1385-Pos Board B277
Super-Resolution Imaging of AKAP79/150 Signaling Complexes using Stochastic Optical Reconstruction Microscopy (STORM)
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Our previous FRET and functional studies suggest AKAP (A-kinase-anchor- ing-protein) 79/150 to be crucial in modulation of ion channel activity and neu- ronal function, by orchestrating important molecules such as protein kinases, phosphatases, G protein-coupled receptors and ion channels into signaling complexes in the plasma membrane. In this study, to directly visualize these AKAP79/150 signaling complexes and interactions between AKAP79/150, ion channels and receptors in sympathetic neurons, we utilized stochastic optical recon- struction microscopy (STORM) with sub-diffraction (~20 nm half width) resolution. STORM uses dyes that can cycle between a dark and a fluo- rescent state thousands of times, thus enabling detection of the precise locali- zation of the center of these scattered spots given by cumulative integration of each cycle. Consistent with previous immunostaining/confocal studies, STORM imaging of fixed Chinese hamster ovary (CHO) cells using fluores- cently labeled antibodies against AKAP150, or KCNQ2 and KCNQ3 subunits revealed the precise plasma membrane localization for the former, and mem- brane and cytoplasmic distribution of the latter, at the single-molecule level. Using multi-color STORM to simultaneous image AKAP150, KCNQ2-3 and G_{G_{q/11}}coupled muscarinic receptors in a subclone of CHO cells, we have identified linker between helices A and B in the C-terminus of KCNQ2 to be the primary site of PIP2- binding, with K452, R459 and R461 shown to interact with the spatial co-distribution of AKAP150 with its signaling partners, and whether loss of AKAP150 causes redistribution of ion channels and receptors.

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Use of Voltage-Sensitive Phosphatase to Investigate the Location of the PIP2-Binding Site on KCNQ2 Channels
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Phosphatidylinositol 4,5-bisphosphate (PIP2) is known to be necessary for the activation of KCN K’ channels. Many studies have investigated the location of the PIP2-binding site on those channels, which have implicated binding to the C-terminus. Our lab has suggested the linker between helices A and B in the C-terminus of KCNQ2 as the primary PIP2 binding site in KCNQ2. We investigated the potential role of those residues in PIP2-channel interactions for KCNQ2. We found mutations at analogous positions in KCNQ2 (K319A, R325A and R327A) to decrease whole cell current amplitudes. However, use of a voltage-sensitive phosphatase from danio rerio, which dephosphorylates almost all PIP2 upon strong membrane depolarizations, revealed the PIP2 affinity of the K319A and R327A mutants to be increased, rather than decreased, as assayed by the rates of current decay upon depolarization to 120 mV, and subsequent current recovery at 30 mV. These results indicate that the decrease of KCNQ2 currents for all the mutants is likely not due to a lower PIP2 affinity for the channels. Indeed, unlike KCNQ1, charge neutralization mutations at the start of the C-terminus seem to augment PIP2-binding to channels, consistent with the previously-identified linker between helices A and B to be the primary PIP2-binding site in KCNQ2 channels.

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Ligand-gated Channels I

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Cleft Closure Mechanism in Gating of Acid Sensing Ion Channel 1A
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Acid sensing ion channels (ASICs) are trimeric proton activated channels which share sequence similarities with Degenerin/Epithelial sodium channels. They are responsible for proton evoked currents in central nervous system of most vertebrates. Their physiological functions include synaptic transmission, sensory perception, learning, and pathophysiology of stroke. Crystal structure of the low pH state of chicken ASIC shows thumb and palm like domains in the extracellular part of each subunit connected through hydrogen bonds between carboxylate residue pairs, thought to be part of proton sensors in ASIC. Based on the low pH structure it’s been hypothesized that these domains would be further apart at high pH due to negative charges and in turn would allow the two domains to move closer, which in turn could trigger changes in the transmembrane segments, causing channel opening. We have used Fluorescence Resonance Energy Transfer(FRET) to study the high pH resting structure of ASIC and determined conformational changes between the thumb and palm domains. Cysteine residues were intro- duced on these domains and tagged with thiol reactive donor and acceptor fluorophores. The recognition site for protease Xa was introduced on either side of the cysteine on the palm domain. This allowed us to perform the FRET measurements in oocytes and HEK-293 cells without purification, as addition of the protease allowed us to quantitatively characterize the back- ground FRET. The FRET constructs were characterized using electrophysiolog- y to establish that they were functional. The FRET based distances showed 3A decrease in distance between thumb domain residue 340 and palm domain residues 130 and 139 upon changing the pH from 6 to 8. In addition in- dustance provides evidence for the hypothesized movement in the extracellular domain of ASIC and also supports the protonation of the carboxylate pairs across these domains.

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An Acidic Ring in the Palm Domain of ASIC1a Facilitates Pore opening
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Acid-sensing ion channels (ASICs) are neuronal cation selective proton- gated ion channels that contribute to nociception, mechanosensation, synap- tic plasticity, learning and memory, and fear conditioning. ASIC subunits have two transmembrane helices, intracellular N- and C- termini, and a large extracellular region organized in discrete domains named the thumb, finger, palm, beta-ball and knuckle. The palm domain is directly connected to the pore-forming transmembrane helices. We combined site-directed mutagene- sis and electrophysiology to examine the contribution of residues Glu79 and Glu416 to ASIC1a proton-gating and desensitization. These acidic residues are located in the core of the palm domain forming a ring-like structure. Individ- ual substitution at these positions to Cys, Lys or Gln shifted the apparent proton affinity of ASIC1a approximately one pH unit toward more acidic values. A similar shift in apparent proton affinity was observed in channels bearing substitutions at both positions, indicating that Glu79 and Glu416 cooperatively contribute to proton-gating. E79C and E416C mutants were modified by MTSET in the closed, but not in the desensitized state. MTSET-modification of E79C increased the magnitude of the response to protons up to six-fold and slowed desensitization by ten-fold, while MTSET-modification of E416C only reduced the response to protons nearly 50 percent. Ala mutagenesis at neighboring positions of Glu79 indi- cates that the covalently modified side chain of Cys at position 79 became closer to residues in the thumb and palm domains during channel gating. Our results suggest that Glu79 and Glu416 facilitate pore opening, and that the palm domain experiences a rotation during proton-dependent activa- tion and desensitization.