

1264-Pos**Escherichia Coli Redox Enzyme Maturation Proteins, TorD and DmsD Interact with GTP as Shown by Native Page Assays**

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The twin-arginine translocation (Tat) system can transport fully folded proteins across the cytoplasmic membrane. Transport is dependent on a twin-arginine motif within a cleavable signal peptide. Substrates for the Tat system include redox proteins, necessary for growth during anaerobic respiration. The transport of the catalytic subunits responsible for N- and S-oxide respiration, DmsAB and TorA are dependent on DmsD and TorD respectively. Both are system specific chaperones termed redox enzyme maturation proteins (REMP) and are predicted to play multiple roles in the maturation and export of redox enzymes, including the known role for binding to the twin-arginine motif. Upon successful translocation and signal peptide cleavage, the REMPs remain in the cytoplasm. The question arises as to what governs binding and release of the signal peptide in order for translocation to occur. Here, we propose that REMPs may function like general chaperones like DnaK/DnaJ which exhibit nucleotide binding and hydrolysis for substrate release. It is speculated that TorD binds GTP or the molybdopterin dinucleotide with low affinity. Furthermore, GTP binding sites have been predicted for *Shewanella typhimurium* TorD (1N1C.pdb) and *Salmonella typhimurium* DmsD (1S9U.pdb) structures. We have developed a native PAGE assay, showing both DmsD and TorD exhibiting different banding patterns in the presence of GTP, while being unaffected by ATP. Preliminary results indicate that the presence of the NTP counter ion Mg²⁺ in addition to GTP does not change the banding patterns. DmsD and TorD share the same highly alpha-helical structure. Therefore we have done structural alignments to the *E. coli* DmsD (3EFP.pdb) structure and mapped the residues predicted to bind GTP to identify targets for mutagenesis. Our work aims to definitively answer the question if these chaperones indeed bind GTP.

1265-Pos**Role of Dimerization in Poly-Ubiquitin Chain Formation**
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The ubiquitin proteolysis pathway is responsible for protein degradation utilizing three enzymes, the ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2), and a ligating enzyme (E3), that respectively activate, transfer and ligate ubiquitin (Ub) onto a target protein. Repeated cycles of this process results in a poly-ubiquitinated target protein that is degraded by the 26S proteasome. How the poly-Ub chains are formed remains unknown. One suggested model involves the dimerization of an E2 enzyme allowing Ub passage and ligation between adjacent E2 enzymes. We are examining this mechanism for the E2 enzymes Ubc1 and HIP2, which contain C-terminal UBA domains that allows for non-covalent Ub binding in addition to a thioester-bound Ub. The dimerization of these E2 enzymes was tested using sedimentation equilibrium and small angle x-ray scattering and showed that both are monomeric. Disulphide-bound E2-Ub complexes were used to mimic the thioester, and these complexes had a weak propensity to dimerize. This was supported in ubiquitination assays that showed a thioester-bound Ub on an E2 could be transferred to a non-hydrolyzable, disulphide-bound Ub molecule on a second E2 enzyme. This suggests weak dimerization is likely sufficient to allow the first step of poly-Ub chain formation. We tested whether the length of the poly-Ub chains on HIP2 stimulates dimerization by creating HIP2-Ub₂ and HIP2-Ub₄ complexes. The ability of these species to dimerize was assessed via sedimentation equilibrium and NMR spectroscopy. The isolated UBA domain from HIP2 was used in competition experiments to determine how it might influence poly-Ub chain formation. This work provides the first structural evidence for poly-Ub chain formation as assembled on the E2 enzyme.

1266-Pos**How Insulin-Like Growth Factor Hormones, IGF1 And IGF2, Engage their Cognate Receptor**

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The type 1 insulin-like growth factor receptor (IGF1R), a trans-membrane glycoprotein, is activated by binding of its cognate growth hormones, IGF1 and IGF2. The IGF family was suggested to play a key role in cancer development and progression, thereby making it a potential target for anti-cancer therapeutic efforts. However, the molecular mechanisms underlying hormone-receptor interactions are unclear, as are the molecular bases for differing affinity of each hormone, due in part to the fact that there have been so far no detailed structural models of IGFs-bound-IGF1R to test. Constructed using a homology model of the IGF1R ectodomain, and the NMR structures of IGF1/2, with the help of an

