Determination of the Stoichiometry Between α and γ1 Subunits of the BK Channel using LRET

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control. These chaperones also communicate with the ubiquitin-proteasome pathway to clear misfolded proteins from the cell. Protein quality control in the eukaryotic cytosol relies on the sequestration of misfolded cytosolic proteins in specific quality control compartments. Our studies of chaperone function provide a framework to understand the link between protein misfolding and human disease.

2695-Symp
Structure and Dynamics of HIV-1 Capsid Assemblies: Insights from an Integrated Approach
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HIV-1 capsids, assembled from ~1,500 copies of the capsid (CA) protein, are an integral part of mature virions. Conical in shape, capsids enclose the viral genetic material (two copies of RNA) together with several proteins that are essential for viral replication. In the assembled state, capsids are remarkably dynamic, with the CA residue motions occurring over a range of timescales from nano- to milliseconds. These motions are functionally important for capsid's assembly, viral maturation, and interactions with host factors. In this talk, an integrated MAS NMR, DNP, molecular dynamics, and Density Functional Theory approach will be presented to probe the functionally important motions in assemblies of CA and their complexes with host factor Cyclophilin A (CypA), as well as assemblies of CA-SP1 maturation intermediates. The role of dynamic allosteric regulation in capsid's assembly, maturation, and escape from the CypA dependence will be discussed. It will be demonstrated that the integration of experimental NMR and DNP methods and theory, at classical and quantum mechanical levels, yields quantitative, atomic-level insights into the dynamic processes that govern the capsid's function.

2696-Symp
Functional Dynamics of Modular Multi-Domain Proteins
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Fundamental processes driving the operation, propagation, and maintenance of cells are carried out by multi-protein machines. Machines require moving parts and in the case of *protein* machinery, the movement of the machine's parts is enabled by making the constituent proteins modular, linking multiple globular domains together with flexible tethers. Flexibility between domains in these proteins is critical to the movement between the steps that provide the overall output of the machine. While clearly important for function, inter-domain flexibility poses a significant challenge for structural analysis because the spatial organization of the domains (i.e. the architecture) is not static but rather time dependent. Our laboratory applies a combination of NMR and SAXS approaches in solution to define the architectural dynamics of modular multi-domain proteins through their trajectory between functional states. In this presentation I will describe our progress in investigating human DNA replication machinery, characterizing the ubiquitous eukaryotic ssDNA binding protein, replication protein A (RPA), that coats template DNA as it is unwound and the DNA-dependent RNA polymerase, DNA primase, which synthesizes the first ~10 nucleotides of primer on the template. Our results show how changes in the architectural dynamics can modulate RPA affinity for ssDNA and provide a model for its function as a scaffold at the core of the DNA replication machinery. They also reveal the trajectory of changes in the architectural dynamics of DNA primase from pre-initiation through elongation and hand-off to polymerase α, suggesting why synthesis of the initial di-nucleotide is the rate-limiting step in initiation and providing insight into changes in configuration and architectural dynamics as it elongates the primer.

Platforms: Voltage-gated K Channels II

2697-Plat
Determination of the Stoichiometry Between α and γ1 Subunits of the BK Channel using LRET
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Large conductance voltage- and calcium-activated potassium channels, BK, are expressed in many mammalian tissues where they play a large variety of physiological roles. BK channels are tetramers of the main α subunit. Its function is modulated by the interaction with two families of tissue-specific accessory proteins, β and γ. Notably, in the absence of Ca^{2+} the γ1 subunit promotes large shifts of the BK conductance-voltage curve towards more negative voltages. However, very little is known about how α and γ1 subunits interact. In particular, what is the association stoichiometry between α and γ1 subunits. Here, we propose a method to answer this question, using lanthanide resonance energy transfer (LRET). The donor was Tb^{+3} that was chelated in lanthanide binding tags (LBT) engineered either into the C-terminal of the α S1 transmembrane segment, or in the N-terminal of the γ1 TM segment, or in both places. The acceptor was a Bodipy dye attached to the pore blocker iberiotoxin. We then used LRET to determine the stoichiometry of the α/γ1 complex using a method that assumes that the kinetics of LRET sensitized emission (SE) of the double-labeled complex is the linear combination of the individual kinetics of the SE in single-labeled complexes. The result is that γ1 associates with α subunit with a maximal 1:1 stoichiometry. In addition, we found that the kinetics of the SE when only γ1 is LBT-labeled is slower than that found when only α is LBT-labeled, suggesting that the accessory subunit is peripherally located in the assembly. We propose that this method is applicable to determine the stoichiometry of association of proteins subunits within heteromultimeric complexes. Supported by FONDECYT grants 1150273 (to R. L.). The CINV is a Millennium Institute.

