formed. Deadlock can result in plateaus in the assembly dynamics, leading to delays in reaching maximum complex assembly and a reduced final complex concentration. While these plateaus have been extensively studied for simple rings, the effect of assembly deadlock on more general structures like stacked rings remains to be fully investigated. In this work, we focused on the case of a stacked homotrimer; this structure contains both three- and four-member rings as substructures, but is simple enough to allow for extensive investigation. Our mathematical models revealed that this structure could suffer from extreme deadlock that significantly reduces the efficiency of assembly. Using a computationally efficient simulation approach, we exhaustively analyzed the parameter space of self-assembly for this case, and found that the number and duration of plateaus in the assembly dynamics depended strongly on the pattern of affinities in this structure. Since these complexes are generally only functional when fully assembled, we hypothesized that existing stacked ring architectures would evolve to utilize the most efficient assembly pathways predicted by our models. Analysis of interfaces in solved crystal structures of stacked homotrimers confirmed this prediction. Our findings have important implications for understanding how assembly dynamics have influenced the structural evolution of large macromolecular machines.

1107-Pos Board B58
Heat Shock Triggers Assembly of tRNA Synthetases into an Active Supercomplex
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Heat shock stimulates cellular production of protein folding chaperones. This response is a reaction to, and likely triggered by, protein misfolding and aggregation. Using a proteome-scale mass spectrometry screen for protein aggregation upon heat shock *in vivo*, we found that many components of the translational apparatus associate into high-molecular weight species. One such component is the ternary yeast multi-tRNA synthetase complex (glutamyl- and methionyl-tRNA synthetases Gus1 and Mes1 with their aminoacylation cofactor Arc1). *In vitro*, the purified complex self-assembles in response to the same temperature shift. Remarkably, the self-assembled aminoacylation complex retains full activity toward $tRNA^{Met}$. We found that assembly is directed by the N-terminal glutathione-S-transferase (GST)-like domains that mediate complex formation under non-shock conditions. Furthermore, the isolated N-terminal GST-like domain of Gus1 suffices to induce the assembly, and can be used to precipitate covalently-linked proteins. The phenomenon is conserved in the N-terminal of Gus1 from a thermophilic fungus that self-assembles near its heat shock temperature. Biophysical studies reveal only modest structural secondary structure changes between the monomeric and self-assembled states. These results indicate that this heat-triggered protein assembly is distinguishable from large-scale misfolding and nonspecific aggregation, and suggest a novel mechanism by which cells sense temperature changes.

1108-Pos Board B59
Chromatographic Assay for Determining the Effects of Microenvironment on Dimerization of Epithelial Cadherin K14E Mutant
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The classical cadherin family is an extensively studied family of calcium-dependent homophilic cell adhesion proteins, which includes epithelial cadherin (ECAD) and neural cadherin (NCAD). ECAD plays essential roles in the formation and maintenance of epithelial tissues. The adhesive interface lies in the first of five similar tandemly repeated extracellular domains (EC1-EC5) and requires exchange of the N-terminal A-strand. The purpose of the experiments presented here is to develop a simple assay for dimerization using purified recombinant protein. A chromatographic assay has been developed for NCAD, which essentially involves *trapping* the protein in a dimeric state thus significantly reducing the exchange rate between states. This allows resolution of monomer and dimer peaks using size exclusion chromatography. No equivalent assay for the study of ECAD dimerization has been established until now. The K14E mutant of ECAD experiences slow exchange between monomer and dimer, although the dimerization affinity is unchanged by the mutation. Studies presented here establish a chromatographic assay for ECAD dimerization affinity using the K14E mutant. Control experiments demonstrate that equilibration requires 4 hours and dimerization occurs in a calcium and protein dependent manner. This assay is useful for determining the effect of microenvironmental factors that will influence dimerization by ECAD in vivo.
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1109-Pos Board B60
Extraordinary Stability of Domain 1 of Neural-Cadherin
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Cadherins are transmembrane proteins responsible for calcium dependent cell-cell adhesion. Classical cadherins have a common structure with five extracellular domains, connected by a 7-residue sequence linker region. Cadherins mediate adhesion via Adherens Junctions by forming a strand-swapped structure between identical protomers from apposing cells. The adhesive interface has been characterized structurally, and biophysical studies have been used to elucidate the forces that stabilize the strand-swapped structure. Upon calcium binding, the swapping of the N-terminal strands between protomers allows for the symmetrical docking of a tryptophan residue required for adhesion. Fundamental questions still remain regarding the striking difference in the calcium-dependent kinetics of dimerization between N- and E-cadherin. This study compares the first extracellular domain of N-cadherin (NCAD1) and E-cadherin (ECAD1). With NCAD1, the binding pocket for calcium is not complete, so dimerization was not expected. However, we observed a dimeric form that was not in exchange with monomer. This dimer was reversibly converted to monomer by heating the protein. Noticeably, NCAD1 was found to unfold at an uncharacteristically high temperature. Results for NCAD1 construct were similar to results for domain 1 in studies of the two-domain construct, NCAD12. Similar studies of ECAD1 show that it is significantly lower stability. In summary, our results indicate that the difference in kinetics of dimerization may be due to a difference in the intrinsic stability of domain 1.
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1110-Pos Board B61
Resin-Embedded Multicycle Imaging of Cells and Isolated Plasma Membranes
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Studies of the plasma membrane protein organization have undergone numerous advances; however, the large number of protein species involved complicates their examination. One approach which has the ability to resolve this complication is that of resin-embedded multicycle imaging (REMI), which stabilizes protein structure and antigenicity while labels are removed and replaced, allowing an arbitrarily large number of proteins of interest to be imaged in the same area. The implementation spearheading this technique is array tomography, which is ideal for many applications, but certain organelles or spatially restricted volumes of interest, such as the plasma membrane, present considerable difficulty. We have developed a method by which cells or isolated plasma membranes unroofed by osmotic shock can be embedded in thin films of acrylic resin (LR White, Lowicryl), making their spatially dependent proteomic information amenable to repeated immunostaining and other methods of analysis. Here we present our latest work in the development of this method.

1111-Pos Board B62
Spectroscopic Study of Full-Length Recombinant Proteoglycan 4 (rhPRG4): Self-Assembly and Interactions with Hyaluronan
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Proteoglycan 4 (PRG4) is a mucin-like glycoprotein present in synovial fluid (SF) and at the surface of articular cartilage. It functions as a critical cartilage boundary lubricant, both alone in a dose-dependent manner and synergistically with the repeating disaccharide hyaluronan (HA). The mechanism of the functional interaction with HA remains to be elucidated, but appears to be non-covalent in nature and be dependent on PRG4 structure and assembly. Furthermore, concentration of both PRG4 and HA, which can vary in disease, may affect the interaction as well. Recently, full-length recombinant human PRG4 (rhPRG4) has become available. The objective of this study was to characterize aggregation of rhPRG4 and investigate its interaction with HA using spectroscopic techniques.
Spectroscopic techniques including uv absorbance, fluorescence, and light scattering, were employed to characterize the aggregation of rhPRG4 and interaction with HA over a range of physiological concentrations. Various dilutions of rhPRG4 in different buffers and their mixtures with different concentrations of HA, with and without other physiological molecules, were examined. rhPRG4 was provided by Lubris.
UV absorbance and fluorescence spectroscopic data demonstrated concentration based aggregation and suggested a non-covalent linear aggregation of rhPRG4 molecules with increasing concentration. However, multiple species were also observed even at sub-physiological concentrations. Dynamic as well as multi-angle light scattering techniques suggested presence of species with various and large (~MDa) molecular weights. These techniques also