Symposium – Neutrons in Structural Biology

Monday, June 4, 2012
SNS, Building 8600, Conference C-156

Abstracts

10:15 a.m.
Introduction to Neutron Scattering in Structural Biology. Dean Myles, 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.
Exploring Membranes Using Neutron Diffraction, Stephen H. White, Dept. of Physiology and Biophysics, University of California, Irvine, CA 92697-4560, USA

Membranes are complex phases because of their thermal motion and the chemical heterogeneity of the phospholipid molecules comprising them. In order to understand how to predict membrane protein structure from sequence, one must understand in molecular detail the interactions of proteins with the complex bilayer. A major difficulty is that membrane thermal motion precludes atomic-level resolution images of fluid membranes or proteins and peptides within them. The best that can be done at present is a 1-D representation in which the 3-D trajectories of the component groups of the lipid—such as the phosphate, choline, and carbonyls—are projected onto an axis normal to the membrane and averaged to yield transmembrane probability distribution functions. I will discuss how combined x-ray and neutron diffraction measurements on an absolute scattering-length density scale can be used to obtain these probability distribution functions. Although these probability functions are extremely useful, 3-D data are essential for examining lipid-protein interactions in detail. Molecular dynamics simulations offer this possibility. Despite the growing number of atomic-resolution membrane protein structures, direct structural information about proteins in their native membrane environment is scarce. This problem is particularly relevant in the case of the highly-charged S1-S4 voltage- sensing domains responsible for nerve impulses, where interactions with the lipid bilayer are critical for the function of voltage-activated potassium channels. We have used neutron diffraction, solid-state nuclear magnetic resonance spectroscopy, and molecular dynamics simulations to investigate the structure and hydration of bilayer membranes containing S1-S4 voltage-sensing domains. Our results show that voltage sensors adopt transmembrane orientations, cause a modest reshaping of the surrounding lipid bilayer, and that water molecules intimately interact with the protein within the membrane. These structural findings reveal that voltage sensors have evolved to interact with the lipid membrane while keeping the energetic and structural perturbations to a minimum, and that water penetrates into the membrane to hydrate charged residues and shape the transmembrane electric field. Research supported in part by PO1 GM86685 from NIGMS and NINDS.

General References

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2. Krepkiy, D.; Mihailescu, M.; Freites, J.A.; Schow, E.V.; Worcester, D.L.; Gawrisch, K.; Tobias, D.J.; White, S.H.; Swartz, K.J., Structure and hydration of membranes embedded with voltage-sensing domains, *Nature*, 462, 473-479 (2009).
3. Schow, E. V.; Freites, J.A.; Gogna, K.; White, S. H.; Tobias, D.J., Down-state model of the voltage-sensing domain of a potassium channel, *Biophy. J.* 98, 2857-2866 (2010).
4. Andersson, M.; Freites, J.A.; Tobias, D.J.; White, S.H., Structural dynamics of the S4 voltage-sensor helix in lipid bilayers lacking phosphate groups, *J Phys Chem B*, 115, 8732-8738 (2011).

1:15 p.m.

Protein Kinase A: A model for multi-subunit/multi-protein intracellular signalling systems, Donald Blumenthal, PhD, University of Utah

Protein kinase A (PKA) is a multi-subunit protein kinase that is regulated by cAMP. The PKA holoenzyme is a tetramer (R_2C_2) consisting of a regulatory (R) subunit homodimer and two catalytic (C) subunits. The C subunits are inhibited by the R subunits in the holoenzyme complex; binding of cAMP to the R subunits leads to activation of the C subunits by relieving inhibitory interactions of the R subunits. There are four homologous isoforms of the PKA regulatory subunit in mammalian cells that are similar in molecular weight and domain organization. Despite their overall similarities, the four R subunit isoforms are quite distinct from one another in terms of their biochemical properties, their roles in normal cell function and in disease, their subcellular localization, and their interactions with substrates and other signalling enzymes. To better understand the structural features that contribute to isoform-specific functions, we have used small-angle x-ray (SAXS) and neutron (SANS) scattering studies to study PKA isoform homodimer and holoenzyme complexes. These studies have revealed remarkable differences in solution structures among the four PKA isoforms, and have also shown significant effects of disease-causing mutations on the solution structure of PKA. On-going small angle scattering studies are aimed at understanding the interactions of PKA with other signalling enzymes in large multi-protein signalling complexes.

