Symposium abstract

Symposium – Neutrons in Structural Biology

Monday, May 23, 2011
SNS, Building 8600, Conference Room C-156

Abstracts

10:15 a.m.

Introduction to Neutron Scattering in Structural Biology. Dean Myles, Neutron Scattering Science Division, Oak Ridge National Laboratory.

Neutron scattering is one of the most powerful research techniques available to scientists studying the structure and dynamics of materials. Crystallography, small-angle scattering, diffraction, and reflectometry are ideal methods for studying atomic and sub-atomic structures, and several types of neutron spectroscopy are used for characterizing material dynamics. Neutron scattering is applicable to the length and time scales intrinsic to biological systems and, unlike other structural techniques, it is uniquely sensitive to hydrogen (and its isotopes). Oak Ridge National Laboratory (ORNL) hosts two of the world's most powerful and advanced neutron scattering research facilities, the Spallation Neutron Source (SNS) and the High Flux Isotope Reactor (HFIR), and is now using these facilities to help understand cellular systems and process over length scales that range from the atomic to the cellular.

11:15 a.m.

Insights into the modular architecture and dynamic remodeling of replication protein A (RPA) from NMR, SAXS, and SANS. Chris A. Brosey, Dalyir I. Pretto, Marie-Eve Chagot, & Walter J. Chazin, Vanderbilt University.

DNA replication and repair rely upon the coordinated action of dynamic, multi-protein complexes. Modular proteins with flexibly linked domains serve as vital components of these assemblies, as their mobile inter-domain organization is uniquely suited to follow the evolving substrate landscapes present during replication and repair. While such dynamic flexibility is essential for DNA processing, it presents many challenges to understanding the action of the multi-protein machinery at replication forks and damage sites.

Replication Protein A (RPA), the primary ssDNA-binding protein in eukaryotes, is an archetypal example of this class of dynamic, modular DNA processing proteins. NMR spectroscopy, small-angle X-ray scattering (SAXS) and small angle neutron scattering are being used to build a picture of the dynamic RPA 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 constructs bound to ssDNA reveal remodeling and alignment of the protein's principal DNA-binding domains, while protein interaction domains remain undisturbed. These findings provide a foundation for describing how modular flexibility facilitates the assembly and progression of DNA processing complexes.

12:15 p.m.

Dynamic propagation of allosteric signals by nanoscale protein motion. Zimei Bu, City College of New York.

NHERF1 is a multidomain scaffolding protein that assembles signaling complexes, and regulates the cell surface expression and endocytic recycling of a variety of membrane proteins. The ability of the two PDZ domains in NHERF1 to assemble protein complexes is allosterically modulated by the membrane-cytoskeleton linker protein ezrin, whose binding site is located as far as 110 Ångstroms away from the PDZ domains. Here, using neutron spin echo (NSE) spectroscopy, selective deuterium labeling, and theoretical analyses, we reveal the activation of interdomain motion in NHERF1 on nanometer length-scales and on submicrosecond timescales upon forming a complex with ezrin. We show that a much-simplified coarse-grained model suffices to describe interdomain motion of a multidomain protein or protein complex. We expect that future NSE experiments will benefit by exploiting our approach of selective deuteration to resolve the specific domain motions of interest from a plethora of global translational and rotational motions. Our results demonstrate that the dynamic propagation of allosteric signals to distal sites involves changes in long-range coupled domain motions on submicrosecond timescales, and that these coupled motions can be distinguished and characterized by NSE.

2:15 p.m.

Biomolecular Structure and Dynamics with Neutrons and Computer Simulation. Jeremy C. Smith, University of Tennessee & Oak Ridge National Laboratory, Oak Ridge, Tennessee.

A review will be presented of how the integration of neutron scattering and computer simulation techniques provides insight into biomolecular structure and dynamics.

