Telluride Science Research Center

Workshop on Protein Dynamics

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ABSTRACTS

EXPERIMENTAL STUDIES OF STRUCTURE, FUNCTION, AND COHERENT OSCILLATIONS IN BIOMOLEUCLES

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Femtosecond coherence spectroscopy (FCS) can be used to prepare and monitor coherent states of biological samples such as heme proteins. Following laser pulse induced ligand photolysis, the (initially planar) heme group is left far from its final product state equilibrium geometry. This leads to coherent oscillations of those modes composing the reaction coordinate for ligand binding and dissociation. Coherence studies, along with "white light" continuum measurements of the spectral dynamics, probe the dissociation, population decay, vibrational coherence and damping of this fundamental biochemical reaction. Investigations of the effect of temperature and sample condition on the coherent motions of the heme and on the ultrafast geminate rebinding of the diatomic (NO) ligand are also described and emphasized. The influence of the surrounding protein material is probed by utilizing heme model compounds and site directed mutants. These studies show that the spectrum of low frequency heme modes can be significantly altered by the coordination of the proximal axial ligand. The model compound investigations also allow the diatomic ligand rebinding barrier to be separated into "proximal" and "distal" contributions that can be separately determined. The NO rebinding studies reveal that NO decouples the rebinding reaction from the heme conformational substates and that the slower geminate phase of NO rebinding results from the return of the NO from a distal (Xe4) cavity.

Vibrational Dynamics of Iron in Biological Molecules

J. Timothy Sage, Northeastern University

Nuclear resonance vibrational spectroscopy (NRVS) is an emerging synchrotron-based technique that reveals the complete vibrational spectrum of a Moessbauer nucleus, based on ultrahigh-resolution X-ray measurements near the nuclear resonance. I will illustrate novel opportunities that this site-selective method provides for characterizing the vibrational dynamics of ^{57}Fe at the active sites of heme proteins, iron-sulfur proteins, and related model compounds. (1) Quantitative data on the frequency, the amplitude, and in some cases, the direction of all iron vibrations provide a uniquely detailed benchmark for modern quantum chemical vibrational predictions, with which they can be directly compared on an absolute scale. (2) Measurements on oriented single crystals of iron porphyrins reveal low-frequency out-of-plane vibrations that we identify with the long-sought heme doming mode, similar to the motion that takes place on oxygen binding to heme proteins. Moreover, the experimental data provide a direct experimental estimate of the force constant for Fe displacement normal to the heme plane and suggest that this Fe motion is an important element in protein control of biological reaction energetics. (3) Comparison with conventional Moessbauer measurements provides an experimental test for the activation of anharmonic dynamics near 200 K.

Vibrational energy relaxation in proteins

John Straub, Boston University

When a protein is excited by ligand binding, ATP attachment, or laser pulses, there occurs vibrational energy relaxation (VER). Energy initially "injected" into a localized region flows to the rest of the protein and surrounding solvent. VER in large molecules (including proteins) itself is an important problem for chemical physics. Even more significant is the challenge to relate VER to fundamental reaction processes, such as a conformational change or electron transfer of a protein, associated with protein function. The development of a accurate understanding of VER in proteins is an essential step toward the goal of controlling protein dynamics.

Here VER of a selected mode in cytochrome c is studied using two theoretical approaches. One is the equilibrium simulation approach, with quantum correction factors, and the other is the reduced model approach, which describes the protein as an ensemble of normal modes interacting through nonlinear coupling elements. Both methods result in similar estimates of the VER time (sub picosecond) for a CD stretching mode in the protein at room temperature. The theoretical predictions are in accord with the experimental data of Romesberg's group. A perspective on future directions for the detailed study of time scales and mechanisms for VER in proteins is presented.

Resonant four wave mixing femtosecond spectroscopy as a probe of protein dynamics

Ralph Jimenez, JILA

Femtosecond electronic spectroscopy has been used to characterize the fluctuations of many cofactor-containing proteins. Nonlinear methods such as photon echo spectroscopy provide the ability to measure the spectrum of motions on femtosecond through nanosecond timescales and beyond. I will introduce the photon echo technique, survey published work by a few groups, and draw as many connections as possible between nonlinear femtosecond experiments and other methods such as MD simulations and NMR relaxation measurements.

