**Symposium Abstracts**

**Past, present and future structure-function studies of carbonic anhydrase**

Prof Robert McKenna
*IDS Biochemistry and Molecular Biology, University of Florida and Biochemistry and Molecular Biology, College of Medicine, Gainesville, Fl*

The carbonic anhydrases (CA) comprise of a group of well-studied and distinct gene families (α,β and γ) of mostly zinc-metalloenzymes that catalyze the hydration of carbon dioxide. These ubiquitous enzymes, present throughout virtually all living organisms, including animals, plants, algae, bacteria, and archaebacteria, are involved in a vast number of physiological processes and therefore are a uniquely broad and interesting family of enzymes, since the reaction they catalyze is linked to respiration, acid-base homeostasis, photosynthesis, and other biosynthetic pathways. The mammalian α-class of CA is comprised of 14 expressed isozymes (CA I – XIV) with varying tissue distributions and catalytic activity. Human CA II is the most extensively studied of these isozymes, as it has widespread tissue distribution and because it is the most efficient of the HCAs with a catalytic turnover rate of $10^6$ $s^{-1}$.

From a basic science stand-point, HCA II is an excellent model system for the study of proton transfer, the rate limiting step in CA catalyzes. Recent studies, combining high-resolution x-ray and medium resolution neutron crystallography, with molecular dynamics and kinetic studies have yielded a new level of understanding of the proton transfer mechanism.

In addition, from an applied science application, the broad involvement of CA in physiological processes makes it an attractive drug target in the treatment of human diseases such as glaucoma and cancer, and the possible control of mosquito populations and the human pathogens they carry.

Both current basic science and application studies of CA will be discussed and the possible future directions of CA studies will be considered.

**A conformational switch autoregulates the scaffolding protein NHERF1**

Prof Zimei Bu
*Biomolecular Structure and Function; Fox Chase Cancer Center, Philadelphia, PA*

Scaffolding proteins are molecular switches that control diverse signaling events. A particularly important example is the scaffolding protein NHERF1, which assembles and regulates the localization and intracellular trafficking of a number of important membrane proteins. At its N-terminus, NHERF1 begins with two modular protein-protein interaction domains-PDZ1 and PDZ2-and ends with a C-terminal (CT) domain. The CT domain binds to ezrin, which, in turn, interacts with cytosekeletal actin. Previously we have shown that ezrin binding to NHERF1 increases the binding capabilities of both PDZ domains. Our solution small angle neutron scattering and NMR experiments reveal the autoregulated intramolecular domain-domain interactions, as well as much longer range conformational changes in NHERF1 upon activation by ezrin binding. The results provide a structural explanation, at both mesoscopic scales and atomic resolution, of the allosteric control of NHERF1 by ezrin as it assembles protein complexes. We propose that this long-range allosteric regulation of NHERF1 by ezrin enables the membrane-cytoskeleton to assemble protein complexes that control cross-talk and regulate the strength and duration of signaling.

**Concerted neutron scattering and molecular dynamics simulation studies of membrane protein structure and dynamics**

Prof Douglas Tobias
*School of Physical Sciences, University of California, Irvine*

Membrane proteins perform a wide variety of important physiological functions, including energy production and signal transduction. High-resolution structures of membrane proteins determined by crystallographic techniques provide some clues to their function, but they generally do not provide information on the surrounding membrane. This is unfortunate because it is becoming increasingly evident from a growing body of evidence that specific protein-lipid interactions play an important role in membrane protein structure and function. In the first part of this lecture, I will show how neutron diffraction experiments employing selectively deuterated samples can be used in conjunction with molecular dynamics simulations to gain insight into the structure of membrane proteins in lipid bilayers, as well as protein-lipid and protein-water interactions, using the voltage-sensing domain from a voltage-gated ion channel as an example.

