targeting riboflavin binding protein (RFBP), which acts as a model protein for the riboflavin receptor. By characterizing the binding interactions between riboflavin dendrimer gold nanoparticle conjugates and RFBP, the efficacy of this platform for a targeted approach of drug delivery can be predicted more accurately. Atomic Force Microscopy (AFM) was used for biological imaging studies of these riboflavin-dendrimer complexes conjugated with gold-nanoparticles. using a systematic ''building block'' approach, the size distribution of riboflavin dendrimer gold nanoparticle conjugates was mapped. Changes in height upon binding to RFBP and subsequent removal by competitive binding ligands demonstrate that this method could present a novel approach to screening the binding of drugs to drug targets.

2858-Pos Board B13

Enforced Unfolding and Mechanical Properties of the Importin-Beta and Importin-Beta-Binding-Domain Complex

Andreas Russek, Helmut Grubmüller.
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Importin-beta plays an important role in material exchange between the cell nucleus and the cytoplasm. In order to transport large molecules through the nuclear pore complex it has to bind tightly to the importin-beta-binding (IBB) domain of its cargo.

To probe the mechanical properties of this binding, enforced unbinding was investigated by single molecule atomic force microscopy (AFM) experiments in which the cargo was pulled out of the complex. To structurally characterize the unfolding pathway, we have performed force probe molecular dynamics (MD) simulations at different constant loading rates on the importin-beta/IBB domain complex.

Depending on the applied loading rate, three different unbinding pathways were observed. using time dependent rate theory, these pathways were characterized in terms of the rupture rate, rupture length and the barrier height of the complex, which allow direct comparison with AFM data.

We hypothesize that the elastic properties of the involved proteins control binding and unbinding of this complex.

2859-Pos Board B14

Investigating the Electrostatic Role of a Critical Arginine for the Catalysis of E. Coli ADP-Glucose Pyrophosphorylase

Angela Mahaffey, Saleh Aiyash, Miguel Ballicora.
Loyola University-Chicago, Chicago, IL, USA.

ADP-glucose pyrophosphorylase (ADP-Glc PPase) is the regulatory enzyme of the pathway for starch synthesis in plants and glycogen in mammals and enteric bacteria. It exists as a 200 kDa homotetramer ($\alpha 4$) in enteric bacteria, and as a heterotetramer ($\alpha 2\beta 2$) in plants. In both *in vivo* and *in vitro* the substrates (Glucose 1-Phosphate; Glc-1P and Adenosine 5'-Triphosphate; ATP) are converted into a glucose donor ADP-Glucose and a pyrophosphate (PP*i*) via the ADP-Glc PPase enzyme. It has been noted that some residues are conserved in homotetrameric bacterial ADP-Glc PPases, but are not in some plant forms. One of them is Arginine-32 (R32) in the *Escherichia coli* ADP-Glc PPase. To explore the overall role of this residue and evaluate the structural and electrostatic importance of the Arginine's guanidinium group, we replaced it with Lysine (K, -amino group), Alanine (A, - methyl group), Cysteine (C, -sulfide group), Glutamic (E, - carboxylate group), Glutamine (Q, -amido group) and Leucine (L, -hydrophobic side chain) via site directed mutagenesis. We overexpressed the enzymes, purified them to homogeneity, and measured their kinetic properties. The Specific Activity (U/mg) for the mutants were as follows: WT (90.56), R32A (1.65), R32C (0.57), R32E (0.04), R32K (5.81), R32L (0.65) and R32Q (1.37). Currently, the properties of the R32H (Histidine, -imidizoleum ring) mutant are being investigated. Our results clearly indicate that this guanidinium group of the Arginine-32 residue is critical for catalysis. Modeling of the *E. coli* enzyme suggests that the two (2) nitrogen atoms of the guanidinium group may interact with the β and γ phosphates of the ATP, helping in the positioning of the substrates, via electrostatic interactions, and making the PP*i* product a more stable leaving group.

2860-Pos Board B15

Ubiquitin Modulates Tollip Function in the Endocytic Pathway

Sharmistha Mitra, Cynthia Alicia Traughber, Stephanie Gomez, Daniel Capelluto.
Virginia Tech, Blacksburg, VA, USA.

