known to interact with elongin B and C and cullin 5 to form E3 ubiquitin ligase complexes, targeting creatine kinases (CK) for ubiquitin mediated degradation. Using isothermal titration calorimetry (ITC) we found the interaction between ASB9 and CK to be largely entropic, with some residues between 19-34 in ASB9 constituting a major portion of ASB9's binding interface. The ARD alone and the N-terminus + ARD of ASB9 have the same entropic contribution to the binding free energy with CK. However, when the first 18 amino acids are removed from ASB9, the entropy of binding to CK is lowered by an order of magnitude. We performed molecular dynamics simulations to rationalize the interaction thermodynamics and explore the structural ensembles of ASB9 (1-252) and CK. These simulations were clustered and dockings were performed on their centroids. Remarkably, residues 23-34 docked into the active site of CK. The docking also found that CK residues 180 to 203 constitute a part of the binding interface, which agree with our hydrogen deuterium exchange mass spectrometry (HDXMS) studies. We plan to precisely characterize the residual dynamics of ASB9 within the complex using NMR spectroscopy interpreted in the light of accelerated molecular dynamics.

3485-Pos Board B213

Sequence Analysis and Biophysical Characterization Reveals the Presence of a Long Disordered Region in the CapA Membrane Protein from F. Tularensis

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The *capA* gene (FTT_0807) from *Francisella tularensis* subsp. tularensis SCHU S4 encodes a 44.4 kDa integral membrane protein composed of 403 amino acid residues. The gene is part of an apparent operon with genes encoding two other membrane proteins, CapB, and CapC. Together, these proteins have been proposed to be involved in the biosynthesis of a polymeric-based capsule in this bacterium[1,2]. Membrane topology and secondary structure predictions show that CapA may have two transmembrane helices located at the N- and C-termini and a very large hydrophilic domain which is predicted to be mostly α-helical. We have overexpressed the *capA* gene in *E. coli* as a His_6-tagged fusion with a folding reporter green fluorescent protein (GFP). Dynamic light scattering indicated that the purified CapA-GFP was highly monodisperse with a size that was dependent upon the protein concentration and choice of detergent. Circular dichroism showed that CapA-GFP was stable over a wide range of pH values. The CapA-GFP protein requires a high ionic strength to keep its secondary structure, indicating that electrostatic interactions are playing a key role in the stability of the protein. Analysis of the sequence by disorder predictors reveals that the CapA membrane protein contains a long disordered region of approximately 60 residues, suggesting that the ionic strength dependence arises due to electrostatic interactions involving the disordered region. Specifically, the addition of salt might minimize unfavorable intra- or inter-molecular electrostatic repulsions in the disordered region, allowing the formation of α-helices. Proteolysis experiments using proteinase K also confirmed that this disordered region is highly solvent exposed, which may indicate that it may play a role in binding to other proteins.

3486-Pos Board B214

Structural Basis of Multiple Sequential LC8 Sites: Insights from Interactions of Lc8 with Pac11

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Cytoplasmic dynein is a large multi-subunit protein complex that is responsible for retrograde transport of diverse cellular cargoes along microtubules. Dynein is comprised of heavy chain subunits responsible for motor activity, and intermediate chain (IC) and light chain subunits for cargo attachment and regulation. Dynein also binds to dynactin which is essential for most dynein activities. Dynein light chain LC8 is conserved across species and is proposed to dimerize and stabilize IC but its effect on dynein regulation remains unclear. To examine the role of LC8 in complex with IC, we used LC8, IC and dynactin homologs in *Saccharomyces cerevisiae*, Dyn2, Pac11 and Nip100, respectively. Backbone assignments of Pac11 1-87 and secondary chemical shifts show that the N-terminal 24 residues adopt a long helical structure, which are followed by a short nascent helix for residues 28-38. The two recognition motifs for Dyn2 have small propensity to form β-strands while the rest of the molecule is disordered. NMR titration experiments identify the exact residues of Pac11 involved in binding to both Dyn2 and Nip100. The energetics of binding of Dyn2 to two sites on Pac11 and its effect on Nip100 binding were determined by ITC. Comparison of the thermodynamics of this interaction with the Pac11 peptide that contains a single Dyn2 site, Drosophila IC with a single LC8 site and Drosophila IC with two sites suggests that the role of multiple sites in Pac11 is not simply to form a stable IC/light chain assembly but to also restrict the conformation of Pac11 so that it binds Nip100 with higher affinity. These studies suggest a novel role for protein disorder in controlling the interplay of light chain binding and dynein regulation and function.

3487-Pos Board B215

Combining NMR and Computer Simulations to Evaluate Cdc25B Protein Flexibility

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Cdc25B is a phosphatase involved in cell cycle checkpoints and has become an important drug target because it is overexpressed in several types of cancer. Crystal structures indicate that the C-terminus of the Cdc25B catalytic domain is in close contact with the active site. On the other hand, computer simulations and bioinformatic predictors suggest a large conformational flexibility in this region. In order to obtain detailed insight on the dynamics of the Cdc25B C-terminal segment in solution, we produced isotopically enriched protein samples for nuclear magnetic resonance (NMR) data acquisition. Agreement between backbone dihedral angles obtained from the crystal structure and predicted from NMR chemical shifts (CS) suggest consistency between the crystal and the solution structure of Cdc25B. Comparisons between experimental backbone CS and values back-calculated from long molecular dynamics (MD) simulations (total aggregate MD time of 10 us) suggest significant conformational flexibility in several Cdc25B regions. In particular, the greater amplitude of CS fluctuations for the terminal residues in comparison with the non-terminal ones is consistent with higher flexibility at the terminal regions. For instance, Ser373 located at the N-terminus populates two different states characterized by a 13Calpha CS of 58 and 59.5 ppm. These fluctuations are mainly associated with changes in psi and chi1 dihedral angles observed along the MD trajectory. Our results illustrate the power of the combination of NMR and MD simulations in order to obtain a faithful description of the conformational distribution of proteins in solution.

3488-Pos Board B216

How Electrostatics Influences the Conformational Disorder and Dynamics of the Sic1 Protein: A Single-Molecule Study

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In yeast, the cyclin-dependent kinase inhibitor Sic1 is a disordered protein which interacts with a single site on its acceptor Cdc4 only upon multi-phosphorylation of its dispersed sites. To gain insight into the multi-phosphorylation dependence in Sic1-Cdc4 interaction, the conformational properties of the disordered Sic1 N-terminal targeting region were studied using single-molecule fluorescence spectroscopy.

At least two Sic1 conformational populations with different sensitivities to charge screening by non-denaturing salts and ionic denaturants were identified. As described by the polyelectrolyte theory, the chain dimensions decrease monotonically with salt concentration and roll over at high denaturant, although a scaling factor of 1.2 indicates that Sic1 is not accurately described as a random chain. Fluorescence correlation analysis shows that Sic1 structure fluctuations occur on fast (10-100 ns) and slow (10-100 ms) ranges, with the fast phase being absent at low salt. Our data provides direct evidence that intrachain charge repulsions are significant for the conformational landscape of Sic1, and support the role of electrostatics in determining the size and shape of intrinsically-disordered proteins.