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 thiolester, 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-Ub_2 and HIP2-Ub_4 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

calculated from kinetic data. The differences in thermodynamic parameters measured for the cyclic and reduced peptide explain epitope mapping data obtained by NMR.

1270-Pos

Creating Steroidal Ligand-Receptor Pairs for Behavioral Studies of Androgen Receptor

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Heterologous agonist-receptor pairs designed after high resolution structural studies have yielded insights into specificity of signaling by protein kinases and G-protein coupled receptors. We are extending this concept to the realm of steroid nuclear receptors with a goal to learning the behavioral effects of steroid activation of the androgen receptor in the brain. We are designing a steroidal ligand-hormone receptor pair such that the novel ligand does not bind to endogenous androgen receptors (AR), and the designer receptor does not bind to endogenous androgens. We have synthesized novel steroids that do not bind endogenous AR. Since steroid binding affects the folding of the receptor, prediction of amino acid mutations in the androgen receptor that are needed to accommodate the steroidal ligand is difficult. To combat this issue, we are using a genetic selection in S. cerevisiae to reveal gain of function activity from a library of about 10 ⊥ 9 randomly mutated AR ligand binding domains. In this experiment, yeast strains containing a hormone-inducible HIS3 gene select for full length AR mutants that are active in the presence of our novel steroids.

1271-Pos

Molecular Dynamics Study of 2-Arachidonoyl-Sn-Glycero-3-Phosphoinositol (2-AGPI) and Lysophosphatidylinositol (LPI) in a POPC Bilayer and their GPR55 Docking Sites

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GPR55 is a Class A G protein-coupled receptor that has been shown to be activated by cannabinoids (Henstridge et al. FASEB J, 2008). The receptor is expressed in several mammalian tissues including several brain regions. It has been reported that GPR55 is also activated by LPI found in rat brain (Oka et al. BBRC, 2007), with 2-AGPI having the highest activity (Oka et al. J Biochem, 2009). Since 2-AGPI and LPI are lipids and are shown to interact with the membrane-embedded GPR55 receptor, we undertook a study of the location and conformations they can adopt in a phospholipid bilayer, as well as, of their interaction modes with GPR55. To this end, 2-AGPI and LPI were added to a fully hydrated, pre-equilibrated POPC bilayer (28 waters/lipid; 72 lipid molecules with 36 in each leaflet) and their behavior in POPC was studied using the NAMD2 molecular dynamics software package (NPAT ensemble; P 1atm, T 310K) with the CHARMM27 parameter set including data for polyunsaturated lipids, and the TIP3P water model. The MD studies place the 2-AGPI and LPI headgroups in the water-lipid interface with the inositol moiety either upright and solvated in water or bent and buried in the POPC headgroups. Extensive ligand inter- and intramolecular hydrogen bonding contributes to the inositol location and conformation. 2-AGPI's acyl tail is extremely flexible and prefers compact to moderately extended conformations, whereas LPI's tail prefers more extended ones. Following these MD studies, the ligands were docked in a GPR55 model in the TMHs 1,2,3, 6 and7 region using K2.60 as the primary interaction site. [Support: NIH RO1 DA023204 (MEA) and KO5 DA021358 (PHR)]

1272-Pos

Diminished Cooperativity: Comparing Linker Lengths in Synaptotagmin I C2A Domain

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Exocytosis of neurotransmitters is triggered by the initial influx of Ca2+. Synaptotagmin I is known to bind Ca2+ and the phospholipid membrane to modulate this process. The exact mechanism for this information transduction, however, is not well known. Synaptotagmin I contains two binding domains, C2A and C2B, that are tethered to a neurotransmitter containing, lipid vesicle with a flexible linker region. The wild type C2A domain acts as a Ca2+ dependent trigger by binding the calcium ions in a cooperative manner. We seek to understand the role that the linker region has on the binding properties of the protein. To do this, a shortened construct (amino acids 141-267 verses amino acids 97-265 of the previously studied long construct) has been utilized as a probe to examine the effects of the truncated linker region. Ca2+ and phospholipid binding assays have been carried out and monitored via steady state fluorescence to make a thermodynamic comparison between the two constructs. Binding partition functions have been derived for this purpose and clearly show the diminished linkage relationship between the binding sites of the shortened construct. This material is based in part upon work supported by the National Science Foundation under CAREER-MCB 0747339

