extracellular acidification (% increase for step current: 132 ± 5 vs. 58.4 ± 4.2, pH 6.8 vs. 7.4, P < 0.01; % increase for tail current: 115 ± 3 vs. 45.2 ± 3.2, P < 0.01). In contrast, alkalosis (pH 8.4) weakened the drug-induced hERG current enhancement (% increase for step: 30.3 ± 3.1 vs. 58.4 ± 4.2, pH 8.4 vs 7.4, P < 0.01; % increase for tail: 21.9 ± 2.3 vs. 43.2 ± 3.2, P < 0.01). The measured bath drug concentrations were the same at normal pH, acidosis, and alkalosis. Hypokalemia (1 mM K⁺) did not affect drug-induced hERG current enhancement but hyperkalemia (10 mM K⁺) attenuated the drug-induced hERG current enhancement (increase for step: 41.1 ± 1.6% vs. 58.4 ± 5.9%, pH 7.4 vs. 5 mM K⁺, P < 0.01; increase for tail: 31.5 ± 13.3% vs. 43.2 ± 3.2%, P < 0.05). These results demonstrate that hERG current enhancement by A-93152 is modulated by extracellular potassium concentrations and hyperkalemia.

2332-Pos Board B318
Studying Potassium Conductance in Isolated Mouse Heart Using Thallium Flux Assay
Ian Moench, Anatoli N. Lopotin.
Inward rectifier potassium channels IK₁ underlie major resting ionic conductance in the heart. A wealth of research on IK₁ has been conducted on isolated cells using voltage-clamp techniques but studying IK₁ in intact hearts is limited to ion flux assays. Additionally, in the past, flux studies were primarily performed in beating hearts thus eliminating the possibility of measuring any resting conductance. We have implemented a novel approach based on recently discovered thallium-dependent fluorescence of a low-affinity calcium-sensitive dye (BTC). Langendorff-perfused isolated mouse hearts were (i) loaded with membrane permeant BTC and (ii) stopped using verapamil and lidocaine. Despite being non-invasive and stable, BTC was found to permeate the heart (thus silencing voltage-dependent potassium conductances). (iii) 1 mM thallium was then applied and (iv) fluorescence of BTC captured using a camera. In all hearts the time course of the relative increase in fluorescence, ΔF/Δt, was characterized by (1) well-defined fast (tau=50+150 sec) and (2) highly variable slow components. The total initial rate of ΔF was significantly increased in transgenic hearts with upregulated IK₁ (up to 2-fold vs control). The initial rates of both components of ΔF were strongly IK₁ dependent. Both, transgenic down regulation of IK₁ and blockade of IK₁ with Ba²⁺ increased in transgenic hearts with upregulated IK₁ (up to 2-fold vs control). These results demonstrate that hERG current enhancement but hyperkalemia (10 mM K⁺) attenuated the drug-induced hERG current enhancement (increase for step: 41.1 ± 1.6% vs. 58.4 ± 5.9%, pH 7.4 vs. 5 mM K⁺, P < 0.01; increase for tail: 31.5 ± 13.3% vs. 43.2 ± 3.2%, P < 0.05). These results demonstrate that hERG current enhancement by A-93152 is modulated by extracellular potassium concentrations and hyperkalemia.

2333-Pos Board B319
Phospholipid Regulation of Purified and Reconstituted Human Inward Rectifier (Kir) Channels
It is becoming increasingly clear that membrane lipids are critical determinants of ion channel function, but previous studies have provided limited and largely qualitative information on how lipids influence Kir channel function because they typically utilize cell-based systems where membrane composition is unknown and cannot be precisely controlled. Recent breakthroughs have enabled the purification of human Kir2.1 channels (D’Avanzo et al., Protein Expr Purif. 2010 May;71(1):115-21) that can now be reconstituted into liposomes of defined composition to determine quantitative and mechanistic regulation of channel activity. With the use of "RB" flux assays and patch clamp on giant proteoliposomes we now definitively show that these channels are directly activated by PIP(4,5)P₂ and that PIP(4,5)P₂ is absolutely required in the membrane for Kir channel function, with half maximal activity at ~0.1% PIP(4,5)P₂. Activation of Kir2.1 by PIP₇ is also highly selective for PIP₂; PI(4,5)P₂ does not activate channels and PI(3,4,5)P₃ activates the channels minimally. We further demonstrate that Kir2.1 has a previously unresolved secondary non-specific requirement for anionic phospholipids. Kir2.1 channels are activated by PG, PS, PA, PI, or Cardiolipin with half-maximal activation at ~5%, in the presence of 1% PIP(4,5)P₂, but not by PC, EPC. No activation was observed by anionic phospholipids in the absence of PIP(4,5)P₂. Patch clamp analysis reveals that both unitary conductance and open probability are elevated by increasing membrane POPG concentration-unlike PIP(4,5)P₂ which only increases open probability. Collectively, these data demonstrate quantitative dependence of human Kir channel activity on PIP(4,5)P₂ in membranes of defined composition, and reveal a previously unrecognized secondary requirement for anionic lipids.

