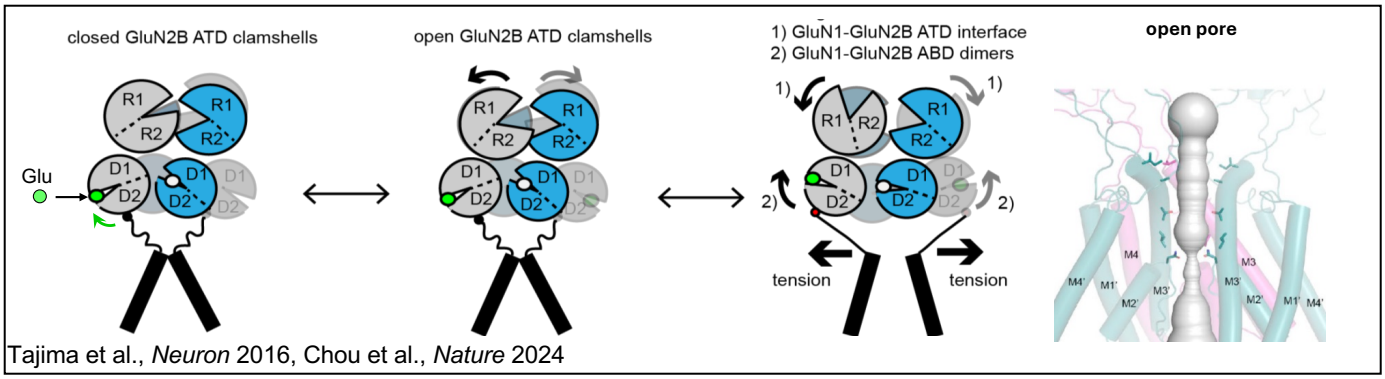
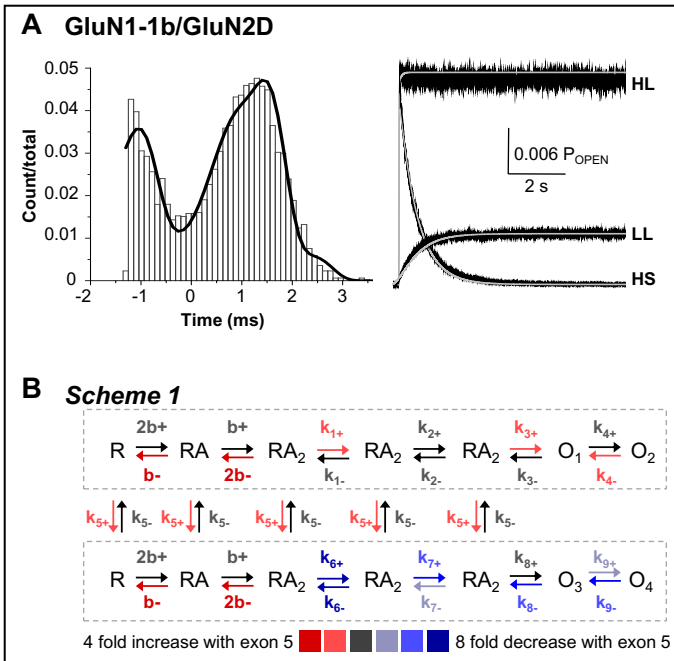
Mechanism by which the NMDA receptor pore opens



Tajima et al., *Neuron* 2016, Chou et al., *Nature* 2024

NMDA receptors are ligand-gated channels that catalyze the transmembrane flux of cations in response to the binding of the neurotransmitters glutamate and glycine. The architecture of the NMDA receptor is becoming understood with recent crystallographic and cryo-EM data, which unambiguously show that the NMDA receptors contain a large extracellular region comprising four dimers of eight bilobed clamshell-like domains that are connected by short polypeptide linkers. Four of these clamshell-like domains harbor an agonist binding site deep within their cleft. These four bilobed domains that comprise the agonist binding sites are connected by short linkers to a non-selective cation pore, which appears similar in structure to an inverted potassium channel. Receptors are tetrameric assemblies of two glycine-binding GluN1 and two glutamate binding GluN2 subunits, which occur as four unique gene products that each endow the receptor with distinct properties.



