prefers to open to the intermediate and large conductance states. In the presence of the willardiine partial agonists, the channel opens more frequently to the smallest and intermediate conductance states. Kinetic modeling using maximum interval likelihood rate optimization revealed two time constants in each open state and at least three in the closed state for the partial and the full agonists. These data suggest the mode of channel activation is similar for both glutamate and willardiine compounds with varying rates of activation. Supported by NIH NS049223.

2533-Pos Board B503

Energetics of the Cleft Closing Transition and the Role of Electrostatic Interactions in Conformational Rearrangements of the Glutamate Receptor Ligand Binding Domain

Michael J. Yonkunas, Tatyana Mamonova, Maria Kurnikova.
Carnegie Mellon University, Pittsburgh, PA, USA.

The ionotropic glutamate receptors are localized in the pre- and postsynaptic membrane of neurons in the vertebrate central nervous system. Activation by the principal excitatory neurotransmitter glutamate allows the ligand binding domain to change conformation, communicating opening of the transmembrane channel for ion conduction. The free energy of the GluR2 S1S2 ligand binding domain (S1S2) closure transition was computed using a combination of thermodynamic integration and umbrella sampling modeling methods. A path that involves lowering the charge on E705 was chosen to clarify the role of this residue. A continuum electrostatic approach in S1S2 is used to show E705, located in the ligand binding cleft, stabilizes the closed conformation of S1S2. Molecular dynamics simulations reveal: (1) in the closed conformation, in the absence of a ligand, S1S2 is somewhat more closed than reported from X-ray structures; (2) a semi-open conformation characterized by disruption of a single cross-cleft interaction differing only slightly in energy from the fully closed S1S2; (3) the fully open S1S2 conformation exhibits a wide energy well, sharing structural similarity to the apo S1S2 crystal structure. Hybrid continuum electrostatics/MD calculations along the chosen closure transition pathway reveal solvation energies, as well as electrostatic interaction energies between two lobes of the protein increase the relative energetic difference between the open and the closed conformational states. By analyzing the role of several cross-cleft contacts and binding site residues we demonstrate how S1S2 interactions facilitate formation of the closed conformation of the ligand binding domain. A molecular model of the full GluR2 receptor is currently being constructed to reflect a consistent physical and biochemical picture based on an evolutionary comparison and all available biophysical data.

2534-Pos Board B504

Hinge and Twist Rigid Body Domain Motions in Ionotropic Glutamate Receptor GluR6 and the Hydrogen Bond Interactions that Switch Them On and Off

Rodney E. Versace, Marco Ceruso.
City College of New York, New York, NY, USA.

Ionotropic glutamate receptors are tetrameric ligand-gated ion channels found in pre and postsynaptic cell membranes of the central nervous system. There are three pharmacological classes of ionotropic glutamate receptor, namely N-methyl-D-aspartate (NMDA); alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA); and kainate receptors. Ionotropic glutamate receptors play an important role in neuronal development and synapse plasticity as well as in higher order processes such as memory and learning. Ionotropic glutamate receptors are also implicated in several neurological and neurodegenerative disorders such as epilepsy, Parkinson's and Alzheimer's diseases. Only the structure of the isolated ligand binding domain is known: it comprises two lobes that enclose a ligand binding cleft. Using this isolated domain it has been possible to extract a great wealth of information concerning the relationship between functional and structural states of the receptor. Here, we characterize the intrinsic conformational dynamics properties of the ligand-binding domain of GluR6, a kainate receptor, in the absence of glutamate. Notably, we identify three inter-lobe hydrogen bonds interactions that govern and regulate the opening of the binding cleft via two distinct mechanisms: an hinge-like and a twist-like rigid-body domain motion. The computational studies reveal how the interplay between these interactions promotes either one or the other form of rigid-body motion. Moreover, the pattern of evolutionary conservation of these inter-lobe interactions suggests a putative role in the differential functional properties of the distinct ionotropic receptors classes.

2535-Pos Board B505

Purification and crystallization of iGluR Amino Terminal Domains

Janesh Kumar, Mark Mayer.
NIH, Bethesda, MD, USA.

