site-directed mutagenesis studies in combination with patch-clamp experiments. Our results suggest that the efficacy of the agonists is closely coupled to the energetics of the C-loop of the receptor. With the presence of different ligands in the binding sites, the C-loop closure energy profiles given by potential of mean force (PMF) calculation reveal interesting differences. The results suggest that the combination of modelling and patch-clamp experiments for partial agonists can be a powerful approach to deciphering the atomistic details of glycine receptor activation.

2771-Pos Board B463
Towards Structural and Functional Determination of a Non-Desensitizing α1 Glycine Receptor
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Glycine receptors (GlyR's) are inhibitory ligand-gated receptors in the nicotinicoid receptor superfamily. GlyR's mediate neurotransmission in CNS and are typically activated by glycine. GlyR is implicated in pain signaling to the brain. In order to better understand the silencing electrical activity of the brain and also the structure and function of GlyR in its open state, ivermectin (IVM) sensitive GlyR channels are developed as IVM is shown to stabilize GlyR in its non-desensitizing state. Double mutant F207A/A288G in α1 human GlyR has been shown to increase IVM sensitivity and reduce/remove sensitivity for glycine. We are developing photo crosslinking methodologies linked with mass spectrometric analysis on systematically generated single Cys mutations in IVM sensitive GlyR to enable us to study state-dependent structure of GlyR in the open state. Studies on GlyR in its open state will provide distance constraints that can be used in computational models to better the structure in its open state (non-desensitizing) and can help perform comparative studies with a desensitizing GlyR.

2772-Pos Board B464
Methods for Identification of State-Dependent Crosslinks for Structural Determination of Membrane Proteins
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Determining the structure of membrane proteins is critical to understanding how they function. Though various techniques exist to elucidate structural information, often times they lack the ability to determine allosteric movements associated with the dynamic nature of the proteins. Development of a method that affords the possibility of mapping changes in structure associated with the complicated allosteric mechanisms of membrane proteins is a valuable tool to better understanding the structural basis of protein function. The glycine receptor (GlyR) is a ligand-gated ion channel associated with inhibition of signal propagation in the central nervous system. Single-Cys mutant homomeric human α1 GlyR is overexpressed in insect cells, purified, and reacted with a methanethiosulfonate-benzophenone heterobifunctional crosslinker containing an alkyne tag. After state-dependent photoactivation in the presence or absence of modulatory ligands, inter- and intra-subunit crosslinks may be isolated by proteolysis, reduction, click chemistry and affinity chromatography. Verification and quantitation of crosslinked species may be sensitively identified by fluorescent tagging and use of a HPLC-microfluidic-laser induced fluorescence (LIF) system. Structural information can be derived through the implementation of multi-dimensional mass spectrometry to identify sites of crosslinking and relative distances which can be used to refine computational models in state specific manner. Development of this method, using GlyR as a model system, will allow for structural determinations to be made on any protein and thus allow for a better understanding of the dynamic nature of allosteric proteins.

