmetry. The two distinct pathogenic SOD1 dimers in the asymmetric unit in space group P312 pack in a six-fold “honeycomb” arrangement (top right) in alternating bilayers such that each subunit is related to the others by both crystallographic and pseudosymmetric axes of rotation. The convoluted path leading to the eventual final structure will be described in the hope that others will not have to endure the trials and tribulations we experienced in this process. In the end, the observed differences in pathogenic SOD1 structure, and the insight into CCS action and fALS etiology derived from them, would have been missed completely had the twinning issues not been detected and handled properly.



Crystal Structure of *Medicago truncatula* UGT85H2 - Insights into the Structural Basis of a Multifunctional (Iso)flavonoid Glycosyltransferase

Lenong Li, Luzia V. Modolo†, Luis L. Escamilla-Trevino,
Lahoucine Achnine, Richard A. Dixon, and Xiaoqiang Wang

Plant Biology Division, Samuel Roberts Noble Foundation,
2510 Sam Noble Parkway, Ardmore, OK 73401, USA

(Iso)flavonoids are a diverse group of plant secondary metabolites with important implications for plant, animal and human health. They exist in various glycosidic forms. Glycosylation, which may determine their bioactivities and functions, is controlled by specific plant uridine diphosphate glycosyltransferases (UGTs). A new multifunctional (iso)flavonoid glycosyltransferase, UGT85H2, was identified from the model legume *Medicago truncatula* with activity towards a number of phenylpropanoid-derived natural products including the flavonol kaempferol, the isoflavone biochanin A, and the chalcone isoliquiritigenin. The crystal structure of UGT85H2 has been determined with molecular replacement using program Phaser and the structure of UGT71G1 (PDB ID: 2ACV) as a search model, and refined at 2.1 Å resolution to an R factor of 19.7% and R_{free} of 24.2%. UGT85H2 displays a similar GT-B fold observed previously for UGT71G1, and consists of two N- and C-terminal domains with similar Rossmann-type folds. The structure of UGT85H2 reveals distinct structural features which are different from those of other UGTs and related to the enzyme's functions and substrate specificities. Structural and comparative analyses revealed the putative binding sites for the donor and acceptor substrates which are located in a large cleft formed between the two domains of the enzyme, and indicated that Trp360 may undergo a conformational change after sugar donor binding to the enzyme. Further substrate docking combined with enzyme activity assay and kinetic analysis provided structural insights into this substrate specificity and preference.

Allosteric Motions in Structures of Yeast NAD⁺-Specific Isocitrate Dehydrogenase

Alex Taylor, Gang Hu, Lee McAlister-Henn, and P. John Hart

*Department of Biochemistry and the X-ray Crystallography Core Laboratory
University of Texas Health Science Center, San Antonio, TX, USA*

Mitochondrial NAD⁺-specific isocitrate dehydrogenases (IDHs) are key regulators of flux through biosynthetic and oxidative pathways in response to cellular energy levels. The affinity of the yeast IDH enzyme for isocitrate is enhanced upon the binding of AMP and diminished by ATP and NADH. This metabolite-mediated control contributes to the inverse relationship between rates of energy production by oxidative pathways and glycolysis. Under conditions of energy sufficiency, *i.e.* when relative cellular ratios of [ATP]/[AMP] and of [NADH]/[NAD] are high, flux through the tricarboxylic acid (TCA) cycle is attenuated at the level of IDH, rates of glycolysis increase, and the tricarboxylic acids, citrate and isocitrate, are diverted into biosynthetic pathways. Unlike the homodimeric bacterial enzymes that contain two equivalent IDH subunits, yeast IDH is composed of four IDH1 and four IDH2 subunits that are 47% identical. Each IDH1 and IDH2 subunit associates to form a heterodimer, and four heterodimers assemble into the biologically relevant ~300 kDa heterooctamer. IDH2 has retained the catalytic isocitrate/Mg²⁺ and NAD binding sites, while IDH1 has diverged to bind isocitrate and AMP, thereby acting as a regulatory subunit. Although well-characterized kinetically, the molecular basis for allosteric regulation of eukaryotic IDH enzymes has been elusive because, until now, no structures of eukaryotic IDH enzymes were known. To further our understanding of regulatory mechanisms, we have determined the X-ray crystal structures of the functional yeast IDH heterooctamer in the presence and absence of regulatory ligands. The results suggest that the eukaryotic enzymes are exquisitely tuned to ensure that allosteric activation occurs only when concentrations of isocitrate are elevated. This fine-control stands in stark contrast to the on-off regulation of non-allosteric bacterial isocitrate dehydrogenases via phosphorylation.

