reconstruction methods (Zhang and Hinshaw, 2001, Chen et al., 2004). Crystal structures of the GTPase and PH domains from various species have been fitted to the structure of the ΔPRD dynamin phospholipid tube in its constricted and non constricted states (Mears et al., 2007). The PRD interacts with the SH3 domains of several proteins involved in signaling pathways. We are using cryo-EM and a single particle approach to solve the structures of the full length protein-lipid tubes in the constricted and non-constricted states. In both states, we have observed a decrease in the number of subunits per turn compared to previous structures of ΔPRD dynamin tubes. This suggests that the presence of the PRD changes the arrangement of the dynamin domains around the phospholipid tube. Further evidence of a direct interaction between the GTPase domain and the PRD is provided by simultaneous immunogold labeling of the two terminal domains.

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Structural Basis For HIV-1 DNA Integration in the Human Genome

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Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein that requires the lens epithelium-derived growth factor (LEDGF), a cellular transcriptional coactivator. In the presence of LEDGF, integrase forms a stable complex *in vitro* and importantly becomes soluble by contrast with integrase alone which aggregates and precipitates. Using cryo-electron microscopy (EM) and single-particle reconstruction, we obtained three-dimensional structures of the wild type full length integrase-LEDGF complex with and without DNA. The stoichiometry of the complex was found to be $(integrase)_4$-$(LEDGF)_2$ and existing atomic structures were unambiguous positioned in the EM map. *In vitro* functional assays reveal that LEDGF increases integrase activity likely in maintaining a stable and functional integrase structure. Upon DNA binding, IN undergoes large conformational changes. Cryo-EM structure underlines the path of viral and target DNA and a model for DNA integration in human DNA is proposed.

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Structural Studies of a Phycobilisome

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Phycobilisomes are protein complexes present in cyanobacteria and red algae; they are involved in light harvesting and conduction of light and the aim of this study has been to understand the high efficiency observed in these processes The structure of the phycobilisome from an eukaryotic algae *Gracilaria chilensis* was studied by biochemical methods in order to obtain intact phycobilisomes and to obtain their phycobiliprotein components, phycoerythrin, phycocyanin and allophycocyanin. The structure of phycobilisomes has been studies by electron microscopy and electrophoresis and by theoretical methods; the structure of phycobiliproteins has been studied by protein crystallography and because they are chromophorylated, their properties also were studied by absorption and emission spectroscopy. We have also built a theoretical docking model for an antenna formed by two units of phycoerythrin and two units of phycocyanin. This model was used to obtain the k_T for the transfer in resonance of the light; the pathway of the light was calculated through the antenna. An evaluation of the model was performed by comparison with the electron microscopy images. The effect the protein environment on the spectroscopic properties of the chromophoric groups was also considered and analysed.
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Quantification of the Exchange of Subunits from Membrane Protein Complexes Using Foerster Transfer Recovery (FTR)

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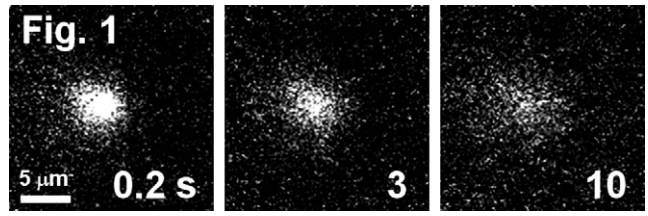
To quantify the exchange of subunits of membrane protein complexes cells expressing CFP/YFP-tagged phospholamban (PLB) were observed by total internal reflection fluorescence (TIRF) microscopy. We performed YFP-selective photobleaching of spots, lines, or larger regions of interest on the basal surface of the cells. This resulted in enhanced CFP fluorescence, indicating CFP-YFP fluorescence resonance energy transfer (FRET). Subsequent spatial broadening of this ''pseudo-photoactivated'' CFP fluorescence was analyzed as a measure of the lateral diffusion of PLB complexes away from the target region. In addition, exchange of bleached and unbleached YFP-PLB from complexes restored FRET over time. This process of Foerster transfer recovery (FTR) was taken to indicate the rate of exchange of fluorescently-labeled subunits of the membrane protein complex. Diffusion and exchange processes were quantified by image analysis using a custom MatLab application for 2-dimensional Gaussian fitting. In addition to its application to FTR, this approach may be useful for cytoplasmic proteins as a way of quantifying dynamic membrane recruitment and lateral diffusion on the plane of the bilayer. Fig. 1 shows the diffusion of acceptor-photobleached CFP/YFP-PLB complexes from a target region, followed by subunit exchange.