Ultrafast Protein Dynamics with Biological Mutation

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Protein dynamics is a complex process and the current challenge is to break down its complexity into elementary processes which act on different time scales and length scales. We integrate *femtosecond spectroscopy, molecular biology techniques,* and *computational simulations* to follow the system evolution in real time and thus elucidate the complex dynamics with unprecedented detail. Here, we report two important biological systems of protein surface hydration and light-driven DNA repair by photoenzyme (photolyase). With femtosecond temporal and single-residue spatial resolution, we mapped out the global water motion in the hydration layer using tryptophan residue to scan the protein surface with site-directed mutagenesis. The obtained results reveal the ultrafast nature of surface hydration dynamics and provide a molecular basis for protein conformational flexibility, an essential determinant of protein function. By altering chemically and structurally important residues of photolyase with mutation, we identified key residues in catalytic reactions and followed the entire functional evolution of DNA repair. We resolved a series of ultrafast processes including active-site solvation, energy harvesting and transfer, and electron hopping and/or tunneling. These results elucidate the crucial role of ultrafast dynamics in biological function efficiency and lay bare the molecular mechanism of DNA repair at atomic scale.

Protein Dynamics of Hemoglobin and Myoglobin: Time-resolved resonance Raman study

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Protein dynamics are intimately connected to the structure/function relationship of biological systems. The molecular mechanism of cooperativity in oxygen binding of hemoglobin (Hb) is one of the classical problems in this aspect. The binding of small molecular ligands to the hemes in Hb is a highly localized perturbation. Nonetheless, this localized perturbation initiates a sequence of propagating structural events that culminates in a change of quaternary structure. Myoglobin (Mb) is structurally very similar to a subunit of Hb and serves as a model system for the tertiary relaxation processes. In this talk, I will discuss the structural dynamics of Hb and Mb following ligand dissociation from the heme.

Resonance Raman (RR) spectroscopy is a versatile spectroscopic technique for studying the structure of proteins. For Hb and Mb, Raman bands of the heme moiety are selectively enhanced when the excitation wavelength around 400 nm is employed. On the other hand, when the excitation wavelength is tuned between ~195 and 260 nm, strong resonance Raman scattering from the peptide backbone and aromatic amino acids provides vibrational information on local protein structure and environmental changes. Thus, we can selectively obtain structural information for the heme moiety and protein by tuning the excitation wavelength for RR measurements. We have constructed time-resolved visible and ultraviolet RR spectrometers to study protein dynamics of Hb and Mb. Protein dynamics upon ligand dissociation was investigated by examining temporal changes of RR spectra of some specific parts of the proteins. The RR bands due to the heme vibrations showed almost instantaneous changes upon the ligand dissociation. On the other hand, frequency shift in the picosecond time range was observed for a stretching mode of iron-histidine bond, which is the only covalent linkage between the heme and the protein. RR bands of tryptophan and tyrosine residues also showed temporal changes in the picosecond time region. Based on the time-resolved RR data, I will discuss the sequence of propagating structural events which is driven by the ligand dissociation. The following topics will be focused on.

1. Protein dynamics of Hb
    a. Dynamics of Hb and its isolated chains
    b. Dynamics of Hb encapsulated in porous sol-gels
2. Protein dynamics of Mb
    a. Dynamics of Mb and its mutant which lacks covalent linkage between the heme and the polypeptide
    b. Ultrafast protein dynamics upon ligand dissociation revealed by picosecond time-resolved ultraviolet RR spectroscopy

Unveiling Functional Protein Motions with Picosecond X-ray Crystallography and Molecular Dynamics Simulations.