An Excursion from Ser/Thr to Tyrosine Kinase Activity Underlies the Threshold Behavior of MAPK Modules

John M. Humphreys,¹ Seung-Jae Lee,¹ Haixia He,¹ Prashanti Madhavapeddi,² Yue Chen,¹ Yingming Zhao,¹ and Elizabeth J. Goldsmith¹

¹ Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75390-8816

² AstraZeneca India Private Ltd., Bellary Road, Hebbal, Bangalore - 560024 India

How MAP kinase modules (comprised of a MAP3K, a MAP2K and a MAPK) induce switch-like sigmoid responses has remained unclear. The switch depends upon four kinase reactions, two each on the activation loops of MAP2Ks and MAPKs, to activate first a dual specificity MAP2K, then a Ser/Thr MAPK. Here we show that the dual specificity of the central MAP2Ks depends upon phosphorylation status, like dedicated Tyrosine and Ser/Thr kinases. The MAP2K MEK6 adopts different conformations as it becomes phosphorylated, the first is active as a Ser/Thr kinase, but inactive toward its substrate MAPK p38a. The second is competent as a Tyrosine kinase, the first step in MAPK activation. Thus, the dual-specificity of the MAP2K is used to establish a precise order to the kinase reactions catalyzed by the MAP kinase module, taking a phosphorylation-directed detour from Ser/Thr to Tyrosine kinase activity that creates extra thresholds to MAPK activation.

Crystal Structures of GSI- α Glutamine Synthetase from *Bacillus subtilis* bound to ATP with glutamate and the ATP analogue, AMP-PCP

David Murray, Ryan Watkins, Maria Schumacher, and Richard Brennan

*Department of Biochemistry and Molecular Biology, MD Anderson Cancer Center,
Houston, TX*

Glutamine synthetase (GS) catalyzes the two-step, ATP-dependent reaction converting glutamate and ammonia into glutamine, ADP and inorganic phosphate. Glutamine synthetases are divided into two major categories in bacteria: GSI- α and GSI- β . GSI- β is found in high G+C gram positive bacteria, proteobacteria, and others and is controlled by protein adenylylation. GSI- α is found in low G+C gram positive bacteria, Euryarchaeota, and some thermophilic bacteria and is regulated by glutamine and AMP dependent feedback inhibition. This feedback inhibition is dependent upon the glutamine bound GSI- α forming a complex with the TnrA transcription factor, which in turn cannot repress GlnR, the repressor of the gene encoding GSI- α ?The structure of GSI- α in *B. subtilis* has been solved in its APO form, bound to the ATP analogue AMP-PCP, and with both ATP and glutamate at 2.55, 2.74, and 3.10 Å resolution, respectively.

The structure GSI- α was solved in its functional dodecameric state, with each subunit containing 444 amino acids (5328 residues in total). Between 440 and 444 residues were built into each subunit. This structure was solved by molecular replacement, using the *M. tuberculosis* GSI- β as a search model. The structure of *B. subtilis* GSI- α has two domains, a large C-terminal domain containing the active site and a smaller N-terminal domain. The binding pocket has a bifunnel structure with ATP entering the “top” and glutamate and NH_4^+ entering at the “bottom” and “side”. Two residues seem to be most important in binding ATP and AMP-PCP: Y201 and R331. The tyrosine and arginine form a π -stacking interaction with the adenine ring, with R331 forming additional electrostatic interactions with the phosphate groups. Glutamate is held in place by a histidine and arginine residue and by the gamma-phosphate of ATP. Early analysis of the difference maps indicates the presence of the gamma-glutamyl phosphate intermediate.