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14. L. PETRIDIS, S.V. PINGALI, V. URBAN, W. T. HELLER, H. M. O' NEILL, M. FOSTON, A. RAGAUSKAS & J C. SMITH. Physical Review E. In press.
15. E. BALOG, D. PERAHIA, J.C. SMITH & F. MERZEL, Journal of Physical Chemistry B. In Press.

3:15 p.m.

Probing the Structure of Carbonic Anhydrase using X-ray and Neutron Crystallography. Robert McKenna, University of Florida, Gainesville.

Carbonic anhydrase (CA) is a ubiquitous metalloenzyme that catalyzes the reversible hydration of CO_2 to form HCO^{3-} and H+ using a Zn–hydroxide mechanism. The first part of catalysis involves CO_2 hydration, while the second part deals with removing the excess proton that is formed during the first step. Proton transfer (PT) is thought to occur through a well ordered hydrogen-bonded network of waters that stretches from the metal center of CA to an internal proton shuttle, His64. These waters are oriented and ordered through a series of hydrogen-bonding interactions to hydrophilic residues that line the active site of CA. Combined X-ray and Neutron studies were conducted on wild-type human CA isoform II (HCA II) in order to better understand the nature and the orientation of the Zn-bound solvent, the charged state and conformation of His64, the hydrogen-bonding patterns and orientations of the water molecules that mediate PT and the ionization of hydrophilic residues in the active site that interact with the water network. Several interesting and unexpected features in the active site were observed which have implications for how PT proceeds in CA.

From a basic science stand-point, HCA II is an excellent model system for the study of PT, the rate limiting step in CA catalyzes.

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.

4:30 p.m.

The Study of Biological Model Membrane Systems with Neutrons. John Katsaras, Neutron Scattering Science Division, Oak Ridge National Laboratory.

Unlike X-rays, neutron scattering lengths are different for isotopes of the same element. This is because neutron scattering is the result of nuclear forces, and the neutron scattering length can experience a dramatic change in magnitude and phase as a result of resonance scattering. Importantly, in the case of hydrogen its scattering length is negative even at energies far from its resonance energy. When it comes to biological material inherently rich in hydrogen, dramatic changes in scattering amplitudes are achieved through the substitution of H for its isotope D, selectively tuning the sample's contrast *in situ* with minimal or no changes to its native structure. Over the past few years we have studied a number of biologically relevant membrane systems using neutron scattering techniques. During the course of the seminar I will expand on the following:
Neutron studies of deuterated cholesterol incorporated into 1,2 diarachidonoyl phosphatidylcholine (PUFA) bilayers found cholesterol sequestered inside the membrane, in contrast to its "usual" position with the hydroxyl group located near the lipid/water interface. [1,2] Most recently, neutron experiments demonstrated the preference of cholesterol for different lipids. [3,4]

Neutron diffraction studies performed on aligned, self-assembled bilayers of Na-, Ca- and Mg-salt forms of lipopolysaccharides (LPS) isolated from *P. aeruginosa* PAO1 show that water penetrates Ca2+-LPS bilayers to a lesser extent than either Na+- or Mg2+-LPS bilayers. [5] We propose that this difference in water penetration is the result of calcium "compacting" the LPS molecules in the outer/inner core region of smooth LPS bilayers.

One of the most important parameters needed to accurately describe bilayer structure and lipid-lipid and lipid-protein interactions in biomembranes is lipid lateral area, A. In addition to playing a key role in describing membrane structure, knowledge of lateral lipid area is central to molecular dynamics (MD) simulations. Neutrons are playing a key role in accurately determining lipid areas and influencing the refinement of MD force fields. [6]

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Poster Session

Evolution of structure and dynamics for a family of disordered proteins. Wade Borcherds, U. South Florida.

