well as determining how their interaction regulates differentiation. Multiparameter fluorescence detection nowadays allows direct observation of molecular processes at the single molecule level.
The obtained evolutionary conserved multi-protein complex is located in the subapical region (SAR) of embryonic epithelia and plays a central role in the maintenance of epithelial cell polarity, morphogenesis and survival of photoreceptor cells in Drosophila melanogaster. The complex is composed of the four proteins DPATJ and DLin-7, Stardust (Sdt), and Crumbs (Crb). The scaffold protein Sdt contains two subsequent L27 modules, which mediate the interaction with DPATJ and DLin-7 through their L27 domains. The trans-membrane protein Crb binds to the PDZ domain of Sdt with its cytoplasmic tail.
Here we use single-molecule fluorescence resonance energy transfer (FRET) in order to understand better quantitative parameters and spatial dynamics of the complex. We show that free DLin-7 exists in two major conformations, a high-FRET, folded state, and a second low FRET, unstructured state, which allows us to assign the L27 domain in DLin-7 as an "extended" disordered region.
Upon formation of a complex with Stardust, the proportion of folded DLin-7 molecules increases. Depending on the Stardust/DLin-7 ratio different heteromers are formed. The presence of DPATJ further increases the fraction of DLin-7 bound to Stardust, indicating that the L27-domains of all three proteins contribute to a positive cooperativity. Thus, L27 domains are versatile modules ideally suited to provide flexibility of protein complexes.

368-Pos Board B247
Conformational Flexibility of the GM2 Activator Protein Loop Regions Investigated By Site Directed Spin Labeling EPR Spectroscopy
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The GM2 activator protein (GM2AP) is an essential component in the degradation pathway of neuronal gangliosides. GM2AP is a required accessory protein for the hydrolytic conversion of GM2 to GM3 by a water soluble hydrolase. The X-ray structure of GM2AP reveals a β-cup topology with multiple conformations of the protein within the unit cell. Because the crystal structures show different conformations of the putative membrane binding loops, we have utilized site-directed spin labeling to investigate conformational flexibility of these loops for protein in solution and bound with GM2 ligand. As such, a series of single and double CYS mutants (still with original 8 CYS in 4 disulfide bridges) have been generated and spin labeled with MTSL. EPR spectra of spin labeled GM2AP were collected with and without GM2 ligand, and no significant changes in the EPR lineshape were seen. EPR spectra were simulated for spin labels located in the loop regions and reveal multiple component fits, while those in the backside of the β-cup beta strands have single component fits. For certain sites in the mobile loops, spectra were acquired as a function of temperature. From these lineshape simulations, the activation energy for the conformational change has been determined. The SDSL EPR results indicate that the multiple conformations observed in the crystallographic unit cell are populated in solution and represent conformational flexibility of the protein; which is not necessarily altered by binding to lipid ligands. Additionally, spin labeled protein was analyzed by mass spectrometry to confirm proper formation of the four disulfide linkages and addition of only one spin label at the mutant cysteine.

369-Pos Board B248
Single-Molecule Protein Conformational Dynamics and Molecular Interaction Dynamics under Enzymatic Reactions
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Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. Subtle conformational changes play a crucial role in protein functions, and these protein conformations are highly dynamic rather than being static. Protein-molecular interactions define the enzymatic reaction potential surface, pathway, and dynamics. The single-molecule protein-protein interaction dynamics reveals the nature of the molecular complex formation and recognition that are critical for an enzymatic reaction to occur. We applied AFM-enhanced single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detections. We obtained the rates for single-molecule conformational active-site open-close fluctuation and correlated enzymatic reactions. We have demonstrated a specific statistical analysis to reveal single-molecule FRET anti-correlated fluctuations from a high background of fluorescence correlated thermal fluctuations. Our new approach is applicable to a wide range of single-molecule FRET measurements for protein conformational changes under enzymatic reactions.

370-Pos Board B249
Slippage Between Noncovalently Bound Filaments Of Self-assembling Peptide
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Much attention has been given to the rupture of noncovalent chemical bonds in protein dynamics, and Bell's model has become widely used. In many cases, however, unbinding or unfolding requires that multiple energy barriers be overcome in parallel, in coordinated failure events. We examine one such system, slippage between ß-sheet filaments of the self-assembling peptide RAD16-II ([RARADADA]2). RAD16-II forms amphiphilic ß-sheet filaments, with alanine side chains forming the hydrophobic surface. In an aqueous environment, filaments are found in pairs, with their hydrophobic faces placed together. We examine slippage between two filaments using steered molecular dynamics simulations. We observe that alanine side chains on one ß-sheet filament form a rectangular array, and the alanine side chains of the opposing sheet occupy the interstices. For slippage to occur, these methyl groups must jump from one interstice to the next. Since the alanines in one ß-sheet are elastically linked, this failure occurs in a cooperative manner. Slippage of a single alanine side chain correlates with slippage of its immediate neighbors, and a dislocation propagates across the bound surface within a few picoseconds. We present a one-dimensional, coarse-grained model based on Langevin dynamics, that incorporates the basic elements of this system: multiple elastically linked particles each residing in an energy well and overcoming an energy barrier under applied force. The coarse-grained model shows good agreement with molecular dynamics results and provides a useful platform for studying coordinated failure events. [Supported by the NHLBI, EB003805.]