2698-Plat
Resolving the BK Channel Voltage Sensor Activation Transition with Relative Atomic Coordinates Under Physiologically-Relevant Conditions
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Large-conductance, voltage- and Ca^{2+}-gated K^+ channels (BK, Slo1) are potent regulators of cellular excitability. Our understanding of BK structure and Ca^{2+}-dependent gating was recently greatly advanced by cryo-EM-derived structures; however, the mechanism of voltage-dependent BK activation remains unclear. We applied the new optical approach, distance-resolving Voltage Clamp Fluorometry (drVCF), to determine the distances between transmembrane helices in the BK voltage-sensing domains (VSDs), in human channels expressed in *Xenopus* oocytes under the cut-open oocyte voltage clamp.
In this implementation, drVCF was enhanced with atomic "GPS", to determine the distance and orientation of position 203 (S4) relative to surrounding helices. We found that, at rest, the Cα atom of position 136 (S1) is 7.5 [6.8,8.5] Å (mean, 95% C.I.) away from ^{203}Cα. This distance increases upon activation to 13.7 [13.1,14.7] Å. In both states, ^{136}Cα is nearer ^{203}Cα than ^{203}Cβ; that is, the W203 side-chain points away from S1. Relative to the W203 Cα-Cβ axis, ^{136}Cα lies at 138° [128°,147°] at rest and 163° [157°,170°] upon activation. ^{145}Cα (S2) is 6.8 [6.2,7.6] Å away from ^{203}Cα at rest, diverging to 13.1 [12.5,14.1] Å upon activation. S2 also sits "behind" W203: 146° [113°,166°] at rest; 147° [132°,158°] upon activation. Finally, ^{19}Cα (S0) is 15.6 [14.1,17.0] Å away from ^{203}Cα at rest and 20.3 [18.5,22.3] Å in the Active state. W203 points more towards S0 (Resting: 48° [38°,66°]; Active: 92° [72°,115°]) than S1 and S2.
Using these spatial constraints, acquired from conducting human channels in a cellular environment and physiologically-relevant conditions, we constructed a model of voltage-dependent BK activation. Together with the cryo-EM-derived information on BK structure, Ca^{2+} sensing and gating, the two models combined present a first view of BK channel dual activation by ligands and membrane depolarization.

2699-Plat
Gating of BK Channels: Roles of the C-LINKER and a Potential Hydrophobic Gate
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Large conductance Ca^{2+}-activated K^+ channels (BK channel) are synergistically and independently activated by Ca^{2+}, Mg^{2+} and membrane depolarization. However, the structure basis of the gating of the channel is still unclear. A "passive spring model" was previously proposed, which suggests that pore opening is induced by mechanical pulling of the pore domain by the Ca^{2+} sensing domain (RCK domain) via the linker between RCK and the pore (C-linker). However, recently solved EM structures of BK channel reveal that the C-linker has similar structure and position between metal-bound and metal-free states and is thus unlikely the spring. Here, we will report our recent results from atomistic simulation and experiment that together support a more active role of the C-linker in pore-gate coupling of BK channels. In addition, we