Protein motions occur over many decades of time, from femtoseconds to seconds and longer. It is well established that fast (picosecond to nanosecond) atomic fluctuations are usually required for protein function. A large number of experimental and simulation studies have led to the conclusion that the environment of a protein has a profound influence on its dynamics. In the second part of this lecture, I will describe how temperature-dependent neutron spectroscopic measurements on selectively deuterated samples, combined with molecular dynamics simulations, can be used to unravel the coupling between the motions of proteins and their environment on picosecond-nanosecond timescales. Using the maltose binding protein as an example, I will show that soluble protein motions are directly coupled to their aqueous solvent. Using the protein bacteriorhodopsin in its native purple membrane as an example, I will show that the situation is more complicated in membranes, where protein dynamics couple directly to both water and lipid motions.

Suggested reading:

1. D. Krepkiy, M. Mihailescu, J. A. freites, E. V. Schow, D. L. Worcester, K. Gawrisch, D. J. Tobias, S. H. White, K. J. Swartz. Nature 462, 473-479 (2009).
2. D. J. Tobias, N. Sengupta, M. Tarek. Faraday Discuss. 141, 99-116 (2009).
3. K. Wood, A. Frölich, A. Paciaroni, M. Moulin, M. Härtlein, G. Zaccai, D. J. Tobias, M. Weik. J. Am. Chem. Soc. 130, 4586-4587 (2008).
4. K. Wood, M. Plazanet, F. Gabel, B. Kessler, D. Oesterhelt, D. J. Tobias, G. Zaccai, M. Weik. Proc. Natl. Acad. Sci. 104, 18049-18054 (2007).

**Small-angle neutron scattering to investigate polyglutamine aggregation in Huntington's disease**

Christopher Stanley
*Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, TN*

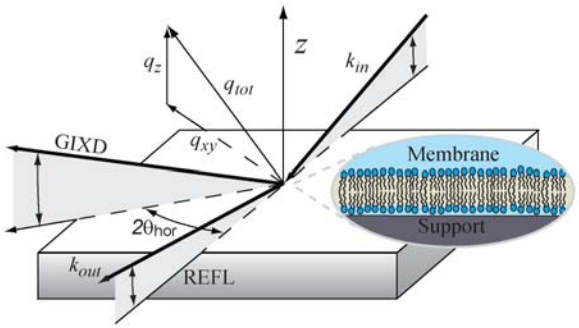
The presence of an abnormally expanded polyglutamine (polyGln) sequence in huntingtin protein ultimately results in β-sheet-rich fibrillar aggregates, a hallmark of Huntington's disease. Current challenges are to map out the polyGln aggregation pathway by identifying the various precursor structures and establish their pathological roles. We are using time-resolved small-angle neutron scattering (SANS) to probe the aggregates formed by peptides having the protein context of huntingtin exon 1 (HD protein) and with varying polyGln lengths. SANS is a particularly useful technique for following structural changes on the nanometer length-scale in solution. From the time-resolved

scattering data, we obtain snapshots of the polyGln structures as the kinetics reaction ensues, which yields quantitative information on the size and shape of precursors and the internal structure of the resulting fibrils. This research is providing new insights into the pathway of polyGln aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

**Emergent Structures in Models of Biological Membranes**

Prof. Tonya Kuhl
*Department of Chemical Engineering and Materials Science and Department of Biomedical Engineering, University of California, Davis, Ca*

Over the past several decades, supported lipid bilayers have been used as model systems of cellular membranes, to investigate various membrane interactions, and as platforms for development of bio-sensors. Precise structural characterization by neutron and x-ray scattering offers a wealth of insight into membrane organization, self-assembly, and domain formation as well as how membranes respond to changes in their environment, e.g. temperature, protein binding, etc. In this talk, I will discuss some recent advances in our understanding of supported membranes, their use in biotechnology applications, and highlight the importance of scattering techniques.