MD-assisted Monte-Carlo approach, we present the first experimentally consistent all-atom structural models of IGF1/IGF1R and IGF2/IGF1R complexes. Our models are notable because each hormone remains stably bound in independent 36-ns long explicit-solvent molecular dynamics (MD) simulations. The asymmetric structural relaxation of the apo-IGF1R homology model in a 30-ns MD equilibration facilitated the computational docking of each hormone. Our predicted complexes are significant because we observe simultaneous contacts of each hormone with the site 1 (formed by L1 and CR), and site 2 (formed by L2, and the fibronectin domains), of the receptor, suggesting cross-linking of receptor subunits. Interestingly, we observe differences in recognition of each hormone by IGF1R, because IGF1 interacts relatively strongly with L1 and CR (IGF1R), whereas IGF2 has stronger interactions with L2 and the fibronectin domains. Our simulations also provide direct evidence in favor of previously suggested electrostatic complementarity between the C-domains of IGF1/2 and the CR-domain of the receptor. Additionally, we provide detailed hormone-receptor contacts that are consistent with earlier mutagenesis studies.

1267-Pos**Investigation of the Cu(II)-Binding Properties of Alpha-Synuclein**
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Parkinson's disease is a chronic, progressive, and often fatal neurodegenerative disorder that affects 1 in 100 individuals over the age of 60. The hallmark of Parkinson's disease is modified dopaminergic neurons termed Lewy Bodies, of which the protein Alpha synuclein is the primary fibular component. Epidemiological studies correlated long-term metal exposure (such as in an industrial setting) with an increased incidence of fatal Parkinson's disease. Fibril assays indicated that certain metals, notably Cu(II), Fe(III) and Al(III) increase the rate of in vitro alpha synuclein fibril formation. Our work seeks to elucidate the stoichiometry, affinities, chelating residues and binding motifs of Cu(II) to alpha synuclein. Using EPR spectroscopy to analyze recombinant alpha synuclein, mutants and synthetic peptides we have determined a heretofore unknown Cu(II) binding motif. This motif may provide insight into a possible explanation for the increase in the rate of alpha synuclein fibril formation.

1268-Pos**Thermodynamic and Hydrodynamic Characterization of the Interaction Between DmsD and the DmsA Twin-Arginine Leader Peptide**

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The system specific chaperone DmsD plays a role in the maturation of the DMSO reductase enzyme through its interaction with the twin-arginine leader peptide of the catalytic subunit DmsA, prior to its assembly into the holo-enzyme. A pocket of residues, clustered together within the structure of DmsD, has previously been shown to be important for binding a fusion protein composed of 43 of the 45 amino acid residues of the DmsA leader peptide however the region of the DmsA leader peptide that interacts with DmsD has not been identified. Various portions of the DmsA leader peptide were synthesized and assayed for binding to DmsD using isothermal titration calorimetry. A peptide composed of 27 amino acid residues near the C-terminus of the DmsA leader sequence was found to bind to DmsD and subsequently used to characterize the thermodynamics of binding of each of the DmsD variant proteins previously shown to be important for binding to the DmsA leader peptide. Size exclusion chromatography and native-PAGE were used to determine the effect of peptide binding on multimeric state and electrophoretic mobility for each of the variant proteins. In the presence of the peptide, wild type DmsD migrates faster on native-PAGE but remains monomeric while some DmsD variant proteins undergo oligomerization while still changing to a the same faster migrating form on native-PAGE. The electrophoretic mobility, multimeric forms and thermodynamics of peptide binding of mutations in the chaperone leader-binding site are compared.

1269-Pos**Binding of Antibodies to Continuous Antigenic Epitopes**

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Continuous, or linear, antigenic epitopes are common to proteins and peptides. The accessibility of continuous epitopes often depends on protein/peptide conformation and its proximity to disulfide bridges. Temperature dependence of the equilibrium binding constants and the kinetic rates were studied for anti-BNP mAb 3-631 by means of fluorescence spectroscopy. This antibody recognizes a relatively short amino acid sequence in the loop between cysteines 10 and 26 of human B-type natriuretic peptide.

Thermodynamic parameters including changes in the free energy, enthalpy and entropy measured at equilibrium are in a good agreement with the parameters