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An hydrophobic mismatch between protein length and membrane thickness can lead to a modification of protein conformation, function, and oligomerization. To study the role of hydrophobic mismatch, we have studied the change in mobility of transmembrane peptides in model bilayers. The studied peptides possess an hydrophobic helix of various length $d\pi$, and the hydrophobic thickness, h, of the bilayers can be tuned at will. For each mismatch value, using Fluorescence Recovery After Pattern Photobleaching (FRAPP), we precisely measured the diffusion coefficient D of the embedded objects and gained access to their apparent size. This enables us to observe the orientation or oligomerization state of the peptides versus their concentration, and discover that the effects of positive and negative mismatches on diffusion are highly asymmetric. For bilayers thinner than $d\pi$, the diffusion coefficient decreases and the derived characteristic sizes are larger than the peptide radii. As suggested by previous studies, the peptides should accommodate by tilting, and this scenario was confirmed by ATR-FTIR spectroscopy. As the membrane thickness increases, the value of the diffusion coefficient increases: the peptides raise (i.e. their tilt is reduced) and reach an upright position and a maximal mobility for $h \approx d\pi$. Using accessibility measurements, we show that when the membrane becomes too thick, the peptide polar heads sink into the interfacial region. Surprisingly, this "pinching" behavior does not hinder the lateral diffusion of the transmembrane peptides. But it creates interactions between the embedded peptides, and collective behaviors emerge. For low peptide concentration, the transmembrane anchorage of the peptide is broken as the bilayer is swollen. For intermediate concentrations, we observed the arrangement of small monodisperse clusters, while polydisperse macro-domains are formed at higher peptide density, leading to spontaneous and reversible formation of "vesicles".

1170-Plat
The Gating Mechanism of Yeast Aquaporin Studied by Molecular Dynamics Simulations
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Aquaporins are membrane proteins responsible for the permeation of water and other solutes through the cell membrane. They arrange in a tetrameric conformation, where each monomer acts as a highly efficient single-file water channel. The x-ray structure of the yeast aquaporin (Aqy1), recently found at a very high resolution (1.15 Angstrom), revealed the conformation of the extended N-terminus - an unusual feature within the family of aquaporins - occluding the water pore. In contrast, functional assays with spheroplast of P. pastoris showed a substantial increase in the water transport activity when Aqy1 was present compared to an assay where Aqy1 was deleted, indicating that Aqy1 is a gated water channel. Here we address the question of a putative gating mechanism of Aqy1 by using molecular dynamics simulations. Our findings suggest that Aqy1 may be regulated by both phosphorylation of a serine residue (Ser107) or membrane-mediated mechanical stress. Both possibilities lead to similar opening transitions after a local rearrangement of the residues Tyr31, Leu189, Ala190 and Val191, located in the gate of the pore. We observed that there is a principal collective motion causally involved in these gating transitions, and that is possible to attain reproducible opening events along this collective coordinate. The simulation results are therefore consistent with a mechanism in which both phosphorylation and mechanosensitive gating can trigger the channel opening. Aqy1 regulation may help yeast to survive rapid freezing and thawing, and sudden osmotic changes.

Platform Y: Protein Dynamics I

1171-Plat
Protein Similarity Derived Solely from Molecular Dynamics
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The dynamic motions of many proteins are central to their function. It therefore follows that the dynamic requirements of a protein are evolutionary constrained. In order to assess and quantify this, one needs to compare the dynamic motions of different proteins. Comparing the dynamics of distinct proteins may also provide insight into how protein motions are modified by variations in sequence and, consequently, by structure. The optimal way of comparing complex molecular motions is, however, far from trivial. The majority of comparative molecular dynamics studies performed to date relied upon prior sequence or structural alignment to define which residues were equivalent in 3-dimensional space. Here we discuss an alternative methodology for comparative molecular dynamics that does not require any prior alignment information. We show it is possible to align proteins based solely on their dynamics and that we can use these dynamics-based alignments to quantify the dynamic similarity of proteins. Our method was tested on 10 representative members of the PDZ domain family. As a result of creating pairwise dynamics-based alignments of PDZ domains, we have found evolutionarily conserved patterns in their backbone dynamics. We compare the results to other recently developed methods.

1172-Plat
Structure Fluctuations in Proteins and their Relationship to Amino Acid Propensities
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The spectrum and scale of fluctuations in protein structures affect the range of cell phenomena, including stability of protein structures or their fragments, allosteric transitions and energy transfer. The study presents a statistical-thermodynamic analysis of relationship between the sequence composition and the distribution of residue fluctuations in protein-protein complexes [1]. A one-node-per-residue elastic network model accounting for the nonhomogeneous protein mass distribution and the inter-atomic interactions through the renormalized inter-residue potential is developed. Two factors, a protein mass distribution and a residue environment, were found to determine the scale of residue fluctuations. Surface residues undergo larger fluctuations than core residues, showing agreement with experimental observations. Ranking residues over the normalized scale of fluctuations yields a distinct classification of amino acids into three groups: (i) highly fluctuating - Gly, Ala, Ser, Pro and Asp, (ii) moderately fluctuating - Thr, Asn, Gln, Lys, Glu, Arg, Val and Cys (iii) weakly fluctuating - Ile, Leu, Met, Phe, Tyr, Trp and His. The structural instability in proteins possibly relates to the high content of the highly fluctuating residues and a deficiency of the weakly fluctuating residues in irregular secondary structure elements (loops), chameleon sequences and disordered proteins. Strong correlation between residue fluctuations and the sequence composition of protein loops supports this hypothesis. Comparing fluctuations of binding site residues (interface residues) with other surface residues shows that, on average, the interface is more rigid than the rest of the protein surface and Gly, Ala, Ser, Cys, Leu and Trp have a propensity to form more stable docking patches on the interface. The findings have broad implications for understanding mechanisms of protein association and stability of protein structures.
1. A.M. Ruvinsky and I.A. Vakser. arXiv:0907.5021v1

