1300 ± 309 versus 225 ± 84/μm^2). Three characteristics of these particles are similar to the tetrameric CaV1.1 channels of skeletal muscle: 1) diameter, only slightly smaller than that of CaV1.1; 2) unusual height indicated by the platinum free ''shadow''; 3) square, slightly scalloped outline of the same shadow in some images. It is reasonable to postulate that the ''large and tall'' particles represent tetrameric Orai1 channels clustered in correspondence with cortical Stim-bearing ER junctions. These observations introduce a new approach to visualize individual unlabeled Stim and Orai molecules in situ. Funded by NIH RO1 HL-48093 (CFA) and NS-14609 (MDC).

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Electronic Viscosity Affects Diffusion through Membrane Electric Fields Near Channel Pores

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A polar water molecule will interact more frequently with the charge distributed over kosmotropic (Na^+. Ca^{2+}) ionic surfaces compared to the dipole of adjacent waters. Ionic charge is spread over the combined surface area of the ion-water complex. Additional water molecules will continue to join this complex and bind tightly until the surface charge density of the ion-water complex is equal in strength to the molecular dipole charge density of water. Electronically, the hydrated ion looks like water at its surface. When an electric field applies a force (F) to ions in solution, Stoke's Equation states that ions will migrate through a medium of viscosity (η) with velocity (v) proportional to radius (r): $F=6\pi\eta rv$. Driving calcium ions at low electric fields (<300 V/cm), ion velocity was measured using capillary electrophoresis. Assuming the viscosity of water, the effective radius of hydrated calcium is 0.334 nm, yielding a volume that corresponds to 5.09 water molecules. At high electric fields (>500 V/cm), the calcium hydration shell is stripped and its radius is 0.1 nm. The resulting 3-fold drop in radius should correspond to a 3-fold increase in migration velocity if the viscosity is unchanged, but hydration stripping produces only a 33% increase in velocity. Therefore, the viscosity must increase by 2.56 times that of water. Unlike the comparable charge densities of hydrated ions and water, the stripped ion carries its exposed charge through a sea of molecular dipoles. We attribute the observed submaximal rise in migration velocity to this 'electronic viscosity'. As the membrane electric field at kosmotropic ion channel pore entrances is sufficiently strong to strip an ion, it is our position that permeation models should start from a dehydrated ion, albeit one whose diffusion is limited by electronic viscosity.

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Thermodynamic Comparison of Dysferlin C2A Wild Type, and C2A V-1 Domains, to the Synaptotamin I C2A and C2B Domains

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Through regular use the plasma membrane or, sarcolemma, of myocytes undergoes a number of physical disruptions which in order for the cell to survive must be effectively and rapidly repaired. Currently, it is believed that small lesions in the cellular membrane are repaired through a calcium dependent mechanism which patches the lesion through the rapid exocytosis of intracellular vesicles. Synaptotagmin I is a transmembrane protein which has been shown to play an integral role in vesicular fusion in neurons. Dysferlin is highly homologous to Synaptotamin I which has led to speculation that it plays a critical role in the final steps of vesicular fusion leading to the formation of a membrane patch over the lesion. Dysferlin consists of a single transmembrane region linked to seven C2 domains and two DYSF domains. A C2 domain is a structural motif that consists of an eight strand β-sandwich; these domains are involved in binding both calcium and phospholipids. In the present study, the stability of the wild type Dysferlin C2A domain and an isoform of the C2A domain, V-1, are compared to the stabilities of the C2A and C2B domains of Synaptotagmin I. By comparing these domains we have gained valuable insight into the physiological function of Dysferlin. In the absence of ligand the free energy of denaturation at 37°C of the Dysferlin C2A domains was found to be 0.17 ± 0.02 and 0.51 ± 0.02 in (kcal/mol) for the wild type and V-1 isoform respectively, compared to 2.32 ± 0.05 and 1.74 ± 0.9 in (kcal/mol) for the Synaptotagmin I C2A and C2B domains.