Ion Channels and Disease I

2773-Pos Board B465
Learning the Kinetics of Amyloid β Pore in Alzheimer's Disease Pathology
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Increased synthesis of self-aggregating amyloid beta (Aβ) peptides caused by abnormal processing of amyloid precursor protein (APP) is a hallmark of Alzheimer's disease (AD) pathogenesis. Aβ mediates it's effect by disrupting the integrity of cell membrane and interacting with plasma membrane channels. The soluble form of Aβ aggregates into calcium (Ca^{2+}) - permeable pores in the membrane. Aβ pores promote uncontrolled increase in the cytoplasmic Ca^{2+} concentration by allowing Ca^{2+} influx to the cell, in addition to enhancing the activity of Ca^{2+}- permeable channels on the plasma membrane and intracellular compartments. The Ca^{2+} influx through Aβ pores upsets the otherwise fine-tuned micrometer-sized elementary Ca^{2+} release events and whole-cell Ca^{2+} response. The disrupted Ca^{2+} signaling in turn has the potential to alter cell function in many ways.
Here, we have used computational modeling in conjunction with TIRF microscopy to study the function of Aβ pores in AD cells. TIRF microscopy was used to image Ca^{2+} flux through thousands of Aβ pores in parallel at the millisecond scale and single channel resolution. The fluorescence time-series from individual pores was idealized by extending the Maximum Likelihood-based method developed for separating signal from baseline in noisy quantal data (Bruno et al. 2013, Biophys. J. 105:68). The idealized data was used to developed data-driven models for the kinetics of Aβ pore at different stages of it's life. In addition to providing deep insights into the kinetics of Aβ pore, this study demonstrates that the massive imaging data obtained from thousands of channels in parallel using TIRF microscopy can be utilized for single molecule modeling in the same manner as electrical patch-clamp data. Employing the optical patch-clamp data for Markov chain modeling has the added advantage of the experiments being done under physiological conditions.

2774-Pos Board B466
Investigating How Aβ and αSynuclein Oligomers Initially Demage Neuronal Cells
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Scanning Ion Conductance Microscopy (SICM) allows simultaneous measurements of various important structural and functional parameters with nanometre resolution on living cells. In addition, the pipette allows defined dosing of substances to cells. Amyloid beta 42 is a well known component of extracellular amyloid plaques in association with Alzheimer's disease. Similarly, alpha synuclein is the main component of intracellular Lewy bodies in Parkinson's disease. The initial mechanism by which these proteins cause cellular cytotoxicity remains elusive. Solutions containing oligomers of these bio molecules were locally delivered, via the SICM pipette, to the surface of neurons and glial cells in a quantitative fashion. The resulting Ca^{2+}-influx was monitored over a 10 min period and taken via an EMCCD camera. These experiments provide new insights into the molecular mechanism by which protein oligomers initially damage cells and how many oligomers are required.

2775-Pos Board B467
Acute Effects of β-Amyloid (1-42) Oligomers on Rat Pyramidal Entorhinal Neurons
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Deposition of beta-amyloid peptide (Aβ) in senile plaques is the hallmark of Alzheimer disease neuropathology. Soluble Aβ oligomers block voltage-gated ion channels as P/Q type calcium channels (Nimmrich et al., 2008) as well as the transient potassium current (Liudyno et al., 2012) and calcium dependent potassium channels, BK type (Yamamoto et al., 2011). Exposure to Aβ may cause a loss of cellular calcium homeostasis, but the mechanism by which this occurs is uncertain. In this work we evaluated the acute response of rat pyramidal entorhinal neurons to oligomers formed from purified Aβ1-42 in both entorhinal cortex slice preparations and isolated pyramidal neurons in culture using current- and voltage-clamp conditions. Exposure to Aβ oligomers but not monomers increased the input resistance and enlarged the action potential in the slice preparation, whereas in culture neurons produced a reversible inhibition of the inward potassium current generated by voltage ramps from −70 to 70 mV in symmetric potassium conditions. This current is generated by inward rectifier potassium channels as well as the leak potassium channels, it was blocked by barium, arachidonic acid, bupivacaine and extracellular pH acidification, suggesting that TASK type K2P channels are targets of the toxic effects of Aβ. Blockage of potassium channels by Aβ could lead to prolonged cell depolarization, thereby increasing calcium influx.
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2776-Pos Board B468
Disregulation of Calcium Homeostasis Connected with Familial Alzheimer's Disease
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Familial Alzheimer's disease (FAD) caused by mutations in presenilin-1 (PS1) gene in approximately 50% of cases and in Amyloid precursor protein (APP)