2:15 p.m.

Enzymes for carbon sequestration: Neutron crystallographic studies of carbonic anhydrase, Zoe Fisher, LANL

Carbonic anhydrases (CAs) are ubiquitous enzymes that catalyze the reversible hydration/dehydration of carbon dioxide/bicarbonate. As such, there is enormous industrial interest in using CA as a bio-catalyst for carbon sequestration and biofuel production. Human CA II is the best studies and fastest of any CA characterized so far and there is a lot of interest in using the human enzyme for applications. However, to ensure cost effective use of the enzyme under harsh industrial conditions (high/low pH, elevated temperatures, etc.) we need isoforms that are stable and that remain highly active. Studies were initiated to better understand the catalytic mechanism and active site details using joint neutron and X-ray diffraction. We obtained two neutron structures of wild type human carbonic anhydrase II at pH 7.8 and 10.0. Comparative analysis revealed interesting differences in hydrogen bonding and water network orientations that suggest pathways for proton transfer, the rate limiting part of catalysis. Unique features observed by using neutrons: 1) Tyr7 is deprotonated at pH 10 and may have a role in proton transfer at high pH; 2) the Zn-bound solvent is water (D2O) at both pH 7.8 and 10.0; 3) His64, the proton shuttling

residue, is observed in multiple conformations that correspond to different charged states; 4) A pH-dependent switch in the water network hydrogen bonding patterns that support proton transfer.

3:30 p.m.

The utility of SANS to investigate challenging protein systems: from disorder to aggregation to hydration, Christopher Stanley, Biology and Soft Matter Division, Oak Ridge National Laboratory

Certain proteins contain important structural features that are difficult to obtain by conventional techniques. Two such examples are the dynamic ensemble of intrinsically disordered proteins (IDPs) and the transient aggregate species formed by amyloid-like proteins. In these cases, small-angle neutron scattering (SANS) is particularly useful for directly capturing details of these structures in solution. We are using SANS combined with computations to assess the structural flexibility of an IDP pair that undergoes coupled folding-binding upon interaction. We also employ time-resolved SANS to monitor huntingtin aggregate formation related to Huntington's disease, a genetic neurological disorder. In addition, neutron contrast can be exploited to probe protein hydration by osmolytes, and we are currently developing this method toward discerning the internal structure of huntingtin fibrils. These various applications of SANS are providing new insights into these ill-defined protein structures.

4:30 p.m.

Neutrons, X-rays and Simulations: How we use them to determine the structure of biomembranes, John Katsaras, Biology and Soft Matter Division, Oak Ridge National Laboratory

Scattering techniques, in particular electron, neutron and X-ray scattering have played a major role in elucidating the static and dynamic structure of biologically relevant membranes. Neutron and X-ray scattering are similar in that both techniques are capable of providing dynamical and structural information. However, whereas X-rays are scattered primarily by electrons, neutrons are fundamental particles scattered primarily by their interaction with atomic nuclei. Although the scattering "ability" of X-rays increases in a simple way, with atomic number, in the case of neutron scattering this depends in a complex manner on the nucleus' mass, spin and energy levels. Since neutrons interact uniquely with different nuclei, including with the various isotopes of elements, this fashion of neutrons interacting with atoms allows for the powerful and commonly used contrast variation method. In the case of biological samples inherently rich in hydrogen (^{1}H), the classic example is the substitution of ^{1}H for its isotope deuterium (^{2}H). This method for selectively tuning the sample's "contrast" is used to accentuate or nullify the scattering from particular regions of a macromolecular complex. Importantly, in combination with X-ray scattering and computational simulation, neutron scattering techniques have enabled new areas of study. The seminar will describe three recent model membrane studies of relevance to biology, where neutrons played a key role in revealing physical insight, and possibly even, biological insight.