Structural Biology of the *E. coli* stress response proteins: YedU and YciF

Aditya Hindupur, Deqian Liu, Yonghong Zhao, Henry D. Bellamy, Mark A. White, and Robert O. Fox

Department of Human Biological Chemistry & Genetics University of Texas Medical Branch, Galveston, TX

Bacteria are capable of responding to a variety of stress conditions. These conditions include heat, osmotic shock, acid/alkali. The heat shock response results in production of a variety of proteins ranging from the molecular chaperons GroEL, ClpA, ClpB, to specific proteins whose functions are now being elucidated. Here we report the structure and potential biochemical function of two such proteins: YedU and YciF. The yedU mRNA is induced 31-fold on heat shock and is enhanced in an hns-deletion strain that is known to derepress the stress response. It is a 31 kDa protein that forms dimers in solution. The structure shows alpha-beta characteristics and is a member of the Class I glutamine amidotransferase superfamily that includes proteases, catalases and transcriptional regulators. YciF is an 18.5 kDa protein found as a dimer in solution. The protein is conserved across eubacterial species. The structure of the apo-protein was solved at 2.0 Å resolution. The protein forms 4 helix bundle with an additional 5th helix. Structurally YciF was found to be close to the archaeal diiron protein rubrerythrin. Another archaeal protein sulerythrin shows similar features to YciF in lacking a rubredoxin domain found on rubrerythrin.

Structural Mechanism of Organic Hydroperoxide Induction of the Transcription Repressor OhrR

Kate J. Newberry¹, Mayuree Fuangthong², Skorn Mongkolsuk² and Richard G. Brennan¹

¹*Department of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX;* ²*Laboratory of Biotechnology, Chulabhorn Research Institute, Lak Si, Bangkok 10210, Thailand*

OhrR, a member of the MarR family of transcriptional regulators is widely conserved amongst Gram negative and Gram positive bacteria, and activates expression of the *ohr* peroxidase gene in response to organic hydroperoxides (OHP), but not hydrogen peroxide. The soil bacterium *Xanthomonas campestris* (Xc) contains an OhrR that is a specific sensor of lipid hydroperoxides, such as linoleic acid hydroperoxide, that are highly toxic compounds generated by the plant host defense response. These lipid hydroperoxides are sensed via oxidation of a conserved cysteine residue near the N-terminus of the protein. The recently described crystal structures of *Bacillus subtilis* OhrR in both the apo- and DNA-bound forms describes the structure a single cysteine containing OhrR in the reduced form and although these structures give valuable insight into the DNA-binding mechanism of the MarR family of transcriptional regulators, the atomic details of the mechanism of oxidation-induced derepression by either family of OhrR remains unclear. We have recently determined the crystal structures of both reduced and oxidized OhrR, each to 1.9Å resolution. In the reduced form the disulfide forming cysteine residues C22 and C127 are 15.5Å apart. Upon oxidation by OHP, OhrR undergoes a dramatic structural rearrangement of helices ? 5 and ? 6 that brings residues C22 and C127 into proximity, thereby allowing disulfide bond formation and a reconfiguration of the dimer interface, which disrupts DNA binding. Moreover, these structures in combination with *in vivo* and *in vitro* studies on a series of Xc OhrR point mutants provide an OHP-induction roadmap that is likely utilized by the vast majority of the MarR family.

Segrosome structure revealed by ParR-centromere complex

Maria A. Schumacher¹, Tiffany C. Glover¹, Anthony J. Brzoska², Slade O. Jensen², Thomas Dunham¹, Ronald A. Skurray² & Neville Firth²

¹Department of Biochemistry and Molecular Biology, University of Texas, M.D. Anderson Cancer Center, Unit 1000, Houston TX 77030, U.S.A.

²School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006, Australia

The stable inheritance of genetic material depends on accurate DNA partition. Plasmids serve as tractable model systems to study DNA segregation because they require only a DNA centromere, a centromere-binding protein and a force-generating ATPase. The centromeres of partition (*par*) systems typically consist of a tandem arrangement of direct repeats. The best-characterized *par* system contains a centromere-binding protein called ParR and an ATPase called ParM. In the first step of segregation, multiple ParR proteins interact with the centromere repeats to form a large nucleoprotein complex of unknown structure called the segrosome, which binds and recruits ParM filaments. pSK41 ParR binds a centromere consisting of multiple 20-bp tandem repeats to mediate both transcription autoregulation and segregation. Here we report the structure of the pSK41 segrosome revealed in the crystal structure of a ParR-DNA complex. In the crystals, the 20-mer tandem repeats stack pseudo-continuously to generate the full-length centromere with the ribbon-helix-helix fold of ParR binding successive DNA repeats as dimer-of-dimers. Remarkably, the dimer-of-dimers assemble in a continuous protein super-helical array wrapping the DNA about its positive convex surface to form a large segrosome with an open, solenoid-shaped structure. Cryo-EM studies of the complex reveals a circular helical structure with a diameter identical to that seen in the crystal structure, supporting the segrosomal crystal structure model. Importantly, this helical superstructure suggests a mechanism for ParM capture and subsequent plasmid segregation.