**Poster Abstracts**

**P1. Insights into the Modular Architecture and Dynamic Remodeling of Replication Protein A (RPA) upon ssDNA-Binding from NMR Spectroscopy and SAXS.**

Chris. A. Brosey[1], Susan Tsutakawa[2], Dalyir I. Pretto[1], Marie-Eve Chagot[1], Kavita Dorai[3], and Walter J. Chazin[1]

*[1]Departments of Biochemistry and Chemistry, Center for Structural Biology, Vanderbilt University, Nashville, TN 37232-8725, USA; Deparment, [2]Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA, [3]Indian Institute of Science Education and Research (IISER) Mohali, MGSIPAP Complex, Sector 26, Chandigarh, 160019, India.*

As the primary ssDNA-binding protein in eukaryotes, Replication Protein A (RPA) serves to shield ssDNA templates from degradation or chemical modification while the DNA double helix is unwound and exposed for purposes of replication or biochemical repair. Because of this protective role, RPA also acts as a gateway for numerous DNA processing proteins requiring access to the ssDNA. RPA's ability to accommodate such a broad substrate landscape during replication and repair is thought to arise in part from its modular and versatile domain architecture. Using NMR spectroscopy and small-angle X-ray scattering (SAXS), we have begun to build a picture of RPA's dynamic architecture and the fundamental alterations that occur upon binding ssDNA. Our results reveal a hierarchy of inter-domain mobility within RPA that is specifically correlated with domain function (e.g. ssDNA binding or protein-protein interaction). Studies of intact RPA and multi-domain fragments bound to ssDNA demonstrate remodeling and alignment of the protein's principal DNA-binding domains upon interaction with ssDNA, while protein interaction domains remain undisturbed. These results provide a foundation for understanding ssDNA-induced remodeling in intact RPA and how such structural changes facilitate RPA's role in modulating the assembly and disassembly of multi-protein DNA processing complexes.

**P2. Mechanism of Cellular Uptake of HIV-TAT Peptide**

Xiaochao Chen

*The University of Edinburgh, UK*

The poor permeability of cell membrane and low bioavailability *in vivo* are two major barriers to the development of therapeutic molecules. Several strategies have been designed to improve cellular uptake of those molecules. Recently, HIV-TAT peptide have attracted much interest due to their apparent ability to cross the cell membrane and deliver various biomolecules into cells, including oligonucleotide, DNA, siRNA, peptides and proteins as well as liposomes. HIV-TAT peptide is derived from the transactivator of transcription (TAT) protein from the human immunodeficiency virus 1 (HIV-1), which contains six arginine and two lysine residues, possesses a high net positive charge at physiological pH. Although TAT peptide has been successfully employed for the delivery of a wide variety of compounds into cells, the mechanism of cellular uptake still remains a subject of lots of controversies. Previous data have suggested that the uptake pathways can be categorized into two groups: energy-independent direct translocation and energy-dependent endocytosis. Now no cellular uptake pathway appears more convincing than another one, but a remarkable agreement has been established that an initial electrostatic interaction between the cationic residues of HIV-TAT peptide and anionic plasma membrane constituents are the prerequisite for the successful cellular uptake.

More accurate measurements such as Neutron diffraction and X-ray diffraction need to be performed to confirm the accurate cellular uptake mechanism of HIV-TAT peptide.

**P3. Design and optimization of liposome based biosensors**

Navneet Dogra and Punit Kohli

*Department of chemistry and biochemistry, Southern Illinois University, Carbondale, IL 62901*

Liposomes are of great interest to the scientific community for their use to convey vaccines, drugs, enzymes or other substances to target cells or organs and sensing of molecules and various particles. Conjugated polydiacetylene (PDA) liposomes have environmentally sensitive optical and chemical characteristics which make them excellent sensors for the detection of virus, proteins, DNA, toxins, biologically relevant molecules like glucose. In this presentation we will present the preliminary results, design and optimization of sensitive and selective chemical and biochemical sensors based on Fluorescence Resonance Energy Transfer (FRET) mechanism. The high sensitivity of PDA liposomes can also be utilized for detecting pore formation with the interaction between enzymes and liposomes. The controlled pore formation through the liposome bilayer can be utilized in drug delivery systems. The main objective of the present studies is to enhance our understanding of underlining mechanisms liposome behavior to external stimulations.

