synchronized activation of multiple (2–4) RyRs basically depends on the existence of FKBP12.6. Notably, FKO cells displayed fractional \( \frac{[\text{H}]}{[\text{R}]} \) of 0.3–0.8 pA. This finding provided direct evidence for the partial opening of a single RyR in situ in the absence of FKBP12.6. The suppression of fractional \( \frac{[\text{H}]}{[\text{R}]} \) events in wild-type cells demonstrated that FKBP12.6 played a key role in coordinating the allosterism among RyR subunits. Taken together, our study demonstrated for the first time that FKBP12.6 mediates both inter- and intra-molecular coordination of RyR gating in intact heart cells.

1079-MiniSymp
Single Molecule Fluorescence Study of the B. Thuringiensis Toxin Cry1Aa Reveals Tetramerization
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Pore forming toxins comprise a class of potent virulence factors that attack their host membrane in a 2- or 3-step mechanism. After binding to the membrane, the toxins enter the membrane by means of monomeric or oligomeric form. Here, we used single subunit counting, to determine the number of oligomers contained in a Cry1Aa toxin as a function of the concentration. Cry1Aa is one of the 6-endotoxins of B. thuringiensis, a soil bacteria that is, because of its specificity for certain insect larvae, used as a biological alternative to chemical pesticides. Cry toxins were purified and fluorescently labeled in their monomeric form. Lipid vesicles of various compositions were incubated with labeled toxin monomers, and supported bilayers formed from the mixture. We recorded the fluorescence intensity over time of distinct fluorescence intensities in the supported bilayer and determined their photobleaching behaviour. As single fluorophores bleach in a single step, the number of steps corresponded to the minimal number of monomers in each oligomer. The oligomerization state showed a concentration dependence which was consistent with both a concentration dependent association rate between monomers and a concentration dependent distribution on the lipid vesicles during incubation. Statistical analysis of the results showed that the toxins enter the membrane in their monomeric form and diffuse laterally to form tetramers, which appears to be the pore forming unit.

1080-MiniSymp
Improved Genetically Encoded Voltage Sensitive Optical Probes Detect Action Potentials and Subthreshold Events
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We improved a genetically encoded voltage sensitive optical probe, a conjugate of the Ecliptic GFP and CiVSP, by modifying the fluorescent protein and its insertion site in the voltage sensitive phosphatase. The signal size is increased from ~1% to ~35% for 100 mV depolarization steps. The improved probes’ dynamics is fitted by the double exponential equation, with fast Tau-on of 10 ms and fast Tau-off of 20 ms. The signal size and dynamics enable the probes to detect single action potentials and subthreshold electrical events in individual cultured neurons with high reliability. These probes advance the possibility of fully optical recording and control of neuron activity.

1081-MiniSymp
Using Voltage-Clamp Fluorometry Technique to Study the Mechanism of the Cooperativity Between Hv Channel Subunits
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In the immune system, the activity of voltage-gated proton channels has been shown to play a key role in the charge compensation for the electron extraction by NADPH oxidase during respiratory burst in phagocytes. In addition, this channel was also found in many other cell types including lung airway epithelia cells, sperms and high metastatic breast cancer cells, in which they have been implicated in the pathophysiology of asthma, pH dependent spermatogenesis and tumor metastasis, respectively. It was not until 2006 that two independent groups discovered the genes coding for the voltage-gated proton (Hv) channels. It was shown that the Hv channel is a dimer and that there is a strong cooperativity between two subunits during activation of this channel. However, how the two subunits in Hv channels cooperatively activate the channel and to what extent this cooperativity affect the channel activation are unknown. In this study, we investigate the detailed molecular mechanism of cooperativity in the dimeric Hv channel by using the combination of two electrodes voltage clamp (TEVC) and voltage clamp fluorometry (VCF). We measure the voltage sensor movement and pore opening of each subunit in a linked-dimer that has two subunits with different activation voltage ranges. Thereby we can determine how one subunit affects the other subunit during voltage sensor movement and pore opening.

Platform: Membrane Transporters & Exchangers I

1082-Plat
Intracellular Proton Access Mechanism of the CLC-e1 Cl-/H+ Exchanger
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CLC-e1 is a bacterial CLC Cl-/H+ exchanger that catalyzes 2:1 exchange of Cl⁻ and H⁺. E148 is the pH-dependent external gate of both Cl⁻ and H⁺ transport and E203 mediates proton transport from intracellular solution to the protein interior. Substitution of non-protonatable residues on either glutamate uncouples the exchanger by completely abolishing H⁺ transport. However, it is not clear how protons in the intracellular solution gain access to E203, which is buried under a cytoplasmic “lid” formed by the protein’s N-terminal helix. Truncation of the N-terminal 29 residues removes this lid and preserves 2:1 coupling of Cl⁻ and H⁺ and absolute transport rate. This result implies that proton transport from intracellular solution to E203 is not rate-limiting in the transport cycle, and that protons are somehow facilitated in their movement to the buried E203. In order to discover proton facilitators, we examined polar residues near E203. Nonpolar mutants of Q207, S446 and R403 only slightly inhibit H⁺-transport rate. However, mutations on the protonic glutamate, E113, and disruption of a nearby salt-bridge (E117I and R209I) decrease H⁺-transport rate ~5-fold. These residues appear to form a water-filled conduit for proton access to E203. Interestingly, nonpolar mutants of E202, located near the dimer interface and near to intracellular solution, show 30-100-fold reduced H⁺-transport rates compared to wildtype. All mutations here preserve H⁺-coupled Cl⁻ transport, although those with severely reduced rates display somewhat higher Cl⁻/H⁺ stoichiometry, indicative of Cl⁻ slippage. One or two crystallographic water molecules were found between E203 and E202. We propose that these water molecules form an access pathway for H⁺ from bulk water, which is blocked by bulky, hydrophobic amino acids at E202.

1083-Plat
Transient Water Channels Connecting the Cytoplasmic and Extracellular Glutamate Gates in CLC-ec1
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CLC-ec1, a bacterial member of the CLC family, exchanges chloride ions and protons across the cellular membrane. A key step of the transport cycle of CLC-ec1 is the transfer of protons between the extracellular and cytoplasmic gates, E148 and E203, respectively. These residues are 15 Å apart, and in death of any intermediate titratable groups needed for proton shuttling. Hence, it is an open question as to how protons shuttle between the two gates. Proton hopping through water chains provides a possible mechanism in this regard, having been investigated in numerous computational studies, given the lack of experimental structural data on water. Two possible chains, either involving Y445 or side-chain rotation of E203, have been proposed based on searching algorithms and short molecular dynamics (MD) simulations. We herein propose another water chain characterized through extended (0.42 μs) MD simulations of CLC-ec1 dimer. The water chain forms frequently (once every 50-100 ns) but transiently (lasting for ~1–2 ns). Neither Y445 nor the side-chain rotation of E203 is needed for water chain. The presence of the water chain, however, coincides with significant side-chain conformational changes of F199 and F357 around the chloride-binding site and F208 and F219 at the dimer interface remote from the ion permeation pathway. We further performed a 0.25 μs simulation of monomeric CLC-ec1, which has been shown to be structurally identical to the dimer, but with a halved activity. We show that water chains don’t form as readily in the monomeric simulation as was observed in the dimer simulation; side-chain conformations of F199, F357, F208 and F219 are also different from the dimer. Our study supports the idea that both local and long-range factors could be important for the CLC-ec1 activity.