Epigenetic control of transcription through histone demethylation by LSD1

Mischa Machius,

Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, 75390.

Histone methylation regulates diverse chromatin-templated processes, including transcription. The recent discovery of the first histone lysine-specific demethylase (LSD1) has changed the long-held view that histone methylation is a permanent epigenetic mark. Many transcriptional corepressor complexes contain LSD1 and CoREST that collaborate to demethylate mono- and dimethylated H3-K4 of nucleosomes. LSD1 is a flavin adenine dinucleotide (FAD)-dependent amine oxidase that demethylates histone H3 Lys4 (H3-K4). We have determined the crystal structure of the LSD1-CoREST complex. LSD1-CoREST forms an elongated structure with a long stalk connecting the catalytic domain of LSD1 and the CoREST SANT2 domain. LSD1 recognizes a large segment of the H3 tail through a deep, negatively charged pocket at the active site and possibly a shallow groove on its surface. CoREST SANT2 interacts with DNA. Disruption of the SANT2-DNA interaction diminishes CoREST-dependent demethylation of nucleosomes by LSD1. The shape and dimension of LSD1-CoREST suggest its bivalent binding to nucleosomes, allowing efficient H3-K4 demethylation. This spatially separated, multivalent nucleosome-binding mode may apply to other chromatin-modifying enzymes that generally contain multiple nucleosome binding modules. In order to elucidate the mechanism by which LSD1 achieves its substrate specificity, we have further determined the crystal structure of LSD1 with a propargylamine-derivatized H3 peptide covalently tethered to FAD. H3 adopts three consecutive gamma-turns, enabling an ideal side-chain spacing that places its N terminus into an anionic pocket and positions methyl-Lys4 near FAD for catalysis. The LSD1 active site cannot productively accommodate more than three residues on the N-terminal side of the methyllysine, explaining its H3-K4 specificity. Dysregulation of histone acetylation and methylation leads to the silencing of tumor suppressor genes and contributes to cancer progression. Inhibitors of enzymes that catalyze the addition and removal of these epigenetic marks thus have therapeutic potential for treating cancer. Because LSD1 belongs to the family of FAD-dependent amine oxidases, certain inhibitors of monoamine oxidases (MAOs), including the clinically used antidepressant trans-2-phenylcyclopropylamine (PCPA; tranlycypromine; Parnate), are also capable of inhibiting LSD1. We have characterized the kinetic parameters of the inhibition of LSD1 by PCPA and determined the crystal structure of LSD1-CoREST in the presence of PCPA. PCPA forms a covalent adduct with FAD in LSD1 that is distinct from the FAD-PCPA adduct of MAO B. Our study provides the basis for designing more potent inhibitors of LSD1 with therapeutic potential, either derived from the LSD1-bound H3 peptide or derived from PCPA, exploiting the unique structural characteristics of the LSD1 substrate-binding site.

Domain-domain flexibility leads to allostery within the cAMP Receptor Protein (CRP)

James E. Knapp, Mark A. White, James C. Lee

Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX 77555

CRP is a homodimeric protein with each subunit having cAMP-binding and DNA-binding domains connected by a short hinge region. Each subunit has a high affinity cAMP and low affinity cAMP binding sites. CRP is an excellent model system to study allosteric protein regulation because there is a direct relationship between protein dynamics and cooperative cAMP and DNA binding. This relationship was demonstrated by hydrogen-deuterium exchange and adiabatic compressibility experiments on a series of mutations. Both experiments measure global dynamics. These results showed that binding energetics ($\Delta\Delta G$) between the binding of the first and second cAMP to each sample is correlated to the global protein dynamics¹. Furthermore, the energetics of DNA binding (ΔG) follows the same trend¹. The crystallographic analysis of WT in the presence of differing concentrations of cAMP shows that DNA binding in solution is correlated with flexibility of the domain-domain hinge motion as judged by the difference in the hinge angle between NCS related subunits. Similarly, the structural analysis of the D53H and S62F CRP mutations shows that mutation induced changes in the energetics of DNA binding and cooperative energetics of cAMP binding correlate with the domain-domain flexibility of each protein. Thus, the crystallographic work presented here is consistent with the hypothesis that the domain-domain dynamics mediates allostery within the CRP system.

