Protein Structure

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Protein Structure Initiative-Materials Repository (PSI-MR): An Open Shared Public Resource for Structural Genomic Plasmids

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The Protein Structure Initiative Materials Repository (PSI-MR; http://www.hip.harvard.edu/PSIMR) was established in 2006 at the Harvard Institute of Proteomics (HIP) with the mission of providing centralized storage and distribution of information and samples for the 100,000 protein expression plasmids created by PSI researchers. These plasmids are an invaluable resource that allows the research community to dissect the biological function of proteins whose structures have been identified by the PSI. Researchers can search for and request clones in the PSI collection through the Plasmid Information Database (PlasmID: http://plasmid.med.harvard.edu/PLASMID). PSI plasmids are linked to the PSI Structural Genomic Knowledgebase (PSI KB) which facilitates cross-referencing of a particular plasmid to protein annotations and experimental data. Thus far over 25,000 PSI plasmids are in process at the MR, and nearly 9,000 are already available from PlasmID. In addition to distributing materials, the MR has sought to simplify the MTA process in order to decrease the time it takes for institutions to deposit or receive plasmids. To achieve this goal, the MR pioneered two documents, the depositor's agreement, which sets forth the terms enabling the MR to distribute deposited plasmids from outside institutions, and the expedited process MTA, which eliminates the need for researchers to wait for their institutions to sign an MTA. In the future, the MR will maintain a similar scope and mission of continuing to make PSI plasmids and data available to researchers and increasing the expedited MTA network so that researchers can receive PSI plasmids without delay.

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From The Polymer Nature Of Proteins To The Evolution Of Protein Function

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We develop a new view on evolution of protein function which is based on the key role of a polymer nature of protein polypeptide chains in determining structure and evolution of proteins. Polypeptide backbone flexibility establishes a restriction on the size and shape of basic structural units of proteins. These elements, closed loops, are formed by the returns of the protein backbone and have a characteristic size of 25-35 amino acid residues. The closed loops also possess elementary functions, which they bring together in the protein globule forming a functional site. An elementary function is defined by one or few residues involved into function, and it is encoded in the sequence of the loop by the specific signature. Biological function of the globule is built as a combination of few elementary functions which provide necessary sequence of chemical reactions occurring in the functional site. Our model delineates connections between different protein structures based on partitioning them into elementary functional loops (closed loops with a functional signature). The computational procedure for deriving sequence/structure prototypes of elementary functional loop seeks for the primordial prototypes of contemporary elementary functional loops. By considering prototypes of elementary functional loops and their presence in various protein folds, we create a graph of evolutionary connection between different protein functions. We demonstrate, for the first time, that it is possible to reconstruct how biological functions of contemporary proteins emerged as a combination of elementary nes. We explore an evolution protein function and show how current diversity of proteins evolved by utilizing elementary functional loops. We plan to use our model in theoretical predictions of outcomes from the directed evolution experiments and in the de novo design of protein folds with desirable biological function.

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Predictions of Protein Circular Dichroism Calculated by the Dipole Interaction Model and Compared to Synchrotron Radiation Circular Dichroism Experiments

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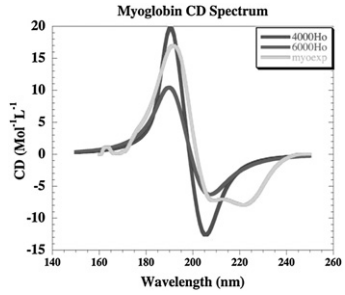
The determination of accurate secondary, tertiary and quaternary structures of macromolecules is vital for the study of intra- and interbimolecular interactions important for drug discovery, probing enzyme mechanisms and other functional protein complexes. Despite the recent advances in protein structural determination methods like X-ray crystallography, there is still a need to evaluate the accuracy of the model structures used in macromolecular interaction prediction. This is especially important where accurate three dimensional structures of the proteins are absent. Synchrotron radiation circular dichroism (SRCD) is an emerging technique sensitive not only to secondary structure but also teriary and quatertary structure. Theoretical calculations of circular dichroism (CD) using the dipole interaction model have successfully predicted CD for a variety of peptides and monomeric proteins. Herein, the dipole interaction model predicts CD for proteins in monomeric, dimmeric or tetrameric forms and is compared to experimental SRCD spectra. This is an attempt to evaluate the homology models. Preliminary results show comparable CD spectra between the dipole interaction model predictions and SRCD data for small proteins like myoglobin.