Poster Session

Structural Analysis of *H. jerocina* Cel7a using X-Ray Crystallography, Annette Bodenheimer[1], Matt Cuneo[2], Paul Schwarz[1], Helen He[2], Hugh O'Neill[2], Dean Myles[2], Flora Meilleur[1,2]

[1]Molecular and Structural Biochemistry Department, North Carolina State University, Raleigh, NC

[2]Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN

Cel7a, also known as cellobiohydrolase 1 (CBH1), is a well-studied hydrolase responsible for the break down of cellulose into cellobiose. Once the lignin of the plant is chemically separated from the cellulose, Cel7a catalyzes the first step in cellulose conversion into ethanol. The efficiency of Cel7A in hydrolyzing crystalline cellulose makes this a key enzyme in the conversion of biomass into biofuels.

The cel7a structure consists of a large catalytic domain (CD), capable of cleaving cellulose, a flexible linker and a small cellulose binding domain (CBD), required for efficient degradation of the cellulose by the CD. Cellulose is threaded through the long tunnel like active site of the CD, where it is hydrolyzed into cellobiose and exits through the other side.

The CD and the CBD structures have been determined by NMR and X-ray crystallography, respectively. We have undertaken the X-ray crystallographic analysis of the full length Cel7a from *Hypocrea jeronica* . The crystals diffract to 1.5 Å and belongs to the space group C2, with unit cell parameter a = 152.1 Å, b= 44.9 Å, c= 57.6 Å and β =101. We will describe our preliminary analysis of the *H. jerocina* cel7a structure.

HyP - Predicting hydrogen atom positions from electron density maps, Matthew Bronstad and Ho Leung Ng, University of Hawai'i – Manoa.

Determining the positions of hydrogen atoms is essential to understanding protein structure and function. However, experimental validation by neutron or ultrahigh resolution x-ray crystallography cannot be applied for most proteins studied. We present a computational method, HyP (Hydrogen Prediction), for detecting hydrogen atom positions by quantitative sampling of x-ray crystallographic electron density maps. This method is sensitive at resolution ranges up to 1.6 angstroms, which includes 12% of all protein structures in the PDB. In a data set of structures up to 1.0 angstroms, our method detects electron density peaks at rotameric positions for 60% of 428 alanine residues, consistent with hydrogen atoms. In the resolution ranges of 1.0-1.19, 1.2-1.39, and 1.4-1.59 angstroms, HyP detects potential hydrogen atoms at rotameric positions for 57, 56 and 49% of alanine residues from a total of 1537, 958, and 2601 alanine residues, respectively. We also compare our predictions with ultrahigh resolution x-ray crystal structures.

Dihydrofolate Reductase thymidylate synthase of *Trypanosoma cruzi*- A potential drug target, Debasish Chattopadhyay[1], S. Velu[1], S. Ananthan[2], A. Rosowsky[3]

1University of Alabama at Birmingham, Birmingham, AL, 2Southern Research Institute, Birmingham, AL, 3Dana Faber Cancer Institute, Boston, MA

Dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolate to tetrahydrofolate. Since tetrahydrofolate serves as a cofactor for the thymine biosynthesis, for which there is no alternate pathway, DHFR activity is essential for survival of cells. Drugs targeting DHFR are therefore extensively used in cancer chemotherapy. Moreover, antifolate drugs are used in antimicrobial therapy and have been remarkably successful as anti-malarial agent. As a potential target for developing drugs for treatment of

Chagas' disease we are conducting a comprehensive structure-activity analysis of the DHFR enzyme of the protozoan parasite Trypanosoma cruzi. We showed that at low nano-molar concentration a lipophilic antifolate drug inhibits the growth of the parasite. Co-crystal structures of the enzyme with high affinity inhibitors have been determined to probe the active site of the parasitic enzyme and identify features that may allow development of selective inhibitors. Moreover, we are examining the structural and functional role of the20 residues long amino terminal extension, which distinguishes T. cruzi DHFR domain from its human counterpart. Results of our investigation will be presented.

How Adenylate Kinase Plays for Moving a Phosphoryl Group, Young-Jin Cho, Lien Phung, Jordan Kerns, Roman Agafonov, and Dorothee Kern, Department of Biochemistry and Howard Hughes Medical Institute, Brandeis University

Adenlylate kinase (Adk) is a ubiquitous phosphotransferase by transferring terminal phosphoryl group of ATP to corresponding AMP resulting in 2 ADP's in equilibrium: ATP+AMP D ADP+ADP. The catalysis is occurring in the closed conformational state while substrates are released in the open state, which is consistent with its dynamic property. However, detailed mechanism of this catalysis has been less informed.

