in this study) expressing the hBEST1 using Total Internal Reflection Fluorescence Microscopy (TIRFM). In addition to stoichiometry, we have also tried to determine the interaction among different members of bestrophin family by checking the colocalization of EGFP tagged BEST1 and tt-tomato tagged BEST2, BEST3 or BEST4. We find that all four members of the bestrophin family are tetramers and that BEST1 does not interact with any other members in the family.

2796-Pos Board B566
Drosophila Bestrophin 1 is a Swell Activated Chloride Channel
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Clathrin channels activated by cell swelling or volume increases are ubiquitous. Although important in many physiological and pathological functions, their molecular identity is uncertain. To identify new channel candidates and critical determinants in the ‘swelling’ pathway we have completed an unbiased genetic screen in Drosophila S2R+ cells. Using an anion-sensitive YFP as a reporter of chloride swell-sensitive (Clswell) channel activity, we have confirmed that drosophila Bestrophin 1 (dBest1) is a leading candidate for a Clswell. RNAi specific to dBest1 eliminated the drosophila Clswell current, as did over-expression of a dominant negative form of dBest1. Why is this channel sensitive to swell while its mammalian homologs are not? We have used chimeras between dBest1 and the swell-insensitive mBest2 channel to identify the essential components of the channel necessary for swell activation. Swell sensitivity can be conferred to the mammalian homolog with the switch of a single domain. dBest1 is the only known channel clearly responsive to swell, and its activation depends on the protein’s intrinsic properties.

2797-Pos Board B567
CLIC1 Functional Expression in the Plasma Membrane Correlates with Human Glioblastoma Aggressiveness
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Glioblastomas are brain tumors composed by two cell types: cancer stem cells (CSCs), which are a small population able to self-renew and generate progeny, and bulk cells, consisting of a larger population committed to a precise fate. Glioblastomas are aggressive tumors because of CSC brain infiltration efficiency and their resistance to chemotherapies. Therefore, CSCs represent the most tumorigenic cells found in glioblastomas. Several forms of glioblastomas exhibit a level of Chloride Intracellular Channel 1 (CLIC1) expression higher than in normal brains. CLIC1 mainly localizes in the cytoplasm and in the nucleoplasm and, in stress conditions, it is able to translate into plasma and nuclear membranes where it acts as a Cl- permeability. Four human glioblastoma biopsies were cultured in a CSCs selecting medium. By knocking down CLIC1 expression using siRNA lentiviral infection (siCLIC1), we found that CLIC1-deficient cells migrated about 50% less efficiently than cells treated with siRNA for luciferase (siLUC, control) in Boyden chamber assays. To determine whether this phenotype results from the lack of CLIC1 plasma membrane expression, CLIC1-mediated currents were estimated by using a specific inhibitor (IAA94, for luciferase (siLUC, control) in Boyden chamber assays. To determine whether deficient cells migrated about 50% less efficiently than cells treated with siRNA lentiviral infection (siCLIC1), we found that CLIC1- deficient cells.

2798-Pos Board B568
Mutation of the Highly Conserved Pore-Lining Leucine Residue Increases Agonist Sensitivity of a Glutamate-Gated Chloride Channel
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The glutamate-gated chloride channel (Gluci) is an invertebrate, ligand-gated anion channel of the Cys-loop receptor family. It is activated by the endogenous neurotransmitter L-glutamate and by the antiparasitic drug ivermectin. The crystal structure of the Caenorhabditis elegans GluCI alpha homopentamer (3.3A° resolution; Hibbs & Gouaux, 2011) shows the location of the glutamate binding site, the separable ivermectin site, and the highly conserved leucine residue at the 9’ position of the pore-lining M2 transmembrane domain. Mutation of this L9’ residue in other Cys-loop receptors dramatically increases agonist sensitivity. Using whole-cell patch clamp, we found that six of seven mutations at this position (L9’S, A, F, I, L, V, but not G) increased the glutamate sensitivity of the heteromeric Gluci channel by factors of 5- to 80-fold. Beta-branched amino acids (Ile, Thr, Val) gave the greatest reductions in EC50. Analysis of such chain properties revealed that side-chains destabilizing the native structure of this L9’ residue in other Cys-loop receptors dramatically increases agonist sensitivity. Except for WT and the L9’F mutation, we were unable to detect glutamate- and/or ivermectin-induced changes in membrane potential using a fluorescent membrane potential assay (FlexStation, Molecular Devices), probably because the spontaneous activity of the mutant channels obscured the fluorescence changes. However, whole-cell patch clamp and fluorescent membrane potential experiments confirmed that the L9’F mutation increased both the glutamate and ivermectin sensitivity of the Gluci channel. Increasing GluCl sensitivity to ivermectin will benefit its use as a neuronal silencing tool.

2799-Pos Board B569
Characterizing ATP Permeation through mVDAC1 using Markov State Models (MSMs)
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Voltage-dependant anion channels (VDACs) are transmembrane proteins found in high abundance in the outer mitochondrial membrane of all eukaryotes. VDAC mediates the transfer of metabolites such as ATP, ADP, and NADH between the cytoplasm and the intermembrane space. The open state is selective for anions, while the closed state is cation selective. The determination of the high resolution X-ray crystal structure of VDAC from mouse (mVDAC1) (Ujwal et al, PNAS 2008) has made it possible to study the molecular workings of this channel in unprecedented detail. Previously, we used continuum electrostatic calculations to show that mVDAC1 is anion selective suggesting that the X-ray crystal structure represents the open state of the channel (Choudhary et al. JMB 2010). However, the hallmark of the open state is high ATP flux (~1-2 million ATP molecules per second), and therefore, we set out to determine the conduction state of the channel with regard to ATP. To do so, we are using a series of fully atomistic, unbiased molecular dynamics simulations to construct a Markov State Model (MSM) of the permeation process using a modified version of the MSMBuilder2 software suite (Beauchamp et al. JCTC 2011). The permeation of ATP through the channel is represented by transitions between a series of discrete, well-populated conformational states. Analysis of the converged MSM using transition state theory, allows us to extract the dominant permeation pathways and compute mean first passage time (MFPT) for transition through the channel.

2800-Pos Board B570
Pathway Reconstitution of Abscisic Acid Hormone Activation of SLAC1 Anion Channels via Novel ABA Signaling Protein Kinase
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The plant hormone abscisic acid (ABA), is produced in response to drought stress in plants and mediates stomatal closure. SLAC1 is a key guard cell 

 hears. 2008; Negi et al, Nature 2008). Though SLAC1 expression in oocytes alone showed no activity, calcium-dependent protein kinase 21 (CPK21), CPK23, and OPEN STOMATA 1 (OST1) have been shown to enhance anion currents. Here we have focused on the following key questions: Can functional ABA activation of SLAC1 anion channels be reconstituted? Can a new protein kinase, that functions in vivo in ABA-induced stomatal closure, mediate ABA activation of SLAC1 channels? Can a new protein kinase be strongly activated SLAC1-mediated currents and phospholipid-dependent proteolytic activation of SLAC1 activity in oocytes. Moreover, we successfully reconstitute a complete ABA signaling pathway demonstrating ABA-induced activation of SLAC1 channels including co-expression of ABA receptors (Park et al Scien 2009; Ma et al Scien 2009), and the newly identified protein kinase.