2334-Pos Board B320
Biomolecular Simulations of Kir Channel Gating and Membrane Phospholipid Interactions
Phillip J. Stansfeld, Mark S.P. Sansom.
Inwardly rectifying potassium (Kir) channels are integral membrane proteins that permit eflux of potassium out of a cell. There are now multiple structures of these channels, existing in a number of different conformational states. Nevertheless, in all of these structures the gate located at the inner transmembrane helix bundling/crossing remains closed. In this study we have applied molecular simulations to investigate the dynamics of an extensively validated Kir6.2 channel homology model on the microsecond timescale. Whilst this timescale is insufficient to characterise the full gating process, it provides a means for understanding potential structural changes of the channel in its membrane environment. We have extended this to a multi-scale approach by using coarse-grained (CG) simulations to investigate significant protein-lipid contacts, in particular with phosphatidylinositol containing lipids (eg PIP₂), which are known to be involved in channel gating. In addition, we have applied dynamics importance sampling (DIMS) to Kir channel structures and homology models to investigate potential pathways adopted by the channel as it transitions from one state to another. Using these structures, it is possible to explore structural mechanisms for Kir channel gating and hypothesise roles for molecules that modify this process, such as ATP and PIP₂.

2335-Pos Board B321
PIP2-Binding to an Open State Model of Kir1.1 Probed by Multiscale Biomolecular Simulations
Phosphatidylinositol bisphosphate (PIP₂) is an activator of mammalian inwardly rectifying potassium (Kir) channels. We have used multiscale simulations, via a sequential combination of coarse-grained and atomic molecular dynamics to explore the interactions of PIP₂ molecules within the inner leaflet of a lipid bilayer membrane with possible binding sites on both open and closed states of the Kir1.1 (ROMK) channel. Coarse-grained simulations of the Kir1.1 channel containing lipid bilayers identified the PIP₂-binding site on each channel. These models of the Kir1.2-channel complexes were refined by conversion to an atomistic representation followed by molecular dynamics simulation in a lipid bilayer. The binding site in the closed state agrees with previous mutagenesis data of Kir1.1 as well as with previous modeling studies of related Kir channels. Intriguingly, analysis of the open state model reveals a differential interaction of PIP₂ with key residues thought to be involved in PIP₂ activation of the channel. These models will serve as a framework for the functional validation of PIP₂ interactions with Kir1.1 and provide a fresh insight into how PIP₂ stabilizes the open state of the Kir channel.

2336-Pos Board B322
Molecular Dynamics Simulations of PIP₂-Driven Kir Channel Activation
Xiaoyu Meng, Hongxing Zhang, Diomedes E. Logothetis, Meng Cui.
Inwardly rectifying K⁺ (Kir) channels are gated by the signaling phospholipid phosphatidylinositol-bisphosphate (PIP₂). The molecular mechanism of how PIP₂ interacts with Kir channels and induces its structural transition from the closed to the open state remains unclear. We used computational approaches, molecular docking and molecular dynamics (MD) simulations, to model the PIP₂-driven Kir channel activation based on crystal structures of a Kir3.1 chimera (BaKir3.1, PDB entry: 2QKS). The BaKir3.1 serves as a valid structural and functional model of Kir3.1 channel behavior (Nishida et al., 2007; Leal-Pinto et al., 2010). BaKir3.1 was crystallized in two conformers, dilated and constricted forms, which differ mainly in the conformations of the cytosolic G-loop gate. We built four channel systems including dilated and constricted BaKir3.1 channels with and without PIP₂ present in the explicit POPC membrane environment, and performed 100ns MD simulations, respectively. We monitored the interactions between PIP₂ and the channel during the simulations that showed C-alpha RMSD stability after 50ns. Several key residues in the Slide helix and the B-loop were identified to form hydrogen bonds and/or salt bridges with PIP₂. Average radii of the ion permeation pathway along the channel were calculated during the 95-100ns simulation interval. Both systems in which PIP₂ was present became wider in the G-loop regions along the ion permeation pathway compared to their counterpart systems in the absence of PIP₂. In the “dilated + PIP₂” system, the MD simulations identified in one subunit of the channel an outward rotation on the residue Phe181, that is located in the helix bundle-crossing (HBC) gate of TM2. The relationship of the channel-PIP₂ interacting residues and their effects on the conformations of the G-loop and HBC gates are actively pursued through a systematic examination of hydrogen bond network patterns and principal component analysis.