binding of the Gz subunit and therefore act as regulators of cell signaling. Ric-8a is a cytoplasmic-receptor-independent-GEF that is known to interact with the C-terminal regions of subunits, possibly in a similar manner as GPCR's. In this study, domain and long-lived segmental motions involved in Gz subunit binding of the GEF Ric-8a and the subsequent nucleotide exchange was probed using single-molecule fluorescence spectroscopic methods.

2258-Pos Board B28
Direct Characterization of Protein Oligomers and their Quaternary Structures by Single-Molecule FRET
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Determining the oligomeric state of a protein under physiological conditions is vital for fully understanding the functions of the protein. Here we demonstrate that a single-molecule method, called alternating-laser excitation (ALEX), can directly distinguish among oligomeric forms from monomers to tetramers and determine their quaternary structures. Using this method, we found that RecR, a recombinator mediator protein, forms a stable dimer and that weak C-terminal region interactions cause pairs of dimers to combine to form ring-shaped tetramers. The measured dissociation constant of dRecR tetramer (12 ± 2 μM) decreased by more than three orders of magnitude (6 ± 2 nM) in the presence of RecO. From these findings, we propose that tetramer RecR encloses DNA through an opening between adjacent C-terminal domains and that RecO binds to the C-terminal domains of RecR to stabilize the ring-shaped tetramer. This work proves that ALEX is extremely useful for determining protein oligomeric forms and their quaternary structures under physiological conditions.

2259-Pos Board B29
Structural Modeling of PSD-95 and other MAGUKs using Single-Molecule FRET
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In order for information to be correctly propagated between cells, it is necessary for both the sending and receiving cells to have organized molecular machinery so that the transmitted signals can be accurately received and processed. By conjugating protein interaction domains, multidomain scaffold proteins form a framework for maintaining and modulating these junctional communications. Scaffolding proteins have been traditionally conceptualized as "beads on a string" with unstructured linkers allowing each domain to be independently oriented, but recent data on multidomain supramolecules suggests that the fundamental functional units of scaffold proteins may be larger multidomain complexes. Membrane-associated guanylate kinase (MAGUK) proteins are multidomain scaffold proteins that are present at epithelial tight junctions and within the postsynaptic densities of neurons. MAGUK proteins contain both PSD-95/Dlg/ZO-1 (PDZ) and Src Homology (SH3) domains, as well as an enzymatically inactive guanylate kinase domain. While the high-resolution structures of the isolated domains of the canonical MAGUK, PSD-95, have been solved, the organization of these domains remains unknown. In combination with rigid body docking and all-atom molecular dynamics simulations, the single-molecule FRET measurements between each of five domains allowed for the determination of the domain positioning in full-length PSD-95. The "ground" state configuration of PSD-95 was found to contain two stable multidomain subunits that while connected by a flexible linker do not appear to interact. A comparison of measurements within each of these partitions with homologous measurements in other MAGUKs showed that domain orientations can vary among this family of scaffold proteins. These findings represent the first unambiguous assignment of domain positioning in a full-length scaffold protein and provide insights into potential allosteric coupling between domains in MAGUK proteins.

2260-Pos Board B30
Examinations of Antibody Structure using Single Molecule Förster Resonance Energy Transfer
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The crystalizable fragment (Fc) region of IgG antibodies interacts with a variety of molecules in the immune system. Some of these interactions are negatively impacted by the removal of the oligosaccharides bound to the antibodies in the Fc region. In order to study the structure of the Fc region of IgG antibodies, we have mutated a surface-exposed serine residue to a cysteine, allowing the antibodies to be labeled with thiol-reactive dye molecules and studied via single molecule Förster resonance energy transfer (FRET). We have also applied single molecule FRET to the study of the distance between the antigen-binding sites of an antibody. All FRET measurements performed involved the examination of photon bursts from freely diffusing donor-acceptor labeled antibodies, from which a histogram of the conformations present was constructed.

2261-Pos Board B31
Normal Mode Comparison Elucidates Dynein Walking Mechanism Triggered by ATP Hydrolysis
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Motor proteins play important roles in transportation of cargoes in the cell and cell motility and are powered by ATP hydrolysis. Motor proteins are classified into two categories: myosin, kinesin and dynein. Dynein is a microtubule based motor protein and related to cell motility. The structure and motion of dynein has been studied by many scientists and its crystal structure was recently reported. In this study, the swing motion of dynein was investigated by normal mode analysis (NMA) using elastic network model. To test the role of the linker of dynein which can be detached from body (AAA5) due to ATP hydrolysis, we performed NMA of the following two different types of dynein structures: native and artificially modified one with no connection between linker and body. NMA results of the native structure showed a twisting motion at the first mode and bending motions at the second and third modes. While, in case of artificially modified dynein, a bending motion comes to the first mode. It implies that the dynamics of dynein can be controlled by the connectivity between linker and body. Therefore, ATP hydrolysis plays a crucial role in dynein walking mechanism as a trigger of swing motion. In the future work, the large conformational change of dynein during its swing motion will be studied by elastic network interpolation.

2262-Pos Board B32
Changes in Bond-Orientational Order of Residues are Associated with Shifts in Energy Landscapes
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We show that local order, measured not only by the number density distribution around a given node, but also as the geometric preferences of neighbors quantified by bond orientational order (BOO)[1] identifies subtle local structural changes in proteins. We first establish a correspondence between topological and geometrical quantities utilized in protein physics[2]. In the topological case, local parameters are represented as moments of the adjacency matrix. For the geometrical counterpart, local information is encoded in BOO parameters, showing up in series expansion of bond density on a unit sphere. Of special importance, the respective terms in this expansion represent compactness; both clustering coefficient C and third-order rotational invariant W use the coupling of three vectors/edges. We then generalize a topological index[3] that measures the propensity of residues to find alternative routes to communicate with function-related destinations. The average number of alternative n-step paths a given residue generates to its neighbors (equal to 2C for two-step paths), normalized by the reachability of that residue by all others in the structure successfully measures the degree of condeness of motions in a protein.
We finally demonstrate the utility of these concepts by showing that W is a good descriptor for identifying local structural changes between the apo and holo forms of ferric-binding and maltose-binding proteins. In both cases, though the holo form resides close to the apo form on the free energy surface as a weakly populated conformation, BOO changes between the states are clearly detectable. Expanding in terms of BOOs, we offer an alternative method for calculating the free-energy change.