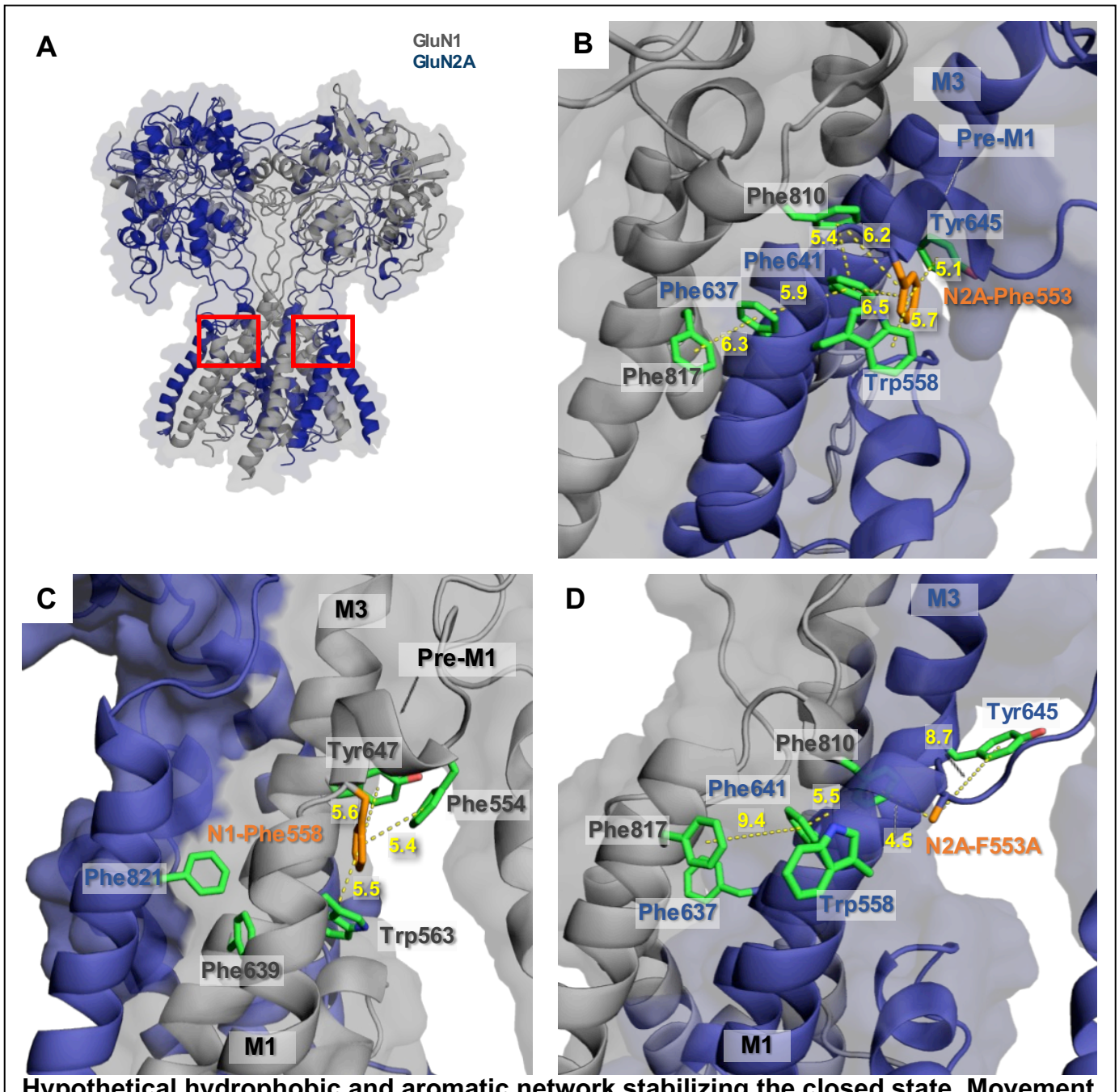
A, Fit of channel closed times from GluN1/2D in an outside-out patch and macroscopic currents activated by 1 mM glutamate for 10 sec (HL), 1 mM glutamate for 10 ms (HS), and 0.0025 mM glutamate for 10 sec (LL) with Scheme 1. **B**, Rates colored based on the change between GluN1+exon5 and GluN1-exon5. Dark red 4-fold increase, light red 1.5-fold increase, light blue 1.5-fold decrease, medium blue 2-5-fold decrease, dark blue 8-fold decrease for exon 5. Vance et al., 2012.

At the single channel level, the complex kinetics that underlie channel function suggest at least three conformationally distinct changes that precede pore opening, which include a very fast step that is almost certainly the brief delay prior to explosive pore dilation. Rapid pore dilation gives the channel-mediated unitary currents recorded in excised patches their characteristic square appearance. The other two steps inferred from the duration of closed periods reflect rate-limiting conformational changes, which functional data and structural data suggest may be controlled in part by the different subunits.

Unitary currents from a GluN1/2A receptor in an outside out patch.

We are studying the contributions of specific domains to receptor gating, with a focus on the parts of the receptor that must undergo the rate-limiting conformational changes we see

prior to opening. We perform single channel and macroscopic recordings and consider these in the context of new structural information. We utilize two forms of perturbations of receptor function to learn about the relationship between structure and key gating steps. These include single channel studies of the mechanism of action of our novel subunit-selective positive and negative allosteric modulators, which bind near the linkers that connect the agonist binding domain to the channel gate and dramatically alter NMDA receptor function. We also have identified a large number of human mutations at three closely spaced interacting elements that we think are involved in channel gating. We are testing specific structural hypotheses about how channel gating proceeds by recording and analyzing the effects of 0, 1, or 2 copies of these mutations in NMDA receptors. These studies could improve our understanding of how channel mutations contribute to neurological disease and how therapeutically relevant modulators change receptor function. Current hypotheses involve the pre-M1 helix and a network of aromatic residues that stabilize the closed channel and rearrange during channel activation.



Hypothetical hydrophobic and aromatic network stabilizing the closed state. Movement of pre-M1 helix destabilizes the network favoring rearrangement to the open state.