**P4. Dynamics in Alzheimer's disease: the role of peptide flexibility on amyloid beta aggregation**

María Antonieta Sánchez Farrán, Janna K. Maranas

*Penn State*

Aggregates of the amyloid beta peptide (Aβ) are thought to trigger brain cell death in Alzheimer's patients. Two different types of Aβ aggregates have been identified: soluble, and insoluble. Soluble aggregates are formed in early stages of peptide association, whereas insoluble aggregates are the final state of aggregation. Interestingly, it is the soluble aggregates, not the insoluble ones, which correlate with disease progression. Despite the relevance of soluble aggregates as a target for Alzheimer's disease, their mechanism of formation is unknown. The role of local flexibility in protein function has recently received attention: in this study we ask if local flexibility plays a similar role in how soluble aggregates form. To answer this question, we perform all-atom molecular dynamics simulations of the wild-type A□ monomer, and two mutated forms that vary in their ability to form soluble aggregates. We find that enhanced flexibility facilitates the formation and availability of nucleation sites by allowing the peptide to more easily access the conformations most favorable to association. Peptides with high flexibility show larger conformational changes than less flexible peptides, the extent of these changes could determine the ability of Aβ to self associate.

**P5. Neutron Crystallography of Ras**

Genevieve Holzapfel

*North Carolina State University*

Ras is a signal transduction molecule involved in a myriad of cell processes including cell proliferation, differentiation, and survival. Ras functions as a molecular switch alternating between a GTP bound active form and a GDP bound inactive form. In the GTP bound state, Ras is able to bind downstream effectors such as Raf, propagating a signal in the cell. GTP hydrolysis and exchange controls the activity of Ras. This is especially important in oncogenesis where 20-30% of human cancers contain mutations that affect the hydrolysis of GTP in Ras. The switch I in the RasGppNHp structure from canonical crystals with symmetry P3221 has a the same conformation as in the Ras/RasGAP complex and gives insight into the mechanism of GAP stimulated GTP hydrolysis. Recently we published an analysis of Ras-GppNHp from crystals with symmetry of the R32 space group. In this crystal form the switch I has the conformation found in the complex with Raf and lead us to propose an

alternative mechanism of GTP hydrolysis. In this mechanism, a bridging water molecule between residue Tyr32 and the gamma-phosphate of the GTP also H-bonds to Gln61. A proton is shuttled from the catalytic water molecule through the gamma-phosphate to the bridging water molecule. The resulting hydronium ion is stabilized by interactions with Gln61 and Tyr32. The resulting positive charge would help stabilize the negative charge that accumulates on the gamma phosphate in the course of the hydrolysis reaction. To better understand Ras hydrolysis, it is necessary to understand the positions of hydrogen atoms in the active site. Neutron crystallography will provide this information. To date we have neutron diffraction after a 2 hour exposure for Ras-GppNHp in both the P3221 and R32 crystal forms. After 18 hours of exposure the crystals with symmetry P3221 diffracts to about 2.5Å. In an attempt to improve diffraction and obtain clearer maps, perdeuterated Ras has been expressed, purified and crystallized in both the P3221 and R32 space groups. These results support the feasibility of obtaining a neutron diffraction data set for Ras that can be used to refine the current understanding of Ras GTP hydrolysis.

**P6. The mechanism of IPPase catalyzed phosphoryl transfer**

Ronny C. Hughes[1], Leighton Coates[2], Joseph D. Ng[1]
*[1]Laboratory for Structural Biology, Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 35899; [2]Oak Ridge National Laboratory, Neutron Scattering Science Division, 1 Bethel Valley Rd, Oak Ridge, TN, 37831*

A model for the catalytic cycle of the Family I Inorganic Pyrophosphatase (IPPase) from the sulfur-reducing hyperthermophilic archaeon *Thermococcus thioreducens* (Tt-IPPase) is proposed in providing new insight into proton transfer mediated, metal-assisted enzyme catalysis. IPPase is a metal-dependant, multimeric enzyme that catalyzes the breakdown of pyrophosphate into two molecules of orthophosphate. Presently, many aspects of the catalytic process remain unclear or have been difficult to validate due to the lack of knowledge pertaining to hydrogen positions in the active site. Seventeen X-ray crystallographic structures of the enzyme have been determined at ultra-high resolution in complex with substrate, product, and in the presence of various metal cofactors and reactive species analogues. Large volume crystals (>6 mm3) of the enzyme suitable for neutron structural studies have been obtained in effort to determine the precise location of hydrogen atoms within the active site needed to complete and validate a mechanistic model for Tt-IPPase catalysis.

