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$1300 \pm 309$ versus $225 \pm 84/\mu m^2$). 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).

**528-Pos Board B297**
**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 ($\eta$) 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.

**529-Pos Board B298**
**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 $\beta$-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 $0.17 \pm 0.02$ and $0.51 \pm 0.02$ in (kcal/mol) for the wild type and V-1 isoform respectively, compared to $2.32 \pm 0.05$ and $1.74 \pm 0.9$ in (kcal/mol) for the Synaptotagmin I C2A and C2B domains.

**530-Pos Board B299**
**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.

**531-Pos Board B300**
**Spectroscopic Study $Ca^{2+}$ Induced Changes in the Structure, Dynamics and Stability of Dream Protein and its Mechanism of DNA Interaction**
**Khoa N. Pham**, Jaroslava Miksovska.
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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 $\mu$M) whereas weaker interactions were observed at at 25°C). Very weak binding with Kd = 91 $\mu$M and Kd = 200 $\mu$M 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.

**532-Pos Board B301**
**Modulation of the KV4.3-KchIP3 Interactions by $Ca^{2+}$ and NS5806**
**Walter Gonzalez**, Jaroslava Miksovska.
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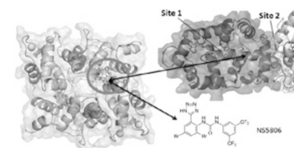
Here we report how the interactions between Kv4.3 T1 domain and KChIP3 are modulated by $Ca^{2+}$ binding to KChIP3 as well as by the ITO activator NS5806. The affinity of KChIP3 for Site 1 of Kv4.3 T1 domain was measured to be $KdCa^{2+}$= 9 $\mu$M, whereas binding to Site 2 shows a large $Ca^{2+}$ dependence with Kdapo= 126 $\mu$M and $KdCa^{2+}$= 22 $\mu$M. Moreover, NS5806 binds to KChIP3 with Kd = 5 $\mu$M in a $Ca^{2+}$ independent manner, and lowers the $Ca^{2+}$ affinity of both EF-hand in KChIP3 from 1.1 $\mu$M and 3.7 $\mu$M to 3.6 $\mu$M and 7.7 $\mu$M. NS5806 also lowers the affinity between KChIP3 and Site 1 to 18 $\mu$M 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

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Brownian Dynamics. We modeled the carboxylate-rich selectivity filter of calcium channels with 8 independent half-charged oxygens confined in the central region of the pore. We computed ions' trajectories self-consistently evaluating the electrostatic forces acting on the ions at every timestep. Such forces were evaluated solving Poisson's equation with a Boundary Element Method to deal with dielectric boundaries, called Induced Charge Computation method (ICC). A transmembrane potential was included as a spatially constant component of the electric field, a good approximation to a fully consistent treatment, see Crozier et al. (Biophys. J. 81:3077) and Hollerbach and Eisenberg (Langmuir, 18:3626). Boundary conditions for ionic concentrations in the intra- and extra-cellular domain were imposed by a Grand Canonical-Monte Carlo algorithm. We simulated different concentrations of $CaCl_2$ added to NaCl solution only on one side of the membrane. Ion permeation was investigated under physiological conditions, using different sub-millimolar calcium concentrations and different transmembrane potentials. Channel selectivity and conductance were determined by electrostatic forces, steric repulsion due to charge crowding, and gradients of concentration and potential.

**534-Pos Board B303**

**Real-Time Modulation of Zebrafish Cone Phototransduction by Whole-Cell Delivery of zGCAP3 and of its Monoclonal Antibody**

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Regulation of excitation and adaptation in photoreceptors of the vertebrate retina strongly depends on the cytoplasmic $Ca^{2+}$ concentration and its interplay with $Ca^{2+}$ sensor proteins like recoverin, calmodulin and the activating proteins (GCAPs) of guanylate cyclase (GC) (Scholten and Koch, 2011). Of the four GCAP isoforms exclusively transcribed in cones (zGCAP3, 4, 5 and 7), we investigated the physiological function of zGCAP3 in green-sensitive cones of zebrafish, by recording the effect on the photoresponse waveform by cytosol injection of exogenous zGCAP3 (to simulate ''real time'' protein over-expression), and its monoclonal antibody (to simulate protein knock-down). To identify a suitable antibody we screened several hybridoma fluids with respect to specificity and affinity towards zGCAP3, using immunoblotting and surface plasmon resonance (SPR) spectroscopy. The global fitting of an overlay of SPR sensorgrams obtained with increasing antibody concentrations gave a $Ca^{2+}$-independent $K_D$ of 12 nM for the interaction of zGCAP3 with the antibody. Exogenous proteins were incorporated with a precise timing in the zebrafish cone cytosol by an internal perfusion system coupled to a pressure-polished patch pipette (Benedusi et al. 2011). Typical whole-cell recordings lasting even more than 20 min did not show any significant change in light sensitivity, dark current amplitude, response kinetics and light adaptation, proving also that the enzymatic cascade was not perturbed by the recording protocol. Injection of anti-zGCAP3 caused the complete shutdown of the dark current, indicating that zGCAP3 plays a major role in regulating GC. Injection of purified zGCAP3 did not alter the photoresponse, indicating that the target GC was already saturated with endogenous zGCAP3.