Intrinsically Disordered Proteins (IDPs) are frequently found in vital cellular pathways including transcriptional activation and signal transduction. There is however a dearth of atomic models available for IDPs, hampering insight into how the structural ensemble is specified by the amino acid sequence. Nuclear Overhauser Effect (NOE) data was collected from the intrinsically disordered transactivation domain of a series of p53 (p53TAD) orthologues to investigate the conservation of dynamic behavior. The data suggest the presence of recurring but variable structural elements among the orthologues in the region aligning with the ubiquitin ligase MDM2 binding domain of human p53TAD, and to a lesser extent in the region correlating to the RPA binding domain of human p53TAD. The data also show significant variation in the backbone dynamics at these regions. The recurrence of these structural features across evolutionary time as suggested by the NOE data gives some credence to the idea that transient secondary structures within IDPs are constrained, though with possibly varying degrees of dynamic behavior. Future atomic modeling of the structural ensembles of these homologues should allow for a better understanding of the effect of residue similarity on the final structural ensemble in IDPs.

Crystal structure of mutant D68N archaeal inorganic pyrophosphatase at 1.5 Å - A preliminary analysis of an active site variant. Manavalan Gajapathy, Ronny C. Hughes, Joseph D. Ng, Laboratory for Structural Biology, Department of Biological Sciences, University of Alabama in Huntsville.

Crystal structure of D68N inorganic pyrophosphatase is an active site variant of inorganic pyrophosphatase (IPPase) from a sulfur reducing hyperthermophilic archaeon *Thermococcus thioreducens (1).* IPPase is a metal dependent Family I IPPase which catalyses the breakdown of pyrophosphate into two phosphate molecules. To understand its catalytic mechanism, which is presently unknown , an active site variant (D68N) was cloned, expressed, purified and characterized. D68N IPPase retains 9.9% activity when compared to its wild type. Crystals were obtained by sitting drop vapor diffusion. The crystals were measured to be 0.5mm in the longest dimensions. X-ray analysis shows the crystals to be monoclinic belonging to the space group C2. The unit cell parameters measured to be a=103.99 Å, b=93.95 Å, c=113.82 Å and b=98.15. The crystals diffracted to a maximum resolution of 1.54 Å. The structure was determined by molecular replacement using the wild-type IPPase as the target model.

Structural studies of the DNA binding domain of chromatin remodeler Chd1. Katherine Jenkins, John Hopkins University.

Abstract: Nucleosomes are the basic organizational element of genomic DNA in eukaryotes. Chromatin remodelers are ATP-driven motors that move and restructure nucleosomes, processes that are critical for properly regulating gene expression. Some remodelers, such as Chd1, use a DNA- binding domain to space nucleosomes relative to each other and to sense the length of the linker DNA in between nucleosomes. How Chd1 moves nucleosomes and how the DNA-binding domain of Chd1 participates in nucleosome sliding is not known. We are seeking structural information about the interaction of the DNA-binding domain of Chd1 with DNA in order to gain insight into the mechanism of sliding, and specifically the DNA-binding domain's role in sliding. Currently, we are in the process of refining the structure of the DNA-binding domain of Chd1 in complex with a 12 bp dsDNA oligo. I will be seeking further structures of the complex varying the sequence and length of the DNA oligo.

Actinide Tetracyanoplatinates. Branson Maynard, Richard Sykora, and Anne E. V. Gorden.

Pseudo 1-D Pt···Pt interactions are a major structural feature of compounds containing the square planar tetracyanoplatinate anion.[1] The unique spectroscopic and emission properties of these compounds originate from the Pt···Pt interactions and can be modified through changes in the Pt···Pt separation.[1,2] The lanthanides offer the ability to tune the Pt···Pt spacing of the pseudo 1-D chains by taking advantage of the lanthanide contraction.[3,4] Tuning the properties of the actinide tetracyanoplatinate analogs is not straightforward, as the atomic radii of the actinides do not contract in a predictably linear trend. However, the availability of multiple oxidation states of several 5*f* elements may offer other pathways to tune the properties of this class of compounds. This also provides a new means for probing the chemistry of the *5f* elements. Described here is the synthesis and structural characterization using small molecule X-ray single crystal diffraction of the recently reported actinide tetracyanoplatinates, general formula $An_x[Pt(CN)4]_y$.[5]

Peptidyl-tRNA Hydrolase in Complex with Peptidyl-tRNA. Mary C Mays, U. of Alabama, Huntsville.