**P7. Alphavirus adsorption to mosquito cells as viewed by fracture labeling with complementary thin-sections**

Joseph Kononchik
*North Carolina State University*

Sindbis Virus (SV), the prototype alphavirus in the family togaviridae, infects both mammalian and insect cells in its sylvatic cycle. The ability of SV to infect significantly different biochemical environments suggests there may be a common mode of entry into each cell type. Previous studies show that up to 4 hours post infection cells are permeable to small ions (Koschinski et al., 2005). This permeability is blocked by rare earth metals, which can block ion transfer through small pores in the plasma membrane. Through thin-section electron microscopy, SV has also been shown to bind to the plasma membrane and lose its electron dense core (Paredes et al., 2004). These data suggest that SV can dock at the membrane and release its cargo (RNA) in to the cell. Using freeze-fracture replicas, thin-sections and antibody labeling the data presented herein illustrate virus associated with intramembrane particles (IMPs) on U4.4 cells with and without the presence of Bafilomycin $A_1$. These data show that SV requires active V-type ATPase for virus entry, but not for virus adsorption onto cells. It also suggests that the IMPs associated with SV may be the V-type $H^+$-ATPase. These IMPs are likely the docking site of the virus, and may also be used in pore formation during infection.

**References**

Koschinski, A., Wengler, G., and Repp, H. (2005). Rare earth ions block the ion pores generated by the class II fusion proteins of alphaviruses and allow analysis of the biological functions of these pores. *J Gen Virol* **86**(Pt 12)**,** 3311-20.; Paredes, A. M., Ferreira, D., Horton, M., Saad, A., Tsuruta, H., Johnston, R., Klimstra, W., Ryman, K., Hernandez, R., Chiu, W., and Brown, D. T. (2004). Conformational changes in Sindbis virions resulting from exposure to low pH and interactions with cells suggest that cell penetration may occur at the cell surface in the absence of membrane fusion. *Virology* **324**(2)**,** 373-86.

**P8. Establishing a Standard for Hydrogen Atom Sites in Proteins--Gamma-Chymotrypsin.**

Louis Lazar
*Brandeis University*

Determining the protonation state of an ionizable residue in a protein is traditionally derived from indirect biochemical data or computational approaches. Ideally, X-ray crystallographic data should provide protonation information, but the resolution is generally not achievable. Consequently, neutron diffraction data would seem to be the best source of such information. Gamma-chymotrypsin will be used as a model enzyme to assess the change in hydrogen positions in the active site as pH is varied. Chymotrypsin has been chosen because its kinetics are well characterized, the crystals are stable over a large pH range, and kinetics in the crystal have been shown to be identical to that in solution. To date, we have collected data at pH 5.6, a pH value at which the enzyme is inactive. Clear density for hydrogen (deuterium) positions has been observed. We will also collect data at pH 7.0, a pH at which the enzyme is active, as well as pH ~9.0, to further analyze changes in protonation state, particularly in the active site. We will assess changes in protonation states upon pH change, paying particular attention to the residues of the catalytic triad, His 57, Asp 102, and Ser 195. These data will be correlated with atomic resolution (<1.2 A) X-ray structures.

**P9. A novel gating mechanism for the Arabidopsis Nodulin-like intrinsic protein 7;1 boric acid transporter in pollen.**

