The CB_1 allosteric modulator, ORG27569, has the paradoxical effect of increasing the equilibrium binding of CP55,940 (an orthosteric agonist), while at the same time decreasing its efficacy. ORG27569 also acts as an inverse agonist. We have previously used computational methods, synthesis, mutation, and functional studies to identify ORG27569's binding site in the THM3/TMH6/TMH7 region (Shore et al., *ICRS*, **2012**). In this site, ORG27569 promotes an active-like conformation of the CB_1 receptor, explaining ORG27569's ability to increase CP55,940's equilibrium binding. This site explains ORG27569's ability to antagonize CP55,940's efficacy in three complimentary ways: 1) ORG27569 sterically blocks movements of the second extracellular loop that have been linked to receptor activation, 2) ORG27569 sterically blocks a key electrostatic interaction between the third extracellular loop residue K373 and D2.63$^{(176)}$, and 3) ORG27569 packs against TMH6, sterically hindering movements of TMH6 that the Farrens lab have shown to be important to receptor activation. Additionally, we identified a key interaction between ORG27569's piperidine ring nitrogen and K3.28$^{(192)}$ that is required for ORG27569 to act as an inverse agonist.
Using our model of ORG27569 docked in our active state model (in the presence of CP55,940), we designed, synthesized, and functionally characterized 4 analogs of ORG27569 that were designed to test our model and have improved interactions with the receptor. The analogs were functionalized with 3 different goals: 1) to form electrostatic interactions with D6.58$^{(366)}$, 2) to form an aromatic stack with F3.25$^{(189)}$, or 3) to test packing with TMH6-7. Our strategy to form new interactions with D6.58$^{(366)}$ was the most successful, resulting in an analog that is more potent than ORG27569. Interestingly, none of the analogs acted as inverse agonists, suggesting the potential therapeutic promise of CB_1's allosteric site.

2100-Pos Board B119
Structural Model of K^+ Channel Activation by the Beta-Gamma Subunits of G-Proteins
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The first known effectors of the βγ subunits of heterotrimeric G-proteins (Gβγ) were the G-protein-gated inwardly rectifying K^+ (GIRK) channels which underlie acetylcholine-induced decrease in heart rate (IK_{ACh}). How Gβγ subunits specifically regulate the conformations of their effector proteins to alter activity is not understood at a molecular level. Although several GIRK crystal structures have been published, attempts to co-crystallize Gβγ have failed precluding knowledge of the reciprocal interactions between the two proteins.
We have employed a computational approach that combines several known methods in protein-protein docking to produce experimentally testable models of the protein complex. The best scoring model of the GIRK1-Gβγ complex predicted a ~1800 $Å^2$ interaction surface that includes key interactions of the channel's LM and DE loops with Gβ residues that are known to interact with the helical N-terminus of Gα-GDP in the structure of the inactive heterotrimeric G-protein. The channel-Gβγ interactions predicted by the model could be disrupted by mutation of one protein and rescued by additional mutation of reciprocal residues in the other protein. Channel activity was found to be stimulated by Gβγ interactions that enlarged the cleft between the LM and DE loops of the channel and stabilized the LM loop in a ''raised'' position seen in the ''open intracellular gate'' conformation of the GIRK1 crystal structure. GIRK4 displayed differences from the GIRK1 with respect to the pattern of responses to Gβγ mutants but the physiologically relevant heteromeric GIRK1/4 channel behaved similarly to GIRK1. The proposed site of action of Gβγ in the channel's DE-LM cleft is also shared with alcohols and is consistent with a previously described cascade of PIP_2-driven changes in intramolecular interactions of the channel leading to stabilization of the open conformation of its intracellular G-loop gate.