Ribosomes frequently stall during protein synthesis leaving toxic peptidyl-tRNAs in the cell. Peptidyl-tRNA hydrolase (Pth) cleaves the peptide:tRNA ester bond; this enzymatic activity is required for cells to deal with the pollution of peptidyl-tRNAs. Pth is also highly conserved across bacterial species and has no essential eukaryotic homolog making it a high value drug target. To date, no structure of Pth in complex with peptidyl-tRNA has been solved. Herein we report potent Pth inhibition from novel tropical plant extracts and small angle neutron scattering from the Pth:peptidyl-tRNA complex. Coupled with NMR chemical shift perturbation mapping, we hope to better understand Pth, its interaction with binding partners, and mechanism of inhibition.

A New Look at an Old interaction: Developing a Model for PAI-1: Vitronectin Associations. Letitia N. Olson*[1], Cynthia B. Peterson[1], [1]Biochemistry Cellular and Molecular Biology, University of Tennessee, Knoxville, Knoxville, TN, 37996, USA, lolson@utk.edu.

Human Plasminogen Activator Inhibitor 1 (PAI-1) belongs to the serpin (serine protease inhibitor) family. It is an antifibrolytic, proinflammatory, and proadhesive protein. PAI-1 is a metastable serpin that favors the its latent state due to its unstructured Reactive Center Loop (RCL). PAI-1 is stabilized from this latency conversion by its cofactor, a glycoprotein called Vitronectin (VN). Research has shown that PAI-1 and Vitronectin form a 4:2 (PAI-1:VN) intermediate complex which assembles into higher order forms in the extraellular matrix (ECM). However, the exact details of the complex assembly are unclear. The presence of two PAI-1 binding sites on VN led to the assumption that two PAI-1 molecules bound VN at the two separate sites. We have used small-angle neutron scattering on mixtures of protonated vitronectin and perdeuterated PAI-1 to probe the 3D organization of this complex. The data reveal a PAI-1:PAI-1:VN interaction, in which the serpin interacts with itself. In order to further probe this hypothesis, PAI-1 mutants that are deficient in VN binding were created. These binding "knock out" mutants also include a single cysteine that can be labeled with a chromphore. These mutants are mixed with VN and wtPAI-1 in a 1:1:1 ratio to test for the formation of a 2:1 complex that incorporates the labeled mutant. More recently we have also begun to explore the effect of osmolytes on the region of VN that is responsible for binding to PAI-1. This region contains an Intrinsically Disordered Domain (IDD) that we will evaluate using SANS to determine if the binding of PAI-1 affects the formation of structure in the IDD. This study of the assembly mechanism provides insight into the formation of the adhesive, matrix-localized forms of the PAI-1/VN complex. This knowledge will also aid in developing new models that will provide insight into the way PAI-1 and VN complex formation affects the structure and function of each.

In-situ Time Resolved Dilute Acid Breakdown of Lignocellulosic Biomass Isolated Cellulose. Jonathan Reyles, UTK.

Lignocellulosic biomass embodies the vast majority of biomass on Earth making it a clear candidate for conversion to renewable bioethanol. There is however a caveat: lignocellulosic biomass shows significant recalcitrance towards the structural breakdown into sugars that are necessary for fermentation to bio-ethanol. Recalcitrance makes it a cost-ineffective feedstock. To eliminate recalcitrance, chemical pretreatment of lignocellulosic biomass is the remediating option. It is intended to work by overcoming recalcitrance. Accordingly, it improves the efficiency of subsequent enzymatic hydrolysis of cellulose. Chemical pretreatments commonly have elevated temperatures and/or extreme pH conditions that dissolve and remove the non-crystalline pectin and hemicellulose polysaccharides and detach lignin from the cellulose microfibrils. However, subsequent re-precipitation of lignin on cellulose surfaces following pretreatment impedes effective conversion of cellulose to fermentable sugars (enzymatic hydrolysis). Recently, we were successful in performing in-situ small-angle neutron scattering (SANS) studies during the dilute acid pretreatment of native switchgrass. To elucidate the morphology of the cellulose/lignin composite in switchgrass, in-situ SANS investigation were employed on extracted holocellulose too. Collected data is currently being processed and analyzed. The structural information obtained from these studies will facilitate in optimizing biomass pretreatment protocol necessary for efficient lignocellulosic biomass conversion to biofuels.

