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Dynamical Mechanisms of Glutamate Receptor Gating and Sub-Conductance

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Ionotropic glutamate receptors (iGluR) are responsible for initiation of excitatory signal transmission in the central nervous system (CNS). They play important roles in synaptic plasticity and long-term potentiation, while their dysfunction causes variety of diseases including neurodegenerative diseases and epilepsy, therefore they are potential targets for drug design. Glutamate receptors exhibit a distinctly modular multi-domain structure, in which truncated proteins containing only the ligand binding domain (LBD) and the transmembrane domains (TM) form the smallest functional glutamate gated channels. These homo-tetrameric AMPA subtype glutamate receptors gating and desensitization occurs on millisecond time-scales, and thus, these are the smallest and fastest working ligand-gated ion channels. In this work we report on extensive multi-microsecond molecular dynamics (MD) simulations of the AMPA receptor gating and regulation. The simulations were designed to allow the receptors to inter-convert between their various functional states, such as open and closed TM channel, and ligand-bound, active and desensitized LBD domain. We have used a variety of machine learning (ML) methods and systematic feature selection techniques to characterize which specific features of the LBD structure are responsible for transducing the signal for channel opening. We have observed multiple events of partial closures of the channel correlated with the LBD cleft closure degree from which we are able to draw conclusions on specific mechanism of allosteric coupling between the LBD domains monomeric and quaternary structures, as well as the ion channel state of the TM domains. Our study is the first one that was able to successfully design a simulation for opening and closing of the TM ion channel in response to the state of the LBD domain and characterize thus far elusive sub-conductance states.

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Molecular Mechanism of pH Regulation on TMEM16F Lipid Scramblase and Ion Channel

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TMEM16F, a dual functional ion channel and phospholipid scramblase, is known to be important in blood coagulation, skeleton development and HIV infection. Recent structural and functional studies greatly advanced our understanding of the molecular architecture of the TMEM16F and its Ca²⁺-dependent activation mechanism. Nevertheless, how TMEM16F is regulated by other intracellular factors is still not clear. Here we show that both TMEM16F channel and scrambling activities are strongly influenced by intracellular pH (pH_i). Low pH_i attenuates while high pH_i potentiates both TMEM16F channel and scrambling activities. Our mutagenesis results explicitly demonstrate that the pH effects on TMEM16F are caused by protonation and deprotonation of the Ca²⁺ binding sites, which in turn reduces and enhances Ca²⁺ binding affinity to TMEM16F, respectively. Our findings thus uncover a new regulatory mechanism of TMEM16F, which will facilitate our understanding of TMEM16F lipid scramblase and ion channel under physiological and pathological conditions.

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Life in the Fast Lane- Binding to Glutamate Receptors

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Ionotropic glutamate receptors (iGluRs) are large transmembrane protein complexes found in neuronal synapses. iGluRs transduce chemical signals carried by the neurotransmitter glutamate into electrical impulses. The binding of neurotransmitter molecules to the ligand-binding domains (LBDs) of these receptors drives the opening of transmembrane pores, allowing cations to flow across the cell membrane to trigger the generation of a nerve impulse in the postsynaptic neuron. Activation of the receptor is dependent on allosteric coupling of conformational changes at the binding site to changes at the ion channel gate. iGluRs mediate excitatory responses at the vast majority of synapses in the brain, and they play key roles in learning and memory. iGluRs are

implicated in neurological disorders such as epilepsy, depression, dementia, and pathological pain. Using both enhanced sampling and equilibrium all-atom molecular dynamics simulations, we have determined the binding mechanisms of the natural agonists glutamate, glycine, and D-serine to the isolated LBDs of AMPA, kainate, and NMDA receptors, the major families of functional iGluRs. Glutamate was found to bind to the AMPA and kainate receptor LBD via a “guided-diffusion” mechanism, in which charged residues on the surface of the protein form metastable interactions with the ligand to help guide it into its binding pocket. For NMDARs, which require both glutamate and glycine or D-serine for activation, glutamate was found to bind via guided diffusion, whereas glycine was found to bind via “unguided diffusion”. The physiologically important NMDA receptor co-agonist D-serine can bind via both guided and unguided diffusion, although guided diffusion is heavily favored. Electrophysiological recordings demonstrate that eliminating the metastable binding sites in the AMPA receptor slows activation and deactivation, consistent with slower glutamate binding and unbinding. These results suggest that binding pathways have evolved to accelerate iGluR responses at synapses.

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Using a Network of Single Site Specific Mutations and Crosslinking Mass Spectrometry (CXMS) to Refine the Structure and Dynamics of the Human Alpha 1 Glycine Receptor (GLYR)

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A network of site-specific single Cys-mutations coupled with CX-MS can be used to elucidate a more refined structure of GlyR and obtain a more definitive understanding of pentameric ligand-gated ion channel (pLGIC) allostery. Each Cys-mutant is introduced into an $\alpha 1$ homomeric Cys null background (C41S/C290A/C345S), or in the same background with F207G/A288G mutation that allows non-desensitizing GlyR activation by ivermectin (IVM). State-dependent crosslinking with methanethiosulfonate benzophenone to a single thiol of purified, vesicle reconstituted GlyR are conducted after enriching the receptor in different allosteric states: resting (no ligand), open (F207G/A288G + IVM), or desensitized (excess glycine). Digested peptides are analyzed via liquid chromatography mass spectrometry to identify sites of intra- and intermolecular crosslinking. Tandem MS of mass-shifted precursor ions further refine these distance constraints. Independent comparative studies targeting different single Cys GlyR (M287C, K116C, K206C) provides evidence of allosteric changes between the three states, as well as direct topological information of unresolved regions, most notably the M3-M4 loop, in other high-resolution structures of pLGICs.

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Cryo-EM Structure Determination and Model Fitting of the Proton-Gated Ligand-Gated Ion Channel glie at Multiple pH States

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Pentameric ligand-gated ion channels (pLGICs) play central roles for signal conduction in the nervous system, where closely related anionic or cationic channels exhibit a broad range responses to various neurotransmitters. In addition to the primary agonist, pLGICs are also highly sensitive to allosteric modulation, which makes them highly interesting model systems to understand conformational transitions and state-specific stabilisation - in particular bacterial homologs such as the pH-gated GLIC channel from *Gloeobacter violaceus*. It has however been surprisingly difficult to characterise both the various states as well as the conformational transitions between them. Here, we present a series of new high-resolution cryo-EM structures of the GLIC channel obtained at a range of different pH values, with local resolutions of 3-5 Å. These structures show interesting differences to previous X-ray structures, including a significant bias towards non-conducting transmembrane pores at all pH values, while the overall orientation of the pore-lining helices and ECD vary more between structures. To improve the structural modeling, we combined the 3D density reconstruction with a newly developed framework for Bayesian density fitting of models in molecular dynamics simulations, which enables us to rapidly achieve high-resolution structural models, to determine pH-dependent structural differences, and not least to assess the flexibility of different parts of the structure. This provides new insight into the structure of ligand gated ion channels under different conditions, it raises interesting questions about the