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We have developed the method of picosecond time-resolved Laue crystallography and used this technique to investigate structural dynamics in biological macromolecules at ambient temperature. Time-resolved snapshots of myoglobin following flash photolysis of the CO adduct were determined with 150 ps time resolution and < 2 Å spatial resolution. The structures reveal numerous sites in which CO becomes transiently trapped, as well as correlated motion of the protein side chains. When a single point mutation was introduced in a position near the binding site (L29F), the departure of CO from the primary docking site was significantly accelerated. Dramatic differences in the correlated protein displacements in wild-type vs. L29F Mb were found, which provide a structural explanation for these kinetic differences. The time-dependent structural changes have been stitched together into a movie that literally allows us to watch this protein as it functions. This movie depicts the average structure of an ensemble of intermediates, not a single molecule. To gain single-molecule insights into mechanisms of protein function, a joint analysis of all-atom molecular dynamics (MD) calculations and picosecond time-resolved X-ray structures was performed. Ensemble-averaged MD simulations of the L29F mutant of myoglobin following ligand dissociation reproduce the direction, amplitude, and timescales of crystallographically-determined structural changes. This close agreement with experiments at comparable resolution in space and time validates the individual MD trajectories. From numerous single-molecule trajectories, we identify and structurally characterize a conformational switch that directs dissociated ligands to one of two nearby protein cavities. This unique combination of simulation and experiment unveils functional protein motions and illustrates at an atomic level relationships among protein structure, dynamics, and function.

The Effect of Dynamics on Protein-Ligand Interactions

Markus Meuwly

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(July 14, 2005)

With recent advances in both, experiment and computer simulations, it has become possible to follow in detail how small molecules interact with proteins. This is of particular interest because small ligands can be used to probe the interior of proteins or interfaces between the protein and the solvent. In this presentation I will focus on two systems for which the dynamics of the ligand plays a different role.

One of the paradigm system for protein-ligand interaction is myoglobin interacting with small molecules such as O_2, CO, and NO. All three systems have been extensively studied both experimentally and theoretically. Detailed information is available for structural, spectroscopic and energetic aspects of the interaction between CO and Mb. With refined and carefully parametrized electrostatic models it has become possible to understand a variety of experimental observations.

The situation is quite different for insulin. Although insulin is a physiologically very important hormone, its active structure is not yet known. Recent experiments have suggested that insulin interacts quite favourably with glucose. Using computational docking studies and molecular dynamics simulations it was possible to show that the preferred interaction site of glucose corresponds to the one inferred from experiment. In addition, the binding site is not only found statically but also dynamically stable.

The role of environment on conformational transitions in peptides and proteins

Charles L. Brooks III, The Scripps Research Institute

In this talk I will discuss some of the affects of environmental conditions, e.g., pH, presence of membranes, etc. on conformational equilibria and folding (or unfolding) of peptides and proteins. Specific examples will focus on the role of pH in modulating helix content in peptides and proteins that form amyloid fibrils and the role of the membrane environment in controlling peptide conformation and assembly thermodynamics. With this work we will introduce new implicit models for the treatment of membrane environments as well as the techniques that enable pH-coupled conformational transitions to be examined.

Insights into protein-protein and protein-ligand association from end-point free energy calculations.

Jessica Swanson, University of California, San Diego

The prediction of absolute binding affinities with end-point free energy methods will be discussed. These methods combine molecular mechanics energies from explicit solvent simulations with continuum solvation energies to measure the free energies of the end-points of a binding reaction, the bound and free states, and thus the change in free energy upon molecular association. A clear connection between statistical thermodynamics and end-point methods will be presented, highlighting several of the key methodological challenges: measuring the association free energy, arising from one molecule's loss of translational and rotational freedom from the standard state concentration; measuring the conformational free energy, due to both molecules' change in conformational freedom; and ensuring compatibility between implicit and explicit solvent models. Several methods for measuring the association free energy directly from a molecular dynamics simulation will be discussed. Results for protein-protein and protein-ligand systems suggest that the entropic cost of binding a small molecule can be substantially less than that of a large protein. Finally, a discussion of the need for additional linkage to experimental studies will be initiated.