Tian Li
*University of Tennessee Knoxville*

Functional analysis shows that there are two subfamilies of aquaporin: aquaporin which transport water specifically, and the aquaglyceroporins which can facilitate conduct of glycerol, urea, and other small molecules in addition to water. Structure analysis indicate that all the aquaporin adopt 'hourglass' conformation with 6 transmembrane alpha-helices linked by 5 loops. There are two constraints: two highly conserved domains with 'NPA motifs' are located on loop B and loop E and a tetrad of amino acid residues that contains aromatic residues and a conserved Arginine referred as Ar/R region. In my research, I focus on the structure and function of one plant MIP: AtNIP7;1. AtNIP7;1 belongs to the second subgroup of plant nodrolin-26 like intrinsic protein with AtNIP5;1 and AtNIP6;1. The functions of AtNIP5;1 and AtNIP6;1 have already been elucidated that both of them can conduct boric acid in order to transport boron to the shoot tissue. In order to understand the molecular basis for the unique function of AtNIP7;1, *Xenopus* occyte system is used for the water or other solutes uptake assay and computational biology improves the understanding of structural complexity and functional diversity. Boric acid uptake assay didn't show apparent boric acid conductivity in AtNIP7;1. Homology modeling on AtNIP7;1 provides an idea that the Y81 probably is the key residue blocking the hole by observation of up and down conformation of Y81, and mutation of Y81 to C facilitates boric acid transportation by boric acid uptake assay. Molecular dynamics simulation shows another residue---R220, which belongs to Ar/R region, adopts two conformations: up and down which could interact with Y81 by hydrogen bond. These two residues Y81 and R220 could be the very candidates involving in the gating mechanism of AtNIP7;1.

**P10. BCL::EM-FOLD: PROTEIN FOLDING TOOL FOR MEDIUM RESOLUTION DENSITY MAPS**

Steffen Lindert[1], Rene Staritzbichler[1], Nils Wötzel[1], Nathan Alexander[1], Mert Karakas[1], Phoebe Stewart[2], Jens Meiler[1]

*[1]Chemistry Department, Vanderbilt University; [2]Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center*

Both cryo-electron microscopy (cryoEM) and X-ray crystallography frequently yield medium resolution (6 – 10 Å) density maps of large macromolecular assemblies. Generally no atomic detail is resolved in these density maps, making it impossible to deduce the protein structure from the density map alone. We present a novel computational protein folding algorithm that aids in the interpretation of medium resolution density maps from cryoEM and X-ray crystallography. BCL::EM-Fold folds the amino acid chain *de novo* into density rods observed for α-helices at sub-nanometer resolution. This is done by incorporating the experimental data as restraints. The placement of helices is restricted to regions where density rods are observed in the density map. BCL::EM-Fold has been benchmarked with ten highly α-helical proteins of known structure that have between 250 and 350 residues. Starting with knowledge of the true secondary structure for these ten proteins, the method can identify the correct topology within the top scoring ten models. With more realistic secondary structure prediction information, the correct topology is found within the top scoring five models for seven of the ten proteins. Subsequent high-resolution refinement for the successful proteins using the density as a restraint in Rosetta creates models with RMSDs as low as 2.5 Å. The algorithm has been used to build an atomic model for large parts of human adenovirus protein IIIa. This protein, for which there is no high resolution structure, is predicted to be highly α-helical. It is resolved in a 6.8 Å resolution cryoEM adenovirus structure as a bundle of 14 α-helical density rods.

**P11. Exploring the Trafficking, Activity, Conformational Changes and Folding for a Model GPCR**

Andrea N. Naranjo[1], Michelle A. O'Malley[2], Anne Skaja Robinson[1]

*[1] Department of Chemical Engineering, University of Delaware, [2] Department of Biology, Massachusetts Institute of Technology*

G protein-coupled receptors (GPCRs) are integral membrane proteins vital for cellular signaling and constitute one of the major drug targets. Despite their importance, relatively little information is known regarding to their signaling mechanism, structure and folding compared to soluble proteins. Biophysical techniques in general have not been widely apply to study GPCRs mainly because these receptors are difficult to express in high yields and are difficult to solubilize due to their seven hydrophobic transmembrane domains. In addition, techniques such as thermal and chemical denaturation, employed to study protein folding, may translate poorly to the characterization of membrane proteins and may lead to sample aggregation.

We have successfully purified a human adenosine receptor, $A_{2A}$ ($hA_{2A}R$), in high yields from yeast and reconstituted it in a mixed-micelle environment retaining the ligand-binding activity of this membrane protein. In this work, we will describe several biophysical approaches to characterize the $A_{2A}R$ and studies aimed to understand structural features in $A_{2A}R$ that allow its successful expression and purification from heterologous systems.