Here, we present several X-ray crystallographic structures of Adks, which represent physical and chemical processes during enzymatic catalysis. A different degree of ATP-lid opened structure with substrates bound gives insights into the dynamic property of the Adk. A variety of conformations of ligand in the AMP-site in comparison to the static form of matching ligand in the ATP-site suggest the struggling toward correct catalysis by optimizing arrangement of the ligands. The location of cofactor, Mg^{2+} is now confirmed by anomalous signal from Co^{2+} ion. Also important major residues are mutated and investigated by acquiring high- resolution structures. Transition state analogue structure in conjunction with other crystal structures in different states suggests the mechanism of phosphoryl transfer.

Moreover, room temperature diffraction data were collected and analyzed suggesting a various kinds of ligand conformations toward catalytically favored position in the AMP-site while one conformation was preferred in the ATP site. In addition, Adk from different species (E coli and Photobacterium Profundum piezophile) are crystallized and compared.

Biochemical and Biophysical Insights into *Azotobacter vinelandii* NifS:NifU Association During Fe-S Cluster Assembly, Christopher L. Colbert[1], Jaim Simoes de Oliveira[2], Patricia dos Santos[4], Kerri K. Martin[2], Valerie Cash[2], and Dennis R. Dean[2]

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Iron-Sulfur (Fe-S) clusters are required for all fundamental biochemical processes, including respiration, photosynthesis, and nitrogen fixation. These cofactors can be simple 2Fe-2S clusters found in ferredoxins or arranged to construct extremely complex clusters, such as the P-cluster and the FeMo cofactor found in nitrogenase. It is remarkable that given the simplistic building blocks of Fe-S cluster that the biological assembly of these clusters is rather complex.

In the early 1990's, NifS and NifU, the two proteins primarily responsible for the biological assembly of the initial Fe-S clusters involved in the maturation of nitrogenase in *Azotobacter vinelandii*, were characterized[1,2]. NifS was characterized as a pyridoxal-5'-phosphate (PLP) dependent cysteine desulferase[1], and NifU was identified as a Fe-S cluster scaffold protein[3,4]. While the individual functions

of these proteins have been elucidated and it has been shown that they function in conjunction, the mechanism by which NifS and NifU associate to assemble Fe-S clusters remains elusive.

Here we present our biochemical and biophysical analysis of the association of NifS and NifU. First, we present analytical ultracentrifugation (AUC) data indicating that NifS and NifU associate into a complex containing 2 NifU subunits and 2 NifS subunits. Furthermore, our AUC experiments and affinity pull-down assays demonstrate residues 1-130 of NifU (the "IscU domain") mediates NifU:NifS association. Finally, we present biochemical evidence showing that [2Fe-2S] clusters are assembled in our complexes, that both NifU and its "IscU domain" alone provide protection from alkylation to the NifS active site Cys325, and that NifS is catalytically active in this complex. Based on this evidence, we postulate a mechanism by which the persulfide intermediate formed on Cys325 of NifS is protected from non-specific interactions and loss of the sulfur to the solvent when in complex with NifU. Future experiments will analyze the intermediate sequential steps of Fe-S cluster assembly using X-ray and Neutron scattering methods

References:

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2. Fu, W., et *al.*, (1994) *Biochemistry*, 33, p. 13455.
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The Dependence of Membrane Raft Size on Lipid Composition: A Small-Angle Neutron Scattering Study, Frederick A. Heberle, Robin Petruzielo, Jianjun Pan, Paul Drazba, Norbert Kucerka, Gerald W. Feigenson, and John Katsaras