371-Pos Board B250
Multiple Channels of Structural Relaxations in Functional Proteins
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We elucidate the physics of the dynamical transition via 15 ns long molecular dynamics simulations at a series of temperatures (spanning 160 - 280 K) where the protein retains its native structure. By tracking the energy fluctuations, we show that the protein dynamical transition is marked by a cross-over from a piecewise stationary to stationary set of processes that underly the dynamics of protein motions in the water environment.
We find that a two-time-scale function captures the non-exponential character of backbone structural relaxations. One is attributed to the collective protein motions and the other to local relaxations. The former is well-defined by a single-exponential, nanosecond decay that is operative at all temperatures. The latter, on the other hand, is described by a large number of single-exponential motions that display a distribution of time-scales. Though their average remains on the order of 10 ps at all temperatures, the distribution markedly contracts with the onset of the dynamical transition. Interestingly, the collective motions are shown to impose bounds on the time-scales spanned by the local dynamical processes, although they are not directly involved in the transition.
The piecewise stationary character below the transition implicates the presence of a collection of sub-states whose inter-communication is restricted. The ineffectiveness of these sub-states to influence the overall relaxation time is shown to require a wide distribution of local motion time-scales, extending well beyond that of nanoseconds. At physiological temperatures, on the other hand, local motions are confined to time-scales faster than nanoseconds. This relatively narrow window makes possible the appearance of multiple channels for the backbone dynamics to operate, providing alternative routes for protein functionality.

372-Pos Board B251
Solvent Bridging Determines The Molecular Architecture Of The Unfolding Transition State Of A Protein
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Protecting osmolytes are ubiquitous in nature, where they play a vital role stabilizing intracellular proteins against adverse environmental conditions. While the solution thermodynamics of protein/osmolyte mixtures has been well characterized, information is lacking on how osmolytes influence the transition state structure and dynamics of proteins. Here we demonstrate a combination of single molecule force-clamp spectroscopy and solvent substitution that directly identifies the role of protecting osmolytes in the unfolding transition state structure of a protein. We measure the effect of osmolyte substitution on the rate of forced unfolding the I27 titin module. From the force dependency of the unfolding rate for each osmolyte we determine ΔG_U, the height

of the activation energy barrier, and Δx_U, the distance to the transition state. For all protecting osmolytes we measure ΔG_U increases, demonstrating that the I27 protein is stabilized. More striking is the measurement of Δx_U. Unfolding the I27 protein in water gives a $\Delta x_U = 2.5$ Å, a distance similar to the size of a water molecule. Water molecules have been identified as integral components of the unfolding transition state of the I27 protein, forming a solvent bridge between two β-strands. By varying osmolyte molecule size we rigorous test this solvent bridging hypothesis. We find that Δx_U correlates with osmolyte size for molecules ranging in size from 2.5 Å to 5.6 Å. However, for larger molecules (> 5.6 Å) Δx_U remains unchanged relative to the value measured in water, suggesting these osmolytes do not participate in solvent bridging in the transition state. These studies uniquely probe the length scales over which solvent molecules can modify the molecular architecture of the unfolding transition of a protein, an area which remains beyond the reach of other experimental techniques.

373-Pos Board B252

Site-Specific Folding Dynamics of Isotopically Labeled Peptides Studied by Time-Resolved Infrared-Spectroscopy

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Peptides with well-defined secondary structure are ideal model systems for study of protein folding dynamics for specific, unique structures. IR techniques provide the necessary time resolution as well as have structural sensitivity, which arises from coupling of sequential residues, normally evidenced as a splitting or frequency shift of the amide centered transitions. The amide I region, mainly the C=O stretching vibrations of the polypeptide backbone, is the prime target band for secondary structure. Isotopic labeling of individual amide 13C=O groups can induce site-specific frequency shifts and provide insight into local structure. A nanosecond laser is used to excite the solvent and induce a fast temperature jump (~10 C), and relaxation dynamics are probed with a diode laser tuned to selected, structurally sensitive wavenumbers across the amide I absorption. Site-specific dynamics have been monitored for the thermal unfolding of an isotopically labeled beta-hairpin peptide, a 12-mer tryptophan zipper peptide, which has a hydrophobic core formed by four Trp residues, by use of cross-strand coupled 13C=O labeled variants [1]. Data for single labeled peptides provided a control. Mutants of this sequence with just two Trp residues were introduced to destabilize the hairpin selectively near the termini or near the turn. Differences in kinetic behavior have been found for the loss of beta-strand and the gain of disordered structure. The isotope-edited kinetics vary with labeling position along the hairpin backbone and the mutations show consistent patterns depending on position. Our data supports a multistate folding mechanism for this hairpin structure. Similarly obtained data for other model peptides provide useful basis for interpretation of the observations.

