1117-Pos Board B68
An Equilibrium Model for the Combined Effect of Macromolecular Crowding and Surface Adsorption on the Formation of Linear Protein Fibrils
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The formation of linear protein fibrils has been previously shown to be enhanced by volume exclusion or crowding in the presence of a high concentration of chemically inert protein or polymer, and by adsorption to membrane surfaces. An equilibrium mesoscopic model for the combined effect of both crowding and adsorption upon the fibrillation of a dilute tracer protein is presented. The model exhibits behavior that differs qualitatively from that observed in the presence of crowding or adsorption alone. The model predicts that in a crowded solution, there exist critical values of the volume fraction of crowder or intrinsic energy of tracer-surface interaction about which the tracer protein undergoes an extremely cooperative transition - approaching a step function - between existing almost entirely as a slightly self-associated species in solution and existing almost entirely as a highly self-associated and adsorbed species.

1118-Pos Board B69
Aggregation of Claudins and Formation of Tight Junctions: A Coarse-Grained Molecular Dynamics Study
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Tight junctions are dynamic structures that consist of a number of membrane proteins and their cytoplasmic counterparts. Claudins are one the major components of tight junctions that control the transport of ions and small molecules in paracellular pathways. The recently solved crystallographic structure of claudin-15 shows a membrane protein consisting of four transmembrane helices (TM1-TM4) and a large extracellular domain (ESC). Claudin monomers polymerize in membrane to form tight junction strands, which are connected to similar strands in neighboring cells and glue two cells together. We have used the structure of claudin-15 to investigate the polymerization and assembly of claudins in lipid membranes with molecular dynamics simulations. The simulations revealed that the linear arrangement of claudins as observed in the crystal is not stable in the membrane. Individual claudins rotate in the membrane to form new contacts between TM3 and ECS resulting in a curved configuration with a diameter of 100A. To obtain more insight about the side-by-side interaction of claudins in the membrane, 16 claudins were placed in a lipid bilayer in a 4x4 matrix arrangement and their diffusion was studied in coarse-grained simulations. In these simulations, claudins form linear configurations consisting of 2-8 claudins stabilized by interactions between TM2 or TM3 segments and ECS. These results show that the side-by-side interaction of claudins in the membrane occurs through two interfaces. The presence of two interaction surfaces might in fact be a key factor in stabilization of long and flexible tight junction strands in the cell. In addition, head-to-head interaction of claudins across two neighboring cells, which is essential for tight junction formation, was studied. In the presence of trans interactions, claudins show a slower dynamics in each membrane and form shorter strands.

1119-Pos Board B70
Lateral Interactions Affect Cadherin Binding Kinetics and Function
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Adhesion protein interactions between cell membranes (2D) are indispensable for the formation and maintenance of tissues in multicellular organisms. However, binding and organization of membrane proteins within these interfaces is not well understood at the molecular level. To address this question, we use cell-cell adhesion proteins Cadherins as our model. Cadherins form adhesive interactions by binding to identical proteins on opposite cells, but are also hypothesized to form clusters on a single membrane. The protein-protein bonds proposed to stabilize these ''lateral'' interactions were not detected in any solution binding studies (3D), and their existence as well as their relevance for establishing cell-cell junctions and transducing signals are not clearly known. We investigated the relevance of these lateral interactions using quantitative micropipette measurements of cadherin-mediated cell-cell binding kinetics. We found that classical E-cadherin exhibits kinetics that exhibit two distinct kinetic processes. This 'biphasic' kinetics is not consistent with kinetic models based on solution binding data, and suggested that one of the kinetic steps might be due to clustering of the confined proteins. In support of this interpretation, mutating the proposed lateral interaction sites eliminated the kinetic step that we attributed to clustering. We also demonstrated the functional significance of this putative lateral clustering step, by quantifying the leakiness of cell-cell junctions to macromolecules. Cell junctions formed by the cadherin mutants were leakier than WT E-cadherin. Wound healing assays also showed that cells expressing the mutants migrated faster than cells with WT protein. Together, these data reveal that cadherins in ''2D'' environments undergo additional interactions than in solution, and these alter the assembly of intercellular junctions. These findings might also be relevant to similar class of adhesion proteins, like nectins and CAMs.

Enzymes and Protein Dynamics I

1120-Pos Board B71
Active Role of the Substrate during Catalysis by the Therapeutic Enzyme L-Asparaginase II
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Bacterial type II L-Asparaginases (ASPII) have been used for over four decades to treat acute lymphoblastic leukemia, yet a full reaction mechanism remains unknown. ASPII enzymes catalyze the deamidation of both asparagine (Asn) and glutamine (Gln), which results in the formation of aspartate (Asp) and glutamate (Glu) respectively, and the by-product ammonia. Proposed ASPII mechanisms to date have yet to explain the absolute requirement of a substrate α-carboxyl group, and clearly identify the role of the catalytic threonines T12 and T89. Here, we study the reaction mechanism of asparagine degradation by ASPII through ab initio molecular dynamics (MD) simulations. We selected a reduced system from the substrate-bound enzyme obtained by classical MD, and explore different initial reaction pathways by driving the system in steered simulations. Our results show that direct nucleophilic attack by T12 produces a highly unstable substrate-enzyme intermediate, as the stabilization provided by the nearby protons (i.e., the ''oxyanion hole'') is insufficient to sustain the high energy state. We find that the substrate-enzyme intermediate can be stabilized by first protonating the substrate's amide oxygen through the K162-T89 proton bridge. Furthermore, the α-carboxyl of the substrate acts as a proton acceptor for the hydroxyl sidechain of T12 during nucleophilic attack. We conclude by showing that a complete deamidation mechanism may require a sequence of several nucleophilic attacks by both T12 and T89, with K162 playing a critical role as a proton buffer during the course of the reaction.

1121-Pos Board B72
Co and No Binding in Inducible Nitric Oxide Synthase
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Nitric oxide synthases (NOSs) are homodimeric heme enzymes that catalyze the oxidative degradation of L-arginine (L-Arg) to nitric oxide (NO). Three structurally similar isoforms have been identified in endothelial cells (eNOS), neuronal tissues (nNOS) and in macrophages (iNOS). Different from eNOS and nNOS, iNOS is not present in resting cells but is expressed upon inflammatory and immunologic stimulation. As this isoform has been implicated in the pathogenesis of various diseases, there is a growing need for potent and highly selective inhibitors.
The targeted development of potent inhibitors that are highly specific for iNOS requires detailed insights into the interaction between the ligand, the heme and the surrounding protein matrix on the molecular level. Therefore, we have investigated ligand and substrate binding in iNOSoxy using Fourier transform infrared (FTIR) spectroscopy in combination with temperature derivative spectroscopy (TDS). The physiological ligand O2 was replaced by carbon monoxide (CO) and NO. Both CO and NO allow us to exploit their excellent properties as spectroscopic probes. Previous studies on CO and NO migration in myoglobin have shown distinct differences in CO and NO migration despite their similar sizes.

1122-Pos Board B73
Intact Protein Analysis by Mass Spectrometry to Characterize the Truncated Hemoglobin THB1 from Chlamydomonas Reinhardtii
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THB1 is one of twelve genes encoding hemoglobins in *Chlamydomonas reinhardtii*. Although previous work [1] has correlated the expression of *THB1* to nitrate metabolism through the NIT2 gene regulator, the exact function of THB1 is unknown. Current work investigates possible enzymatic roles by