Mechanism of proton transport from crystallographic structures of the nine states of the bacteriorhodopsin photocycle. J. K. LANYI, Dept. Physiology & Biophysics, University of California, Irvine, CA 92697, U.S.A.

In the last few years we have been able to trap the K (*1*), L (*2*), M_1 (*3*), M_2 (*4*), M_2' (*5,6*) and N' (*7*) intermediates of the bacteriorhodopsin photocycle in crystals, and determine their structures by x-ray diffraction to 1.43-1.62 Angstrom resolutions. With models proposed earlier for N and O from crystallography of non-illuminated mutants (*8, 9*), structures are now available for the initial bacteriorhodopsin state (*1*, *10*) as well as the eight intermediate states. We applied stringent criteria for evaluating whether the often small changes in the electron density maps are in the data or come from the refinement, i.e., are they real and meaningful? The structures reveal the reasons for protonation of the retinal Schiff base by Asp85, proton release to the extracellular membrane surface, the switch event that allows reprotonation of the Schiff base from the cytoplasmic side, side-chain and main-chain motions initiated in the cytoplasmic region, formation of a single-file chain of hydrogen-bonded water molecules that conducts the proton of Asp96 to the Schiff base, and reprotonation of Asp96 from the cytoplasmic surface. The refined models describe in atomic detail how the transformations of the photoisomerized retinal change its interaction with wat402, Asp85, and Trp182, and how the displacements of main-chain and functional residues, and the water molecules sequestered in the extracellular and cytoplasmic regions facilitate the transfer of a proton from one membrane surface to the other. The observed changes can be summarized as a detailed atomic-level model for the transport in this pump, that describes it as the gradual relaxation of the distorted retinal that causes a cascade of displacements of water and protein atoms that spreads to the rest of the protein and results in vectorial proton transfers to and from the Schiff base. Such local-global coupling of conformational changes may be the general principle for how ion pumps and receptors function (*11*).

1. Schobert, Cupp-Vickery, Hornak, Smith & Lanyi. *J. Mol. Biol*. 321:715-726, 2002.
2. Lanyi & Schobert. *J. Mol. Biol*. 328:439-450, 2003.
3. Lanyi & Schobert. *J. Mol. Biol*. 321:727-737, 2002.
4. Luecke, Schobert, Cartailler, Richter, Rosengarth, Needleman & Lanyi. *J.Mol.Biol*. 300:1237-1255, 2000.
5. Luecke, Richter & Lanyi. *Science* 286:255-260, 1999.
6. Lanyi & Schobert (in preparation)
7. Schobert, Brown & Lanyi. *J. Mol. Biol*. 330:553-570, 2003.
8. Subramaniam & Henderson. *Nature* 406:653-657, 2000.
9. Rouhani, Cartailler, Facciotti, Walian, Needleman, Lanyi, Glaeser & Luecke. *J.Mol. Biol*. 313:615-628, 2001.
10. Luecke, Schobert, Richter, Cartailler & Lanyi. *J.Mol.Biol*. 291:899-911, 1999.
11. Lanyi & Schobert. *Biochemistry* 43:3-8, 2004.

Coherent Control of Retinal Isomerization in Bacteriorhodopsin

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Abstract: The primary processes of biological systems are best described within a quantum description that appropriately captures the wave properties of matter and importance thereof on the length scale of the reaction coordinate. It remains an intriguing question whether or not quantum coherence effects play a role in biological functions and to what degree. This question has been resolved to a certain degree by exploiting the pulse protocols of coherent control to essentially make a molecular interferometer on the molecular frame of reference to test the coherence properties of the reaction coordinate of retinal in bacteriorhodopsin. Not only have conserved coherence propagation been observed but the degree of intrinsic isomerization could be manipulated with shaped pulses using a genetic learning feedback. This finding is the first to fully demonstrate constructive and destructive interference pathways in biological systems and provides new fundamental insight into the transition state region of biological systems.