Ubiquitylation is a highly controlled post-translational modification of proteins, in which proteins are conjugated either with monoubiquitin or polyubiquitin chains. Ubiquitin modifications on target proteins are recognized by ubiquitin-binding domains, which are found in several effector proteins. In this study, we describe the function of the Toll-interacting protein (Tollip), which is an effector protein in the innate immune signaling pathway and an adaptor protein for endosomal trafficking. We have previously demonstrated that the central C2 domain of Tollip preferentially interacts with phosphoinositides and that this association is critical for membrane targeting of the protein. Remarkably, we found that ubiquitin modulates Tollip's lipid binding. We have observed an ubiquitin dose-dependent inhibition of binding of Tollip to phosphoinositides and it does so specifically by blocking Tollip C2 domain-phosphoinositide interactions. This led us to hypothesize that the Tollip C2 domain is a novel ubiquitin-binding domain. In addition, we have biophysically characterized the association of the Tollip CUE domain to ubiquitin and identified key interacting residues. The Tollip CUE domain reversibly binds ubiquitin with low micromolar affinity at a site that overlaps with that corresponding to the Tollip C2 domain. We have also found that ubiquitin binding to dimeric Tollip CUE domain induces a drastic conformational change in the protein, leading to the formation of a heterodimeric Tollip CUE-ubiquitin complex. These data suggest that ubiquitin binding to the Tollip C2 and CUE domain and ubiquitin-mediated dissociation of CUE dimer reduces the affinity of the Tollip protein for endosomal phosphoinositides, allowing Tollip cytoplasmic sequestration. Overall, our findings will provide the structural and molecular basis to understand how Tollip, as an endocytic adaptor protein, is modulated by ubiquitin and determines the fate of polyubiquitinated cargo for endosomal degradation.

2861-Pos Board B16

Avidity of Scaffolding Interactions Studied on Inverted Membrane Sheets Using the Synaptic Scaffolding Protein PICK1

Simon Erlendsson, Thor Seneca Thorsen, Mette Rathje, Kenneth Lindegaard Madsen, Ulrik Gether.
University of Copenhagen, Copenhagen, Denmark.

Maintaining membrane proteins at the right place at the right time is crucial to cellular function. To support such organization a broad spectrum of regulating proteins from the cytosol bind, recruit and arrange the membrane proteins into specific structures. PICK1 is an abundant scaffolding protein interacting with more than 30 different receptors, transporters and ion channels, embedded in lipid membranes. PICK1 contains a single N-terminal PDZ domain and forms homodimers via its central membrane bending and curvature sensing N-BAR domain thus forming a functional unit with two PDZ domains. The PICK1 PDZ domain binds many different PDZ peptide ligands such as GluA2, mGluR7 and the dopamine transporter with no apparent conserved binding motif. The wide span of affinities seen for such proteins raises the question of the importance of the affinity for the isolated domain interactions in context of the overall avidity and the functional effects of these interactions.

We have established a system based on the previously published supported membrane sheet system to study the binding of PICK1 to a cell membrane expressed ligand to determine the avidity for ''on membrane interactions''. Secondly, we address the functional effects of lowering the affinity of the PDZ domain interactions on the functional effects of PICK1.

We demonstrate a dramatic increase in the binding Kd for the oligomeric interaction compared to affinities previously reported in non-native condition binding assays. Furthermore, we show that the interaction is only facilitated by functional PDZ domain. We also observe a significant change in Bmax for lower affinity ligands indicating that the increase of the PDZ affinities might rely on a cooperative binding mechanism of the PICK1 homodimer, which is consistent with results showing that the homodimer binds tighter than the monomer.

2862-Pos Board B17

Membrane Protein Interaction Studies using Microscale Thermophoresis

Sameer K. Singh[1], Julian M. Glück[1], Luis Möckel[1], Yu-Fu Hung[2], Dieter Willbold[1,2], **Bernd W. Koenig**[1,2].
[1]Research Center Jülich, ICS-6, D-52425 Jülich, Germany, [2]University Düsseldorf, IPB, D-40225 Düsseldorf, Germany.

A common challenge in biophysical studies of membrane proteins is the choice of an adequate model membrane or membrane mimetic. Commonly used mimetics (detergents, liposomes) often suffer from well known limitations (adverse modification of protein structure, inhomogeneous protein distribution, etc.), thus prohibiting extensive studies on membrane proteins. However, the recently developed 'nanodisc' membrane mimetic sytem has helped alleviate some of these shortcomings. Nanodiscs are self assembled proteolipid particles, wherein two copies of an apolipoprotein A-I derived recombinant membrane scaffold protein (MSP) clasp a lipid patch, and seal the hydrophobic edge of the bilayer from water. The incorporation of a membrane protein into the