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homodimers. Eight of the C to A single mutants contained a maleimide-reactive thiol in their ectodomains, whereas the wt-mAno1 and the C357A and C631A mutants lacked a free ectodomain thiol. We conclude from this finding that only eight of the cysteine residues are accessible in the ectodomain and that these eight cysteine residues are involved in the formation of four intrachain disulfide bonds per wt-mAno1 protomer. Also all the 28 C to A double mutants that comprise all pairwise combinations possible with the eight extracellularly accessible cysteine residues assembled efficiently as homodimers. However, in contrast to the single mutants, only a very few of the double mutants exposed a free thiol group. We suggest that the six ectodomain cysteines remaining in each of the double mutants can efficiently re-arrange in a manner that prevents in most cases the formation of only one disulfide bond.

341-Pos Board B110
Characterization of the N-Glycosylation on HCN2 Channels
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Hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) belongs to the Cyclic Nucleotide-Gated (CNG) channel superfamily, which has been found to control pacemaker activity in the heart and brain. HCN2 was shown to undergo N-linked glycosylation. However the effect of the glycosylation on the expression and function of HCN2 channels is still under debate. In this study, we use biochemical modification, confocal microscopy and flux assay to investigate the role of N-glycosylation on the membrane trafficking and function of HCN2 channels. We showed that HCN2 mutant N380Q is not N-glycosylated. Confocal microscopy and Western Blot are used to ascertain the subcellular localization of N380Q mutant. By using PNGase-F digestion, WGA lectin beads isolation, and antibody affinity purification from plasma membranes, we showed that N-glycosylation on HCN2 channels is heterogeneous. One step further, we reconstituted HCN2 channels onto liposomes and established a cation flux assay to study the function of reconstituted HCN2 wild type and N380Q mutant. The result generated in this study will answer the question of whether the N-glycosylation is critical for the function of HCN2 channels. The method developed here will provide a platform, which could be applied for studying the functions of other CNG channels.

342-Pos Board B111
Mechanisms of Channel Activation in GLIC
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GLIC is a pentameric proton-gated ion channel with an EC_{50} at pH of 5.0. It remains unclear which regions of GLIC are responsible for channel activation. In this study, we explore two potential regions by combining mutagenesis/functional experiments with computations using the Perturbation-based Markovian Transmission (PMT) model. The first region involves inter-subunit salt bridges between the C-loop (E177 and D178) and β5 (D91) of the complementary side. The second region (N152, D153, and D154) was part of the cavity identified crystallographically for anesthetic ketamine binding (Pan, Chen, *et.al,* Structure, 20, 1463, 2012). Mutations in the first region (D91N, E177Q, D178N) removed salt bridges and resulted in an increase of the EC_{50} (decreased pH to 4.8), suggesting the importance of the salt bridges in stabilizing the open-channel conformation, as confirmed by the less closed C-loop in the X-ray crystal structure. The mutation in the second region (N152C) resulted in a decreased of the EC_{50} (increased the pH to 5.4), presumably due to more flexibility for the protonation of D153 and D154. Using the PMT model, which provides time dependent information on the pathway of signal propagation, we found that perturbations to the first and second regions propagated through two different paths to the pore-lining TM2. The perturbation to the first region travels mainly from loop C, down β10 and β7 to the Cys-loop, and on to TM1 and TM3, which together affect TM2. The perturbation in the second region starts in the middle of the F-loop, travels down along the F-loop into the TM2-TM3 linker, and then to TM2. Taken together, our results reveal residues important to channel activation and allosteric signal pathways. Supported by NIH (R01GM066358, R01GM056257, R37GM049202, and T32GM075770).

343-Pos Board B112
The Inactivated Bacterial Potassium Channel Conducts Water
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The potassium channel KcsA was reported to conduct water at an extraordinarily high rate, which implied a 20-fold increase in mobility of water molecules inside the channel as compared to bulk water [1]. A conformational state of the channel that is permeable to water, but closed to ions would have resulted in an underestimation of channel number, and thus, in an overestimation of water mobility. Here we tested whether the recently described C-type inactivated state of KcsA may represent such a conformation. Therefore, we reconstituted a KcsA mutant in which the inactivation was suppressed with an E71A mutation in the pore helix. KcsA labeling with a fluorescent dye allowed determination of the number of reconstituted channels per vesicle by fluorescence correlation spectroscopy. It was equal to the number of open channels, as revealed by fusing part of these vesicles with planar lipid bilayers. We subjected the remaining part of these vesicles to stopped flow experiments. This approach allowed unitary water permeability calculation of the non-inactivating KcsA variant. It is roughly an order of magnitude smaller than the single channel permeability of the wild type channel, suggesting that the open but inactivated potassium channel is a selective water channel.
[1] Saparov, S. M., and P. Pohl. 2004. Beyond the diffusion limit: Water flow through the empty bacterial potassium channel. Proc. Natl. Acad. Sci. U. S. A. 101: 4805-4809.

344-Pos Board B113
Preliminary Structure/Function Studies of the Human Voltage-Gated Proton Channel
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Voltage-dependent H^+ (Hv) channels mediate proton conduction into and out of cells under the control of the membrane voltage (V_m) and the transmembrane pH gradient (ΔpH). Hv channels are unusual compared to voltage-dependent K^+, Na^+ and Ca^{2+} channels in that Hv channel genes encode a voltage sensor domain (VSD) without a pore domain. Purification of Hv channels to homogeneity and reconstitution into synthetic lipid liposomes demonstrated that the VSD by itself supports H^+ flux. In addition to this unique biophysical behavior, recent research has revealed many important physiological roles for Hv channels in immunity, human male fertility and cancer. An experimentally determined structural model of human Hv channels would greatly contribute to our understanding of how the channel is regulated by V_m and ΔpH and may reveal the conduction pathway for protons through the channel. Furthermore, a structural model could allow for rational development of pharmacological contraceptive or anti-cancer agents. Here, I will be presenting preliminary work towards the biochemical, functional and structural characterization of purified human Hv channels.

345-Pos Board B114
Structural Basis for Ion Permeation Mechanism in Pentameric Ligand-Gated Ion Channels
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Structural studies of ion permeation in pentameric ligand-gated ion channels (pLGIC) need higher resolution crystal structures. We have solved the structure of an open form of GLIC, a prokaryotic pLGIC, at 2.4 Å. It reveals two clearly resolved water pentagons with one sodium ion between them: a self-stabilized ice-like water pentagon at the level of Ser 6' and a second wider water pentagon at the level of Thr 2', in continuous exchange with Glu −2'. Computational methods predict that the position of the ice-like ''tight'' pentagon of water is very sensitive to the position of the hydroxyl group of Ser 6'. Electrophysiology on GLIC wild-type and Ser 6' mutants, including single-channel recordings, confirm that the side chain hydroxyl of Ser 6' is crucial for ion translocation. Simulations that pull a cation through the pore suggest that the ion remains hydrated during permeation and that residue 6' actively contribute to ion translocation by reorienting its side chain when the ion is going through the pore. Generalization of these findings to the pLGIC family is proposed.