gene in 13 % cases. It was found that FAD genes mutants affect calcium homeostasis in hippocampal neurons by disrupting Ca^{2+} storage in the lumen of endoplasmic reticulum (ER). In our study we used electrophysiological recordings with a patch-clamp technique in whole-cell mode and calcium imaging with Fura-2AM to study calcium channels activity, ER calcium load and ER calcium leak. We observed different effects of FAD mutated genes expressions on activity of store-operated and L-type voltage-gated calcium channels in mouse hippocampal neurons, mouse neuroblastoma Neuro2a and human neuroblastoma SK-N-SH cell lines. We found that effects of FAD PS1 mutants on calcium channels were driven by misbalance in activity of ER calcium sensors STIM1 and STIM2. The different effects were connected with calcium sensors STIM1 or STIM2 impaired signal transduction from ER to calcium channels in plasmatic membrane (PM) under control of ER calcium levels. The impaired signal transduction was revealed in live confocal imaging experiments. The enzyme activity of PS1 was involved in regulation of store-operated calcium entry in two ways: the activity of channels was depended on PS1 endoproteolysis level and APP cleavage.

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2777-Pos Board B469

Tau Protein Forms Ion Channels

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Tau is a microtubule associated protein that is intrinsically disordered and accumulates in amyloid fibril deposits alone and in combination with other amyloid forming proteins. We have found that Tau 441 can form ion- permeable channels in planar lipid bilayers under conditions of acidic pH. The channels are irreversibly associated with the membrane and exhibit heterodisperse conductances. Acidic phospholipids enhanced channel activity. The channels are only weakly selective for cations. Zn^{++} does not block the channels. These channels show significant, but incomplete, similarity to the amyloid channels formed by the Alzheimer peptide Abeta, the diabetes associated peptide IAPP, the prion related PrP106-126 ,and other amyloid channel peptides. Channel forming activity may play a pathogenic role in Tau associated diseases such as Lewy Body Dementia, by causing ion leakage in target cell membranes.

2778-Pos Board B470

Alterations in Ionic Currents and Gap Junctional Coupling by Pan-Histone Deacetylase Inhibition

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Dynamic balance of histone acetyl transferase and histone deacetylases (HDACs)is maintained in multiple signaling pathways determining expression and repression of genes. Pan-HDAC inhibitors like Vorinostat(VOR, ZolinzaTM) and Romidepsin (Istodax(r)) have been approved for treatment of peripheral and cutaneous T-cell lymphomas. On rare occasions during clinical trials,HDACi has been associated with symptomatic ECG alterations, cardiac arrhythmias, and even sudden cardiac death for unknown reasons. Rationale of this study is to identify electrophysiological and molecular determinants involved in asymptomatic cardiac abnormalities in patients treated with HDACi during clinical trials. We investigated the effects of VOR on ionic currents and gap junctions responsible for cardiac excitability and propagation. Cultured neonatal mouse ventricular myocytes (NMVM) were treated with 1-5μM VOR overnight and patch clamp experiments were performed to measure the transient (Ito) and steady state (IK) outward K^+, peak inward Na^+ (INa), and peak inward L-type Ca^{2+} (ICa,L) current densities. Dose-dependent decreases in INa density and NaV1.5 protein levels occurred along with +10mV shift in INa activation in myocytes treated with 1-5μM VOR. Conversely, ICa,L density increased significantly without concomitant change in CaV1.2 protein expression levels. Moreover, augmentation of ICa,L byVOR was independent of stimulation with 5μM isoproterenol, suggesting discrete acetylation and phosphorylation dependent mechanisms for modulation of cardiac ICa,L However, VOR did not alter any IK or Ito current densities. Also, VOR dose dependently reduced gap junction coupling and protein expression of connexin43. Pan-HDACi treatment significantly reduces Cx43 protein expression and coupling along with salient alterations in INa and ICa,L densities. These ion channel changes present possible etiologies for cardiac abnormalities in patients. VOR being a pan-HDAC inhibitor, we plan to determine the effects of isoform-selective HDACi on ionic currents and APD in human cardiomyocytes derived from induced pluripotent stem cells.

