By enhancing the genetic code it is possible to incorporate unnatural amino acids (UAAs) with new properties into proteins without restrictions on the site to which they are added. By using the amber suppression method in Xenopus oocytes we have obtained high expression levels with the heterologously expressed voltage-gated Shaker potassium channel (Kv) harboring the fluorescent unnatural amino acid Anap at various key regions. Anap is environmentally sensitive which makes it capable of probing local conformational changes in the channel. In contrast to the traditional post-translational fluorophore labeling technique with thiols-chemistry, we are now capable of probing dynamics on the cytosolic side aswell as within the membrane bilayer. This opens a wide field of structural questions to be addressed since the important dynamics usually resides inside the cell or within the membrane. Anap was thus incorporated on each side of the S4 voltage sensor as well as on the S6 cytosolic gate. With voltage-clamp fluorometry we were able to determine that the four voltage sensors activate independently while the pore opening occurs cooperatively. Against previous models, pore opening required two cooperative movements. We also simultaneously labeled both ends of the voltage sensor S4 and found that dynamics of N- and C-termini differ. The successful use of fluorescent UAAs combined with voltage-clamp fluorometry has made it possible to study internal dynamics in electrogenic membrane proteins and will find widespread application in structural biology.

**2717-Pos Board B409**

**Offsetting the Electric Field Sensed by Kv Channels through Residue Substitutions on Top of S1**


Kv channel subunits consist of 6 transmembrane segments (S1-S6) whereby the S1 through S4 segments assemble into a voltage sensing domain (VSD) that detects the membrane electric field. The positively charged S4 segment forms the main component of the VSD and undergoes the largest reorientations upon a membrane de- or hyperpolarization, generating a transient gating current. The S1-S3 segments surround the S4 and facilitate the latter’s movement across the hydrophobic plasma-membrane. A positive (lysine) and negative (aspartate) charge substitution scan at the extracellular end of the S1 segment in the Shaker-type Kv1.5 channel indicated that this region is sufficiently close to the S4 segment such that it modulates the local membrane electric field. At positions E268, E272, F273 and E276 a charge substitution or charge introduction exerted a surface charge effect and shifted the voltage dependence of channel opening accordingly. Surprisingly, these residues, which modulated the electric field, did not face the S4 in a predicted 3D structure of the Kv1.5 channel in the open state (homology model based on the crystal structure of the Kv2.1/Kv1.2 chimera). This suggests that the introduced charges affect the electrical field around the S4 segment in the closed state only. In conclusion, residues at the top of the S1 segment can state-dependently offset (polarize) the electric field sensed by the S4 segment, supporting that both segments are in close proximity. (This research was supported by fellowship CONACyT 8203936 to EMM & grant FWO-G.0449.11N to DJS.)

**2718-Pos Board B410**

**Alostatic Control of the Inner Activation Gate to the Outer Pore of a Potassium Channel**

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In potassium channels, functional coupling of the inner and outer pore gates may result from energetic interactions between residues and conformational rearrangements that occur along a structural path between them. Here, we show that conservative mutations of a residue near the inner activation gate of the Shaker potassium channel (I470) modify the rate of C-type inactivation at the outer pore, pointing to this residue as part of a pathway that couples inner gate opening to changes in outer pore structure and reduction of ion flow. Because they remain equally sensitive to rises in extracellular potassium, the alterations inactivation rates of the mutant channels are not secondary to modified interaction of the S6 helix. We hypothesize that either the electrostatics or the volume/exposure of the side chains pointing into the cavity can affect this coupling. Accordingly, we have introduced side-chains at position 470 in a Ca2+ - reversible. Raising external K+ to 5-20 mM (in 40 mM Ca2+) largely restores IK. How is Ca2+ suppression and K+ restoration of IK explained? Ca2+ blocks IK through an open channel strongly at negative voltages. We propose that Ca2+ competes with K+ at Site0 (just outside the selectivity filter) where K+ is strongly favored, but Ca2+ occupancy is enhanced at negative voltages. Ca2+ occupancy of Site0 strongly reduces K+ access to and occupancy of Site1 (outmost site of the filter), allowing repulsion among the carbonyls of Site1 to dilate this site, causing C-type inactivation. La3+ has almost the same crystal radius as Ca2+, and is much more potent in suppressing IK: with 10 μM La3+ externally, IK of T449A is largely eliminated (reversibly). We postulate that trivalent La3+ has high affinity for Site0. Neither Ca2+ nor La3+ has strong effects on T449V, which does not exhibit C-type inactivation.

