1076-Pos Board B27
Characterization of PC2 Cterm Calcium-Binding Interaction and its Structural Implications
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Polycystin-2 (PC2) is a Ca^{2+}-regulated Ca^{2+}-channel from the transient receptor potential (TRP) family. Mutations in PC2 can cause autosomal dominant polycystic kidney disease (ADPKD). The C-terminal cytoplasmic tail of human PC2 (HPC2 Cterm) is crucial for channel assembly and function. We have combined biophysical and structural approaches to characterize the Ca^{2+}-dependent molecular mechanism within the C-terminal tail that is involved in channel assembly and functional regulation. We have determined that HPC2 Cterm forms a trimer in solution with and without Ca^{2+}-bound, even though TRP channels are generally tetrameric. We have definitively shown that there is only one Ca^{2+}-binding site in HPC2 Cterm, located within its EF-hand domain. However, its Ca^{2+}-binding affinity is greatly enhanced relative to its intrinsic binding affinity in the isolated EF-hand, possibly due to the positive cooperativity from the trimer interaction. We also employed sea urchin PC2 as a parallel model to study. The sea urchin C-terminal domain construct (SUPC2 Ccore) also trimerizes in solution independent of Ca^{2+}-binding. In contrast, the SUPC2 Ccore contains two Ca^{2+}-binding sites within its EF-hand domain, which exhibit cooperative Ca^{2+}-binding due to internal stabilization. Consequently, trimerization does not further improve Ca^{2+}-binding affinity in SUPC2 Ccore relative to the isolated EF-hand domain. Using both hydrogen-deuterium exchange mass spectroscopy and nuclear magnetic resonance, we have localized the Ca^{2+}-binding sites in PC2 C-terminal tail and mapped the conformational changes induced by Ca^{2+}-binding. We demonstrate that in addition to the direct, local stabilizing effects within the EF-hand, Ca^{2+}-binding also causes conformational changes in the distal coiled-coil domain. This study provides a structural basis for regulation of the PC2 channel by its cytosolic C-terminal domain, with an improved understanding of the functional role of PC2 in regulating intracellular Ca^{2+} signaling.

1077-Pos Board B28
EPR with Rigidly Bound Spin Labels used to Probe the Interaction of Calmodulin with the Ryanodine Receptor
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We have used site-directed spin labeling and EPR spectroscopy to probe the structural dynamics of calmodulin bound to a ryanodine receptor target peptide. Calmodulin binds to a conserved 30-amino acid sequence on the sarcoplasmic reticulum calcium release channel (ryanodine receptor, RyR) and directly modulate its activity in muscle contraction. In order to characterize the structural details of this crucial interaction, a bifunctional spin label was used to rigidly label di-cysteine mutant calmodulin at positions 34 and 38. Solid-phase peptide synthesis was used to create a peptide corresponding to the calmodulin binding site on the ryanodine receptor channel (RyRp, residues 3614-3643 in RyR1) with a TOAC spin label rigidly coupled to the backbone. The intermolecular distance distribution between the two spin probes was determined by EPR, using both continuous wave dipolar broadening and dipolar electron-electron resonance (DEER). The center of the distance distribution was in good agreement with the crystal structure of the complex (18 Å), but the width of the distribution was ~ 1 nm, comparable to that observed previously within calmodulin itself. Thus calmodulin retains significant inter-lobe domain motion, even after RyRp binding. These results are consistent with a model in which calmodulin binds RyRp, bringing its two terminal lobes close together but preventing them from physical contact with each other, allowing flexibility in each of the lobe.

Protein-Small Molecule Interactions II

1078-Pos Board B29
Predicting Peptide Binding Sites on Protein Surfaces by Clustering Chemical Interactions
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Short peptides play important roles in cellular processes including signal transduction, immune response and transcription regulation. Correct identification of the peptide binding site on a given protein surface is of great importance not only for mechanistic investigation of these biological processes but also for therapeutic development. In the present study, we developed a novel computational approach, referred to as ACCLUSTER, for predicting the peptide binding sites on protein surfaces. Specifically, we use the twenty standard amino acids as probes to globally scan the protein surface. The poses forming good chemical interactions with the protein are identified, followed by clustering with the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) technique. Finally, these clusters are ranked based on their sizes. The cluster with the largest size is predicted as the putative binding site. Assessment of ACCLUSTER was performed on a diverse test set of 251 non-redundant protein-peptide complexes. The results were compared with the performance of POCASA, a pocket detection method for ligand binding site prediction. Peptidb, another protein-peptide database that contains both bound structures and unbound or homologous structures was used to test the robustness of ACCLUSTER. The performance of ACCLUSTER was also compared with PepSite2 and PeptiMap, two recently developed methods developed for identifying peptide binding sites. The results showed that ACCLUSTER is a promising method for peptide binding site prediction. Additionally, ACCLUSTER was also shown to be applicable to small molecular binding site prediction and protein-protein interface prediction. Notably, ACCLUSTER is based on physical interactions rather than informatics training, and can easily be extended to other macromolecular systems, including systems like peptide-RNA complexes which lack sufficient structural data and therefore pose challenges to machine-learning based methods.

1079-Pos Board B30
Homology Modeling and Docking Studies Identify Subtype-Specific Characteristics of Melanocortin Receptor Activation
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Melanocortin receptors (MCRs) belong to the family of G protein-coupled receptors (GPCRs), and the five subtypes of MCRs (MC1R, MC2R, MC3R, MC4R, and MC5R) are implicated in diverse physiological processes such as pigmentation, food intake, and energy homeostasis. Because of their physiological importance, MCRs are natural targets for drug development. It is known that synthetic cyclic analogues of natural MCR hormones have the potential for pharmaceutical applications. However, 1) no current structures exist for MCRs and 2) the synthetic cyclic peptides possess little specificity for MCR subtypes, making identification of structural motifs in receptor-ligand binding extremely difficult. Our hypothesis is that MCR activity is determined by specific intermolecular interactions of ligand side chains with amino acid residues of the melanocortin receptor, while the selectivity of the ligands towards different subtypes of MCRs is controlled by conformational restraints of ligand backbones. Identification of general characteristics of MCR-ligand interactions will facilitate intelligent design of modified ligand structures that lead to MCR subtype specificity. To test this hypothesis, we developed homology models of the different MCR subtypes and conducted docking studies of a known agonist (Melanotan-II) and antagonist (SHU-9119) on each MCR model, in order to characterize the respective binding affinities of each receptor-ligand complex. Our results show that agonists and antagonists have a common set of binding characteristics along with interactions that are unique to each ligand that can be directly related to their function. The ability to accurately characterize unique structural interactions between known MCR agonists and antagonists is the first step in developing the ability to design effective, subtype-specific therapeutic agents of melanocortin receptors.

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Quantifying the Thermodynamic Molecular Driving Forces in Protein-Ligand Binding
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The thermodynamic driving forces behind protein-ligand binding are still not well understood. To better understand these phenomena we calculate spatially resolved thermodynamic contributions of the different molecular degrees of freedom for the binding of propane and methanol to multiple pockets on the proteins Factor Xa and p38 MAP kinase, as examples. An end-point method grounded in statistical physics is presented to compute thermodynamic contributions of the bound and free states from canonical ensembles obtained from molecular dynamics simulations. Energetic and entropic contributions of water and ligand degrees of freedom provide an unprecedented level of detail into the mechanisms of binding. We found direct protein-ligand interaction energies to