Characterizing interactions between surfactants and membrane proteins with special emphasis on crystallization. Anvar Samadzoda, Abraham M. Lenhoff, Anne S. Robinson

In spite of the importance of membrane proteins (MPs) in many essential biochemical pathways and cell functions, there are a few structures of this class of proteins in the Protein Data Bank. One of the major bottlenecks to a greater number of solved structures is obtaining well-diffracting crystals. Crystallization remains largely a trial-and-error process, due to the complexity of crystallization solution conditions that require surfactants to solubilize MPs, and also different types of precipitants to induce crystal formation. As such, developing a more systematic crystallization strategy based on insight

into the effect of interactions between solution components can be an important step for obtaining new structures of MPs.

The approach of the current research to improving MP crystallization emphasizes the importance of surfactant phase behavior, self-aggregation in the form of micelles, as well as surfactant interactions with MPs and common precipitating agents, such as poly(ethylene) glycol (PEG). In particular, isothermal titration calorimetry measurements indicate that there is an endothermic interaction between monomers of lauryldimethylamine oxide (LDAO), a surfactant commonly used with MPs, and chains of PEG-4000, a common precipitant. This result gives us a better picture of the kind of interactions and structures that surfactants can form in solution conditions of interest. Additionally, using a model membrane protein system – reaction center from *Rhodobacter sphaeroides* solubilized in LDAO - in microbatch crystallization trials, we have shown that excessive surfactant, even a few millimolar, can have a negative effect on the outcome of crystallization. The effect of excessive surfactant seems to be offset by the presence of methyl-β-cyclodextrin, known to form inclusion complexes with LDAO. This result gives rise to the possibility of chemical modulation of effective surfactant concentration during the process of crystallization by vapor diffusion, where total solute concentration is steadily increased by the loss of water. Precise modulation of surfactant concentration, either chemically or physically, seems to be a key ingredient of developing a successful crystallization strategy. Hence, accurate quantification of surfactant becomes an imperative. Currently, efforts are under way to finalize a microdialysis crystallization set-up for physical modulation of surfactant concentration, whereby variation of saturation and supersaturation phase boundaries with respect to surfactant concentration could be recorded in a systematic manner.

Structurally flexible macro-organization of membrane systems in diatom. R. Ünnep[1], M. Szabó[2], G. Káli[1], G. Nagy[1,3], L. Kovács[2], Y. Miloslavina[2], P. Lambrev[2], L. Rosta[1], and G. Garab[2]; 1) Research Institute for Solid State Physics and Optics, HAS, Hungary; 2) Biological Research Center, HAS, Szeged, Hungary; 3) Institut Laue-Langevin, BP 156, F-38042, Grenoble Cedex 9, France.

Our civilization is facing problems of energy, climate change and growing population. Nature has perfected photosynthesis as a highly efficient and flexible means of converting the light energy of sunshine to chemical energy. The better understanding of these processes might help us in finding solutions to these problems. Little is known so far about the self-organization and regulation of the structure of this membrane system during photosynthesis and under the variation of environmental parameters. We determined characteristic repeat distances of thylakoids in living Phaeodactylum tricornutum (diatom) aligned in a magnetic field. Treatment with different concentrations of sorbitol and high temperatures caused change in the peak positions of the SANS signals characteristic of the membrane repeat distances. We have also reported on light-induced changes in repeat distance, which could be correlated with photosynthetic processes in vivo and which were observed with a time resolution of a few minutes. These multilamellar membrane systems are capable of undergoing reversible reorganizations. These results correlate with the measurement of circular dichroism spectroscopy.