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Ligand Binding and Sickle Hemoglobin Polymerization Kinetics: Implication for Therapies

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Sickle Cell Disease results from a point mutation on the beta subgroups of hemoglobin. When hemoglobin releases its four ligands it changes from a relaxed (R) structure to a tense (T) structure and the mutation causes polymer chains to grow. Typical in vitro experiments measure this through complete photolysis of a COHbS sample with a laser and then quantify the scattered light from growing polymers. However, in vivo, many molecules are partially liganded due to the incomplete transfer of oxygen from red blood cells to the surrounding tissue. Liganded T state molecules could contribute to polymer growth, although until now the effect on the kinetics of fractional saturation was unknown. We examined the effects of introducing NO into COHbS samples. The strong binding of NO to HbS keeps its ligand distribution unchanged during the COHbS experiment. We found that the NOHbS caused the polymerization rate to decrease by 50% due to tertiary inhibition of the partially bound T state hemoglobin. We ruled out the possible effects of non-polymerizing R state NO Hb through a flash photolysis experiment, where photolysis curves were analyzed for an initial fast recombination of CO to R state Hb. Only an insignificant possible amount of R state was found ($<3\%$), and could not account for the effects recorded. The effect of partial ligation on polymerization is important in analyzing possible therapies for sickle cell disease. One possible therapy would be to alter the oxygen affinity of Hb, thereby decreasing the number of fractional intermediates and decreasing the number of T state HbS overall.

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Fiber Depolymerization: Fracture, Fragments, Vanishing Times and Stochastics in Sickle Hemoglobin

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Polymerization of sickle cell deoxyhemoglobin (HbS) into stiff fibers lies at the root pathology in sickle cell disease. It induces red cell rigidification, cell membrane damage with myriad pathophysiological consequences, and hemolysis and anemia. The well characterized polymerization kinetics bear intimate relation to pathogenesis, but the role of the less well characterized fiber depolymerization remains to be defined. Its rates may be important in at least 3 ways: i) they govern whether residual polymers fail to dissolve in the lungs and pass into the systemic circulation, facilitating repolymerization; ii) they may affect resolution of vaso-occlusion in sickle cell crises; iii) delayed dissolution might exacerbate cellular damage. Here we observe depolymerization experimentally and develop a theoretical model that encompasses fiber fracture, fragment formation, stochastics and the probabilistic distribution of fiber vanishing times. We use Monte Carlo simulations to show when dissolution is rapid and when slow. Experimentally, we demonstrate fracture in real time and show that dissolution of a fiber does not proceed uniformly in time and space and thus is stochastic. We derive an analytic equation for the distribution of

vanishing times that conforms to Monte Carlo simulations based on a fracture and end-depolymerization model. Our new model, extending previous work (Turner et al, Biophys. J. 91:1008-1013, 2006), characterizes the distribution of fragment lengths during depolymerization as a decreasing exponential that becomes steeper with time. Finally, we propose extensions of the model to characterize dissolution of fiber bundles and gels. The large number of fibers in a gel results in longer vanishing times. Applied to HbS, our model characterizes a pathological process. It is potentially applicable to other linear polymers that depolymerize by fracture and end-depolymerization and in which normal function depends on cyclic polymerization and depolymerization.

398-Pos Board B277

A Repulsive Electrostatic Mechanism For Protein Translocation Through Type III Secretion System: Insights From Pulling Simulations Of MxiH Across The Needle Apparatus Of Shigella Flexneri

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Many gram-negative bacteria inject effector proteins to the host cell through the type III secretion injectosome that comprises of a basal body, a needle, and a tip. In the context of understanding the protein translocation mechanism, steered molecular dynamics (SMD) simulations have been performed to translocate an MxiH needle protein through the needle pore of *Shigella flexneri*. The needle apparatus is modeled implicitly to increase the computational efficiency. The energetics deduced from the SMD trajectories indicates that the translocation of MxiH is not favorable, which in fact, mimics the transportation of a chloride ion across the apparatus whose pore is highly electronegative in nature. Detailed analyses of structurally known proteins that pass through the injectosome reveal considerable electronegative patches on their surface. Further, the basal region has an electronegative pore. Intriguingly, similar feature is found in the flagellar filament of propelling flagellar secretion apparatus. Based on these observations, we propose a repulsive electrostatic mechanism by which the effectors/substrates pass through the type III injectosome and flagellar apparatus. This mechanism gains support from the fact that the transportation of a protein across these nanomachinery requires ATPase that provides the energy to overcome the initial electrorepulsive barrier.