1173-Plat
Direct Observation of Ligand Dynamics in Cytochrome *C* Using Time-Resolved FTIR Spectroscopy
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Horse heart cytochrome *c* (cyt c) has emerged as a paradigm for the study of protein folding. The folding of reduced cyt *c* induced by photodissociation of CO from the CO-bound unfolded protein has been studied extensively. Following a nanosecond light pulse, four transitions have been resolved with time constants of approximately 1-5, 50-100, 200-500, and 1000-10,000 μs. While originally thought to be associated with CO rebinding to two different partially folded states of cyt *c*, the two slower process are now understood to reflect the bimolecular reassociation of CO followed by religation of the His18, which by the base elimination mechanism is induced to dissociate after CO photolysis. Thus, the two slower time constants turn out not to report on protein folding, but instead reflect the complexity of heme ligation. The two faster time constants have been attributed to ligation at the heme center by protein side chains. Here, to unambiguously determine the post-photodissociation steps involving CO, we monitored the CO vibration following photodissociation with step-scan FT IR spectroscopy. We find that like the slower timescale processes, the 50-100 μs timescale process is associated not with protein dynamics, but with CO ligand dynamics. The data clearly demonstrate that whatever the origins of the spectral changes, they clearly involve CO rebinding or changes in the environment of an already bound CO ligand. In addition to these fast dynamics, we also find multi-phasic CO rebinding on timescales of 1-100 s. The dependence of the associated amplitudes on denaturant concentration suggests that a unique species exists at intermediate denaturant concentrations, consistent with a folding-unfolding process of the protein driven by CO dissociation. This may

represent the first evidence for the long sought-after protein folding process triggered by photo-induced CO dissociation.

1174-Plat

Phosphorescence from Single Tryptophan in Amorphous Solid Human Serum Albumin Exhibits Solvent-Protein Dynamics Slaving

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The physical properties of amorphous biomolecules are important to the texture and stability of low-moisture foods, the stability of pharmaceuticals, the permeability of edible films, and the viability of organisms during anhydrobiosis. Protein stability is often improved via the inclusion of small-molecule excipients during freeze-drying and organisms overproduce sugars such as sucrose or trehalose during anhydrobiosis. The effect on internal protein dynamics caused by substitution of a protein's surface water molecules with small sugar molecules is unclear. To explore this question, we have analyzed tryptophan phosphorescence decays of human serum albumin (HSA) in the dry amorphous solid state. Phosphorescence is an ideal approach, as the long-lived triplet state of tryptophan is sensitive to the long time-scale molecular motions of proteins in the dry state. Human serum albumin (HSA) was chosen because it contains a single, buried tryptophan residue and thus can provide information on the local dynamics of a specific site in the interior of the protein. Amorphous protein films were prepared by spreading concentrated solutions of HSA with and without sugar onto quartz slides, followed by rapid drying and extensive desiccation. Phosphorescence intensity decays were collected and fit with multiple exponential functions. From the average lifetime of these fits the rates of non-radiative decay (kNR) of the triplet state were calculated; kNR is dependent on the microviscosity of the site and is thus a measure of molecular mobility of the HSA tryptophan site. At all temperatures this measure of molecular mobility was lower in the films containing sucrose. Break-point analysis of a kNR Arrhenius plot revealed two temperature regimes with a transition occurring at the glass transition temperature of sucrose. Research supported in part by the National Research Initiative of USDA-CSREES.

1175-Plat

Structural Dynamics of Light-Driven Proton Pumps

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In a recent publication (Andersson et al. (2009) *Structure*. 17(9):1265-75), we applied the emerging technique of time-resolved wide-angle X-ray scattering (TR-WAXS) to visualize the structural dynamics of two light-driven proton pumps, namely bacteriorhodopsin and proteorhodopsin, in real-time. Direct structural information was obtained over a time course of 360 ns to 100 ms. Our results establish that three conformational states are required to describe the respective photocycles of both proteins. Significant motions of the cytoplasmic half of helix F and the extracellular half of helix C are observed prior to the primary proton transfer event, which increase in amplitude following proton transfer. These results both simplify the structural description that have emerged from a range of biophysical techniques and reveal shared dynamical principals for proton pumping. Moreover, the measured magnitudes of the helical movements associated with the bacteriorhodopsin photocycle are larger than those anticipated by intermediate trapping studies. This demonstrates the effect of a crystal lattice on protein dynamics and shows the advantage of direct measurements in solution at room temperature.