Characterizing Structural Polymorphism in Fungal Prion HET-s by X-ray Fiber Diffraction. William Wan, Wen Bian, Michele McDonald, Gerald Stubbs.

Prions are aberrantly folded infectious proteins which propagate their infectious folds by inducing the refolding of normal globular proteins. This refolding process generally leads to the formation of chemically homogenous, unbranched, fibrillar aggregates called amyloid. Prions have been implicated in a number of neurodegenerative diseases called the transmissible spongiform encephalopathies (TSEs) while other amyloids have been implicated in a number of diseases such as Alzheimer's disease, Parkinson's disease and type II diabetes. However, in lower organisms such as yeast and fungi, a number of functional prions have been found. In the filamentous fungus *Podospora anserina*, the prion form of the HET-s protein is used in a normal self non-self recognition process known as heterokaryon incompatibility. HET-s prions exhibit a relatively intricate fold on the order of complexity with the folds of TSE-related prions, making them a suitable model system for studying the mechanisms of infectivity.

Large fibrillar aggregates are particularly difficult to study because they are not ammenable to common structure determination techniques such as solution NMR and X-ray crystallograpy. In order to study these systems, we have used X-ray fiber diffraction to determine structural details about HET-s fibrils. We have found that under certain conditions, HET-s is able to take on multiple stable folds. These results will allow us to determine the interplay between prion structure and infectivity.

Dynamic Orientation of Ubiquitylated PCNA In Solution and Its Implication in Translesion Synthesis. Chris Weinacht[1], Junjun Chen[1], Yongxing Ai[1], Susan E. Tsutakawa[2], Ivaylo Ivaynov[3], John A. Tainer[2], Zhihao Zhuang[1]; [1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE, 19716; [2]Life Science Division, Lawrence Berkeley National Laboratory, Berkeley, California, 94270; [3]Department of Chemistry, Georgia State University, Atlanta, Georgia 30302.

Translesion synthesis (TLS), a mechanism utilized by cells to synthesize past DNA lesion, is evolutionarily conserved in organisms from prokaryotes to eukaryotes. Ubiquitylation of proliferating cell nuclear antigen (PCNA) plays an important role in eukaryotic TLS. The molecular details of how ubiquitylation of PCNA regulates TLS are not fully understood. One obstacle in studying eukaryotic TLS resides in the difficulty of preparing sufficient amount of ubiquitylated PCNA. We developed a chemical approach for ubiquitylation of PCNA. The chemically ubiquitylated PCNA is functionally equivalent to the native ubiquitylated PCNA in effecting polymerase switch. We also demonstrated the strict requirement of PCNA ubiquitylation for polymerase exchange. Moreover, our study revealed a surprising degree of mobility of ubiquitin moiety on PCNA. To further determine the structure of ubiquitylated PCNA in solution, we combined small angle X-ray scattering (SAXS) and multi-scale computational modeling. We identified three positions for ubiquitin along the axis of the PCNA homotrimer. We argue that these flexible conformations are important for exposing the hydrophobic surface of ubiquitin needed to bind the TLS polymerase. However, despite this high degree of flexibility, there are preferred positions on the PCNA ring with which ubiquitin interacts. We find that the ubiquitin occupies a preferred position on the back face of the PCNA ring as observed in the X-ray crystal structure. We also find the ubiquitin occupies a position on the side of the PCNA ring as suggested by the simulation. Finally a significant portion of the population is in a state where the ubiquitin moieties are in a flexible position in which the ubiquitin is attached to Lys-164 of PCNA but is not otherwise interacting with the PCNA. An understanding of this flexibility will be critical for understanding the recruitment and switch of TLS polymerase Pol η.

