qualitative indicators of dynamics and the quantitative methods of filtered fluorescence correlation spectroscopy (fFCS) and dynamic photon distribution analysis (PDA), can be extended and applied to three-color FRET experiments. Specifically, in three-color pulsed interleaved excitation experiments (PIE) with multiparameter fluorescence detection (MFD), the multidimensional information of color, lifetime and anisotropy offers superior contrast to separate different conformational states. This increases the robustness of the analysis and the sensitivity to minor conformational changes that are otherwise not detectable in two-color FRET.

2361-Pos

Structural Studies of the Fc Region of Murine Immunoglobulin G Antibodies using Single Molecule FRET

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The glycosylation of antibodies is known to influence their ability to instigate an immune response. The biantennary sugars present in the crystallizable fragment (Fc) region of antibodies are thought to stabilize the conformation of the Fc region so it can successfully bind to its receptors, thereby initiating an immune response. Prior studies have suggested that sugar removal leads to an increase in the flexibility of the Fc region. Other results show a collapse of the Fc region upon sugar removal. Either of these conformational alterations would impact receptor binding, thereby explaining the decreased immune response observed upon sugar removal. To examine the structure of the Fc region of murine immunoglobulin G (IgG) antibodies, click chemistry was used to attach dye molecules to azide-modified sugars in the Fc region. In addition, the enzyme EndoS was used to cleave the majority of the sugars from the Fc region. This enzyme leaves behind a single *N*-acetylglucosamine moiety, which was modified with an azide group and then reacted via click chemistry with dye molecules. These dye-labeled glycosylated and deglycosylated fully intact IgG antibodies were then examined using single molecule FRET to observe the structural impact of sugar removal.

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Binding Free Energy Analysis of Programmed Cell Death Protein PD1 to its Ligand PD-L1

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Immune checkpoint inhibitors targeting programmed cell death protein receptor PD1 and the ligand PDL1 represent a step forward for treatment of previously difficult-to-treat cancers such as non-small cell lung cancer, metastatic melanoma, and renal cell carcinoma. However, the same degree of benefit has unfortunately not been seen in glioblastoma, the most common and deadliest of glial tumors with a median patient survival between 10 to 20 months. Despite promising pre-clinical data, anti-PD1 monoclonal antibody *nivolumab*-failed to reach its primary efficacy endpoint in a recent phase III randomized clinical trial, CheckMate-143. One possible reason for failure in glioblastoma is poor diffusion of large monoclonal antibodies across the blood-brain barrier. Detailed characterization of the PD1-PDL1 complex may aid in development of a small molecule inhibitor that would overcome the size limitation. We investigated the binding free energies involved in PD1-PDL1 complex formation with a molecular dynamics approach and identified the specific amino acid sites important for PD1-PDL1 complex formation. Five simulations of 500 ns each were performed for the binding domains of wild-type (PDB: 4ZQK) and high-affinity mutant (PDB: 5IUS) PD1-PDL1 complexes in an explicit-water environment with AMBER force fields. Binding free energies estimated by Molecular Mechanics Generalized Born Surface Area and entropy calculated by normal mode analysis confirmed high-affinity PD1 mutant receptor had a stronger binding free energy to PD-L1 than wild-type PD1 receptor. Among residues of the high-affinity PD1 mutant receptor, I^{132}, I^{134}, T^{78}, E^{70}, H^{68}, and E^{136}contribute the most free energy difference between bound and free states and are implicated as the most important residues for stable complex formation. We suggest that ability of PD1 to bind stably to PD-L1 would be most vulnerable to interference at these sites.

2363-Pos

Determining Native-State Dynamics of Mitoneet using Hydrogen Exchange Mass Spectrometry

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The protein mitoNEET (mNT) is a protein first described in 2004 that binds the type- 2 diabetes drug pioglitazone. The homodimeric protein is encoded by the gene CISD1 and has been implicated to play still undefined role(s) in type-2 diabetes and Parkinson's Disease. Like all NEET proteins, the mNT monomer possesses a CDGSH domain with three cysteine and one histidine residue coordinating a [2Fe-2S] cluster. Removal of this cluster by treatment with acidic conditions induces mNT unfolding. While there are numerous crystal structures in the Protein Data Bank, little is known about *holo* mNT dynamics and stability or the unfolding of *apo* mNT. Here we apply hydrogen exchange mass spectrometry (HXMS) to *holo* and *apo* mNT to detect dynamics ranging from local breathing to global unfolding under native-state conditions. With mild shifts in pH and temperature, our N-HXMS approach can detect partially unfolded intermediates, extract the free energy of unfolding and/or the rate constants of unfolding and folding for the native state and intermediates. In addition, we can simultaneously monitor *holo* and *apo* states of mNT due to their differences in mass. By systematically comparing their HXMS behavior, we will gain insight into the connection between mNT dynamics and function.

Posters: Protein-Small Molecule Interactions

2364-Pos

The Effects of Ligand Structure on Protein-Multimodal Ligand Interactions

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Many important biophysical phenomena are driven by the structuring of water around solutes. Solutes perturb the structure (*i.e.*, the packing and orientational organization) of water molecules in the vicinity - the extent of the perturbation depending on the nature of solute and strength of solute-water interactions - resulting in solute-solute interactions that are strongly mediated by water. Although the role of water in mediating solute-solute interactions has been well characterized for simple systems, such as small ions or simple hydrophobic solutes, most biological systems are larger and more complex molecules that contain regions of mixed charge, hydrophobicity, hydrogen bonding, and other modes of interaction. Here, we focus on the role of water in governing the behavior of small, flexible molecules containing multiple modes of interaction (e.g., charge, hydrophobicity, and hydrogen bonding interactions) and how this behavior impacts their interactions with proteins. In particular, we study a set of multimodal chromatographic ligands which are commonly used for protein separation applications. We characterize the conformational and hydration preferences of each ligand in water including water density fluctuations in the solvation shell. By decomposing ligand contributions intramolecular versus water-mediated parts, we quantify the role of water in ligand conformational equilibria. We also perform molecular simulations of multimodal ligands in aqueous solutions with different proteins. We find that regardless of which region of the protein surface the ligand binds to, its conformational preferences in free solution are maintained upon binding. Finally, we identify ligand design characteristics that lead to differences in the overall strength of protein-ligand interactions. This work provides a basis for characterizing the role that water plays in governing protein-ligand interactions as well as serves to guide the design of new multimodal ligands for protein separations.

2365-Pos

Peptide Assisted Supramolecular Polymerization of the Anionic Porphyrin Meso-Tetra(4-Sulfonatophenyl)Porphine

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The photoelectronic properties of porphyrins have been harnessed by nature to power such widely known processes as photosynthesis. When excited by light, many porphyrins release electrons; however, without an efficient or directed method to transport them, this energy is typically limited to facilitating local enzymatic reactions. We investigated a series of peptides containing partial and complete porphyrin binding regions (PBRs) for their ability to promote meso-tetra(4-sulfonatophenyl)porphine ($TPPS_4^{2}$-) to form electrically conductive structures known as J-aggregates. While aggregation has previously been shown to occur in the absence of peptide, this requires very acidic conditions ($pH < 1$) and the resultant aggregates are too small and irregular to effectively conduct electrons. Our approach uses amphiphilic peptides containing complete PBRs (Lys_3) as well as partial (Lys_1 or Lys_2) regions located at the peptide termini. Our goal is to induce the concurrent binding of TPPS to the incomplete domains of two peptides, forming a bridge between them. Binding was assessed in aqueous solution by monitoring the soret and Q-bands of $TPPS_4^{2}$-) across