[1] Hauser, K., Krejtschi, C., Huang, R., Wu, L., Keiderling, T.A., J. Am. Chem. Soc. 130 (2008) 2984- 2992.

374-Pos Board B253

Linear Response of Biomolecules To External Perturbations: Revisit Induce-fit

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Atoms in proteins, viewed as interlinked hubs, communicate with each other in complying with governed physical forces. The deviations of their positions from the mean, known as fluctuations, are essential in mediating functionally relevant biological processes maintaining one's daily life. The coupled fluctuations between pairs of atoms, the fluctuation covariance, can be determined analytically by Normal Mode Analysis, or numerically by MD simulations. Our study considers how this network of atoms reacts in response to external perturbations. Effects of such perturbations are exemplified by ligand- or (another) protein-induced conformational changes as well as the appreciated structural distortion of crystalline structures from its solution conformers. We assume the response of the system has linear departure from the mean under small perturbations on the Hamiltonian at equilibrium state. The formulated linear response theories, either time-dependent or -independent [1], says that the positional change of a given atom i is the accumulative sum of fluctuation covariance ij, at unperturbed state, multiplied by the force exerted on atom j. The time-dependent response function determined from MD simulation of carbonmonoxy myoglobin is used to track time-dependent conformational changes upon photo-dissociation of CO. The consequently obtained understanding of perturbation propagation is compared with experimental results. Also, the structural distortion of X-ray-characterized ubiquitin from its solution conformer can be well explained by the induce-fit theory, in a wider sense, while population-shift does not account for such a deviation.

[1] Ikeguchi M, Ueno J, Sato M, and Kidera A. (2005) Phys Rev Lett, 94, 078102.

375-Pos Board B254

Dynamic Allostery In Proteins

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Allostery or signalling between two sites of a molecule is a widely present phenomenon in nature. Classically it is believed that the communication between distant sites proceeds via a series of conformational changes. Recently allosteric proteins without conformational change have been observed. This cannot be accounted for with the classical theory and therefore we have built a model that explains the long distance signalling in such cases. We believe that the signalling proceeds via a change in dynamic behaviour of the protein and we illustrate the feasibility of such an explanation on a model system, the Catabolite Activator Protein (CAP).

CAP displays negative cooperativity without conformational change upon binding two cAMP ligands. We have built a coarse grain model of multiple slow and fast modes and demonstrated how negative cooperativity can arise without conformational change. The slow, global modes are responsible for the allosteric behavior and result in a purely entropic contribution to the allosteric free energy (ΔΔG). Multiple slow modes need to be included to achieve the experimentally observed free energy values. In the real system compensating entropic and enthalpic terms are observed. Fast modes, despite being localized can couple to the slow modes and assist allostery. We show that they account for the split of ΔΔG into the entropic and enthalpic parts. The value of ΔΔG is proportional to the number of slow modes. The size of the compensating enthalpic and entropic terms increases with number of enslaved fast modes. We can therefore estimate how many slow and fast modes are taking part in the allosteric signaling.

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Impact of Hofmeister Salts on Structural Dynamics of Photoactive Yellow Protein

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Water is known as the lubricant of life. Without water, most proteins would lose their biological activities. Extensive studies have been carried out on how aqueous solutions with high concentration salts alter the stability and solubility of proteins. Such effects are thought to be mediated largely via salt-water interactions and water-protein interactions. This classic research field is known as the Hofmeister Series. We report the effects of Hofmeister salts on the structural dynamics of proteins. Photoactive yellow protein (PYP), a bacterial blue light photoreceptor protein, is employed as a model system in this study. Time-resolved FTIR spectroscopic techniques were used to probe the protein structural changes of PYP in response to blue light excitation. Our data demonstrate that high concentration salt solutions have profound effects on functionally important motions of PYP, including (1) the light triggered proton transfer pathway in the active site and (2) the large conformational changes associated with PYP receptor activation. We will discuss the significance of our study in relation with protein crystallization, and with other properties of the Hofmeister series.

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Linking Enzyme Conformational Dynamics To Catalytic Function With Single-molecule FRET

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Many enzymes endure sizable conformational remodeling on a timescale comparable to their catalytic cycle. In adenylate kinase (AK) from E. coli, this involves large-amplitude rearrangements of the enzyme's lid domain, which may be critical to the enzymes' catalytic function. We applied high-resolution single-molecule FRET developed in our laboratory to follow AK's domain movements on its catalytic timescale. This was achieved by recording and analyzing data photon by photon to rigorously account for counting noise, background, and cross talks. By utilizing a maximum entropy-based approach to remove photon-counting noise, the enzyme's entire conformational distribution was quantitatively recovered without a presumed model. Armed with precise single-molecule FRET dynamics measurements and comprehensive bulk kinetic