This work was supported by a grant to Z.Z. from National Science Foundation (MCB-0953764).

Chen J., Ai Y., Wang J., Haracska J., Zhuang Z. Chemically ubiquitylated PCNA as a probe for eukaryotic translesion DNA synthesis (2010) Nature Chemical Biology. 6:270.

Creation of Tubular □□Helical Assemblies through Seven-Helix Coiled Coils. Chunfu Xu, Emory University.

Native coiled-coil sequences can form large bundles composed of four or more helices in which the helix packing arrangements define continuous channels throughout the supermolecular structure. If the number of helices within the assembly and the inter-helical packing arrangements could be controlled, then one might be able to tailor the channel dimensions and chemistry to facilitate binding of specific classes of guest molecules. To better understand the self-assembly of α-helical peptides and to explore the potential applications of coiled-coil peptide assemblies to selectively encapsulate small molecules in the channel, a nanoscale peptide fibrous assembly was designed based on the seven-helix coiled-coil motif. Various characterization methods including Electron Microscopy, Analytical Ultracentrifugation, Solid-state NMR, and Fluorescence Spectroscopy have been employed to investigate the structure of the nano-fibrous assembly and its ability to encapsulate guest molecules.

Computational study of the first picoseconds following photoexcitation of GFP. Yao Xu, Ramachandran Gnanasekaran and David M. Leitner, Department of Chemistry and Chemical Physics Program, University of Nevada, Reno, 89557 U.S.A.

Unknown atomistic and molecular details exist in many photochemical reactions in proteins such as green fluorescent protein (GFP), which has been observed to undergo excited state proton transfer (ESPT) in about 10 ps. The ESPT follows photo-excitation of the GFP chromophore, which is surrounded by about 10 confined water molecules. To understand the mechanism of excited state dynamics of GFP and the role of the confined water, we have examined time-dependent energy coupling of the electronic states between the protein matrix, the chromophore, and the confined water. The autocorrelation functions of these energy differences exhibit a fast, roughly 100 fs, relaxation at early times, followed by a much slower non-exponential relaxation over several picoseconds.

Macromolecular Crowding Effect on the Structure of Unfolded Outer Membrane Proteins. Cui Ye[1], Maria Wu[2], Qian Chai[1], Meng Zhong[1], Yinan Wei[1]*; [1]Department of chemistry, University of Kentucky, Lexington, KY 40506, [2]Paul Laurence Dunbar High School, 1600 Man O' War Blvd, Lexington, KY 40513.

The biogenesis of outer membrane proteins (OMPs) in Gram-negative bacteria is a multi-step process involving the synthesis of the polypeptide chain in the cytosol, the translocation of the chain first through the inner membrane, and

then through the periplasmic space, and finally the insertion and folding of the protein into the outer membrane. Recent studies demonstrated that the macromolecular crowding condition mimicking the environment in living cell has an obvious impact on protein folding. Here, we use Ficoll 70 as the crowding agent to investigate the macromolecular crowding effect on the residual structure of four OMPs (OmpA, OmpF, OmpG and OmpT) in the unfolded state using several techniques including circular dichroism spectroscopy (CD), fluorescence spectroscopy and limited protease digestion. We found that unfolded OMPs maintained residual secondary structure in aqueous solution, and this structure changed in the presence of Ficoll 70. In addition, the peak shift of Trp fluorescence spectra of three OMPs (except for OmpA) in the presence of Ficoll 70 indicated that the micro-environment of aromatic residues changed, suggesting a structural variation of OMPs. Furthermore, through limited protease digestion experiment, we found that structures of OmpA and OmpG were more resistant to chymotrypsin digestion in the presence of Ficoll 70, suggesting a more compact structure under the crowding condition. We conclude that “unfolded” OMPs actually retained residual structure, and the macromolecular crowding condition affected the structure of unfolded OMPs.