1390-Pos Board B282
Ligand-Gated Ion Channel Opening and Closing Mechanism from Molecular Simulations
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Pentameric Ligand Gated Ion Channels comprise key receptors for neurotransmitters including acetylcholine, GABA, and serotonin. They are thus targets for many anesthetics, alcohol, and other drugs such as antipsychotics and antidepressants. The channels typically open after physiologic ligand binding to a site in the extracellular domain, but the channels are also highly susceptible to allosteric modulation at sites in the transmembrane domain, the mechanism utilized by many drugs. To better understand ligand and drug action, we are studying ion channel gating using Gloebacter violaceus (GLIC) channels. GLIC is a prokaryotic homologue believed to share all the important characteristics of metazoan channels, but with several structures available. We have previously(1) shown a single closing event for GLIC at neutral pH in molecular simulations. Here, we have employed ensemble molecular dynamics simulations to systematically explore the conformational dynamics of the channel, starting from both open and locally-closed conformations. We observe a large number of both opening and closing events. We have also simulated multiple functional mutants of the GLIC channel and observe shifts in opening or closing propensity that agree well with the functional data. Mutants strongly biased towards opening remained open for greater than one microsecond in our simulations. Based on our results, we generate a structural model for which portions of the channel can close sufficiently to restrict water and ion flow.
(1) Samuel Murail, Rebecca J. Howard, James R. Trudell, Edward Bertaccini, Erik Lindahl, Tracing the Closing of a Ligand-Gated Ion Channel in Atomic Detail: An Unconstrained Four-Microsecond Simulation of GLIC Leads to a Closed State Remarkably Similar to ELIC, Biophysical Journal, Volume 102, Issue 3, Supplement 1, 31 January 2012, Pages 113a-114a, ISSN 0006-3495, 10.1016/j.bpj.2011.11.639. (http://www.sciencedirect.com/science/article/pii/S0006349511019874)

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Spectroscopic Investigation of Agonist-Induced Rearrangements of Cyclic Nucleotide-Modulated Ion Channels
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Cyclic nucleotide-gated (CNG) and hyperpolarization activated cyclic nucleotide-modulated (HCN) ion channels are activated by the direct binding of cyclic nucleotides, e.g. adenosine 3',5'-cyclic monophosphate (cAMP), to a conserved, cytoplasmic domain. The structure of the cyclic nucleotide binding domain (CNBD) is similar to those found in other cyclic nucleotide-activated proteins, including the kinases PKA and PKG, the transcription factor CAP, and the guanine nucleotide exchange factor Epac. The core of this structure contains an eight-stranded β-barrel followed by two helices (the B and C helices). Cyclic nucleotides initially bind to residues in the β-barrel. Subsequent to binding, the C helix of HCN and CNG channels undergoes a translation toward the binding pocket as well as a stabilization of its helical structure. This conformational rearrangement is coupled to opening of the ion channel pore. We have extended our previous studies using transition metal ion fluorescence resonance energy transfer (tmFRET) in the purified C-terminal domain of HCN2 to demonstrate that the B helix also reorganizes relative to the β-barrel subsequent to agonist binding. Furthermore, we have used electron paramagnetic resonance (EPR) on the spin-labeled HCN2 C- terminus to investigate the reorientation and stabilization of the CNBD induced by cAMP binding. These studies further extend our knowledge of the conformational changes in the CNBD of HCN and CNG channels and may provide a general picture of the activation of other families of cyclic nucleotide-regulated proteins.