**2720-Pos Board B412**

**Shaker Kv Channel’s Sugar Remotion in Real-Time**

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Voltage-gated K+ channels (Kv) are tetramers, each subunit containing six transmembrane segments (S1-S6). These segments make two functionally and structurally independent domains: an ion conduction pore (S5-S6), and voltage-sensor domains (S1-S4). Like many transmembrane proteins, Kv channels undergo extensive posttranslational modifications, with a significant percentage of the mature protein mass being composed of glycany moieties. Shaker Kv channels are glycosylated at two asparagine residues (N259 and N263) located in the first extracellular loop. Mutating asparagine residues to glutamine abolishes glycosylation but does not prevent cell surface expression of functional channels. Using the same population of Shaker Kv channels, we studied the functional consequences of toxin binding before and after deglycosylation by PNGase F. Binding properties of Hanotoxin, GxTx and Agotoxin remained unaltered by removing glycosylation moieties, suggesting that sugars attached to the channels are not intimately interacting with regions of the voltage sensor domains and the pore where these toxins bind. We also studied ionic and gating currents, before and after deglycosylation with PNGase F. Opening kinetics were substantially slowed down by deglycosylation, as reported previously in many members of the Kv family. Total charge distribution mediated by the voltage sensors of the non-conductive W434F mutant Shaker channels shifted only ~3-4 mV towards positive potentials after deglycosylation. Overall our data provides direct proof to understand how glycosylation contributes to Kv channel function.

**2721-Pos Board B413**

**Locking the Open State of a Voltage-Dependent Concatemer Potassium Channel with Metal Bridges**

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Voltage-gated K+ (IK) channels are homotetramers. Each subunit is formed by six transmembrane segments, S1 to S6. The permeation pathway is formed by S5, the P-region and S6 from each subunit, that assemble around a central canal through which K+ permeate. In response to voltage changes, the voltage sensor domain (S1-S4) undergoes conformational changes that are coupled to the opening and closing of an intracellular gate located at the inner end of S6. It is known, that a cysteine at position 476 in S6 (near the gate) traps the mutant channel in the open state when Cd2+ is added to the intracellular solution. This behavior is caused by a metal bridge between the cysteine at 476 of one subunit and a native histidine at 486 in an adjacent subunit. We constructed a Shaker K+ concatemer that has all 4 subunits linked at the DNA level. When all four V476 is mutated to cysteine, intracellular Cd2+ locked the channels open as in wild-type channels. We are presently developing concatemer channels with one, two and three metal bridges to assess one by one their contribution to the opening of the channels. Because the metal bridges can be disrupted by a H486Y mutation, this approach should allow us to discern the assembly of a functional concatemer channel in the membrane.

**2722-Pos Board B414**

**Mutations in the Cavity Affect the Rate of Slow Inactivation in Shaker K+ Channels**


It has been reported earlier that the activation and inactivation gates of Shaker channels are coupled, and this coupling might be mediated by a rotational motion of the S6 helix. We hypothesize that either the electrostatics or the volume/hydrophobicity of the side chains pointing into the cavity can affect this coupling. Accordingly, we have introduced side-chains at position 470 in a membrane de- or hyperpolarization, generating a transient gating current. This behavior is caused by a metal bridge between the cysteine at 476 of one subunit and a native histidine at 486 in an adjacent subunit. We constructed a Shaker K+ concatemer that has all 4 subunits linked at the DNA level. When all four V476 is mutated to cysteine, intracellular Cd2+ locked the channels open as in wild-type channels. We are presently developing concatemer channels with one, two and three metal bridges to assess one by one their contribution to the opening of the channels. Because the metal bridges can be disrupted by a H486Y mutation, this approach should allow us to discern the assembly of a functional concatemer channel in the membrane.