3359-Pos
Iris-Like Mechanism of Pore Dilation in the CorA Magnesium Transport System
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Magnesium translocation across cell membranes is essential to numerous physiological processes. Three crystal structures of the CorA magnesium transport system have recently revealed a surprising architecture, with a bundle of giant α-helices forming a 60-Å-long pore which extends beyond the membrane before widening into a funnel-shaped cytosolic domain. The presence of divalent cations in putative intracellular regulation sites suggests that these structures correspond to the closed conformation of CorA. To examine the nature of the conduction pathway, we performed 110-ns molecular dynamics simulations of two of these structures in a lipid bilayer with and without regulatory ions. Results show that a 15-Å hydrophobic constriction straddling the membrane-cytosol interface constitutes a steric bottleneck whose location coincides with an electrostatic barrier opposing cation translocation. Structural relaxation induced by the removal of regulatory ions leads to concerted changes in the tilt of the pore helices, resulting in iris-like dilation and spontaneous hydration of the hydrophobic neck. This simple and robust mechanism is consistent with the regulation of pore opening by intracellular magnesium concentration and explains the unusual architecture of CorA.

3360-Pos
Human Copper Transporter 1: Model-Structure, Function and Motion
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Copper is an indispensable nutrient for functioning of various cell processes. Human CTR1 (hCTR1) is a member of the eukaryotic copper transporter family, essential for copper uptake in human cells and have been also implicated in cellular sensitivity to some chemotrapy drugs. We constructed a Cα-trace model of the transmembrane region of this trimeric transporter using cryo-electron microscopy and evolutionary data. The model-structure was supported by mutagenesis data, and provided a structural perspective of the roles of the evolutionary conserved and essential sequence motifs, MxxxM of TM2 and GxxxG of TM3. Specifically, Met150 and Met154 of the MxxxM motif, situated at the narrow pore entrance, were suggested to serve as both selectivity filter and extracellular gate. To gain further insight into dynamics and cooperativity of hCTR1, we investigated the structural fluctuations of the model-structure using elastic network models. The analysis revealed that the most prominent hinges correspond to residues of the known sequence motifs, indicating their importance for protein functional motion. Moreover, we identified a role for TM2 in coupling between the three monomers of the TM region via rotational symmetry. Of the two main structural fluctuations modes, the slowest mode introduced structural changes mainly at the cytoplasmic, wide end of the pore, whereas another highly cooperative fluctuation manifested the activation of the extracellular pore entrance coupled to motion at the cytoplasmic ends.

3361-Pos
GPR35: Study of Class a GPCR Sequence Divergences using Conformational Memories
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GPR35, a recently deorphanized Class A G-protein coupled receptor, shows prominent expression in immune and gastrointestinal tissues (Wang et al. JBC, 2006), with additional expression in pancreatic islets, skeletal muscle, and lung (Horikawa et al. Nat. Genet. 2000), brain and spinal cord. An endogenous ligand of GPR35, kynurenic acid, is one of the major metabolites of the kynurenine pathway; a pathway in which the main route of tryptophan catabolism has been associated with important physiological roles in the brain. Compared with prototypical Class A GPCRs such as the beta-2-adrenergic receptor (β2AR; Cherezov et al. Science 2007), GPR35 has several major sequence differences: (1) in TMH4, GPR35 lacks the Pro at 4.60; (2) in TMH2 there is a Pro shift from 2.59 to 2.58; and, (3) in TMH5, there is an additional Pro at position 5.43. In order to study the consequences of these sequence divergences on the GPR35 structure, we used the Monte Carlo/simulated annealing program, Conformational Memories (Whitnell et. al, J. Comput. Chem. 2007). Each helix was built using the standard phi and psi angles for TMHs, −62.9°/-41.6° (Ballesteroes et al. Meth. Neuro. 1995). CM calculations revealed that the extracellular end of GPR35 TMH4 diverges from that of the β2AR, as the bend, wobble angle and face shift was (26.2°,40.0°,48.0°) compared to the average of 105 CM structures for GPR35 TMH4 (18.04° ± 4.95°, −123.39° ± 58°,1.63° ± 11.83°). The GPR35 one residue N-terminal TMH2 proline shift created a face shift of 53.04° ± 27.21° compared to 96.8° for the β2AR TMH2. The additional proline in GPR35 TMH5 resulted in a bend and face shift of (12.76° ± 4.74°, 47.43° ± 22.74°) vs. (6.0°, 80.1°) for the β2AR. [Support: NIH RO1 DA023204 (MEA) and KO5 DA021358 (PHR)]