Investigations of polypeptide topology and rotational diffusion in aligned membranes by ^{2}H and ^{15}N solid-state NMR spectroscopy

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A solid-state NMR approach which allows for the accurate determination of the tilt and rotational pitch angles of peptides reconstituted into uniaxially oriented membranes will be presented. The method works with transmembrane or in-plane oriented peptides that have been labelled with 3,3,3-$^{2}H_3$-alanine and ^{15}N-leucine at two selected sites. Proton-decoupled ^{15}N and ^{2}H solid-state NMR spectroscopy at sample orientations of the membrane normal parallel to the magnetic field direction have been used to characterize the tilt and rotational pitch angle of several peptides in considerable detail.

When the same samples are inserted into the magnetic field at 90 degrees tilted alignments provide valuable information on the rotational diffusion constants in membranes and thereby of the association and size of peptide complexes within the membrane environments. Whereas monomeric transmembrane peptides exhibit spectral averaging and well-defined resonances, larger complexes are characterized by broad spectral line shapes. In particular the deuterium line shape is sensitive to association of a few transmembrane helices. In contrast, the formation of much larger complexes affects the ^{15}N chemical shift spectrum.

The biological systems investigated by us using solid-state NMR spectroscopy include antibiotic peptides, polypeptide channels, signal sequences, DNA transfectants, Alzheimer fibrils, colicins and proteins involved in apaptosis.

References:

Aisenbrey Ch. & Bechinger, B. Tilt and rotational pitch angles of membrane-inserted polypeptides from combined ^{15}N and ^{2}H solid-state NMR spectroscopy *Biochemistry* 43, 10502-10512 (2004)

Aisenbrey Ch. & Bechinger, B.Investigations of peptide rotational diffusion in aligned membranes by ^{2}H and ^{15}N solid-state NMR spectroscopy, *The Journal of the American Chemical Society* 126, 16676-16683 (2004)

Reviews:

Bechinger, B., Kinder, R., Helmle, M., Vogt, T.C.B., Harzer, U., & Schinzel S., Peptide structural analysis by solid-state NMR spectroscopy *Biopolymers - Peptide Science*, 51, 174-190 (1999)

Bechinger, B. Understanding Peptide Interactions with the Lipid Bilayer: A Guide to Membrane Protein Engineering*Current Opinion in Chemical Biology* 4, 639-644 (2000)

Bechinger, B. Solid-state NMR investigations of interaction contributions that determine the alignment of helical polypeptide domains in biological membranes, *FEBS Letters* 504, 161-165 (2001)

Bechinger, B. and Sizun, C.The alignment and structural analysis of membrane polypeptides by ^{15}N and ^{31}P solid-state NMR spectroscopy, *Concepts in Magnetic Resonance* 18A, 130-145 (2003)

Bechinger, B. Structure and function of lytic peptides *Critical Reviews in Plant Sciences* 23, 271-292 (2004)

Bechinger, B. Solid-state NMR spectroscopy to study peptide alignments in membranes, *Biochimica Biophysica Acta* 1666, 190-204 (2004)

Toward the elucidation of structure and dynamics of membrane protein complexes by NMR

Gianluigi Veglia, University of Minnesota

Membrane protein complexes regulate a myriad of cellular functions, including ion transport, signal transduction etc. We present the application of both solution and solid-state NMR techniques to the elucidation of the interactions between Ca-ATPase (or SERCA, Sarco(endo)plasmic Ca-ATPase) and its endogenous inhibitor, phospholamban (PLB). The SERCA/PLB complex is central to the regulation of heart muscle contraction and relaxation cycle. Naturally occurring mutations disrupt SERCA/PLB interactions, impairing heart function. Solution NMR studies reveal a conformational switch of PLB from lipid-bound to enzyme bound state. In addition, 2D PISEMA and CPMAS experiments carried out on the SERCA/PLB complex reconstituted in mechanically oriented lipid bilayers show that substantial topological changes occur upon portein-protein interactions. These results pave the way for the application of these techniques to study other large membrane protein assemblies.

NMR Strategy for Membrane Proteins-ligands Interactions

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Membrane proteins play crucial roles in many biological events, such as signal transduction processes, immune systems, and cellular recognition, and also are main target proteins in drug developments. Therefore, the identification of the interfaces of ligands-membaran proteins complexes provides deep insights into theses research areas.