2101-Pos Board B120
Construction of a GPR18 Receptor Model using Conformational Memories
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The putative cannabinoid receptor, GPR18, is a member of the Class A subfamily of G-Protein Coupled Receptors (GPCRs). GPR18 binds both lipid-like and small molecule ligands, including NAGly and abnormal-cannabidiol (Abn-CBD) (Kohno et al., BBRC 2006; McHugh et al., Br J Pharmacol 2012). In order to explore the nature of GPR18/ligand interactions, we constructed models of the GPR18 inactive (R) and activated (R*) states, using the μ-Opioid receptor (MOR) crystal structure as template (Manglik et al., Nature 2012). The Monte Carlo/simulated annealing method, Conformational Memories (CM) (Whitnell et. al, J. Comput. Chem. 2007) was used to study the accessible conformations of three GPR18 transmembrane helices (TMHs) with important sequence divergences from the MOR template: TMH3 (P3.36 vs. M3.36 in MOR), TMH4 (L4.54 vs. S4.54 in MOR which participates in a hydrogen bond network that produces a significant bend in TMH4 MOR), and TMH7 (DVILY vs. NPVLY in MOR). We also used CM to calculate the accessible conformations for TMH6 (CFMP vs. CWTP in MOR). This allowed the choice of TMH6 conformers appropriate for the GPR18 R and R* models. All CM calculations used ideal helices as starting points with standard φ (−63°) and ψ (−41.6°) backbone dihedrals. TMH7 calculations also used an ideal helix as starting point, but with a 3(10) helix geometry in the DVILY region. Extracellular and intracellular loop geometries were calculated using Modeller v9.1. Energy minimizations of the resultant R and R* models were performed using the OPLS2005 all atom force field in Macromodel 9.1 (Schrodinger, 2006). The resultant GPR18 R and R* models were used to determine key residues for ligand docking.

2102-Pos Board B121
Aquaporins within a Tetramer Exhibit Different Structural Conformations: An in Silico Study of the Human Aquaporin 5
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Aquaporins are protein channels located across the cell membrane with the role of conducting water or other small sugar alcohol molecules (aquaglyceroporins). The high-resolution X-ray structure of the human aquaporin 5 (HsAQP5) exhibits an important feature: the entire tetramer is crystallized, i.e., the tetramer is not obtained by rotating the monomeric structure around the main axis of the tetramer. Hence, by means of molecular dynamics simulations we conducted a study on the importance of the protein-protein coupling within an aquaporin tetrameric structure and characterized the structural behavior of the human AQP5. We found that different conformations within the tetramer lead to a distribution of monomeric channel structures, which can be characterized as ''open'' and ''closed''. Both the extracellular (where the selectivity filter is located) and the cytoplasmic ends of a channel sample ''closed'' states. In the former region, this can be characterized by a strong narrowing and much lower water permeation rates. In the cytoplasmic end's ''closed'' state water passage is completely blocked by a gating mechanism characterized by the translation of the His67 residue inside the pore. While removing the crystallographic lipid occluding the central pore of the tetramer has no influence on the gating system, the protein-protein coupling might play an important role in regulating its mechanism. Furthermore, our calculated permeation rate of a fully ''open'' channel was found to be in very good agreement with the experimental value.

2103-Pos Board B122
Temperature Controlled Helix-Helix Interactions in Desk Minimal Sensor
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DesK is a temperature sensing transmembrane protein that acts as a molecular switch to regulate membrane fluidity as a function of temperature change. The full function of DesK is modelled by a chimeric construct, denominated minimal sensor (MS), formed by the upper and lower halves of transmembrane helices 1 and 5, respectively. The signalling by MS has been explored experimentally providing data for modelling studies. Resulting from this, the current view is that it forms a dimer, switching its conformation depending on the temperature. To further investigate the molecular details of the switch mechanism, we have developed a new method for exploring the energy landscape of interaction, which allows high throughput screening of transmembrane helix dimers. The results show a clear distinction between helix-helix interactions at high and at low temperatures, providing a molecular basis for the functioning of the minimal sensor. These results form the basis for further experimental exploration, as well as for the rational design of other switching sensors.