3362-Pos
Construction of a μ-Opioid Receptor Model using Conformational Memories
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The μ-opioid receptor (MOR) is a member of the Class A subfamily of G-Protein Coupled Receptors (GPCRs). GPCR activation has been shown to involve a change in the W6.48 χ1 dihedral from *g+* to *trans*. To probe MOR activation, we constructed models of the MOR inactive (R) and active states (R*), using a Beta-2-Adrenergic (β 2-AR) crystal structure template (Cherezov et al, Science 2007; Rasmussen et al, Nature 2007) with two major modifications. First, the Monte Carlo/simulated annealing technique, Conformational Memories (CM) (Whitnell et. al, J. Comput. Chem. 2007) was used to study the conformations of three MOR transmembrane helices (TMH) with important sequence divergences from the β 2-AR template: TMH2, TMH4 and TMH6. Second, the TMH7/elbow/Hx8 region of the β 2-AR was replaced with that of the adenosine A2A crystal structure (Jaakola et. al, Science 2008) due to differences in the number of residues in the elbow region of the MOR vs. β 2-AR. Energy minimizations were performed using the OPLS_2005 force field on the resultant MOR bundles in a three step process and the ligand binding pocket was identified. Docking studies suggested that naloxone, a MOR antagonist, binds in the TMH2-3-6-7 region of the MOR such that the N-allyl group sterically prohibits the movement of the χ1 of W6.48 in the R state, thereby preventing activation of the receptor. Morphine, a MOR agonist, was also found to bind in the TMH2-3-6-7 region of the R state MOR; however no portion of the morphine structure could block the movement of the χ1 of W6.48, thereby producing no impediment for activation. These results are consistent with the pharmacologies of naloxone and morphine. [Support: NIH RO1 DA023905 (PYL) and KO5 DA021358 (PHR)]

3363-Pos
Concerted Motion and Hydration of the Beta-2-Adrenergic Receptor Revealed by Microsecond Time Scale Molecular Dynamics
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The recent crystallographic structures of class A G protein-coupled receptors have shown important differences with their archetypal model, rhodopsin, such as the apparent breaking of the ionic lock that stabilizes the inactive structure. Here, we characterize a 1.02 microsecond all atom simulation of an apo beta-2-adrenergic receptor that is missing the 3rd intracellular loop in order to better understand the inactive structure. The lock rapidly reforms, although there is an activation-precursor-like event where the ionic lock opens for approximately 200ns, accompanied by movements in the transmembrane helices associated with activation. The lock is also found to exist in three states: closed, semi-open with a bridging water molecule, and open. The interconversion of the lock states involves concerted motion of the entire protein. We characterize these states and the concerted motions underlying their interconversion through principal component analysis. These motions are subtle, however, as the structure is found to be remarkably rigid throughout simulation. There is also a rapid influx of water into the protein core along with a slight expansion of the structure relative to the crystal model, leaving the core of the receptor persistently hydrated. We further characterize the structure and dynamics of the internal waters by applying pattern matching methods.

3364-Pos
Activation Pathways of Agonists, Partial Agonists and Inverse Agonist in Beta1 and Beta2 Adrenergic Receptors
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Modulation of cell signaling by ligands of different efficacies via G-protein coupled receptors (GPCRs), depends intrinsically on the effect of the ligand on the dynamics between the multiple conformational states of these proteins. Ligands with different efficacies can remodel the energy landscape of the receptors, thereby perturbing this conformational equilibrium in many ways depending on the nature of the ligand, and the G-proteins that the receptor couples to, thereby conferring functional specificity. Understanding activation dynamics and pathways is vital in designing functionally specific drugs for GPCRs.