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New Spin Label Designed for Double Electron-Electron Resonance Distance Measurements in the Liquid Nitrogen Temperature Range

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Double electron-electron resonance (DEER) measurements of interspin distances in biomolecules are restricted by the spin echo dephasing rates of the paramagnetic centers. Faster dephasing rates limit the longest distance that can be measured and the precision with which the distribution of distances can be measured. Because of the temperature dependence of dephasing rates for currently-used spin labels, the optimum temperature for DEER measurements is 50 to 60 K, which requires the use of liquid helium as the cryogen. A new spin label has been synthesized and characterized, with a structure that is analogous to the commonly-used synthetic peptide TOAC, except that the gem-dimethyl groups are replaced by spirocyclohexyl groups. Because of the absence of methyl groups, the spin echo dephasing rates for this new spin label in 1:1 water glycerol remains approximately independent of temperature up to about 130 K. The spin lattice relaxation rates for the new probe are sufficiently faster at 130 K to compensate for the changes in Boltzmann populations, so the signal-to-noise, longest distance that can be measured, and accuracy of determination of distance distributions in DEER experiments with this probe will be as favorable at 130 K as for currently-used probes at 50 to 60 K. These results indicate that it will be possible to perform DEER experiments with the substantially less expensive liquid nitrogen. Supported by NIH NIBIB EB002807(Denver), NSF CHE-0718117 (Nebraska), NSF/DOE under Grant No. CHE-0087817 and DOE under Contract No. W-31-109-Eng-38 (Indiana).

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Ensemble Dynamics with Orientational NMR Restraints in Solution and Membrane Environments

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We have recently developed various restraint potentials for residual dipolar coupling in solution NMR [1] as well as dipolar coupling and chemical shift in solid-state NMR [2]. In principle, NMR observables are time- and ensemble-averaged, thus the structure determination needs to be based on the average property of an ensemble rather than the collection of multiple independent structure determination. In this work, we have further formulated and implemented the ensemble-average orientational restraint potentials, such as residual dipolar coupling (RDC), dipolar coupling, and peptide plane chemical shift, to explore the flexible nature of proteins embedded in such experimental observables. We will present the numerical accuracy of our implementation as a function of number of replicas during ensemble dynamics and illustrate the efficacy of ensemble dynamics in exploring protein structure and dynamics in solution and membrane environments.

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Molecular Dynamics Simulations Of Escherichia coli Acyl Carrier Protein Containing Fatty Acyl Derivatives

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Acyl carrier protein (ACP) in bacteria and plants is an essential co-factor protein in fatty acid biosynthesis that shuttles covalently bound fatty acyl

intermediates to various enzyme partners. Structures of apo-, holo-, and saturated acyl-ACPs indicate that the acyl groups are housed inside a hydrophobic binding cavity between three largely parallel helices. Molecular dynamics (MD) simulations have previously been used to illustrate the behaviour of various saturated acyl chains attached to ACP, in excellent agreement with experimental data. However, relatively little is known about the fatty acyl derivatives formed during the elongation of the acyl chains, namely the β-ketoacyl-, β-hydroxyacyl-, and enoyl-intermediates. ACP is one of the most abundant proteins in *Escherichia coli* and possesses a large number of interacting enzymes. As such, we seek to understand how the enzymes of fatty acid biosynthesis recognize which derivative is bound by ACP. To address this, we conducted numerous MD simulations of *E. coli* ACP with β-ketoacyl-, β-hydroxyacyl-, and enoyl- intermediates. The unconstrained MD simulations were set up with the attachment either in a solvent exposed or a solvent shielded conformation inside the hydrophobic binding pocket of ACP. We investigate a range of acyl group derivatives attached to ACP spanning from four to eighteen carbon groups in length to develop our understanding of the differences imparted on ACP by various acyl chain lengths. The results of the simulations of fatty acyl derivatives will provide a first look at the manner in which ACP accommodates these binding groups. What these data contribute to the understanding of enzyme:ACP interactions and substrate recognition by fatty acid synthase enzymes will be discussed.

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The Dependence Of Coiled-coil Chirality On Elastic Energy

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Coiled coils are proteins that consist of two or more alpha-helices that wrap around each other to form a super-helical structure. Using a continuum elastic model, a recent paper [1] has shown that the chirality of the super-helical twist is dictated by the chirality of the pattern of hydrophobic residues on each helix only when the bending and twisting energy of each helix is considered. In the absence of any energy cost due to the flexible motions of each helix, they showed that there is a family of structures which are consistent with the hydrophobic pattern. Using a coarse-grained atomistic model for coiled coils that includes the flexible degrees of freedom for each helix, we have carried out monte-carlo simulations to examine how the energy and chirality of coiled coils depends on the strength of the elastic energy. We find that there is an optimal weighting of the elastic energy that leads to the coiled coils adopting the same chirality as the hydrophobic pattern on each helix. We then explored how the chirality of the coiled coil changed under the application of an applied force or an applied torque. Our findings are compared to recent measurements on the mechanics of coiled-coils from single-molecule studies.

[1] S. Neukirch, A. Goriely and A.C. Hausrath, PRL, 100, 038105 (2008).