2104-Pos Board B123
Molecular Basis for the Ion Selectivity of Gap Junction Channels Elucidated by Molecular Dynamics Simulations
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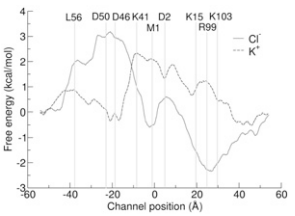
Gap junction channels, formed by 21 types of connexins, are essential for intercellular communications, and their mutations are associated with various diseases. Electrophysiological studies have identified disparate ion selectivity for different connexin channels, but the molecular basis remains unclear.

Several recent molecular dynamics simulations only clouded the picture. We carried out rigorous free-energy calculations using all-atom molecular dynamics simulations for Cx26 and Cx32 hemichannels and junctional channels in explicit membrane bilayers. Our potentials of mean force for cation and anion permeation explain the cation selectivity for the Cx26 channel and the modest anion selectivity for the anion selectivity for the Cx32 channel. For Cx26 (see Figure), pore-lining residues K41/M1(N-terminus) and K15/R99/K103 form energy wells for Cl- and barriers for K^+, while D46/D50 form a barrier for Cl- and a well for K^+. For Cx32, E41 forms a barrier for Cl- whereas N2 forms a barrier for K^+. These results provide a solid foundation for quantitatively rationalizing gap junction channel selectivity and conductance. Supported by the National Research Foundation of Korea (2012R1A1A1012707) and KISTI Supercomputing Center (KSC-2011-C2-44), and by NIH Grant GM88187.



2105-Pos Board B124

Dynamics of Water Inside the Secy Translocon Complex

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In bacteria and archea, the insertion of membrane proteins into the plasma membrane is governed by the SecY translocon complex. Understanding how the SecY translocon distinguishes a transmembrane (TM) segment from a secretory one is therefore of the utmost importance. The process of insertion of a TM segment into the membrane resembles a thermodynamic equilibrium process, which has allowed the determination of a ''biological'' hydrophobicity scale [1,2]. Recent measurements have shown that water-to-membrane partitioning is energetically different from translocon-to-membrane partitioning [3]. A possible explanation is the state of water within the translocon and consequently the strength of the hydrophobic effect. In order to shed light on translocon-to-membrane partitioning, we have investigated water dynamics inside the SecY. We performed molecular dynamics simulations using the crystal structure of SecYE from Pyrococcus furiosus [4] as the initial configuration. Approaching the central region of SecY the hydrogen-bond network between water molecules survives longer than that between bulk water molecules. The rotational motion of water molecules is slowed down and the translational dynamics is characterized by ''anomalous'' diffusion. These results might explain the difference between translocon-to-membrane partitioning and water-to-membrane partitioning. The features we observed are characteristic of water molecules located close to a macromolecule. Being affected by the complex shape and the physicochemical heterogeneity of the macromolecular surface, these water molecules manifest different properties from those of the bulk phase. A TM helix passing through the SecY protein-conducting channel will feel an environment different from that characterizing bulk water.

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2106-Pos Board B125

Insertion Mechanism in E. Coli of Single-Span Membrane Proteins with Far-Downstream Transmembrane Segments

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Single-span membrane proteins (SSMPs) are the most abundant membrane proteins in virtually all organisms. SSMPs with N-terminus outside the cell are referred to as Type I MPs, while those with the N-terminus inside are referred to as Type II MPs. It is generally assumed that insertion occurs co-translationally via the signal recognition particle (SRP) pathway. The positive-inside rule determines TypeI/II MPs. The TM segment of Type I MPs is generally preceded by a cleavable signal sequence to facilitate the Nout-Cin topology. The TM segment of both types of proteins generally occurs early in the amino acid sequence, allowing recognition by SRP as it emerges from the ribosome. Secreted periplasmic proteins have a signal sequence, but are secreted post-translationally through the translocon by the SecA translocase.