¹. Gekko, K., Obu, N., Li, J., Lee, J.C. (2004) "A linear correlation between the energetics of allosteric communication and protein flexibility in the Escherichia coli cyclic AMP receptor protein revealed by mutation-induced changes in compressibility and amide hydrogen-deuterium exchange." *Biochemistry* **43**: 3844-52.

Improving protein crystal quality by selective removal of a Ca²⁺-dependent membrane insertion loop

David B. Neau^b, Nathaniel C. Gilbert^a, Sue G. Bartlett^a, Adam Dassey^a, and Marcia E. Newcomer^a

^aDepartment of Biological Sciences and the ^bCenter for Advanced Microstructures and Devices, Louisiana State University, Baton Rouge, LA

Lipoxygenases (LOX) catalyze the regio- and stereo- specific dioxygenation of polyunsaturated membrane-embedded fatty acids . A Ca²⁺-dependent membrane-binding function was localized to the amino terminal C2-like domain of 8R-lipoxygenase (8R-LOX) from the soft coral *Plexaura homomalla* (Oldham et al., 2005). The 3.2 Å resolution crystal structure of 8R-LOX and spectroscopic data suggested that Ca²⁺ stabilizes two membrane insertion loops. Analysis of the protein packing contacts in the crystal lattice indicated that the conformation of one of the two loops complicated efforts to improve the resolution of the X-ray data. A deletion mutant of 8R-LOX, [delta]40-45:GSLOX, in which the corresponding membrane insertion loop is absent was engineered. Removal of the membrane insertion loop dramatically increased the protein yield from bacterial cultures and the quality of the crystals obtained, resulting in an improvement in resolution better than 1 Å for the diffraction data.

Trash to Treasure: Changing a Poorly Diffracting Frozen Crystal to a High Resolution Data Set

Angela R. Criswell, Kris F. Tesh, A. L. Dowell, Joseph D. Ferrara and J.W. Pflugrath
Rigaku, 9009 New Trails Dr., The Woodlands, TX 77381-5209
Tel: +1 (281) 363-1033 / Fax: +1 (281) 364-3628 / www.Rigaku.com

One of the primary concerns in modern crystallography laboratories is to develop protocols for improving poorly diffracting crystals. Crystals are optimized by changing a number of parameters during crystallization (salt, buffer concentration, pH, additives, etc.). Additionally, post-crystallization techniques include replacing water with additives (PEG, sugars, salts), dehydrating in air or under oil, and cryo-annealing. Much of what is done today is not reproducible and lacks the ability to improve a very poor quality, mounted crystal. In the case of "bad" diffraction, the usual thought is to cut your losses and mount a new crystal; in the worst case, you go back and attempt to grow better crystals. But, this may not need to happen. If there is evidence of better diffraction from previous crystals, or if there are few options due to little available protein, you may wish to "heal" a crystal. The Free Mounting System (FMS) is used to carefully regulate and adjust the relative humidity about a mounted crystal, which changes the water content within the crystal. Previously flash-cooled crystals of very poor quality will be shown to improve as a result of FMS rescue and rehabilitation, even to the point of usable data.

HomeLabs Vs. Synchrotrons: The Facts Will Surprise You

Joseph D. Ferrara

Rigaku Americas Corporation, The Woodlands, USA

This presentation will be divided into two parts. The first part will comprise a review of the current state-of-the-art in home lab x-ray sources from both performance and environmental impact perspectives. In the second part will present the results of data mining of the RCSB PDB (H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne: The Protein Data Bank. (2000) *Nucleic Acids Research*. **28**, 235-242) , and will compare and contrast the productivity of home labs and synchrotrons. The results of the survey will surprise you.