Acidic human fibroblast growth factor 1 (hFGF1) is a major signaling molecule that is heavily involved in cell proliferation, angiogenesis, tumor invasion and metastatic progression. Previous experimental studies have demonstrated that hFGF1 is naturally unstable and that it has a near-physiological denaturation temperature. Heparin (a linear sulfated polysaccharide) is known to stabilize hFGF1 and protect it from thermal and proteolytic degradation. Our study used experimental data to set up a rigorous computational investigation of the hFGF1-heparin hexasaccharide complex.

Three models were simulated for 4.8 microseconds each. Our equilibrium-MD simulations confirmed that the heparin-free monomer is less stable than the heparin-bound monomer. The decreased stability of the heparin-free monomer is due to a conformational change in the heparin-binding region. This conformational change was not observed in the heparin-bound systems. Important interactions that contribute to the stability of the complex were also characterized. K113 and S117 were identified as important residues of the heparin-binding region that contribute to intramolecular hydrogen bonding. Strong intermolecular hydrogen bonding was also observed between R123 and IdoA(4) of the heparin hexasaccharide. We then used a combination of non-equilibrium pulling and bias exchange umbrella sampling simulations to determine the binding free energy of heparin.

Thus far, all published computational studies of the hFGF-1 heparin complex have been based on nanosecond-level simulations. For the very first time, we have used a combination of microsecond-level MD simulations and large-scale enhanced sampling techniques to carry out a more realistic and biochemically relevant assessment of the hFGF1-heparin complex.

**944-Pos**

**Identifying Intermediate States in Prion Protein Folding Pathway: A Possible Precursor to the Misfolded State?**

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Aggregation of prion proteins causes neurodegenerative disorders. The misfolded form of monomeric cellular prion protein leads to toxic protein aggregates rich in β-sheets. Pathological scrapie form of prion protein also catalyzes the conversion of cellular protein to the infectious conformation. Therefore it is very important to understand the mechanism of structural transition of a prion protein from a cellular functional form to the scrapie form. To probe the structures of transient intermediates populated in the folding pathways of prion protein, which can be probable precursors to the pathological misfolded forms, we performed molecular dynamics simulations to probe the folding pathways of prion protein using coarse-grained protein models. The protein populates an intermediate, which is globular and resembles a molten globule like structure where only the $\beta_1$ strand detaches from the rest of the folded protein structure. This observation also supports the NMR studies, which report the partial unfolding of the $\beta_1$ strand. This conformation might possibly be a precursor to the misfolded or the scrapie prion form.

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**945-Pos**

**Disulfide Bonds Modulate Lysozyme Folding Pathways**

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Experiments on lysozyme folding [Dobson *et al.*, 1994; Kiefhaber, 1995] show that it folds in parallel pathways: a slow kinetic pathway with well populated partially folded states and a faster pathway without any intermediates. We studied lysozyme folding using coarse-grained protein models and molecular dynamics simulations. The simulations do show that lysozyme has slower and faster folding pathways as predicted by the experiments. The folding timescale in the fast pathway is on the order of tens of milliseconds as expected for two-state folding globular proteins. In the slower pathway, the folding timescale is higher as the intermediate populated in this pathway is stable for hundreds of milliseconds. The intermediate populated is partially structured where the β domain is fully formed and the α domain is partially formed and there are no inter-domain contacts. These intermediates are kinetically trapped and require rearrangement in order to fold to its native state. The disulfide bonds play a major role in enhancing the lifetime of the intermediate as the conformational space available for the protein to rearrange is constrained due to the presence of the disulfide bonds. The kinetically trapped intermediates are not observed in the folding pathways when the disulfide bonds are absent.

**946-Pos**

**Probing the Chaperone Activity of Erythroid Spectrin**

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Spectrin, the major protein of the RBC membrane skeleton has canonically been thought to only serve a structural function. We have described a novel chaperone-like property of spectrin and have shown that it is able to prevent the aggregation of other proteins such as alcohol dehydrogenase, insulin and free globin chains. Moreover this chaperone activity is pH linked, with spectrin displaying higher oligomeric states and consequently increased chaperone activity at lower pH, with a maxima at pH 4.0. Hemoglobin and other heme proteins are implicated as clients of the chaperone-like activity of spectrin and we have demonstrated that as a consequence of spectrin-heme protein interaction, there is an increase in the enzymatic activity of these heme proteins. The peroxidase activities of hemoglobin, hemoglobin variants, free globin chains, cytochrome-c and hemin increase upon spectrin binding, as does the enzymatic activity of catalase. The $K_m$ values of these heme proteins for $H_2O_2$ decreases in presence of spectrin while the $V_{max}$ values are found to increase. Raman spectroscopy indicates spectrin binding induced conformational changes in the prosthetic heme groups of these proteins as the source of increased enzymatic activity. We have also found that the chaperone-like activity of spectrin competes with its phospholipid/hemoglobin binding ability and phospholipid/hemoglobin bound spectrin is a comparatively weaker chaperone. Non-enzymatic glycation has also been shown to decrease spectrin chaperone potential. We have tried to localize the molecular origin of chaperone-like activity in multi domain spectrin by cloning and investigating individual domains and our current understanding points to the presence of hydrophobic patches on the surface of these domains as the source of the chaperone activity of spectrin, as notably seen in the self-association domain.

**947-Pos**

**Moltenprot: A High-Throughput Analysis Platform to Assess Thermodynamic Stability of Membrane Proteins and Complexes**

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Structural biology has entered a new era, when high resolution analysis of increasingly complex samples was made possible. Still, spectacular technological developments, such as lipidic cubic phase crystallization or direct electron detectors, do not address the primary challenge that any structural biologist faces, namely, how to determine optimal buffer conditions for maintaining stable proteins or protein complexes.

To overcome this issue we developed a software package MoltenProt, which estimates thermodynamic characteristics of protein stability from thermal unfolding assays. The analysis integrates both melting temperature and slope of the unfolding curve to determine standard Gibbs free energy of unfolding. This approach is applicable to proteins of any complexity and origin, consumes minimal amounts of sample and is compatible with most common buffer reagents. Data processing can be massively parallelized, and the whole pipeline is typically done within one week. We demonstrate increased sensitivity and selectivity of MoltenProt analysis compared to standard melting temperature based hit detection and illustrate the utility of the assay by testing the stabilizing properties of 94 widely used detergents on a panel of membrane proteins.

**948-Pos**

**Structural Dynamics of c-Myb DNA-Binding Domain Revealed by DXT and Thermal Analysis**

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