We are studying an unusual class of Type II MPs that lack identifiable signal sequences and whose TM segments can occur hundreds of residues downstream from the N-terminus. One such protein is RodZ, which is a cytoskeletal protein involved in maintaining the rod shape of E. coli. What pathway does this protein use? Several prediction programs and our experimental studies of RodZ with deleted TM segment (RodZ-ΔTM) confirm the absence of a native targeting signal. Proteinase K treatment of spheroplasts reveal that RodZ has Nin-Cout (Type II) topology, consistent with previous studies. Classic N-terminal cleavable signal sequences, such as DsbA or MalE, do not affect the topology of RodZ. We have determined that RodZ insertion is strongly SecA dependent, because RodZ is not incorporated into the membrane under SecA-depletion conditions or in the presence of sodium azide, which is known to inhibit SecA. This result is important, because it is now possible to study the energetics of TM helix insertion by SecA.

2107-Pos Board B126

A Computational Study of H-Ras Nanoclusters in Membrane Domains

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The Ras family of enzymes are guanine triphosphatases (GTPases) that function as molecular switches by cycling between GDP-bound off and GTP-bound on conformational states. Malfunction of Ras proteins due to somatic mutations accounts for about 30% of human tumors. The signaling function of Ras proteins is highly related to their ability to form protein-lipid nanodomains (termed nanoclusters) on the plasma membrane. However, the molecular basis for the formation and distribution of Ras nanoclusters has not been determined. We attempt to address this fundamental issue by studying H-Ras proteins in model membranes and focusing on (1) how multiple Ras proteins oligomerize on the membrane surface, (2) how nanoclustering might be affected by conformational variations, and (3) how nanoclusters might affect the host membrane.

To achieve these goals, we performed coarse-grained molecular dynamics simulations of full-length GDP- and GTP-bound H-Ras in a model membrane. We found that variation in the initial conformation of these two states of H-ras leads to nanoclusters that exhibit different dynamic behaviors. Analysis of protein-protein contacts in the clustered proteins allowed us to map the residues involved in aggregation. By zooming in on the individual residues involved in protein-protein interactions, we found that the two states of H-ras significantly differ in the accessibility and availability of the structural elements that are required for effector binding. Another important observation is that nanoclusters generate positive curvatures on both layers of the membrane. To investigate the mechanisms of these membrane deformations, we performed three dimensional pressure field analyses and determined the surface tension and elastic bending modulus of each monolayer. These results highlight the intricacies of Ras nanocluster formation, which involve both protein-membrane and protein-protein interactions, and pave the way for a better understanding of signal transduction events mediated by Ras clusters.

2108-Pos Board B127

Molecular Dynamics Simulations of Caveolin-1 in Membrane Bilayers

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Caveolin which is found in caveolae is an integral membrane protein. Caveolin is thought to induce membrane curvature and is involved in many crucial cell functions such as endocytosis. Its membrane-embedded domain contains two helices (TM1 and TM2) connected by a three-residue linker, and both its N- and C-termini are exposed to the cytoplasm. Since there is no portion of caveolin that protrudes to the opposite side of the membrane, caveolin is postulated to adopt a horseshoe configuration. Despite considerable efforts, the structure of caveolin in a bilayer remains elusive. This work aims to characterize the structure and dynamics of caveolin-1 (D82 to S136; Cav182-136) in a DMPC bilayer using molecular dynamics (MD) simulations. In Cav182-136, TM1 and TM2 corresponds to A87−F107 and L111−A129, respectively. To explore a sufficiently large configurational space of Cav182-136, 50 independent initial models (10 for each of 45°, 55°, 65°, 75°, 86° TM1-TM2 angles) were built and each of them was placed in a DMPC bilayer with a 0.15M KCl solution using the CHARMM-GUI Membrane Builder. The TM1-TM2 linker (G108, I109, and P110) in each of the systems was randomly placed in between Z = −5 to 5 Å. A 50-ns production was performed for each system using CHARMM, and the results are presented and discussed in terms of (1) the orientation of Cav182-136 and its fluctuation [tilt angle of TM1-TM2 plane, TM1-TM2 angle, TM1 and TM2 tilt angle, insertion depth of the TM1-TM2 linker and four Trp residues: 85, 98, 115, 128], (2) TM1-TM2 contact residues, (3) Cav182-136−bilayer interactions, and (4) bilayer shapes.