Unfolding of the $A_{2A}R$ through thermal and chemical means was monitored via intrinsic tryptophan fluorescence. Although protein unfolding was observed, both techniques led to irreversible aggregation of micelle-reconstituted $A_{2A}R$. Addition of a thiol-reactive fluorescent dye, whose quantum yield increases as $A_{2A}R$ unfolds, was also used in order to achieve clear thermal unfolding transitions. In an effort to reduce the effects of aggregation, we also employed hydrostatic pressure to unfold the tertiary structure of $A_{2A}R$. In summary, we find that the stability of $A_{2A}R$ is slightly affected by ligand addition, stability is retained when disulfide bonds located in the extracellular loop regions are reduced with a chemical reducing agent. Further, we have confirmed that loss of these disulfide bonds also leads to a decrease in receptor ligand-binding activity.

Mutations in the cysteines corresponding to each of the four disulfide bonds exhibit different trafficking patterns and whole cell ligand-binding activities in mammalian cells, HEK293. In the future, the mutated receptors will be expressed and purified from the yeast, *Saccharomyces cerevisiae*, in order to elucidate different trafficking and expression patterns between the two host systems and to conduct the biophysical characterization of these mutated constructs.

**P12. Ultra-High Resolution Analysis of CTX-M Beta Lactamase to study catalysis and discover novel inhibitors.**

Derek Nichols

*University of South Florida*

The resistance to second and third-generation cephalosporins due to the production of beta-lactamase from bacteria continues to pose a serious threat to various regions of the world, particularly in nosocomial settings. Structural analysis of CTX-M 's X-ray crystallographic structure at sub-angstrom resolution has enabled us to study enzyme catalysis as well as perform computational molecular docking. We were able to screen a large database of compounds using the program DOCK to identify potential inhibitors against CTX-M. Interestingly, several novel inhibitors were tested and shown to have Ki values in the low micromolar range. Although ultra-high resolution X-ray diffraction has enabled us to effectively characterize the enzyme's protein-ligand interactions, a more detailed analysis of the proton transfer dynamics is needed to understand the catalytic mechanism by which CTX-M functions. As a result, utilizing neutron diffraction to study the proton transfer mechanism is essential to understanding the enzyme's catalytic function. Furthermore, the determination of ultra-high resolution structures of CTX-M along with the discovery of novel inhibitors against these pathogenic strains is critical in order to combat bacterial resistance.

**P13. The *Pseudomonas aeruginosa* Transcription Factor AmrZ Forms a Repression Complex with Integration Host Factor via DNA Binding and Protein Oligomerization**

Edward E. Pryor, Jr. (1), Elizabeth A. Waligora (2), Yuh-Hwa Wang (1), Daniel J. Wozniak (2, 3), and Thomas Hollis (1)

(1) Department of Biochemistry, Wake Forest University School of Medicine, Winston Salem, NC; (2) Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston Salem, NC; (3) Department of Microbiology, The Ohio State University Medical Center, Columbus, OH

*Pseudomonas aeruginosa* is a common opportunistic pathogen which is a leading cause of nosocomial infections and chronic lung infections in patients with the autosomal recessive disease cystic fibrosis. In order to establish chronic infections, *P. aeruginosa* utilizes a multitude of virulence factors which give the bacteria a selective advantage. These virulence factors include the biosynthesis of the exopolysaccharide alginate, repression of flagellum biosynthesis, biogenesis of type IV pili, and repression of its own transcription. The transcription factor AmrZ has dual roles as both an activator and repressor of genes leading to each of these unique phenotypes.

