regions in cis to ordered domains. Using atomistic simulations and fluorescence studies we characterize the conformational properties of several archetypes of intrinsically disordered sequences in the presence and absence of cis-acting ordered domains. In particular, we quantify the effects of sequence composition, chain length, and sequence patterning of disordered sequences on the inter-domain coupling. The balance between intra-domain and inter-domain interactions can modulate intrinsic conformational propensities of the disordered regions. The circumstances giving rise to convergence toward generic random-coil behavior for disordered regions in cis to ordered domains will be highlighted.

**2154-Plat**

**The C-Terminal V5 Domain of Protein Kinase Cα is a Multi-Functional Intrinsically Disordered Protein Module**

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Protein Kinase C (PKC) family of isozymes regulate a multitude of signaling pathways that control cell growth, differentiation, and apoptosis. The extreme C-terminal V5 domain has been identified as a key player in maturation, activation and down-regulation of PKCs, but the molecular basis of these events remains poorly understood. We report the first large-scale purification and NMR characterization of the V5 domain from PKCα (V5α) and its phosphorylation-mimicking variant. The combined analysis of NMR chemical shifts and circular dichroism data revealed that both V5α constructs are intrinsically disordered protein domains. Unexpectedly, we found that V5α has a propensity to partition into membrane mimetics acquiring a partial helical structure. Our data suggest that V5α anchors its parent enzyme to membranes during the maturation process.

Using NMR techniques, we obtained direct evidence that V5α interacts with another domain of PKCα – the C2 regulatory domain. This interaction is mediated by the phosphorylated hydrophobic motif of V5α and is enhanced by $Ca^{2+}$. Similarly, the affinity of C2 to $Ca^{2+}$ is enhanced in the presence of the phosphorylated hydrophobic motif. These findings indicate that V5α may function as an intra-molecular protein interaction module that sensitizes PKCα to $Ca^{2+}$ ions.

The third aspect of V5α function pertains to its interactions with Pin1, a peptidyl-prolyl isomerase that has been implicated in the down-regulation of conventional PKCs. Pin1 catalyzes proline isomerization of the phosphorylated Ser/Thr-Pro motif. Our preliminary NMR data demonstrate that Pin1 interacts with the hydrophobic motif of V5α, which is a non-canonical site that lacks a proline after the phosphorylated serine. Our data support the hypothesis that V5α serves as a phosphorylation-dependent docking site for Pin1.

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**2155-Plat**

**C-Terminal ERK D- (and F-Like) Domains Link the $Na^+/H^+$ Exchanger NHE1 to ERK2 Phosphorylation and Regulation via Scaffolding**

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Increased activity of extracellular signal regulated kinase (ERK)1/2 plays a central role in cancer pathology. More recently, the membrane protein $Na^+/H^+$ exchanger 1 (NHE1) has also been assigned important roles in cancer development and chemotherapy resistance. Using a combination of fluorescence spectroscopy, heteronuclear NMR analysis, and protein engineering we characterized the interaction of the intrinsically disordered human (h) NHE1 C-terminal cytoplasmic tail (NHE1Cdt) and ERK2 in vitro, and found that NHE1Cdt and inactive ERK2 physically interact through exploitation of both D- and F-like domains of NHE1. Using active kinases we find that NHE1 is specifically phosphorylated by ERK2 and not MEK2 at several sites of importance for regulation of ERK2 activity and/or for NHE1 activity. We additionally found that NHE1 serves as a scaffold for MEK2-induced activation of ERK2. Mutating the D-domains in full-length hNHE1 and expressing wt and mutant NHE1s in epithelial cells lacking endogenous NHE1 evaluated the functional effects of NHE1-ERK interaction. Wt- and D-domain mutant hNHE1s appeared to target normally to the plasma membrane. D-domain mutation however abolished EGF-induced ERK activation, and D domains appeared to be required for full ERK activity. In contrast, net acid extrusion capacity after an acid load was similar in cells expressing D-domain mutant- and wt hNHE1s. In conclusion, ERK physically interacts with hNHE1 and this appears to regulate ERK activity via scaffolding.

**2156-Plat**

**Speed Dating with KIX: A Single Domain that has Many Partners**

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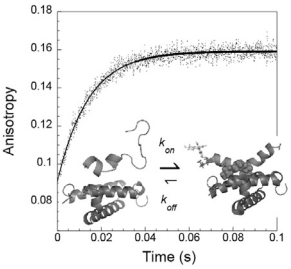
IDPs are overrepresented in processes such as signalling and transcription, where proteins often interact with a range of partners. One much-studied key hub protein is the coactivator CBP/p300, whose folded KIX domain binds to a number of different intrinsically disordered transcription factors. The interaction of KIX with several of its ligands has been well studied by equilibrium methods, and structural information is available for many of the complexes. By careful control and consideration of experimental conditions such as temperature and ionic strength we have been able to perform kinetic studies that reveal the mechanism of the association reaction of KIX with cMyb; the fastest protein-protein interaction yet reported. Furthermore, through comparative studies with several binding partners we shed light on an important outstanding question in the IDP field: what is the advantage of disorder to a protein?




**2157-Plat**

**Molecular Simulations of the Dynamics of Disordered Proteins**

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Intrinsically disordered proteins (IDPs) do not possess well-defined three-dimensional structures in solution under physiological conditions. We develop all-atom, united-atom, and coarse-grained Langevin dynamics simulations for the IDPs α-, β-, and γ-synuclein and microtubule-associated protein tau (MAPT) that include geometric, attractive hydrophobic, and screened electrostatic interactions and are calibrated to the inter-residue separations measured in recent smFRET experiments. We find that all four proteins are disordered with conformational statistics that are intermediate between random walk and collapsed globule behavior and demonstrate close resemblance to the known experimental data. We find that the hydrophobic interactions strongly influence the dynamics of these proteins, and electrostatics only play a role when charges fluctuate over more than several residues as for MAPT. We also investigate the propensity of α-synuclein and MAPT to aggregate and form oligomers, and compare to our previous results with α-synuclein monomers.

**2158-Plat**

**The Protection of Membranes from Cold-Stress: A Structural Study of the Intrinsically Disordered Dehydrin Bound to Micelles and Liposomes**

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Plants produce numerous proteins to respond to cold and drought. One of these responses includes the expression of dehydrins, a family of proteins whose upregulation protects plants from various abiotic stresses. Using the 48-residues K2 dehydrin from Vitis riparia, we characterize the binding interaction and the structural changes that occur as this protein goes from a disordered protein in solution to one that contains weak alpha-helices after binding to liposomes and SDS micelles. We show that the addition of K2 protein to liposomes prevents their fusion after being frozen and thawed. We subsequently use SDS micelles as a membrane mimetic to probe what structural changes occur upon binding. We observe that a maximal change in helical content occurs well before the critical micelle concentration of SDS is reached. A series of ensemble structures were calculated for the free and micelle-bound forms of the protein using several NMR restraints. The conserved regions of the protein, containing several lysines, are shown to interact with the membrane surface, while a poorly conserved region of the protein maintains its high level of flexibility. Using chemical shift perturbation data and paramagnetic relaxation enhancement probes, we suggest which residues may be involved with membrane binding, and present a structural model of the protein bound to a membrane surface.