

**770-Pos Board B525****A Computational Study of Ligand Binding in Chemosensory Ionotropic Glutamate Receptors**Benoite Bargeton<sup>1</sup>, Matteo Dal Peraro<sup>2</sup>, Richard Benton<sup>1</sup>.<sup>1</sup>University of Lausanne, Lausanne, Switzerland, <sup>2</sup>École polytechnique fédérale de Lausanne, Lausanne, Switzerland.

Chemosensation allows animals to detect and respond to myriad chemical signals in their environment indicating food, dangers, kin and mates. We recently characterized a new chemosensory receptor repertoire, the Ionotropic Receptors (IRs), which have derived from ionotropic glutamate receptors (iGluRs). IRs are expressed in chemosensory organs across protostomes, including insects, *C. elegans* and *Aplysia*. Functional characterization in *Drosophila* has shown they mediate recognition of structurally-diverse acids and amines. Like iGluRs, IRs function as ligand-gated ion channels, composed of heteromeric complexes of a canonical IR, important for defining ligand specificity, and a co-receptor (either IR8a or IR25a) (Abuin, Bargeton et al. 2011). Study of the "Venus fly-trap" ligand binding domain (LBD) of IRs provides an important model to understand the molecular basis of chemosensory recognition, and how this has evolved from the glutamate-recognition properties of iGluRs. We have investigated the ligand binding mode of IRs in silico by comprehensive protein modeling using information from the crystal structures of prokaryotic (Mayer, Olson et al. 2001), primitive eukaryote (Lomash, Chittori et al. 2013) and mammalian iGluRs (Sobolevsky, Rosconi et al. 2009). We compared solvent accessibility (cavity volume) and surface charge of ligand binding pockets between published crystal structures and independent homology models of IR LBDs and observed good agreement. We therefore extended our modeling approach to the LBDs bound to their ligands, as defined by in vivo electrophysiological experiments. This analysis deepens our understanding of the emergence of ligand-receptor specificity and thus provides insight into the evolution of the selectivity of an ancient ligand-binding module.

**771-Pos Board B526****Characterizing the Energetic States of a Glutamate Receptor using Umbrella Sampling and Microsecond Molecular Dynamics Simulations**

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Ionotropic glutamate receptor functional states consist of: non-conducting, conducting, and desensitized states that are well characterized by electrophysiological studies. However, the energetics of these states is not well understood. It is known that the interface between monomeric subunits of the tetramer plays a major role in distinguishing these functional states. We have used umbrella sampling and microsecond molecular dynamics simulations of receptor dimers to calculate several free-energetic states of AMPA subtype glutamate receptor ligand-binding domains in the absence of its transmembrane region. Our results show the desensitized conformation as a highly probable conformation during simulations thus characterizing it as a low free-energetic state. We have also developed a model of a transmembrane region of AMPA based on what is known from crystallography of AMPA and potassium channels. Umbrella sampling of this model transmembrane region in the lipid bilayer is being conducted complementary to the ligand-binding domain. We hypothesize that in the full receptor the low absolute free-energetic state is the desensitized state.

**772-Pos Board B527****Differential Effects of Synaptic and Extrasynaptic NMDA Receptors on A $\beta$ -Induced Nitric Oxide Production in Cerebrocortical Neurons**

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Oligomerized amyloid- $\beta$  (A $\beta$ ) peptide is thought to contribute to synaptic damage, resulting in dysfunctional neuronal networks in Alzheimer's disease. It has been previously suggested that A $\beta$  may be detrimental to neuronal health, at least in part, by triggering oxidative/nitrosative stress. However, the mechanisms underlying this process remain to be elucidated. In this study, using rat primary cerebrocortical cultures, we investigated how oligomeric A $\beta$  peptides produce nitrosative stress. Relying on different pharmacological inhibitors, we demonstrate that oligomeric A $\beta$ 1-42 peptide triggers a dramatic increase in intracellular nitric oxide (NO) concentration via a process mediated by activation of NMDA-type glutamate receptors. Considering that synaptic NMDA receptors (sNMDARs) and extrasynaptic NMDA receptors (eNMDARs) may play disparate or even opposing roles in physiological and pathological events in neurons, we explore their respective roles in oligomeric A $\beta$ -induced increases in intracellular NO levels. Using an established protocol for pharmacological isolation of eNMDARs, we discovered that eNMDARs are responsible for the majority of NO production triggered by the exposure to A $\beta$  oligomers.