However, the lack of the appropriate NMR strategy and measurements for larger proteins complex hampers the investigation of ligands-membrane proteins interactions. To address the issue, we proposed the NMR method, cross-saturation measurement[1], which utilizes the TROSY detection and deuteration to a high degree for proteins, for a more rigorous determination of the contact residues of large protein complexes than the conventional approaches, involving chemical shift perturbation and hydrogen-deuterium exchange experiments. Furthermore, we modified the method to overcome the limitations that the cross-saturation method is difficult to apply to protein complexes with a molecular weight over 150 K.[2]

In the present paper, we will show some examples of the application of the transferred cross saturation method to the membrane proteins system.[3,4]

References

1. Takahashi, H., et al. *Nature Struct. Biol.* (2000) 7, 220-223.
2. Nakanishi T., et al., *J. Mol. Biol.* (2002) 318, 245-249.
3. Takeuchi, K., et al., *Structure* (2003) 11, 1381-1392.
4. Takeda, M., et al., *J. Biol. Chem.* (2003) 278, 43550-43555.

Two stories of membrane protein stability and dynamics: The S4 voltage-sensor and the SecY translocation channel.

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The high sensitivity of voltage-dependent ion channels to small variations in the transmembrane electric potential is due to the motion of charged residues located in the so-called S4 transmembrane (TM) domain. The two reported structures of two K+ voltage-gated (Kv) channels from *archae* (KvAP) and mammalian (Kv2.1) organisms indicate that the Arg residues in S4 are exposed to the lipid membrane during. Recent experiments of translocon-mediated insertion into the endoplasmic reticulum membrane have shown that a polypeptide chain with the sequence of S4 in KvAP can be inserted in the membrane. In the first part of my talk, I will present a model, generated from molecular dynamics simulations, that accounts for the stability of S4 in the lipid membrane environment. The recently determined structure for the SecY protein-conducting channel calls for a model of membrane protein integration based on the ability of a nascent peptide chain to probe both hydrophilic and hydrophobic environments, implying that the lateral gate, formed by two TM domains of the translocation channel, is in a highly dynamical state. In the second part of my talk, I will present our efforts to produce a model of SecY in its active oprn-state and characterize its conformational dynamics.

Protein Motions in Catalysis, Binding, and Folding

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NMR spectroscopy is a powerful approach for characterizing protein conformational dynamics on multiple time scales with applications to folding, binding, and catalysis. These applications will be discussed through examples. The villin headpiece domain HP67 is a model for subglobal protein folding. At pH 7.0, the N-terminal subdomain of HP67 exists in equilibrium between folded (98.5%) and unfolded (1.5%) states while the C-terminal subdomain remains stably folded. ATP-binding cassette (ABC) transporters move solutes across membranes and are associated with diseases including cystic fibrosis and multi-drug resistance. Solution NMR spectroscopy of a soluble model ABC, *Methanococcus jannaschii* protein MJ1267, shows that ADP-Mg binding alters the flexibilities of key ABC motifs and induces allosteric changes in conformational dynamics more that 30 Å away from the ATPase active site. Ribonuclease HI enzymes from the mesophilic bacterium *Escherichia coli* and the thermophilic bacterium *Thermus thermophilus* are highly homologous, but differ greatly in thermodynamic stability and catalytic activity. Nuclear spin relaxation measurements identify conformational dynamics on picosecond-nanosecond and microsecond-millisecond time scales that may contribute to these differences.