Membrane rafts are small, transient assemblies of lipid and protein that are proposed to play a role in a variety of cellular processes including protein sorting, vesicular transport, viral entry and exit from cells, and signaling. The composition, size, and connectivity of rafts are all parameters available to the cell for regulating protein contacts, and hence controlling the chemistry that occurs in the membrane. We use small-angle neutron scattering to study unilamellar vesicle samples of a chemically simple model system, for which domain size is controlled by lipid composition. We vary the ratio of the unsaturated lipids POPC and DOPC, together with fixed mole fractions of chain-perdeuterated DSPC (dDSPC) and cholesterol, under conditions which contrast match the solvent to the mean vesicle scattering length density. Under these conditions, the formation of liquid-ordered domains enriched in dDSPC contrast with liquid-disordered domains, causing an increase in scattering at small momentum transfers. The intensity as a function of momentum transfer may then be compared to that of Monte Carlo simulations. We are able to match the model and experimental results to determine the average number of domains that form, and hence, from area conservation, domain size. We also observe a shift in the peak of the scattering intensity with increasing concentration of DOPC, suggesting that lateral domain size can be controlled by the level of unsaturated lipid found in the membrane.

Neutron Scattering Measurements Reveal Slower Cholesterol Transport in Model Lipid Membranes, Li Ge, University of Illinois

Proper cholesterol transport is essential to healthy cellular activity and any abnormality can lead to several fatal diseases. However, complete understandings of cholesterol homeostasis in the cell remains elusive, partly due to the wide variability in reported values for intra- and inter-membrane cholesterol transport rates. Here, we used time-resolved small-angle neutron scattering to measure cholesterol inter-membrane exchange and intra-membrane flipping rates, in situ, without recourse to any external fields or

compounds. We found significantly slower transport kinetics than reported by previous studies, particularly for intra-membrane flipping where our measured rates are several orders of magnitude slower. We unambiguously demonstrate that the presence of chemical tags and extraneous compounds employed in traditional kinetic measurements dramatically affect the system thermodynamics, accelerating cholesterol transport rates by an order of magnitude. To our knowledge, this work provides new insights into cholesterol transport process disorders, and challenges many of the underlying assumptions used in most cholesterol transport studies to date.

Characterization of Protein Dense Phases, Daniel Greene, University of Delaware

The phase behavior of native protein solutions is important in industrial processes such as crystallization and precipitation and can affect other aspects of bioprocessing, especially in the pharmaceutical industry, via the presence of aggregates. Phase behavior is a direct consequence of intermolecular interactions, which can be quite complex in that they depend on many solution variables (e.g., protein concentration, solution composition, temperature, and pH), resulting in a high-dimensional phase diagram. Because of the complexity, complete phase diagrams have been measured for relatively few systems, and very little work has been done to characterize the dense phases obtained other than crystallographic analysis of protein crystals. Understanding the structure of amorphous dense phases will yield information about the mechanism by which they form and thus potentially lead to a "smarter" design of various processes. Using scattering techniques, cryo-EM, and other experimental and computational techniques, the structure of dense protein phases and the underlying processes leading to their formation is investigated.

Crystal structure and DNA binding properties of MafA suggest a multi-step DNA-binding mechanism for Maf proteins, Xun Lu, Gerald Guanga, Cheng Wan, Robert B. Rose, North Carolina State University

Basic leucine zipper (bZIP) proteins constitute a major class of transcription factors in eukaryotes. BZIPs homodimerize or heterodimerize with multiple partners, and recognize diverse DNA-binding sequences, typically consisting of four base pair half-sites. Maf bZIPs distinguish themselves by the presence of an ancillary DNA-binding domain preceding the conventional basic DNA-binding region. Maf factors bind longer consensus DNA sites consisting of a cAMP-responsive element (CRE) or TPA-responsive element (TRE) core flanked by an additional tri-nucleotide TGC sequence. MafA is an oncoprotein, and is critical for insulin gene expression. We determined the crystal structure of a homodimer of the MafA DNA binding domain, excluding the N-terminal transactivation domain, bound to its consensus DNA-binding site with a TRE core. The structure is consistent with the structure of the small Maf protein, MafG, which lacks an activation domain. In the structure MafA makes base-specific contacts to the flanking TGC, but not to the TRE core. However, *in vitro* binding studies utilizing a pulse-chase EMSA revealed that mutating either the core or the flanking TGC dramatically increased the binding off-rate. More surprisingly, adding a short AT-rich sequence 5' to either side of the flanking TGC abrogates the need for the other half-site. Unlike canonical bZIPs, MafA also bound DNA as a monomer. These results suggest a multi-step DNA-binding mechanism. This may include MafA switching from contacting the TRE core to contacting the flanking TGC by a conformational change of protein and/or DNA.

