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.

Starting from the crystal structure of beta2-adrenergic receptor (B2AR), we have used LITiCon computational method to predict the ligand stabilized receptor state with full (epinephrine and norepinephrine), partial (salbutamol and dopamine), and inverse agonists (carazolol) bound. We have calculated the minimum energy pathway going from the inactive to the ligand stabilized state for each of the ligand/receptor complex. The activation pathways derived for all the agonists studied here are in agreement with fluorescence lifetime measurements[1,2]. We have also studied the norepinephrine activation pathway for the mutant m23 avian beta1-adrenergic receptor (B1AR) that has been crystallized, and the wild type B1AR. We show that the activation barrier for activation of the mutant m23B1AR by norepinephrine, is larger than for the wild-type B1AR, which is in agreement with the previous experimental finding that m23B1AR requires higher concentration of norepinephrine for activation[3]. Virtual ligand screening with the salbutamol-stabilized B2AR conformation shows enrichment of non-catechol agonists over norepinephrine-stabilized conformation. Our computational method provides an unprecedented opportunity to understand activation mechanisms in GPCRs.

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3365-Pos

Functionnal Analysis of a Conserved Histidine Residue of the Extracellular Loop of Acid-Sensing-Ion-Channel-1a

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ASICs are non-voltage-gated neuronal sodium channels activated by protons. Activation of ASICs induces a membrane depolarization. ASICs are trimeric assemblies of homologous subunits. The structure of the chicken ASIC1 in its inactivated conformation is known. Each subunit has two transmembrane domains, a large extracellular loop (ECL) and two short intracellular termini. The ECL is the sensor of the extracellular acidity and the target of modulators. Zinc potentiates the acid activation of ASIC2a-containing channels. Two histidine residues are essential for this effect. Only the hASIC1a His163 is conserved in all ASICs and forms H-Bonds with the conserved Asn96 and Gln225 residues. To test their functional relevance, we mutated these and neighboring residues to cys. Most mutant channels showed poor expression in *Xenopus* oocytes suggesting that this part of the ECL is essential for the channel folding. The mutant channels were however functional and were unlike the wild type channel inhibited by Cd^{2+}. The Cd^{2+} IC_{50} values (N96C: $71 \pm 10\mu M$, Q225C: $73 \pm 53\mu M$, H163C: $331 \pm 36\mu M$) suggest together with the structural information that H163 participates in Cd^{2+} chelation in the mutant channels. Covalent modification by the charged sulfhydryl reagent MTSET was used to further investigate the structural and functional role of the His163 and its environment. After MTSET exposure the hASIC1a H163C channel current decreased to $4 \pm 1\%$ and the pH dependence of activation was shifted by -0.24 pH units, while the pH dependence of inactivation was not affected. The unitary conductance of the modified H163C mutant was not different from the conductance of unmodified H163C or wt ASIC1a. These observations suggest a role of this region in the gating, but likely not the pH-sensing of ASIC1a. Our study identifies a conserved region around His163 as critical determinant of ASIC1a expression and function.

3366-Pos

Structural Studies of the Integral Membrane Protein Human LTC4 Synthase by Electron Crystallography

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Human leukotriene C4 synthase (LTC4S), an 18kDa integral membrane protein and member of the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) family, is critical to the biosynthesis of the cysteinyl leukotrienes. LTC4S catalyzes the conjugation of leukotriene A4 with glutathione to produce leukotriene C4. The cysteinyl leukotrienes, in particular leukotriene C4 and its metabolites, are important lipid mediators in inflammation and are central to acute and chronic inflammatory diseases of the respiratory system, in particular bronchial asthma. The structure of human LTC4S was studied by two-dimensional (2D) crystallization and electron crystallography.
When reconstituted into proteoliposomes under rigorously controlled parameters, human LTC4S forms large and well-ordered two-dimensional crystals that retain enzymatic activity. Despite two three-dimensional structure, many questions about the reaction mechanism of human LTC4S remain unanswered. To elucidate these structure/function questions by electron crystallography, we are crystallizing both the wild-type and mutant enzymes. Surprisingly, the number of subunits of human LTC4S is not limited to the trimers earlier observed by both electron and x-ray crystallography.

3367-Pos

Metamorphic Response of CLIC1 Chloride Intracellular Ion Channel upon Interaction with the Membrane

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The Chloride Intracellular Channel (CLIC) family can be characterized as 'metamorphic'proteins; namely, capable of reversibly shifting between two or more different-folded conformations. The CLICs are expressed as soluble proteins but can also auto-insert into the membrane to form active ion channels. Such a conformational transition must involve large-scale structural rearrangement to confer favourable interactions with the membrane. To date, little is known about the process and cellular triggers for CLIC membrane insertion; although a unique feature of mammalian CLIC1 is its ability to undergo a dramatic structural rearrangement from a monomer to a dimer upon oxidation in solution. Whether this oxidation-induced metamorphosis in solution facilitates CLIC1 membrane insertion is unclear. We have sought to characterize the structural response of CLIC1 upon interaction with the membrane. A novel labeling scheme for CLIC1 was devised to enable site-directed-labeling of single native cysteines with either fluorophores or spin labels. Fluorescence resonance energy transfer (FRET) and electron paramagnetic resonance (EPR) revealed that the interaction of CLIC1 with the membrane results in a large-scale inter-domain movement. The two phases for membrane interaction, i.e. association followed by insertion, were also monitored by probing the local environment of an N-terminal transmembrane tryptophan residue (Trp35) using fluorescent quenching. A sucrose-loaded-vesicle sedimentation assay was also used to quantify membrane binding. Our results suggest oxidation of CLIC1 monomer in the presence of the membrane promotes CLIC1 binding and insertion. Our current model for the structural transitions and environmental triggers of CLIC1 membrane-induced metamorphosis will be discussed.

3368-Pos

Structural Analysis of Heptameric Alpha-Hemolysin under Extreme Conditions that Facilitate Nucleic Acid Translocation

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Protein nanopores are under intense investigation as sensors of various analytes, particularly for the rapid analysis of genomic material. In several important applications, notably ultrarapid sequencing, nucleic acids must be analyzed in unfolded single-stranded form. Therefore, conditions were examined that cause the denaturation of double stranded DNA (dsDNA), and single stranded DNA (ssDNA) and RNA with secondary structure. The behavior of the heptameric α-hemolysin (αHL) pore was investigated under alkaline pH conditions (8.0-13.0) and in varying concentrations of urea (0-8 M). The structural stability of the pore was examined by SDS-PAGE, intrinsic tryptophan emission fluorescence (ITFE) spectroscopy and circular dichroism (CD) spectroscopy. The pH studies revealed that the heptamer is resistant to structural change at up to pH 12.0. Electrical recordings revealed that DNA is capable of translocating through the pore at pH 11.7, a value at which its secondary structure is lost [1]. Studies conducted at various urea concentrations revealed that a substantial fraction of the heptamer remains stable in 8 M urea. At urea concentrations above 4.0 M, the secondary structure of single stranded DNA/RNA is denatured [2], permitting translocation.

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3369-Pos

Mtb Membrane Protein Expression , Purification and, Structure Determination : Solid State NMR Approach

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