Why? The answers come from a combination of polymer mixing/demixing and the dominance of polymer/channel interaction for the large but not the small PEGs.

323-Pos Board B123
Exploring the Mechanics and Organization of Elastin Coacervate Structures Using Micromanipulation Tools
Astrid van der Horst, Ming Miao, Nancy R. Forde, Fred W. Keeley, Gijs J.L. Wuite.
Elastin is one of the major structural proteins in tissues such as arteries, ligaments and skin, where it supplies elasticity and resilience. The protein is synthesized as a monomer and assembled into hierarchical fibrillar polymeric structures that last a lifetime.
The elastomeric characteristics of elastin are of interest from a physiological viewpoint. In addition, elastin's unique elasticity, durability, and intrinsic capacity for self-organization make this protein an ideal model system for the development of biomimetic synthetic polymers, *e.g.*, for tissue engineering. In the search for such engineered materials, understanding the molecular basis of the elastomeric properties of elastin and its ability to self-organize is crucial. Thus far, however, this understanding is very limited.
The first step in the self-organization of elastin, both *in vivo* and *in vitro*, is the coacervation of tropoelastin, the protein's monomeric precursor. Although this temperature-induced liquid-liquid phase separation is initially reversible, holding the coacervate above the transition temperature for longer periods of time results in a maturation process after which the coacervate droplets will not go back into solution and touching droplets will not coalesce. The elastin on the outside of the droplets is thought to become semi-organized. There is little definitive information, however, on the extent of this organization.
Micromanipulation techniques such as optical tweezers and atomic force microscopy (AFM), provide unique mechanical access to these protein structures. Here, we investigate the possibility to optically trap and manipulate micrometer-sized coacervate droplets and examine their propensity to coalesce. In addition, we explore the possibility to mechanically probe droplets by AFM. Results of such nanoindentation experiments will provide information on the elastomeric properties of the surface of the droplets during and after maturation and help obtain insight into the physical mechanisms underlying elastin's self-organization.

324-Pos Board B124
Characterizing Induced-Conformational Changes in Intrinsically Disordered Proteins via Multi-Frequency EPR Spectroscopy
Natasha L. Pirman, Eugene Milshteyn, Gail E. Fanucci.
Intrinsically disordered proteins (IDPs) contain little to no secondary or tertiary structure and are often essential in biological systems. Many IDPs undergo a conformational change, where structure is induced upon binding to its target protein. Due to their very nature, structural studies of IDPs are often challenging. Here, we show how a multi-frequency approach to site-directed spin-labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy can be utilized to characterize the mobility and conformational changes of IDPs. We have applied this method to IA_3, which is a 68 residue IDP whose unstructured-to-α-helical conformational transition has been extensively characterized by various biophysical techniques. We monitored the induced conformational change in the presence of the secondary structural stabilizer 2,2,2-trifluoroethanol (TFE), at both X-, and W-band frequencies. Analyses of the X-band EPR spectral line shapes reveal that the data report on global correlation time changes consistent with a two-state model of an unstructured system and the tumbling of a rigid helix; more detailed analyzes of the X-band spectral line shapes can provide site-specific information on the residue level. Analysis of the W-band EPR spectral line shapes, however, more directly reveal site-specific structural changes. Line shape simulations of the data at both frequencies should provide further information on the site-specific conformational changes occurring in the presence of TFE and are currently underway. Using IA_3 as a model system, we show multi-frequency EPR can provide insight into structural changes occurring in IDP systems that are otherwise difficult to characterize.

325-Pos Board B125
Natural Selection of 100% Intrinsically Disordered Biologically Functional Disease Related Pfam Protein Domains
Robert W. Williams, Bin Xue, Vladimir Uversky, A. Keith Dunker.
An analysis is presented of the distributions of predicted intrinsic disorder in biologically functional Pfam domains in 193024 members of the version 23.0 Pfam seed database representing 12456 unique domains, families, and repeats. 616 mammalian members of this set, representing 315 biological functions, are predicted to have 100% intrinsically disordered Pfam domains. The lower phylogenetic domains have smaller proportions of 100% disordered Pfam domains in the following order: mammals > other eukaryota > viruses > bacteria > archaea. Detailed structure-function maps of sixteen examples of these domains presently known to be directly implicated in human disease show that many of these proteins have multiple Pfam domains, and that efforts to crystallize these proteins have been unsuccessful. We also find that about 2% of Pfam domains in Single cell prokaryotes, and about 6% in multiple cell eukaryotes are predicted to be 100% disordered, with one exception, Pneumocystis (pneumonia), with 28% disordered Pfam domains, suggesting that intrinsic disorder is related to the survival of some single cell organisms infecting mammals. It is proposed that shorter 100% disordered functional domains and regions in proteins confer a selective advantage that has contributed to the evolution of more complex organisms and of single cell organisms that they host. The median length of disordered regions in this 100% disorder group is 59 residues. Distributions of disorder in each of these five phylogenetic domains differ at the 0.999 level with p values less than 1x10**-10. When Pfam domains are separated on the basis of their hydrophobicity and percent disorder, we find at least three linearly independent populations, or flavors, of intrinsic disorder, one of which is the completely disordered state.