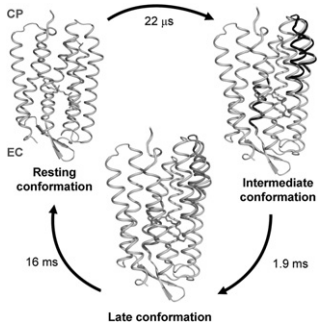

Figure 1. Schematic showing the observed helical rearrangements during the photocycle of bacteriorhodopsin.

1176-Plat

Mechanoenzymatics and Protective Mechanisms of Titins' Catalytic and IG Domains

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The giant titin filament controls many structural and functional properties of the sarcomere. Titin filaments connect M-line and Z-disc of the sarcomere and consist of four regions: the M-line, A-band, I-band, and Z-line. The different domains of titin (mainly immunoglobulin Ig and fibronectin-3 domains, and the catalytic domain titin kinase (TK)) exhibit dramatically different mechanical properties. We used atomistic molecular dynamics simulations to explore the coupling of mechanical stability with the enzymatic activity of titin kinase and the protective properties of Ig-domains. We showed that a unique autoinhibitory mechanism allows TK to act as a molecular force sensor, as relatively low forces already remove the autoinhibitory tail and prime the molecule for ATP binding. At much higher forces, the mechanical stability of Ig27 becomes important: In our studies, extensive dynamic force spectroscopy (DFS), Brownian dynamics, and molecular dynamics simulations worked together to examine mechanical stability of Ig27 under different loading rates. Our results suggest that Ig27 is perfectly suited to act as a molecular force buffer over a wide range of loading rates.

1177-Plat

Dynamics of apoB100-Containing Lipoproteins Determined by Incoherent Elastic Neutron Scattering

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Apolipoprotein B100 (apoB100)-containing lipoproteins (very low density lipoprotein (VLDL) and low density lipoprotein (LDL)) are the principal fat and cholesterol carriers in blood. During metabolic conversion from VLDL to LDL, the particle size decreases (from ~ 80 nm to 20 nm) and lipid composition is changed, however, the amphiphilic apoB100 molecule remains bound to its lipoprotein particle and most likely compensates for structural changes due to its inherent conformational flexibility and dynamics .

Here, we report on motions in the time range of 100 ps to 1 ns in human-LDL, human VLDL and yolk-VLDL, which were recorded by elastic neutron-scattering temperature scans from 20K to 310 K using hydrated lipoprotein powders. The mean square displacement values $\langle u^2 \rangle$ were calculated from the scattering vector dependence of the elastic intensity *I(Q)*. The effective force constants $\langle k \rangle$, which are a measure for the resilience of the particle, were derived from the slopes in the $\langle u^2 \rangle$ vs. T scans. In the low-temperature harmonic regime we found no substantial differences between lipoprotein fractions ($\langle k \rangle$ ~1 N/m). Nevertheless, lipoproteins are softer compared to hydrated myoglobin powder (2 N/m) or purple membranes (1.7 N/m) [1]. Significant differences were observed with increasing temperatures. Both, human and yolk VLDL show two breaks in the scan with a steep increase in $\langle u^2 \rangle$ above 270K, whereas LDL shows a smooth behavior above a dynamic transition around 220K. Accordingly, at physiological temperatures VLDL-fractions are highly soft and mobile ($\langle k'' \rangle$ ~0.08 N/m) as compared to LDL ($\langle k' \rangle$ ~ 0.2 N/m). Sucrose, added as cryoprotectant, significantly modified the dynamics of VLDL, as it confers extreme stability to VLDL over the whole temperature range and substantially suppresses dynamic transitions.

[1] G. Zaccai, Science 288 (2000), 1604-1607

1178-Plat

Valine-Induced Packing Deficiencies of Transmembrane Domains Promote Helix Flexibility and Membrane Fusion

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The helical transmembrane domains of fusion proteins are known to be functionally important and display an overabundance of helix-destabilizing Ile and Val residues. In an effort to systematically study the relationship of helix flexibility and fusogenicity, synthetic LV-peptides were designed whose hydrophobic core consists of Leu and Val residues at different ratios and at different positions (Hofmann et al., 2004; Poschner et al., 2009). The ability of the LV-peptides to fuse membranes increases with the content of helix-destabilizing residues. Molecular dynamics simulations were performed in order to characterize the backbone dynamics of these peptides in membrane-mimicking 80% TFE solvent and to relate the hydrogen-bond dynamics to experimental deuterium/hydrogen exchange kinetics. The analysis revealed that (i) the backbone dynamics of the helices increases systematically with Val content, (ii) that the impact of Val is due to stereochemical constraints within the helical structure and (iii) that side-chain packing mainly determines exchange kinetics. As a consequence, VxxV and VVxVV motifs promote helix destabilization whose relevance for membrane fusogenicity will be discussed.