microscopy. The specific brightness of each particle detected in the images can then calculated for each separate emission channel.
As a first application, we studied the distribution of Bax and Bid on a population of liposomes. These proteins are both pro-apoptotic, promoting pore formation in liposomes, and they are known to interact with one another. We measured the probability that a liposome carried Bax, Bid, or both proteins, allowing us to quantify their colocalization on the same liposome.

258-Pos Board B44
Single-Molecule Investigation of the Flagellar Membrane Signaling Protein Entry Mechanism
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The composition of the flagellar membrane differs from that of the plasma membranes–it is enriched with a unique set of membrane signaling proteins. How these flagellar membrane proteins, which are synthesized in the cytoplasm, enter the flagella across diffusion barriers at the flagellar base has become a significant point of inquiry. Using single-molecule fluorescence imaging methods, we have investigated the intraflagellar transport (IFT) processes of Pkd2-GFP, an important flagellar signaling protein responsible for polycystic kidney disease, and tested two proposed models. In Model I, vesicles carry the proteins to the flagellar base and fuse with the membrane before or after the diffusion barrier to allow the flagellar membrane proteins to enter by lateral diffusion; and, in Model II, the flagellar membrane proteins are bound to the BBSome-IFT-particle machinery (kinesin or dynein motors carry IFT particles, which, in turn, carry BBSomes) at the flagellar base and are carried through the basal body transition fibers into flagella. We have observed Brownian diffusion of Pkd2 at the Chlamydomonas flagellar entry region, suggesting that Model I more accurately describes the entry mechanism. We have further studied the turnaround mechanisms of the IFT machinery at the flagellar tip. Our preliminary results indicate that the IFT machinery does not dissociate, diffuse, and re-associate at the flagellar tip for the return trip to the cell body.

259-Pos Board B45
The Force-Induced Activation of Talin in a Structure-Based Model Simulation
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Talin is a mechanosensitive protein that forges the links between the cytoskeleton and the extracellular matrix and strengthens the focal adhesion under external force. To explore the mechanotransduction of talin, we performed molecular (stochastic) dynamics simulations with a coarse-grained structure-based model (SBM). This model has proven to be a powerful technique to explore the landscape of large scale conformational changes due to the fact that unfolding pathway starts from native conformation, and thus depends more strongly on the protein geometry than detailed energetic information. We preform two types of stretching with constant force and velocity. We used very low constant-force to explore the free energy barrier around transition almost in equilibrium condition. Another important aspect of our study is to interpret the forces in structure-based models in terms of the realistic forces borne by mechanosensitive proteins in physiologically relevant situations, which is done by looking at the balance between the pulling force and the free energy barrier. Since structure-based models expedite the simulation by several orders of magnitude, our work will enable us to explore and interpret the mechanotransduction mechanisms of much larger systems for which all-atom study is unfeasible.

260-Pos Board B46
A Model with Two Elastically Coupled Reaction Coordinates for the Internal Friction of Proteins
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The rates of protein conformational changes are usually not only limited by external but also internal friction, however, the origin and significance of this latter phenomenon is poorly understood. It is often found experimentally that a linear fit to the reciprocal of the reaction rate as a function of the viscosity of the external medium has a non-zero value at zero viscosity, signifying the presence of internal friction. Furthermore, some of the experiments performed at different temperatures indicate that the internal friction follows an Arrhenius-like temperature dependence. To explain these phenomena we suggest a simple model for protein conformational changes in terms of two elastically coupled reaction coordinates, one of which being in contact with the external medium, and the other one experiencing the rough energy landscape of the protein. Our analytical calculations, supplemented with numerical simulations demonstrate that depending on the coupling strength (which is related to the flexibility of the protein) the short-wavelength components of the energy landscape roughness can be observed as an increased apparent internal friction with an apparent activation energy.

261-Pos Board B47
Direct Characterization of Inter-Domain Concerted Motions in Proteins from NMR
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The high resolution description of concerted inter-domain motions in proteins is a major challenge in molecular biophysics. Here we show that residual dipolar couplings (RDCs) measured by nuclear magnetic resonance (NMR) in solution under steric alignment provide information on the degree of concert of the inter-domain motions of two relatively simple representative multi-domain proteins: bacteriophage T4 Lysozyme (T4L) and Adenylate Kinase (AKe). Our strategy simultaneously exploits the structural and shape information contained in RDCs and the impossibility of domain inversion due to steric hindrance and covalent linkage. Exploiting the shape information contained in RDCs appears thus to be a promising strategy to determine how structural and dynamical information is transferred across multi-domain proteins.

262-Pos Board B48
Conformational Flexibility Mediates Resistance of HIV-1 Reverse Transcriptase to Nonucleoside Inhibitors
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HIV-1 reverse transcriptase (RT) plays a critical role in the HIV lifecycle and is a major drug target. Although nonnucleoside reverse transcriptase inhibitors (NNRTIs) have proven effective anti-AIDS drugs, NNRTI resistance mutations appear frequently in patient populations and represent a continuing challenge in the treatment of AIDS. K103N is a common NNRTI resistance mutation that conveys high levels of resistance to multiple NNRTIs including the widely used drug efavirenz (EFV). Because NNRTIs are allosteric inhibitors, mechanisms of both inhibition and resistance may be mediated by conformational dynamics.
Hydrogen/deuterium exchange reveals that the K103N mutation increases the structural flexibility of RT in portions of the polymerase domain of the p66 subunit, which contains the polymerase active site and the NNRTI binding pocket. We also find increased flexibility in parts of the RNAse H domain. Previous H/D exchange studies showed that EFV binding induced significant long-range suppression of molecular flexibility in wild-type RT, suggesting that this rigidification may contribute to NNRTI inhibition. In K103N, EFV binding has negligible effects on molecular flexibility except in the immediate vicinity of the binding site. As a result, K103N-EFV complex is more flexible than wild type-EFV complex throughout most of the structure, including regions thought to be essential for DNA translocation. EFV binding studies by equilibrium dialysis show that, while the binding affinity of the drug is reduced by a factor of 3 for K103N RT compared to wild type, K103N RT is saturated with EFV under the conditions of the H/D exchange experiments. We propose that the retention of essential molecular mobility even when bound to inhibitor contributes to NNRTI resistance in K103N. Our findings suggest that conformational flexibility may play a role in the resistance of other RT mutants to NNRTIs.

263-Pos Board B49
Structural and Dynamic Characterization of Norovirus Protease
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Norovirus is a major etiologic agent of acute gastroenteritis outbreaks often occurring in confined, population-dense communities. Since neither vaccine