1273-Pos

Selectin Mechanokinetics and Two-Dimensional Bond Formation Determine and are Reported by Nano-Motion Dynamic Patterns

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Binding between surface-tethered proteins at cellular interfaces has been considered two-dimensional because of the restricted motion of the two binding partners. Two-dimensional protein interactions between cells are critical for many biological processes, such as leukocyte vascular adhesion via selectins. Experimental measurements have yielded data on the kinetics of selectin bond formation and dissociation. Additionally, computational methods have been employed to integrate molecular and cellular properties to elucidate the factors that influence the dynamics of selectin-mediated rolling. Simulation methods focused on biomolecular properties promise to yield additional novel insights into the molecular component of adhesion with the assistance of measurements from improved assays. We performed an in silico investigation on the effects of the kinetic force dependence, molecular deformation, grouping adhesion receptors into clusters, two-dimensional bond formation, and nanoscale vertical transport on outputs that directly map to observable motions. Statistics describing the motion patterns tied simulated motions to experimentally reported quantities. Distributing adhesive forces among P-selectin/PSGL-1 molecules closely grouped in clusters was necessary to achieve pause times observed in microbead assays. Notably, rebinding events were enhanced by the reduced separation distance following initial sphere capture. The result demonstrates vertical transport can contribute to an enhancement in the apparent bond formation rate. The result also suggests a new mechanism that may be important for the rebinding events characteristic of stable leukocyte rolling. When selectin receptor and ligand are restricted to small, two-dimensional interaction zones during rolling, the resultant wobble was found to be dependent on the confinement model used. Insight into two-dimensional bond formation gained from flow cell assays might also therefore be important to understand processes involving extended cellular interactions, such as immunological synapse formation.

1274-Pos

Single-Molecule Force Spectroscopy of the Interactions Between Platelet Integrin αIIbβ3 and Monomeric Fibrin

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Platelet-fibrin interactions under hydrodynamic blood shear are mediated by the integrin αIIbβ3, but the mechanism of αIIbβ3 binding to fibrin is largely unknown, although interactions with fibrinogen have been extensively studied. We used the optical trap to measure forces required to separate a laser-trapped-bead coated with monomeric fibrin from a pedestal coatedwith purified αIIbβ3. Experiments were performed with recombinant fibrin obtained from thrombin-treated fibrinogen, either wild-type or variants lacking putative integrin-binding sites. Integrin-fibrin interactions manifested as a bimodal force histogram with rupture forces from 20 pN to 140 pN, similar to αIIbβ3-fibrinogen but with somewhat higher binding probability. To test a role of the γ-chain C-terminal 400-411 dodecapeptide, the major αIIbβ3-binding site in fibrinogen, the most abundant fibrin (γA/γA) was replaced with a splicing variant (γ'/γ'), in which the γC-terminus has new amino acids from 408 to 427. Unexpectedly, the lack of the γC400-411 motif did not affect the ability of fibrin to interact with αIIbβ3, suggesting that this structure may not be a major integrin-binding site in fibrin. At the same time, fibrin-integrin interactions were partially inhibited by the γC-dodecapeptide, indicating that the γC400-411 motif still may be involved, perhaps indirectly. Two RGD motifs, one located in the αC region and the other in the coiled-coil connector, were tested as the potential binding sites by using fibrin(ogen) variants αD574E and αD97E. Both of them had a reduced integrin-binding strength and displayed the cumulative binding probability about 2/3 of that of the wild-type fibrin, suggesting that the RGD motifs play a role in the αIIbβ3-fibrin interactions. Free γC-dodecapeptide did not affect the reactivity of the D574E and D97E mutants. The results suggest that the αIIbβ3-fibrin interactions involve the RGD sites rather than the γC400-411 motif.