The crystal structure of the AmrZ protein in complex with the 18bp repression site on the *amrZ* gene illuminates the important interactions in the ribbon-helix-helix domain necessary for DNA recognition. Truncation mutagenesis, along with biochemical, biophysical and in vivo experiments, were utilized to determine that the C-terminal domain of AmrZ is responsible for the formation of oligomeric structures beyond the dimeric state and how these structures regulate gene expression. Lastly, a binding site for the DNA bending protein Integration Host Factor (IHF) has

been determined on the *amrZ* promoter. DNA binding experiments and electron microscopy were used to show that both AmrZ and IHF bind the *amrZ* promoter simultaneously and form a complex. Taken together, the results from these experiments have been used to create a model of how AmrZ and IHF work together to repress transcription of the *amrZ* gene. DNA recognition and binding allows for the interaction of the ribbon-helix-helix domain of AmrZ with the two binding sites on the *amrZ* promoter. IHF then binds the *amrZ* promoter, bending the DNA ≈180$^{o}$. This complex is further stablized by protein-protein interactions formed by the C-terminal domain of AmrZ. The looping of DNA by this complex prevents assembly of RNA polymerase on the promoter leading to transcriptional repression.

**P14. Single-step Immobilization of High Temperature Biocatalysts on Nanofibrous Supports by Reactive Electrospinning**

Christina Tang, Carl D. Saquing, Robert M. Kelly and Saad A. Khan

*North Carolina State University*

Enzymes are highly efficient selective biological catalysts and hyperthermophilic enzymes are of particular interest due to their ability to function at elevated temperatures intrinsic to many industrial processes. In this project, we are immobilizing a model hyperthermophilic enzyme, α-galactosidase from *Thermotoga maritima,* on electrospun nanofibrous supports for potential use in a variety of applications, ranging from increasing nutritive value of animal feed to converting B-type blood to O-type blood, the universal blood type. Immobilization offers inherent advantages: increased enzyme stability, ease of separation of the products so the enzyme can be reused, and lack of product contamination. However, immobilization can also impact the activity of the enzyme. In all cases, the structure of the support material greatly affects the performance of the immobilized enzyme. Fibers have high specific surface area, which increases with decreasing fiber diameter. This makes nanofibers especially promising for enzyme immobilization. Electrospinning is a common technique used to produce nanofibers in which high voltage is used to induce the formation of a liquid jet from a polymer solution. We have electrospun aqueous solutions of polyvinyl alcohol (PVA) and *T. maritima* α-galactosidase with glutaraldehyde, a chemical crosslinking agent, to generate water-insoluble, enzyme-loaded nanofibers of approximately 200 nm in diameter in a single reactive electrospinning process. Using reactive crosslinking eliminates a post-electrospinning crosslinking step, thus accelerating the immobilization method by about 7-fold. After electrospinning, the fibrous structure remains intact when soaked in water. Most importantly, the enzyme retains catalytic activity and its hyperthermophilic characteristics (optimal activity between 90 and 95°C) following the reactive electrospinning process. While the enzyme activity of the immobilized enzyme was about 5-fold lower than the free enzyme, the retained activity was significantly higher than following post-electrospinning treatment using a non-solvent or vapor phase crosslinking technique.

**P15. Creation of an AcrB-GFP Fusion Protein for the Study of the**
**Membrane Protein Assembly and Function**

Linliang Yu, Yinan Wei

*Department of chemistry, University of Kentucky, Lexington, KY 40506*

AcrA-AcrB-TolC is a three component efflux pump of *Escherichia coli.* It allows the extrusion of a wide range of cytotoxic compounds. AcrB is located in the inner membrane of the bacterium. It is a homotrimer and utilizes the proton gradient across the inner membrane to drive the export of drugs. In this study, we created a fusion protein composed of AcrB connected at the C-terminus to the green fluorescent protein (GFP) as a tool for the study of the assembly and function of AcrB *in vitro* and *in vivo*. Gene encoding the AcrB-GFP fusion protein was inserted into the pQE-70 vector to create plasmid pAcrB-GFP. Plasmid pAcrB-GFP was transformed into a strain that is deficient in *acrB (_acrB)* for protein expression. The expression of the fusion protein was observed directly using the fluorescence microscope. The fluorescence of the cells, which correlates with the expression of the fluorescent fusion protein, increased in the presence of AcrB substrates. The *_acrB* strain was very sensitive to drugs. The transformation with pAcrB-GFP increased the drug tolerance of the *_acrB* strain, indicating that the fusion protein is active. Next we will use this fusion protein to illustrate the assembly mechanism of AcrB *in vivo*.