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Computational Modeling of the Structural Mechanism Linking Ligand and Corepressor Binding to Thyroid Hormone Receptors

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Thyroid hormone receptors (TRs) are nuclear receptors with two functional states. Upon binding to the thyroid hormone, TRs recruit coactivator proteins and activate gene transcription. Without the hormone ligands, TRs interact with corepressor proteins and repress transcription. Due to the importance of the thyroid hormone action in embryo development, metabolism, heart rhythm and cholesterol level, the molecular mechanism behind the functional switch has been studied extensively. However, the current available crystal structures of thyroid hormone receptor ligand binding domains (LBDs) are all in the ligand-bound (holo) form, with no revelation on ligand-free (apo) form or transcription corepressor bound form. In order to elucidate the complete apo to holo switch process, we constructed homology models of apo TR from the available apo structures of interrelated nuclear receptors: retinoid acid receptor (RXR) and peroxisome proliferators activated receptor (PPAR). Both models were subjected to energy minimization followed by molecular dynamic (MD) simulation. Analysis of the MD simulations proved that the model based on PPAR was more stable than the model based on RXR. As a result, unlike the prevailing idea that TRs would exert a major structural change in the C-terminal activation helix (AF2) domain upon ligand binding, TRs exhibit only subtle changes at the AF2 domain. Our model predicts that the recruitment of corepressor proteins, which require the relocation of the AF2 domain, is more appropriately portrayed as an induced fit process. Additionally, we constructed homology models of TR LBD in complex with the nuclear receptor interacting domains of corepressor proteins. Molecular dynamic simulation of this receptor-correpressor complex system in the absence or presence of the ligand thyroid hormone has identified correlated conformational changes that may be important for the functional switch.

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Effects Of Reactive Oxygen Species On Cyan Fluorescent Protein

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Recent advances in microscopy techniques and the development of many different colour variants of the GFP family of proteins allow for a more direct analysis of protein function in live cells. The advantage of genetically coded fluorescent protein probes is often offset by their photophysical properties which usually make them very sensitive to cellular environmental changes. Among these, reactive oxygen species (ROS) are an essential part of key cellular processes (mitochondria respiration, apoptosis) and are also involved in the pathogenesis of various diseases (cancer, atherosclerosis, Alzheimer's disease, etc.). We studied the effects of ROS on the cyan fluorescent protein (CFP) *in vitro,* as this fluorescent protein is currently one of the most widely used in protein interaction studies. We studied the fluorescence and absorption changes of recombinant CFP protein using γ-radiolysis for ROS production. γ-radiolysis ROS production allowed us to have an exact control over the radical concentrations delivered unto the protein samples. The radicals used in this study were OH°, O_2^{-} or a mixture of OH° and O_2^{-}. We also determined the chemical modifications that take place upon ROS induced protein oxidation by mass spectrometry. We show that the targets of oxidation are one tyrosine and four methionine residues located on the protein surface and that the chromophore is not likely modified through these oxidation processes.

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Thermodynamic Intermediates of the Alkaline III→IV Transition in Ferricytochrome c Probed by 695 nm Charge Transfer Band

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The 695 nm band in the spectrum of the native state (III) of ferricytochrome c has recently been shown to be composed of different sub-bands which reflect different conformational substates (CS) of the functional pivotal Fe-M80 linkage. In order to explore the influence of the alkaline III→IV transition on this CSs we measured and analyzed the absorption and CD profile of this band as a function of pH between pH 7 and 10 at high (50mM) and low anion concentration for horse heart (hh) cytochrome c. Additional measurements on yeast cytochrome c were performed at low anion concentrations. The titration curves of the two dominating sub-bands are clearly biphasic at low anion concentrations and reflect effective pK-values of 8.5 and 9.65. On the contrary, the titrations curves obtained at high anion concentrations are monophasic; the effective pK being 9.23. The thermodynamic parameters (i.e. pK and the Hill coefficient as a measure of cooperativity) are slightly different for the two CSs. We fitted the data obtained at low anion concentration with a model, which assigns the two effective pK-values to two different alkaline states IV1 and IV2, in which M80 is replaced by K73 and K79, respectively. This model did not reproduce our data well in contrast to an alternative model which assumes only one species with two protonation sites. This suggests that the state populated upon the pK= 8.5 protonation can be interpreted as a thermodynamic intermediate of the III→IV transition. This notion is consistent with our observation, that the 695 nm titration curves of the y-cytochrome c mutants K79R and K73V are still mostly biphasic at low ionic strength.

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X-ray Footprinting at Beamline X28C: A National Resource for Studying Macromolecular Structure and Dynamics

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X-ray footprinting employs intense X-rays produced by synchrotron radiation to generate hydroxyl radicals in solution on microseconds-milliseconds timescales. These hydroxyls radicals undergo stable reaction with solvent accessible sites of macromolecule and produce covalent modifications, which are appropriate to probing macromolecule dynamics under physiological condition. For nucleic acids, one analyzes the pattern of fragments after X-ray exposure by gel electrophoresis; the protected sections that are not cleaved yield a ''footprint''. For proteins, the exposed samples are digested with proteases and analyzed by liquid chromatography- and tandem-mass spectrometry to determine the extent and sites of modification. The data provide detailed structural information to map tertiary contacts of macromolecular interactions, which can subsequently be used as constraints for molecular modeling to generate high-resolution