The glutamate receptor ion channels which mediate excitatory synaptic transmission in the mammalian brain have a unique architecture distinct from that for other ligand gated ion channels. Ten years ago the 1st crystal structure was solved for an AMPA receptor ligand binding domain[1], with members of other iGluR gene families following over the next few years[2-4]. The ligand binding domain is preceded by a large amino terminal domain which controls assembly, but which does not bind neurotransmitter. Despite its key biological role structures of the ATD have not been solved. A major impediment to this is the poor expression of iGluR ATDs in *Escherichia coli*. To address this we screened ATD expression in HEK cells using constructs designed for secretion of soluble proteins and focused on the GluR6 subtype for which we can obtain 4 mg/l of glycosylated protein. The results of crystallization screens and data collection with synchrotron radiation indicate that it will be possible to solve a structure of the GluR6 ATD and explore its role in subtype specific assembly.

1. Armstrong, N., Sun, Y., Chen, G.Q. & Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **395**, 913-917 (1998).
2. Furukawa, H., Singh, S.K., Mancusso, R. & Gouaux, E. Subunit arrangement and function in NMDA receptors. *Nature* **438**, 185-192 (2005).
3. Mayer, M.L. Crystal Structures of the GluR5 and GluR6 Ligand Binding Cores: Molecular Mechanisms Underlying Kainate Receptor Selectivity. *Neuron* **45**, 539-552 (2005).
4. Yao, Y., Harrison, C.B., Freddolino, P.L., Schulten, K. & Mayer, M.L. Molecular mechanism of ligand recognition by NR3 subtype glutamate receptors. *Embo J* **27**, 2158-70 (2008).

2536-Pos Board B506

Structure And Stability Of Ligand Binding Core Dimer Assembly Controls Desensitization In A Kainate Receptor

Charu Chaudhry[1], Matthew C. Weston[2], Peter Schuck[1], Christian Rosenmund[2], Mark L. Mayer[1].
[1]NIH, Bethesda, MD, USA, [2]Baylor College of Medicine, Houston, TX, USA.

Ionotropic glutamate receptors couple free energy of agonist binding to opening and desensitization of a transmembrane ion channel. Central to their function is a structural unit formed by a dimer assembly of the ligand binding domains. The rates of transitions between resting, conducting, and desensitized states is controlled by conformational changes in the dimer. The serendipitous discovery that the GluR2 L483Y mutant blocks desensitization by stabilizing dimer assembly has profoundly influenced understanding of AMPA receptor gating. Paradoxically, GluR5-GluR7 subtype kainate receptors have an aromatic amino acid at the equivalent position, but desensitize rapidly and completely. Using a library of GluR6 dimer interface mutants, we used analytical ultracentrifugation to show that for kainate receptors there is a direct correlation between the rate of onset of desensitization and the stability of dimers formed by ligand binding cores, establishing that the gating mechanisms of AMPA and kainate receptors are conserved. Crystal structures for a series of 5 mutants were solved to reveal the underlying molecular mechanisms. Visualized in the crystal structures is a rich complexity of interactions across the dimer interface, illuminating how small sequence differences within the ligand binding domain function to diversify receptor properties. Our results indicate that even following extensive engineering, the stability of kainate receptor dimers is at most half of that of their AMPA counterparts, and that even if it were possible to generate dimers as stable as those for GluR2 L483Y, these would be insufficient to block kainate receptor desensitization. We show this is because the desensitized state in kainate receptors acts as a deep energy well offsetting the stabilizing effects of dimer interface mutants. Our results reveal how receptors with similar structures and gating mechanisms can exhibit strikingly different functional properties.

Muscle: Fiber & Molecular Mechanics & Structure I

2537-Pos Board B507

Computational Energetic Analysis of Intrafacial Binding Energies in Interpolated Myosin States

Kevin C. Facemyer[1], Michael Carter[1], Marcel Levy[1], Karen Schlauch[1], Christopher M. Herald[2], Josh E. Baker[1], Christine R. Cremo[1].
[1]University of Nevada School of Medicine, Reno, NV, USA, [2]University of Nevada, Reno, Reno, NV, USA.