Dynamic Thylakoid Organization in Cyanobacteria Revealed by Small Angle Neutron Scattering,
Michelle Liberton[1*], Lawrence Page[1*], William B. O'Dell[2*], Hugh O'Neill[2], Volker S. Urban[2‡] and Himadri Pakrasi[1‡, 1]Department of Biology, Washington University, St. Louis, MO 63130
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Cyanobacteria are photosynthetic prokaryotes that convert sunlight into cellular fuel. Knowledge of the thylakoid membrane systems in cyanobacteria is critical to understanding the metabolic processes in these organisms. We used small angle neutron scattering (SANS) to examine *in vivo* the wild-type strain of *Synechocystis* sp. PCC 6803 and a series of mutants with altered phycobilisome (light-harvesting antenna) systems for changes in thylakoid membrane architecture under different light intensities and photosynthetic capacities. In our SANS studies we observed the periodicities arising from the thylakoid membrane layers. Our assignment of the periodicities to lipid membranes is supported by contrast variation studies in which the diffraction peaks were found to have a contrast match point of ~18% D_2O/72% H_2O. By comparing the diffraction periodicities with electron microscopy data, we identified the thylakoid lumen-to-lumen repeat distance for each strain. This distance varied systematically from ~627 Å to ~357 Å in direct correlation with the decreasing size or absence of the phycobilisome complex. Using a prototype LED sample illumination system, we also captured light-induced changes to the membrane periodicities and characterized the reversibility of these changes thus demonstrating a robust flexibility in the membrane system. Comparative measurements after treating cyanobacteria with a photosystem II inhibitor revealed that structural changes are dependent upon a functional photosynthetic apparatus. These results lend a dynamic perspective to the intracellular membrane organization in cyanobacteria cells and demonstrate the advantages of SANS for investigating photosynthetic organisms under physiological conditions.

Water activated shape memory effect in thin wood slivers, Nayomi Plaza1, Joseph E. Jakes2, Don Stone3 and Sam Zelinka2
1 University of Puerto Rico – Mayaguez 2Forest Products Laboratories 3University of Wisconsin – Madison

Biomimetic and smart materials applications have directed scientists' attention towards passive plant actuation. Recently, we discovered a water-activated shape memory twist effect in wood slivers. The effect is studied by means of a torsional pendulum constructed using a wood sliver that is an inch long and has a diameter of approximately 50 μm. Upon wetting, the slivers twist. If unrestrained, the slivers untwist while drying. However, if the sliver is restrained at maximum twist and then dried, a large fraction of the twist is "locked in". After the restraint is removed and the sliver rewetted, it untwists to it pre-wetting configuration. The effects of cellulose microfibril angle and ambient relative humidity are being investigated. Preliminary results have shown that the magnitude of twist and the twist rate increase with higher microfibril angles. Also, slivers conditioned at lower ambient relative humidity twist more and faster than the same slivers conditioned at higher relative humidity when wetted. Both the shape memory fixing factor and the recovery factor increase as the relative humidity is lowered resembling more the ideal case.

Small Angle X-Ray Scattering Restraints to Promote Protein Folding, Daniel Putnam, Brian Weiner, Jens Meiler – Center for Structural Biology Vanderbilt University

Small angle x-ray scattering (SAXS) is an experimental technique used to create low resolution (50 Å to 10 Å) representations of protein structures in solution. Using BCL::Fold, a protein folding algorithm based on the placement of secondary structure elements (SSEs), I created an algorithm BCL::SAXS to convert x-ray crystallographic atomic coordinates to a SAXS scattering profile. The purpose of this research is to develop a method to 1) efficiently calculate SAXS curves from rigid body protein models and 2) calculate saxs curves for proteins containing idealized secondary structure elements, such as those produced by BCL::Fold. The combination of SAXS information with computational methods may be used as a restraint during minimization to enhance native like sampling.

First I present a method for the calculation of accurate SAXS curves based on the Debye formula and a set of scattering form factors. The resulting curves are on par with CRYSOL, the gold standard for SAXS

curve calculations, and can recognize native structures among native-like decoys. Then I present a method for generating SAXS profiles for protein models without explicit side chains, by placing the combined form factor calculation of the side chains at the C-β position of the residue. The curves generated with missing side chains are very similar in morphology to the full atom curves and can filter native-like protein models.