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Voltage-Dependent Membrane Insertion and Translocation of α-Synuclein: Liposome Studies
Ruchika Bajaj, S.D. Zakharov, A.M. Griggs, C. Rochet, W.A. Cramer.
Alpha-synuclein (αSyn), a 140 amino acid protein enriched in neuronal tissue, is implicated in the pathogenesis of Parkinson's Disease (PD), one of the most common neurodegenerative disorders. The membrane appears to be a potential target of αSyn. Studies with oligomeric or aggregated ''protofibrillar'' or amyloid forms of αSyn, in which β-structure is dominant, have been shown to permeabilize membranes in cell-free systems.[1,2] A monomeric αSyn preparation, which could allow determination of a defined structure in membrane provided an alternative perspective.[3] αSyn, disordered in solution, assumes a predominately α-helical conformation when bound to liposomes with an anionic surface and high degree of curvature.[3,4] Using cysteine scanning mutagenesis to probe the membrane interaction of different regions of αSyn, the interaction of monomeric αSyn with membranes has been studied, thus far with mutants that span the polypeptide, from Val3 to Ala124. Two membrane systems, planar bilayer membranes and liposomes, have utilized an array of tagged cysteines to determine voltage-dependent topology of αSyn in membranes. Voltage-dependent membrane insertion into, and translocation across, liposome anionic phospholipid membranes, was studied through the response of the fluorescence probe 7-nitro-2,1-benzoxadiazole (NBD), covalently attached to individual Cys residues, to a potassium diffusion potential generated by addition of valinomycin to K^+-loaded liposomes. Translocation was assessed through quenching of NBD fluorescence by entrapped bromide. A map of the voltage-dependent membrane topology of αSyn was obtained, in response to a potential of −118 mV, in which a segment of αSyn containing Val48Cys is translocated. In liposomes and planar bilayer membranes, translocation with the PD familial mutant E46K was greater than that of wild type. (*Support*: Michael J. Fox Foundation for Parkinson's Research). *References*: (1) Volles et al. 2001; (2) Quist et al. 2005; (3) Zakharov *et al*. 2007, (4) Davidson *et al*. 1998.

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Solvent Dependence of Trialanine Conformers
Siobhan Toal, Omid Amidi, Reinhard Schweitzer-Stenner.
Determining the conformational propensities of amino acid residues in short peptides is increasingly recognized as pivotal for obtaining a reliable picture of the unfolded states of peptides and proteins. In this context, ample experimental evidence indicates that alanine does not exhibit a statistical coil behavior, but rather shows a strong preference for sampling the polyproline II (PPII) region of the Ramachadran plot. Solvation in water has been proposed as the main reason for PPII stabilization. The mechanisms involved in the respective peptide-solvent interactions are still debated. Results from DFT-calculations on dialanine suggest that water stabilizes PPII conformers by forming hydrogen bond bridges between adjacent carbonyl and amide groups. Other models have invoked electrostatic interactions. To explore the solvent dependence of PPII stabilization, we used UV-CD and H-NMR spectroscopy to determine the conformations of cationic trialanine in water, as well as in binary water/glycerol water/ethanol mixtures. All spectra were recorded as a function of temperature ranging between 0°C and 90°C. The resultant dichrosim at 216nm and $^3J(H^{\alpha}H^{N})$ coupling constants were subjected to a simplified two state PPII ↔ β strand thermodynamic analysis. The experimental results indicate that both alcohol co-solvents substantially lower the PPII propensity of alanine in each respective binary mixture. Glycerol seems to be most effective in replacing water in the solvation shell, causing large enthalpic decreases between the two conformers; from ΔH=18.15 kJ/mol in water, to ΔH=12.42 kJ/mol with only a 0.012 mole fraction admixture of glycerol.