1392-Pos Board B284
Single Nucleotide Variant Increases the open Probability of Human ENaC
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The epithelial Na^+ channel (ENaC) mediates Na^+ entry into specific epithelial cells and has an essential role in the regulation of blood pressure and airway surface liquid volume. Rare ENaC mutations have been reported in inherited disorders associated with hypertension or hypotension, and ENaC gene variants have been associated with several human disorders. Recent human genome sequencing projects have revealed a large number of ENaC gene variations in both exons and introns. However, the functional consequences of most variants are unknown. We examined the functional properties of the human ENaC variant γL511Q in the *Xenopus* oocyte expression system. Oocytes expressing αβγL511Q exhibited five-fold greater amiloride-sensitive currents than cells expressing WT channels. Mutant and WT channels had similar levels of surface expression. Single channel recordings with a cell-attached patch revealed that mutant channels had a four-fold higher open probability than WT, but similar unitary currents. The mutant had a significantly reduced Na^+ self-inhibition response, reflecting less reduction of ENaC open probability by extracellular Na^+. Interestingly, the mutant diminished the activating effect of external Zn^{2+} and essentially converted Zn^{2+} from a high-affinity ENaC activator to a low-affinity inhibitor. Furthermore, γL511Q exhibited blunted activation by chymotrypsin. We conclude that γL511Q is a gain-of-function human ENaC variant that is characterized by increased open probability and suppressed responses to extracellular Na^+, Zn^{2+} and chymotrypsin.

1393-Pos Board B285
TNF-α Lectin-Like Domain Derived Peptide Modulates Epithelial Sodium Channel Current
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Sodium absorption by epithial sodium channel (ENaC) is main driving force of lung liquid clearance at birth and lung edema clearance in adulthood. We investigated the molecular mechanism underlying the modulation of ENaC current by TNF-α and TNF-α lectin-like domain derived (TIP) peptides. With the help of the patch-clamp technique we show that TIP peptides caused a substantial increase in amiloride sensitive sodium current through ENaC in human alveolar adenocarcinoma cells (A549), in both whole cells as well as single channel configurations. ENaCs in A549 cells are proteolytically cleaved. This model cell line mimics the ENaC as in edema conditions. We next analyze the effect of TIP peptide in heterologous expression systems. To do so, we transiently transfect hENaC in CHO cells and studied the effect of TIP peptide. Our results show that TIP peptide has direct interaction with ENaC and can modulate the amiloride sensitive sodium current through these channels. In contrast, we barely observe an effect of TIP peptide when applied to the extracellular side of the HNEC cell line RPMI2650. It is widely accepted that two different populations of ENaC are expressed in cells. First, proteolytically cleaved with high open probability called active ENaCs and the second naive with low open probability silent/near silent ENaCs. In RPMI2650 cells ENaC are near silent. To activate these near silent ENaC in RPMI cells we applied Trypsin to the extracellular side and subsequently demonstrate that TIP peptide modulates the sodium current considerably. Our results strongly support a model where modulation of ENaC with TIP peptide AP301 happens in proteolytically active channels and that assaying proteolytic cleavage of ENaC could report on the benefit of therapeutic interventions.
1. Hribar M, et al. (1999) Eur J Immunol 29: 3105-3111

1394-Pos Board B286
Glutamate Receptor Model and Atomistic Simulations; AMPA and NMDA
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Glutamate receptors function as ligand-gated ion channels in the central nervous system and play an important role in excitatory synaptic transmission. To date, there are no fully resolved structures of any Glutamate receptors using any experimental methods such as X-ray crystallography. Since having an atomistic view of the mentioned receptors will help us in better understanding of their structure-function relations we are building atomistic models of the AMPA and NMDA receptor trans-membrane domains. The homology modeling and sequence alignment are based on partially resolved AMPA structure and available potassium ion channels. Molecular dynamics simulations of thus obtained models are then performed with proteins embedded in a fully atomistic lipid. Mechanisms of gating in these channels are then modeled using umbrella sampling and other sampling enhancement techniques.

1395-Pos Board B287
Fast Photoswitching of Tethered Ligands to Study Ionotropic Glutamate Receptor Activation and Desensitization
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Binding of glutamate triggers both the activation and subsequent desensitization of ionotropic glutamate receptors (iGluRs). Matching their role in excitatory neurotransmission, iGluR activation and desensitization are fast processes occurring on the submillisecond and millisecond timescale, respectively. However, little is known about how ligand binding to the four subunits of the tetrameric channel assemblies mediates these processes. Here we address this question using photo-switchable ligands (MAGs) that can be tethered to individual subunits via a cysteine-reactive maleimide group. An azobenzene group serves as photo-switch that allows binding of the glutamate head group in its cis, but not its trans state [1]. This allows us to control ligand binding and