SAXS and SANS on CENP-A nucleosome in solution, Nikolina Sekulic, Kushol Gupta, Greg Van Dyne, Ben E. Black, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA

The centromere is a chromosomal locus necessary for accurate chromosome segregation during cell division. Errors in mitotic centromere function result in chromosome imbalances which are associated with cancer genesis and/or progression. Although centromeres are characterized by highly repetitive DNA sequence, specialized histone - CENP-A, has emerged as an epigenetic mark for centromere specification independently of DNA. It has been proposed that nucleosome, the smallest building unit of chromatin consisting of DNA and histones, will differ if CENP-A replaces canonical H3 histone. Our recent X-ray structure of the subnucleosomal complex containing CENP-A (Sekulic et al, Nature 2010) and the structure of CENP-A nucleosome (Tachiwana et al, Nature 2011) indicate octameric complex with left-handed DNA, similar to canonical nucleosomes. Our model predicts rotation between histones that would result in nucleosome with altered shape and DNA path. It is possible that CENP-A nucleosome samples between several different states, so that only dynamic studies would be successful in identify particularities of this nucleosome. Ongoing studies on CENP-A-nucleosome in solution, combining SAXS and SANS methods have potential to complete picture of CENP-A nucleosome and thus significantly aid our understanding of centromere specification.

Comprehensive analysis of the universal stress protein, Karolina L. Tkaczuk[1,2], Igor Shumilin[1,2], Maksymilian Chruszcz[1,2], Wladek Minor[1,2]

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Universal stress proteins (USPs; members of the PF00582 superfamily [Bateman et al., 2004; Tatusov et al., 2003]) are present in many different organisms, including archaea, bacteria, fungi and plants. In stress conditions such as heat shock, nutrient starvation, presence of DNA-damaging agents or other stress-inducing agents which may arrest cell growth, universal stress proteins constitute a natural biological defense mechanism. While the molecular mechanisms by which USPs act are not well characterized, overexpression of the proteins appears to allow organisms under stress to survive.

In most genomes, multiple USP paralogs are present; the number of copies depends on the organism. For example, *E. coli* produces 6 USP paralogs: UspA, UspC (*yecG*), UspD (*yiiT*), UspE (*ydaA*), UspF (*ynaF*), and UspG (*ybdQ*). UspE is a fusion protein composed of two USP units E1 and E2. In *Arabidopsis thaliana* four copies of the *usp* gene are present.

To date, 18 USP structures have been deposited in the PDB (most of them solved by structural genomics centers). In this work we discuss in detail two USP structures solved by our group (for each apo and cofactor bound forms), from *Archaeoglobus fulgidus* and *Nitrosomonas europaea* respectively. We juxtapose them with other archaeal and bacterial USP homologs, and discuss their similarities and differences in terms of structure, sequential motifs and probable function. We analyze sequence conservation, cofactor binding sites, dimerization patterns, and evolutionary relationships among different types of USPs.

Orientation and Calcium-induced structural changes of calmodulin (CaM) ChARACTERIZED by atomic force microscopy (AFM) and Kelvin probe force microscopy (KPFM), Xiaoning Zhang and Yuguang Cai, Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055.

Calmodulin (CaM) is a protein that composed of 148 amino acid residues. Its conformation changes upon releasing or retaking calcium ions. We characterized this calcium-induced conformational change in the immobilized state by Atomic Force Microscopy (AFM) and Kelvin Probe Force Microscopy (KPFM). First, our AFM topography and KPFM characterization revealed that the conformational change, from CaM (with Calcium binding) to apo-CaM (without Calcium binding), corresponded to a 6.5 ± 0.4 Å increase in the thickness of immobilized Calmodulin monolayer film and a 17.0 ± 0.6 mV decrease in surface potential. In addition, we found that CaM molecules immobilized on surface adopted different orientations even though they have the same height on the same chemical pattern. This orientation difference was distinguished by both AFM phase and surface potential images. Our studies demonstrate the immobilized CaM maintains its activity and can undergo conformational changes. Also, our work provides an approach that can image and quantify the conformational change of immobilized proteins on the molecular level.