

2013

## Calcium Concentration Fluctuations and Subspace Volume Influence Calcium-Regulated Calcium Channel Gating and Subspace Dynamics

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### Recommended Citation

Weinberg, S. H., & Smith, G. D. (2013). Calcium Concentration Fluctuations and Subspace Volume Influence Calcium-Regulated Calcium Channel Gating and Subspace Dynamics. *Biophysical Journal*, 104(2), 367a.

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(HSP)-60. GAT-3 is expressed in unmyelinated axons and glial processes, whereas HSP-60 is present in the cytosol and mitochondria, and is implicated in translocation of mitochondrial proteins from the cytoplasm. Immunocytochemical studies in HEK293T cells showed that MaxiK colocalizes with GAT-3 at the plasma membrane and HSP60 at the cell periphery. These results indicate that MaxiK could be playing a role in modulating GABA release from the presynaptic nerve terminals via GAT-3, and HSP-60 could be involved in translocating MaxiK to the mitochondria. Supporting the latter, we have confirmed the presence of MaxiK in isolated brain mitochondria using immunocytochemistry. Further studies will help to understand the role of MaxiK in modulating GAT-3 or vice versa and the role of HSP-60 in targeting MaxiK to brain mitochondria. Supported by AHA and NIH.

#### 1878-Plat

##### External Architecture of the Large-Conductance $\text{Ca}^{2+}$ and Voltage-Activated $\text{K}^+$ ( $\text{BK}_{\text{Ca}}$ ) Revealed by a Spectroscopic Ruler

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$\text{BK}_{\text{Ca}}$  channels are involved in a large variety of physiological processes and regulatory  $\beta$  subunits are one of the mechanisms responsible of creating  $\text{BK}_{\text{Ca}}$  channel diversity fundamental to the adequate function of many tissues. Regardless the proven importance of this channel little is known about its detailed structure. Here we disclose the external architectural intimacies of  $\text{BK}_{\text{Ca}}$  channels using Lanthanide based Resonance Energy Transfer (LRET) as a molecular ruler to measure intra and intermolecular distances. We introduced a genetically encoded lanthanide binding tag (LBT that binds  $\text{Tb}^{3+}$  with high affinity) at several positions of the external loops of the  $\alpha$  and  $\beta 1$  subunits, and constructed a fluorescent molecule of BODIPY-FL linked to a scorpion toxin, iberiotoxin (Bodipy FL-IbTX), that was used as an acceptor for the LRET interaction with  $\text{Tb}^{3+}$ . These functional LBT- $\text{BK}_{\text{Ca}}$  constructs were expressed in *Xenopus laevis* oocytes that were voltage clamped with two microelectrodes to obtain simultaneously electrical and LRET recordings under physiological ionic conditions. Sensitized emission (SE) recordings from different LBT- $\text{BK}_{\text{Ca}}$  positions had different kinetics indicating different relative positions for each construct. We analyzed SE records with a novel method developed by our group that determines the position of LBT-tagged sites of  $\text{BK}_{\text{Ca}}$  to obtain an external structural map, including the  $\beta 1$  subunit. Interestingly, when the  $\text{BK}_{\text{Ca}}$   $\alpha$  subunit was co-expressed with the regulatory  $\beta 1$  subunit, SE becomes slower, indicating a large conformational change of the  $\text{BK}_{\text{Ca}}$  channel structure. The methodology presented here gives us the first glimpses to the  $\text{BK}_{\text{Ca}}$  channel external surface structure in its different functional states with and without the  $\beta 1$  subunit. Supported by Fondecyt grant 1110430 and NIH grants U54GM087519 and GM030376. CINV is a Scientific Millennium Institute.