Telluride Workshop on Protein Dynamics

Prof. Martin J. Stone
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From NMR Measurements of Protein Dynamics to Thermodynamics: Recent Advances and Future Challenges

The thermodynamics of protein binding interactions and structural transformations are controlled by an intricate balance between many enthalpic and entropic factors. One important factor is the conformational entropy of the protein chain. However, determining the change in conformation entropy upon binding or folding using classical thermodynamic measurements is generally very difficult because this change is accompanied by substantial changes in other entropic terms such as solvent entropy. NMR relaxation methods that are sensitive to the fluctuations of protein structures offer a complementary approach to estimation of conformation entropy. The strength of this approach is that NMR relaxation parameters (and derived order parameters) can be determined at many sites throughout a protein, providing information not only about the net conformational entropy but also about redistribution of entropic fluctuations across the protein structure. On the other hand, NMR methods are not sensitive to all structural fluctuations and conversion of order parameters to conformational entropy is complicated by the possible existence of correlated (synchronized) motions. By analyzing the covariation of order parameters among many forms of the same protein, one can probe the existence of correlated motions in the protein. We have applied this method to study the backbone amide and side chain methyl fluctuations in a series of mutants of a small protein, the B1 domain from protein G. Data indicate that dynamic correlations are indeed present in this domain and that variations in the internal motions make a substantial contribution to protein stability, although they do not correlate directly with stability or structural properties of the mutated amino acid.

Optimal Isotope Labeling_for Protein Structure Determination by NMR: The SAIL method

Masatsune Kainosho

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The SAIL (*S*tereo-*A*rray *I*sotope *L*abeling) technology for protein NMR spectroscopy exclusively utilizes chemically or enzymatically synthesized amino acids, designed to have an optimal stereo- and regiospecific pattern of stable isotopes, from which proteins are obtained by cell-free synthesis. As demonstrated for the calcium-bound form of *Xenopus laevis* calmodulin (CaM, 17kDa) and the cyclodextrin bound maltose binding protein (MBP, 41kDa), SAIL offers sharpened lines, spectral simplification without loss of information, and the ability to rapidly collect the structural restraints required to solve a high-quality solution structure. SAIL is expected to largely eliminate the key limiting factors for detailed solution structure determinations of larger proteins.

Unraveling the unfolded state

Kevin Plaxco, University of California, Santa Barbara

For more than 60 years the random coil model has been the benchmark description of denatured proteins. Here I discuss the validity of this model in light of recent spectroscopic evidence suggesting the significant structure persists in even the most highly denatured proteins. I also discuss recent discoveries regarding the nature of the unfolded state populated in the absence of denaturant.

Oligomerization of the (25-35) fragment of the Alzheimer Aβ peptide

Joan-Emma Shea
Department of Chemistry and Biochemistry, the University of California, Santa Barbara

The Aβ(25-35) peptide is one of the shortest fragments of the Alzheimer amyloid-β peptide to possess neurotoxic properties. This peptide is toxic both in its monomeric form, as well as in an oligomeric state. A characterization of the monomeric species of Aβ(25-35) is key to understanding its toxicity and for the identification of conformations that may act as seeds for further growth into fibrils. We present a replica exchange molecular dynamics study of the conformational space accessible to this peptide in explicit solvent. The peptide is seen to adopt a mostly random coil structure, with a smaller population of structured hairpin conformations. Simulations of dimers indicate that aggregation into larger assemblies can be initiated either from the coil state or from the hairpin configurations, resulting in fibrils of different morphologies.

Terahertz Dielectric Sensitivity to Protein Dynamics

Andrea Markelz, Jing-Yin Chen, Joseph Knab, and Scott Whitmire
SUNY Buffalo

An overview of the terahertz spectroscopy of biomolecules will be given. A brief review of early work done by Fourier transform spectroscopy will lead to a discussion on the technique of terahertz
time domain spectroscopy (TTDS). I will briefly mention applications of TTDS to biomolecular identification. The talk will then focus on our work examining how terahertz dielectric response is sensitive to protein dynamics: conformational change for bacteriorhodopsin, oxidation state for cytochrome c, hydration dependence, and temperature dependent dynamical transitions in hen egg white lysozyme and cytochrome c.

