based on recent studies of ablation of multiple endocrine neoplasia type 1 gene (MEN1, which encodes menin) in mice, identifying a novel means to enhance beta cell regeneration by repressing menin is being pursued as a new avenue for T2D treatment. In particular, it has been shown that menin ablation reverses preexisting glucose intolerance, a hallmark of T2D, suggesting that inhitors of menin would also have this effect. Recent crystal structures of menin in co-complex with short peptide fragments as well as small molecule inhibitors have indicated that it has a deep druggable pocket and is known to form complexes with several proteins via this pocket. These characteristics make menin an ideal target for computational docking studies of lead compounds to optimize binding affinity. In this study, we perform molecular dynamics simulations of the recently solved crystal structure of menin and pursue an ensemble (or multiple protein conformation) docking strategy in order to determine a number of different bound conformations of small molecule inhibitors to this protein. While the insilico docking studies of the inhibitors are performed using a flexible-ligand, rigid protein approach, docking to different snapshots of the protein obtained through the molecular dynamics approach allows for implicit protein flexibility. The different docked poses from the high-dimensional dataset are clustered and the most probable conformations are used to suggest new higher affinity ligands. Efforts to procure these ligands and experimentally determine their binding affinities in order to validate the insilico screen/design are simultaneously being pursued.

296-Pos Board B65

Exploring Kinetic Scenarios for Allosteric Transitions of Calmodulin through Coarse-Grained Simulations

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Although the two domains of Calmodulin (CaM) are topologically similar, they differ in structural flexibility, stability, and Calcium binding affinity. Our coarse-grained simulations of the open-closed conformational transitions in Calcium-free Calmodulin (CaM) suggest kinetic scenarios in which the transition mechanisms for the two domains are distinct as well. Both domains have the same qualitative thermodynamic mechanisms: a ''low temperature'' two-state mechanism in which the domain remains relatively structured throughout the transition, and a ''high temperature'' mechanism involving a partially folded, unstable intermediate in the transition landscape. Under the same simulation conditions, the N-terminal domain (nCaM) exhibits two-state behavior, while the C-terminal domain (cCaM) populates the partially folded intermediate. The simulated transition rate for cCaM is much smaller due to the transient unfolding and refolding along the transition route compared to the two-state transition rate for nCaM. Differences in transition rates for the Calcium-free open/closed transition could explain measured binding rates of Ca^{2+} for the different domains. For example, slower conformational kinetics caused by the partially folded intermediate may bias cCaM towards a conformational selection binding mechanism, while nCaM maintains induced fit binding under some range of calcium concentration. Here, we explore such binding scenarios qualitatively.

297-Pos Board B66

Nanoscale Protein Dynamics and Long-Range Allostery in Cell Signaling

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An emerging point of view in protein chemistry is that proteins are not the static objects that are displayed in textbooks but are instead dynamic actors. Protein dynamics plays a fundamental role in many diseases, and spans a large hierarchy of timescales, from picoseconds to milliseconds or even longer. Nanoscale protein domain motion on length scales comparable to protein dimensions is key to understanding how signals are relayed through multiple protein-protein interactions. A canonical example is how the scaffolding proteins NHERF1 and ezrin work in coordination to assemble crucial membrane complexes. As membrane-cytoskeleton scaffolding proteins, these provide excellent prototypes for understanding how regulatory signals are relayed through protein-protein interactions between the membrane and the cytoskeleton. Here, we review recent progress in understanding the structure and dynamics of the interaction. We describe recent novel applications of neutron spin echo spectroscopy (NSE) to reveal the dynamic propagation of allosteric signals by nanoscale protein motion, and present a framework to analyze the NSE data.

298-Pos Board B67

The Internal Friction of Proteins Determined by MD Simulations

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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. To better understand this phenomenon, we have performed molecular dynamics simulations of a conformation change of trypsin, where we could separately control the friction of the surface and the interior of the protein. Here we present the results of our simulations, and also compare them to the experimental data obtained for the activation of trypsin.

299-Pos Board B68

Force-Dependent Platelet Binding of the Von Willebrand Factor A1-A2 Complex from Atomistic Simulations

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Von Willebrand factor (VWF) is a huge multimeric extracellular protein playing a crucial adhesive role in hemostasis. In response to shear stress, VWF binds to the GP1bα receptor of platelets via its A1 domain, to build a plug that stops the bleeding of an injured blood vessel. Recent experimental studies revealed that the binding of GP1bα is altered by the presence of individual A2 subunits, suggesting that A2, adjacent to A1, interacts with A1, thereby shielding the binding site for GP1bα. In addition, electron microscopy studies measured the distance between the center of masses of A1 and A2, varying from ~4.6 nm, in which the two subunits may be in contact, to ~11 nm, in which the two subunits are not in contact (Zhou YF *et al. EMBO J. (2011) 30: 4098-4111)*. Apart from these distance estimates, little is known on how A1 and A2 interact with each other and thereby modify GP1bα binding, and how this inhibition is sensitive to mechanical forces in flowing blood. We address these issues by using molecular docking, and equilibrium and force probe molecular dynamics simulations. Our study predicts a stable structure of the A1-A2 complex, in which A2 binds to the same site in A1 as GP1bα does, over a time scale of hundreds of nanoseconds. This inhibition is relieved under a stretching force, explaining how A2 can inhibit Gp1bα binding to A1 in a shear-flow dependent manner. Overall, our simulations suggest a new and intriguing mechanism on an intermolecular auto-inhibition that allows a shear-sensitive growth of blood coagulates, which reconciles previous and can be directly tested by future experiments.

300-Pos Board B69

Prestress in Protein Disulfide Bonds Tunes their Stabilities

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Disulfide bonds are ubiquitous covalent links in proteins. By providing a protein fold with additional structural constraints they can tune protein stability and function. We find disulfide bonds to exhibit a varying degree of pre-stress [1]. Using a new Force Distribution Analysis of forces obtained from Molecular Dynamics simulations [2], we identified particularly tensed disulfides, with inter-cysteine forces of 143.34 pN for Cys130-Cys159 in CD4 and 165.47 pN for Cys27-Cys37 in the von Willebrand factor C1 (vWFC1) domain. Remarkably, the order of magnitude of these internal forces is coinciding with those required to unfold or activate proteins, and thus likely to play an important role in the protein's integrity. The pre-stressed disulfide bonds link adjacent strands in the same antiparallel β-sheet, and have been previously classified as 'allosteric disulfide bonds' due to their unusual and unfavourable dihedral configuration [3]. We calculate reduction rates of the two disulfide bonds to increase due to the intrinsic tension by a factor of two to three as compared to the other comparably relaxed disulfide bonds in CD4 and vWFC1. This trend is preserved in a survey over all disulfide bonds in protein structures currently deposited in the Protein Data Bank, with disulfide bonds linking two adjacent strands in a β-sheet featuring a significantly larger sulphur-sulphur bond length on average. The decreased thermodynamic and kinetic stability of