#### 1879-Plat

##### Crystal Structures of the MthK RCK Domain Reveal Allosteric Interactions Among Calcium Binding Sites

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Regulator of  $\text{K}^+$  conductance (RCK) domains form a conserved class of modulatory domains that undergo conformational changes with binding of metal cations and other ligands, to control gating of channels and transporters. In MthK, a prototypical RCK-containing  $\text{K}^+$  channel, each of the channel's eight RCK domains binds multiple  $\text{Ca}^{2+}$  ions to reach the fully-activated state, which can give rise to a complex conformational trajectory. Here we present crystal structures of the MthK RCK domain bound with  $\text{Ca}^{2+}$  in a series of singly-, doubly-, and triply-ligated states. These structures begin to reveal local conformational changes in this RCK domain that may arise from binding of  $\text{Ca}^{2+}$  at individual sites and pairs of sites over a range of ionic conditions, providing insight toward interactions among the sites that may modulate channel gating. Crystals formed at low to moderate  $[\text{Ca}^{2+}]$  show  $\text{Ca}^{2+}$  bound only at a single site, termed C1, determined by residues D184, E210, and E212. In contrast, high  $[\text{Ca}^{2+}]$  (in otherwise identical conditions) results in a new crystal form, with  $\text{Ca}^{2+}$  bound at sites C1, C2 (near residues E248 and E266), and C3 (residues D305 and E326). The mutation D184N, which abolishes  $\text{Ca}^{2+}$  binding at C1, permits  $\text{Ca}^{2+}$  binding at C3 with moderate  $[\text{Ca}^{2+}]$ , suggesting that  $\text{Ca}^{2+}$  binding at C1 inhibits binding at C3. This apparent negative coupling between sites C1 and C3 can be alleviated by the mutation E212Q, which permits  $\text{Ca}^{2+}$  binding at both C1 and C3 and facilitates  $\text{Ca}^{2+}$ -dependent activation. These results suggest a structural basis for allosteric interactions that, in turn, modulate  $\text{Ca}^{2+}$ -dependent gating of the MthK channel.

#### 1880-Plat

##### Targeting the Channel-Calmodulin Interface of Small-Conductance $\text{Ca}^{2+}$ -Activated Potassium Channels

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Small- and intermediate-conductance  $\text{Ca}^{2+}$ -activated potassium channels, activated by  $\text{Ca}^{2+}$ -bound calmodulin, play an important role in regulating membrane excitability. These channels are also linked to clinical abnormalities. A tremendous amount of effort has been devoted to developing small molecule compounds targeting these channels. However, these compounds often suffer from low potency and lack of selectivity, hindering their potentials for clinical use. A key contributing factor is the lack of knowledge of the binding site(s) for these compounds. Here we report our discoveries of the binding pocket for the compounds of the 1-EBIO class, located at the calmodulin-channel interface, by X-ray crystallography. Mutations of the channel, based on the structure data and molecular docking, can effectively change the potency of these compounds. Our results provide insight into the molecular nature of the binding pocket and its contribution to the potency and selectivity of the compounds of the 1-EBIO class.

#### 1881-Plat

##### Calcium Concentration Fluctuations and Subspace Volume Influence Calcium-Regulated Calcium Channel Gating and Subspace Dynamics

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Cardiac myocyte calcium signaling is often modeled using deterministic ordinary differential equations (ODEs) and mass-action kinetics. However, spatially restricted "domains" associated with calcium influx are small enough that local signaling may involve 1-100 calcium ions. Therefore, the question arises: is it appropriate to model the dynamics of subspace calcium using deterministic ODEs or, alternatively, do we require stochastic descriptions that account for the fundamentally discrete nature of these local calcium signals? To address this question, we constructed a minimal Markov model of a calcium-regulated calcium channel and associated subspace. We compared the expected value of subspace calcium concentration and channel open probability (a result that accounts for the small subspace volume and concentration fluctuations) with the corresponding deterministic model (an approximation that assumes large system size and ignores concentration fluctuations). When subspace calcium did not regulate calcium influx, the deterministic and stochastic descriptions agreed. However, when calcium-binding altered channel activity in the model, the continuous deterministic description often deviated significantly from the discrete stochastic model, unless the subspace volume is unrealistically large and/or the kinetics of the calcium binding are sufficiently fast, demonstrating that the calcium concentration fluctuations and subspace volume influence channel gating and subspace dynamics. This principle was also demonstrated using a physiologically realistic model of calmodulin regulation of L-type calcium channels introduced by Yue and coworkers [Tadross, Dick, Yue. Cell 133: 1228-40, 2008]. Additional work will consider the influence of slow and rapid buffers present in the subspace and whether and under what conditions these buffers mitigate the effects of concentration fluctuations on channel gating and subspace dynamics.

## Platform: DNA Replication, Recombination, and Repair

#### 1882-Plat

##### Direct Observation of Stalled Fork Restart and Lesion Bypass via Fork Regression in the T4 Replication System

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The restart of a stalled replication fork is a major challenge for DNA replication. Depending upon the nature of the damage, different repair processes might be triggered; one is template switching that is bypass of a leading strand lesion via a Holliday junction formed by fork regression. using Magnetic Tweezers (MT) to study the T4 bacteriophage enzymes, we have reproduced *in vitro* the complete process of template switching. We show that the UvsW DNA helicase in cooperation with the T4 holoenzyme can overcome leading strand lesion damage by a pseudo stochastic process periodically forming and