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Solid-State NMR Studies of Gas Vesicle Structure

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Gas vesicles are gas-filled organelles that allow algae, bacteria and archaea to adjust their position in the water column for optimal illumination and aeration. The spindle-shaped vesicles are typically ~500 nm long and ~75 nm wide, with ~1.9 nm thick walls and their shells consist exclusively of protein, primarily the highly hydrophobic GvpA monomer (70 residues), with a permeability such that the vesicle is filled with gas of atmospheric composition. Electron microscopy has shown that the GvpA monomers are arranged in a low-pitched helix; infrared spectroscopy shows considerable beta-sheet content, in agreement with results from X-ray scattering; and atomic force microscopy shows beta-strands tilted at an angle relative to the vesicle axis that is consistent with X-ray scattering measurements on partially aligned vesicles. To gain further insight into the molecular structure and interactions that grant gas vesicles their remarkable physical properties, atomic resolution data is required. However, insolubility prevents the use of solution NMR or crystallography, and multiple scattering frustrates high-resolution electron microscopy. Here, we present the results of solid-state NMR experiments aimed at characterizing the structure of GvpA in intact, deflated gas vesicles from Anabaena flos-aquae. Fairly complete dipolar correlation spectra, indicating a largely rigid and well-ordered system, have allowed resonance assignments for ~80% of the protein sequence. These chemical shifts provide evidence for the presence of both beta-strand and alpha-helix elements in the GvpA backbone. Furthermore, certain regions of the sequence present duplicated resonances, which suggest that the basic structural subunit of gas vesicles is an asymmetric GvpA dimer. Finally, molecular mobility and preliminary tertiary structural characteristics are also discussed.

Protein Folding & Stability I

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Sequence-dependent Stability Of The Beta-helical Fold

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The left-handed beta helix is an intriguing structural motif in several known proteins. We attempt to elucidate the factors that contribute to its stability with a theoretical-computational approach. Combining a novel form of coarse-grained molecular dynamics with parallel tempering affords access to the regime of equilibration in short peptides. For example, this method has been used to reproduce important features of the helix-coil transition in polyalanine.
Three-layered beta-helical fragments are formed from various sequences that are superposed upon two backbone templates taken from sections of ideal type-I and type-II beta helices. Native, native-like, and various homogeneous sequences are simulated and their stability analyzed relative to the initial structure.
An all-atom potential energy and associated parameters drawn from the Assisted Model-Building and Energy Refinement package with slight modifications. The effects of aqueous solvent are treated with the generalized Born model and a recently-proposed hydrophobic potential of mean force. Larger conformational changes can be explored by treating non-bonded forces according to Brownian dynamics while simultaneously maintaining molecular geometry with a separate algorithm.

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Investigating the Origins of Fractional ψ-values in Protein Folding Transition States

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ψ-analysis has been used to characterize the inter-residue contacts that define the structure of the transition state ensemble (TSE) for three protein systems. ψ-values identify the degree to which an engineered bi-Histidine metal ion binding site is formed in the TSE. Values of zero or one indicate that the site is fully unfolded-like or native-like, respectively, while fractional values reflect a partial recovery of the binding-induced stabilization in the TSE. This method has been applied to three proteins, protein A (Baxa et al., 2008), Ubiquitin (Ub) (Krantz et al., 2004), and acyl-phosphotase (Pandit et al., 2006). In all three cases, the TSE captures ~70% of the respective native-state topology, as quantified by the relative contact order (RCO) metric. In light of the proposed ''70% rule'', a re-evaluation of the TSE of many small proteins, especially those characterized as polarized by mutational f-analysis, must be considered. While this ''70% rule'' is believed to be a general feature of most small proteins, the potential origin of fractional ψ-values remains to be investigated. All-atom Langevin dynamics (LD) simulations of TS models of Ub are performed with distance constraints on residue pairs having experimentally-determined ψ-values of unity. An analysis of the trajectories indicates that the fractional ψ-values of sites adjacent to unity sites tend to reflect distorted site geometries, while the residues for more distal, fractional values indicate that the sites are able to sample configurations where they are unfolded-like. Nevertheless, the simulations indicate that the unity ψ-values alone are sufficient to generate a TSE with a highly native-like topology. Furthermore, the calculation of f-values based on side-chain-sidechain contacts made in the TSE indicate that experimental f-values can dramatically under-report the amount of structure present even for highly buried residues.

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Electrostatic Interaction In The Unfolded States Of Proteins

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With recent recognition that the unfolded states of proteins play important and diverse roles in protein functions, some advances have been made in developing experimental techniques to help decipher residue-specific interactions. Here we present a molecular dynamics simulation based method that allows direct prediction of electrostatic interactions in the unfolded proteins under native conditions. The theoretical prediction is confirmed by measurements of pH-dependent folding free energies of a small model protein HP36.

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Non-Native Structure in the Unfolded Ensemble of a Prototypical β-Hairpin

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Two of the main challenges of modern molecular biology are the determination of the biologically active conformation of a protein from the information encoded in its amino acid sequence and the understanding of the series of events that brings this sequence to the native state. However, for a complete comprehension of the folding process, it's of fundamental importance not only to characterize the final *folded state* and the pathways that lead a protein there, but also to fully understand the nature of the ''starting point'', *i.e.,* the *unfolded ensemble*.