This effect likely results from the ability of A $\beta$  oligomers to alter the balance of glutamatergic activity between sNMDARs and eNMDARs in neurons, as recently reported by our group. Since sNMDARs outnumber eNMDARs in neurons, eNMDARs appeared to be much more efficient than sNMDARs in stimulating the activity of NOS in response to an A $\beta$  insult. Considering the pronounced role of eNMDARs in neuronal pathophysiology, the effect of this excessive increase in NO could affect various proteins crucial for neuronal and synaptic function and survival. This finding suggests that pharmacological intervention specifically aimed at eNMDARs may provide neuroprotection in Alzheimer's disease by decreasing A $\beta$ -induced nitrosative stress and thus ameliorating neurotoxic pathways that damage synapses.

**773-Pos Board B528****Inter-Subunit Interactions of NMDA Receptor Amino-Terminal Domains Associated with Allosteric Modulation**

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N-methyl-D-aspartate (NMDA) receptors are one of the three main types of ionotropic glutamate receptors. NMDA receptors are obligate heterotetramers typically composed of two GluN1 and two GluN2 subunits. GluN1 subunits can be one of eight different splice variants, and GluN2 subunits are classified as four subtypes A-D. Each subunit is organized into domains: an intracellular carboxy-terminal domain, the transmembrane, pore-forming domain, an extracellular, ligand-binding domain, and an extracellular, amino-terminal domain (ATD). NMDA receptors are unique among glutamate receptors because allosteric modulators bind to the ATD and either inhibit or potentiate the receptor. The unique feature of these modulators is the subunit specific nature of their effect, despite relatively high sequence homology (~56% for GluN2A and GluN2B ATDs). For instance, zinc inhibits NMDA receptors containing different GluN2 subunits with varying affinities and efficacies. Ifenprodil only inhibits receptors containing the GluN2B subtype, and spermine only potentiates receptors containing the GluN2B subtype. Luminescence resonance energy transfer (LRET) on full-length receptors expressed in CHO cells was used to characterize conformational changes associated with the binding of allosteric modulators to the amino-terminal domains. Zinc binding induces a cleft-closure conformational change in the GluN2A ATD, and the lower lobe of the GluN2A ATD moves towards the upper lobe of the GluN1 ATD. There is no change in the distance between the two upper lobes of the GluN1 and GluN2A ATDs. Finally, LRET measurements show that zinc binding to the GluN2A ATD does not allosterically induce any large scale movements within the GluN1 ATD. These findings suggest that the cleft-closure conformational change in the GluN2A ATD upon binding zinc is most likely only propagated down towards the ligand binding domain and ultimately the channel segments.

**774-Pos Board B529****Proton Mediated Conformational Changes in ACID Sensing Ion Channel**

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Acid sensing ion channels (ASIC) are cation channels, activated when there is a reduction in pH. They are involved in key functions including nociception, synaptic transmission and in the pathophysiology of ischemic stroke. Previous crystal structures show a trimeric receptor, with each monomer having a large extracellular domain, shaped like a hand, with finger, palm and thumb domains which in turn connect to the transmembrane segments. Available structures indicate that there are three pairs of aspartate and glutamate residues located in the extracellular region, lining the thumb and finger domains. Previous studies have shown that mutating one or two pairs of the carboxylates leads to a shift in EC50 but not a loss in function, suggesting additional residues are involved in proton sensing. Our results from simulation studies indicate that neutralizing these two pairs alone is not sufficient to reduce the electrostatic potential. However, neutralizing all three pairs of carboxylates eliminates much of the negative electrostatic potential in this region, indicating the role of all three pairs in proton binding and gating of the ion channel. Electrophysiological studies with the triple mutant shows a loss of function, while surface biotinylation and pull down assays show expression of the receptors at the surface. To test the pH mediated structural changes and also the role of these residues in the same, we used Luminescence Resonance Energy Transfer to study the angstrom level changes that occur upon pH reduction from 8 to 6. We see that there is a cleft closure conformational change that occurs between the finger and thumb domain. This motion is lost in the triple carboxylate mutant protein, thus confirming that these residues play an important role in proton mediated conformational change in the receptor and ultimately gating of the receptor.