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Low Resolution Structure of Mitochondrial Rho Protein via SAXS

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The results of a small-angle x-ray scattering (SAXS) study of the mitochondrial rho (miro) protein are discussed. The radius of gyration in solution is found to be 44.4 +/- 0.2 Angstrom from the pair-distance distribution function, in agreement with previous estimations. A DAMMIN reconstruction using 1100 dummy atoms with 3.6Angstrom radius shows a compact conformation featuring several sub-units. This agrees with a sequence analysis suggesting that miro contains at least two GTPase domains and two central EF-hand domains. We also show that within the resolution of our measurements miro remains structurally unchanged in the presence of 3mM Calcium.

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Spectroscopic Study Ca^{2+} Induced Changes in the Structure, Dynamics and Stability of Dream Protein and its Mechanism of DNA Interaction

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Downstream Regulatory Element Antagonist Modulator (DREAM)/Calsenilin/KchIP3 is a multifunctional calcium binding protein that belongs to the EF-hand branch of Neuronal Calcium Sensor family. DREAM associates to Downstream Regulatory Element (DRE) of *prodynorphin* and *c-fos* genes and blocks their transcription in a calcium-regulated manner. Other molecular functions of DREAM involve proteolytic processing of presenilins, modulation of A-type current of potassium channels, and regulation of neuronal apoptosis. Previously, we have studied Mg^{2+} and Ca^{2+} induced changes in structure and dynamics of DREAM and its C-terminal domain. Recently, we have investigated the contribution of individual EF hands (EF-2, EF-3, and EF-4) to Ca^{2+} triggered conformational transition in DREAM by characterizing fluorescence properties of D150N, E186Q, and E234Q mutants. The tryptophan 169 emission and lifetime properties are strongly influenced by Ca^{2+} association to EF-3 whereas the ligand association to EF-2 and/or EF-4 has a minor impact on Trp fluorescence suggesting that Ca^{2+} association to EF-3 is crucial to induced structural changes within the hydrophobic pocket between EF-2 and EF-3. In addition, association of 25-mer oligonucleotide of *prodynorphin* gene to DREAM was characterized using ITC. DNA association to DREAM is temperature dependent. ApoDREAM strongly binds to DynDRE at 35°C (Kd_1 =206 nM, Kd_2 = 24 μM) whereas weaker interactions were observed at at 25°C). Very weak binding with Kd = 91 μM and Kd = 200 μM was observed for DREAM-DynDRE association in the presence of Mg^{2+} and Ca^{2+}, respectively. These results support the mechanism that ApoDREAM strongly binds to DynDRE to block *prodynorphin* gene transcription and the DREAM-DynDRE complex reversibly dissociates upon Ca^{2+} binding.

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Modulation of the KV4.3-KchIP3 Interactions by Ca^{2+} and NS5806

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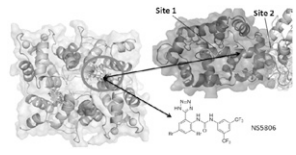
Here we report how the interactions between Kv4.3 T1 domain and KChIP3 are modulated by Ca^{2+} binding to KChIP3 as well as by the ITO activator NS5806. The affinity of KChIP3 for Site 1 of Kv4.3 T1 domain was measured to be $KdCa^{2+}$= 9 μM, whereas binding to Site 2 shows a large Ca^{2+} dependence with Kdapo= 126 μM and $KdCa^{2+}$= 22 μM. Moreover, NS5806 binds to KChIP3 with Kd = 5 μM in a Ca^{2+} independent manner, and lowers the Ca^{2+} affinity of both EF-hand in KChIP3 from 1.1 μM and 3.7 μM to 3.6 μM and 7.7 μM. NS5806 also lowers the affinity between KChIP3 and Site 1 to 18 μM but does not affect the Site 2 interactions. Together, these results indicate that the in vivo and electrophysiological effects observed due to NS5806 may be the results of this drug binding to KChIP3 and affecting its sensitivity to Ca^{2+} as well as Kv4.3 interactions.



533-Pos Board B302

Brownian Dynamics Study of Current and Selectivity of Calcium Channels

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Brownian Dynamics (BD) simulation is a powerful approach to investigate ion permeation properties through protein ion channels. BD does not require the explicit evaluation of the motion of all the particles in the system. Only ions' trajectories are computed. This results in a small computational burden that allows micro-second scale simulations, long enough for the reliable estimate of ionic currents. We studied ion permeation properties and estimated ion currents through calcium channels, using a simplified channel model and