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activated by N-methyl-d-aspartate. Due to their high Ca^{2+} permeability and voltage-dependent channel block by Mg^{2+}, NMDA receptors play a central role in development through stabilization of synaptic connections, as well as in learning and memory by mediating many forms of synaptic plasticity. The mechanisms by which the ion channel of NMDA receptors selects Ca^{2+} for permeation over all other physiological ions, while binding Mg^{2+} and restricting its permeation, are not well understood. We hypothesize that the slightly different radii and electronic properties of Mg^{2+} and Ca^{2+} ions result in drastically different free energy barriers for transition of the ions from a binding site in the selectivity filter toward the intracellular solution. We are applying quantitative theoretical ''bottom up'' approaches to this complex system by combining methods of computational chemistry, molecular mechanics (MM), and bioinformatics. The structure of the NMDA receptor channel is constructed and refined using experimental information, homology modeling and extensive molecular dynamics simulations. We are performing quantum chemical calculations to determine the energy of the transition state of model ligand exchange reactions that mimic divalent ion transition from the selectivity filter of NMDA receptors to water. Quantum calculations are used to parameterize a polarizable molecular mechanics force field for these divalent ion interactions with organic ligands with the further goal to perform MM simulations. Umbrella sampling and thermodynamic integration simulations are used to compute free energies of transfer of divalent ions between water and a model of the NMDA ion selectivity filter as well as free energy barriers for these transitions.

3114-Plat

An Ionic Switch in the Ligand-Binding Domain of Non-NMDA Receptors

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The ionotropic glutamate receptors (iGluRs), activated by the amino acid L-glutamate, account for the vast majority of excitatory neurotransmission in the central nervous system. Given their centrality to the nervous system, it is not surprising that these receptors have been linked to various neurological disorders including epilepsy, Alzheimer's and Parkinson's disease. Based on their sequence, pharmacological properties and function, iGluRs are classified primarily into three subtypes, namely amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), and N-methyl-D-aspartate (NMDA) receptors.

Previously we have reported the identification of a potential ionic switch, or latch, located within the hinge region of non-NMDA receptors. Here we describe extensive molecular dynamics simulations to explore the conformational behaviour of the ionic switch and its influence on the closure or opening of the ligand-binding domain. The position of the switch appears to directly control the conformation of the ligand-binding domain. We discuss the results with respect to the general mechanism of how different ligands are able to induce the same mechanism of domain closure.

3115-Plat

Gating and Stoichiometry of Heteromeric Kainate Receptors

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Kainate-type ionotropic glutamate receptors (KARs) assemble primarily as heteromeric complexes at glutamatergic synapses. In most cases, KAR-mediated synaptic events exhibit slow and variable deactivation kinetics in contrast to the fast gating properties typically observed with recombinant KARs. It is still not clear which factors contribute to the slowing of KAR responses at synapses, and it remains to be understood the low affinity neurotransmitter, L-Glu, triggers prolonged activations of KARs. Here, we investigated the biophysical and stoichiometric properties of recombinant heteromeric KARs assembled from the GluK2 and GluK5 receptor subunits. To do this, we used a combination of outside-out patch electrophysiology to examine functionality and a fluorescent subunit counting technique to assess heteromerization. As expected, the degree of heteromerization with GluK2/GluK5 subunits in individual patch recordings showed a positive correlation with slow deactivation kinetics and responsiveness to the agonist, AMPA. Interestingly, preliminary data from subunit counting experiments suggest that the stoichiometry of heteromeric KARs is fixed. Furthermore, electrophysiological experiments reveal that GluK2/GluK5 heteromers are insensitive to external anions and cations. Since both anion and cation binding sites line the interface between KARs subunits, our data suggest that the process of heteromer assembly affects functionality by disrupting this region of the mature protein.

Platform: Cardiac Muscle

3116-Plat

MYH7-Mutation Associated Allelic Imbalance in Familial Hypertrophic Cardiomyopathy: Molecular Mechanisms and Correlation with Disease Prognosis

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Familial Hypertrophic Cardiomyopathy (FHC) is an autosomal dominant disease of the heart. The severity of the disease ranges from mild cases to sudden cardiac death or progression to heart failure. FHC is mostly caused by mutations in genes encoding for sarcomeric proteins, 30-40% of the patients are affected by missense mutations in one allele of the β-myosin heavy chain (*MYH7*).

To gain further insights into the mechanisms of FHC-progression in heterozygous patients we performed a comparative expression analysis of the wildtype and the mutated *MYH7* allele. We have analyzed samples from *Musculus soleus* and myocardium of genotyped and clinically well-characterized FHC-patients with different *MYH7*-mutations. We demonstrated an unequal allelic mRNA expression for each mutation analysed. The ratios of the mutated mRNA ranged from 29% to 66% in a mutation-specific manner. They were comparable in myocardium and soleus muscle and, importantly, were essentially the same at the protein level. Intriguingly, we observed a correlation between life expectancy and fraction of mutated mRNA or protein. Thus, the allelic imbalance may provide a novel factor underlying the progression of FHC.

Our results suggest that the allelic imbalance is induced by differential regulation of the mutated *MYH7* mRNA-expression. Thus we aimed to identify molecular mechanisms that may account for the mutation-related different mRNA levels. Bioinformatical analysis revealed that mutation V606M disrupts an exonic splicing enhancer site. Additionally, for the mutation R723G severe changes in the mRNA secondary structure were predicted. Alternative splicing variants of the V606-allele and a structure-related increased stability of the R723G-allele thus may provide potential factors inducing altered levels of mutated *MYH7*-mRNA.

3117-Plat

Single Molecule Studies of Recombinant Human α- and β-Cardiac Myosin to Elucidate Molecular Mechanism of Familial Hypertrophic and Dilated Cardiomyopathies

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Hypertrophic cardiomyopathies (HCM) and dilated cardiomyopathies (DCM) are common inherited cardiovascular diseases, often resulting from single point mutations in genes encoding sarcomeric proteins. Genetic and clinical studies have identified several hundred mutations, including severe disease causing mutations in β-myosin heavy chain (MHC). Despite the clinical significance, few single molecule studies exist for mutated β-cardiac myosin, primarily due to difficulties of heterologous protein expression and instrumental limitations. Previous studies have used mouse α-cardiac myosin or biopsies from patients. Those studies are not optimal to understand the molecular mechanism of HCM/DCM because there are significant differences between mouse α- and human β-MHC. Furthermore, biopsy samples from patients are often inhomogeneous mixtures of wildtype (wt) and mutants. This may explain why there have been many inconsistencies between the previous studies.

Here, we demonstrate the first single molecule studies of recombinant human cardiac myosin. We expressed homogenous and fully functional wt human cardiac α- and β-S1 with human light chains bound. Then, we characterized