Protein energy landscapes, solvent α-slaving and hydration β-coupling

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Protein function depends critically on both protein dynamics and structure. It is widely recognized that protein dynamics occur on a high-dimensional energy landscape, where distinct conformations are separated by free energy barriers. Yet the way in which a partition between protein, hydration shell and bulk solvent might be made has not always been fully considered in the context of the energy landscape. It is possible to include solvent α and hydration shell β processes explicitly in the protein energy landscape as temperature-dependent rates determined by the solvent and hydration shell respectively. Three classes of protein dynamics on the energy landscape are evident from a wide range of data: (1) α-slaved protein dynamics, (2) β-coupled protein dynamics and (3) unslaved (vibrational) protein dynamics. One cannot rule out additional classes of protein motion. The rate of α-slaved protein dynamics follow the temperature dependence of the solvent α process, slowed by an entropic contribution from the protein and hydration shell. Similarly to α-slaving, the β-coupled protein dynamics follow the hydration shell β process, but because all β-coupled data we have analyzed depend at least partly on amplitude, there are too few constraints to compute the slowing entropy (although we can bound it). In no case do we require a dynamic protein transition to explain existing β-coupled amplitude data. The empirical decomposition of protein dynamics into different categories suggests a corresponding decomposition of protein dynamical models that may assist in computing protein function.

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Solvent, Hydration, and Protein - Interactions and Motions

By

Robert D. Young

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Proteins exist in a large number of different conformations or conformational substates, described by an energy landscape which depends also on the protein environment. The dynamics in this energy landscape is rich and incompletely explored. Large-scale protein motions, such as the exit of a ligand from the protein interior, follow the dielectric fluctuations in the bulk solvent. The mean-square displacements (msd) from Mössbauer and neutron-scattering experiments probe protein fluctuations on the time scale of the experiment and show that fluctuations in the hydration shell control fast fluctuations in the protein. We call the first type solvent-slaved fluctuations, the second hydration-shell-coupled fluctuations. Solvent-slaved motions are similar to the alpha fluctuations in glasses. Their temperature dependence can be approximated by a Vogel-Tammann-Fulcher relation and they are absent in a solid environment. Hydration-shell-coupled fluctuations are similar to the beta relaxation in glasses. They can be approximated by a Ferry or an Arrhenius relation, are much reduced or absent in dehydrated proteins, and occur in hydrated proteins even if embedded in a solid. They can be responsible for internal processes such as the migration of ligands within myoglobin. The existence of at least two functionally important fluctuations in proteins, one slaved to bulk motions, the other coupled to hydration shell fluctuations, implies that the environment can control protein functions through different interactions. Recent theoretical treatment of the mosaic energy landscapes of liquids applied to protein dynamics sheds light on the solvent-slaved fluctuations. A similar understanding of the hydration-shell-coupled motions is not yet in hand.

The relation between protein and solvent dynamics as studied by neutron scattering and temperature-controlled X-ray crystallography

Martin Weik

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The degree of coupling between protein and solvent dynamics is still a debated issue. In particular, it remains unclear whether dynamical changes in a protein's environment, such as a glass transition, trigger dynamical transitions in proteins which have been revealed and studied by numerous experimental and simulation techniques. Since specific flexibility is vital to protein function, the study of dynamical aspects of proteins and their environment is highly relevant to the understanding of structure-function-dynamics relationships in biology.

We are employing two complementary techniques, neutron scattering and temperature-controlled X-ray crystallography, to address the issue of the dynamical coupling of proteins and their environment. Elastic incoherent neutron scattering allows the determination of atomic mean-square fluctuations on the ns-ps time scale averaged over all hydrogen atoms present in the sample. Consequently, global protein dynamics are assessed if a hydrogenated protein is investigated in D_2O and solvent dynamics is probed if deuterated proteins in H_2O are employed. In particular, temperature-dependent experiments inform about dynamical transitions from harmonic to anharmonic motions in either the protein or the solvent and their relation can be studied. X-ray crystallography, on the other hand, informs about spatially-resolved structural changes as a function of temperature. In this context, intense synchrotron radiation has been discovered to produce specific chemical damage to crystalline proteins that proves to be a valuable tool to monitor structural flexibility. Temperature-dependent structural radiation damage, determined at and below the glass transition of the protein-crystal solvent, suggests that solvent and local protein dynamics are coupled.

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