Benedusi M, Aquila M, Milani A and Rispoli G (2011). Eur Biophys J 40: 1215-23.
Scholten A, Koch KW. (2011). PLoS One 6(8):e23117.

## Excitation-Contraction Coupling I

**535-Pos Board B304**

**Effects of Redox Environment on Calcium Alternans in Isolated Rabbit Cardiomyocytes**

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Cardiac alternans is a multifactorial phenomenon linked to cardiac arrythmias. At the cellular level cardiac alternans is defined by beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (electrical or action potential duration alternans) and Ca transient amplitude (Ca alternans) at constant stimulation frequency. The aim of this project was to characterize the effect of changes in the cellular redox environment on Ca alternans in cardiac myocytes. Single myocytes (from New Zealand White rabbits) were isolated enzymatically by retrograde Langendorff perfusion. Ca alternans were induced by incrementally increasing the pacing frequency (electrical field stimulation) until stable Ca alternans occurred. The frequency at which stable Ca alternans were observed varied from cell to cell and ranged from >1 to 2.5 Hz at room temperature. Global cytosolic Ca transients were measured with Indo-1. In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously measured with the fluorescent Ca indicators Rhod-2 and Fluo-5N, respectively. Confocal microscopy was used to measure Ca sparks with Fluo-4.
Reducing agents dithiothreitol and reduced glutathione partially abolished Ca and mechanical alternans by restoring diastolic Ca and Ca transient amplitudes. A decreased sarcoplasmic reticulum (SR) Ca release flux but not Ca content, together with a decreased Ca spark frequency, suggest that reducing agents normalized alternans through effects on the SR Ca release channel (ryanodine receptor type-2). Addition of a membrane permeant superoxide dismutase mimetic, Tempol, had little effect on Ca alternans, suggesting the possible role of dithiotreitol directly acting on the ryanodine receptor. These data highlight that the redox state of the cell may be important in the generation of Ca and mechanical alternans during oxidative stress.

**536-Pos Board B305**

**Modeling the Effect of Unitary Calcium Current on Neighboring Ryanodine Receptors during Calcium Induced Calcium Release**

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In resting cardiac cells, the open probability (Po) of single ryanodine receptors (RyRs) is very low and consequently little $Ca^{2+}$ is released from the sarcoplasmic reticulum (SR). However, a stochastic RyR opening will cause diastolic local $Ca^{2+}$ release from the SR that can activate neighboring closed RyRs. This inter-RyR $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) may generate diastolic $Ca^{2+}$ sparks. It is known that elevating SR $Ca^{2+}$ load above normal levels dramatically increases spark frequency and increases the unitary RyR $Ca^{2+}$ current. It is this current that acts on neighboring RyRs through CICR. We have developed a simple model based on experimental single-channel RyR $Ca^{2+}$ sensitivity to understand how unitary RyR $Ca^{2+}$ current may control CICR within a group of neighboring RyRs (a $Ca^{2+}$ release unit, CRU). The model predicts how the current carried by an open RyR influences the activity of neighboring RyRs in a CRU. These predictions match published experimental single and clustered RyR channel results obtained in bilayer studies.

**537-Pos Board B306**

**On the Role of Endogenous Calmodulin in Excitation-Contraction Coupling in Skeletal Muscle**

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In skeletal muscle, calmodulin (CaM) regulates excitation-contraction coupling, primarily via modulation of ryanodine receptors. Here we aimed to further our understanding of the role of endogenous CaM in excitation-contraction coupling. Since systemic ablation of CaM in mice is difficult to achieve due to CaM's multiple functions, in vivo gene transfer via electroporation mediated transfection method was used to deliver plasmid coding for both cerulean and short-hairpin (sh)RNA targeting CaM (shRNA-CaM) to study the effect of CaM knockdown in adult mouse *flexor digitorum brevis* skeletal muscle. CaM protein expression levels were significantly reduced in shRNA-CaM fibers, which exhibited no evident morphological changes when compared to the shRNA-control fibers. After confirming the reduction of endogenous CaM expression, we used high-speed confocal microscopy and rhod2-based $Ca^{2+}$ imaging to assess the consequence of CaM knowdown on action potential (AP)-evoked $Ca^{2+}$ signals. Isolated single muscle fibers expressing shRNA-CaM exhibited decreased mean peak amplitude and slowed decaying phase of AP-induced $Ca^{2+}$ transient when compared to the shRNA-controls, indicating compromised $Ca^{2+}$ release and $Ca^{2+}$ uptake. We also used a model for myoplasmic $Ca^{2+}$ binding and transport processes to calculate AP-evoked sarcoplasmic reticulum $Ca^{2+}$ release flux, which demonstrated decreased $Ca^{2+}$ release flux and indicated suppressed $Ca^{2+}$ uptake in shRNA-CaM fibers. Decreased $Ca^{2+}$ release could reflect decreased coupling between Cav1.1 and ryanodine receptor, a reduction in expression of one or both proteins or a decreased store content, whereas a slowed decaying phase is consistent with compromised $Ca^{2+}$ uptake. Our study shows the importance of endogenous CaM in the maintenance of excitation-contraction coupling in adult skeletal muscle and could provide new avenues to further explore the potential role of both CaM-dependent and CaM-independent pathways in skeletal muscle contractility and plasticity. Supported by NIH-NIAMS Grant R37-AR055099.