2779-Pos Board B471

Self-Assembly of the Viral Channel Forming Protein Vpu of Hiv-1 using Coarse-Graining Molecular Dynamics Simulations

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Vpu of human immunodeficiency virus type 1 (HIV-1) which is an 81 amino acid bitopic membrane protein known to homo-oligomerize. It is known for its ability to form channels and to assemble into host factors. The assembly process and the oligomer state of Vpu are not known. Coarse-graining molecular dynamics simulations are performed with full-length Vpu in lipid bilayers. Full-length Vpu is constructed by linking an experimentally derived structural model of the cytoplasm domain with a computationally generated transmembrane domain (TMD). Simulation boxes are generated harboring selected numbers of VPUs. The simulations identify that Vpu assembles via several binding sites into a series of oligomeric states. Most striking, in a simulation of four Vpu's the proteins form a symmetric bundle which is a close match to known channel architectures. The tetramer is followed a dimer formation. Not only TMDs but also cytoplasm domains are involved in the dynamics of self-assembly.

2780-Pos Board B472

Ion-Trapping in HCV P7 Hexameric Bundles - A Molecular Dynamics Simulation Study

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Viral channel forming proteins assemble into homo-oligomers when produced within the infected cells and full fill their role as diffusion-amplifier for ions across subcellular membranes. Alteration of the electrochemical gradients seems to be necessary and in some cases essential for the survival of the virus. Much is known about the structural features of many of the channels, dynamics data about oligomerization, assembly and ion diffusion within the assembled bundles is still lacking.

The dynamics of physiological relevant ions, Na^+, K^+, Cl- and Ca^{2+} are monitored in the vicinity of hexameric bundles of p7 of Hepatitis C virus (HCV), strain 1a. The bundles are generated by a combination of docking approach and molecular dynamics simulations. Ion dynamics is recorded during multi 200 ns MD simulations of 1 M solutions. With a crucial residue, histidine-17, found to point into the lumen of the pore, protonation of this residue is altered and the effect on the ion dynamics monitored. While an 'unprotonated' p7 bundle allows Ca^{2+} to enter the lumen, it is exclusively the Cl- in case of a 'protonated' bundle.

2781-Pos Board B473

Structure and Inhibition of the M2 Proton Channel from the Influenza a Virus

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The influenza A virus M2 proton channel (A/M2) is the target of the antiviral drugs, amantadine and rimantadine, whose use have been discontinued due to widespread drug resistance. Among the handful of drug-resistant mutants of M2, S31N, V27A and L26F were found in more than 99% of the currently circulating viruses. Discovery of inhibitors targeting these M2 mutants has been hampered by the lack of structural information and their limited sizes, polarity, and dynamic nature of their drug binding sites. Nevertheless, using an integrated approach including medicinal chemistry, molecular dynamics simulation, solid/solution-state NMR, X-ray crystallography, and pharmacological characterizations, we have discovered small molecule drugs that inhibit mutant M2 (S31N, V27A and L26F) with potencies greater than amantadine's potency against WT M2. A few compounds exhibiting EC50 around 100 nM are advanced to mice model studies. Structural characterization of S31N drug binding by NMR shows the drug bound in the homotetrameric channel, threaded between the side chains of Asn31. The S31N inhibitors, like other potent WT M2 inhibitors, contain a charged ammonium group. The ammonium binds as a hydrate to one of three sites aligned along the central cavity that appear to be hotspots for inhibition. These drug binding hotspots along the channel axis provide a general model of M2 inhibition that can be used to guide the design of other channel blockers.

2782-Pos Board B474

Mechano-Sensitive Ion Channels (MSCS) Provide Human Breast Cancer Cells with a Sensorium for Mechanical Stress

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Mechanical stress is increasingly recognized as a cancerogenic factor in breast cancer. We investigated the biophysical characteristics of mechano-sensitive