Crystal structure of heterotetrameric NMDA receptor reveals insights into subunit arrangement and function

N-methyl-D-aspartate (NMDA) receptors mediate fast neurotransmission that is pivotal in brain development and basic function including learning and memory formation. They are ligand-gated ion channels that open upon binding of neurotransmitter, glutamate, along with a co-agonist glycine or D-serine (Fig. 1). Dysfunction of NMDA receptors is implicated in neurological disorders and diseases including depression, schizophrenia, Alzheimer's disease, Parkinson's disease, and ischemic injuries associated with stroke. There has been a great enthusiasm toward understanding the structure and function of NMDA receptors at the molecular level owing to their importance in basic neuroscience as well as disorders and diseases. Using **BL41XU** beamline at SPring8 and ID23 beamline at Advanced Photon Source at the Argonne National Laboratory, we obtained the first crystal structure of heterotetrameric NMDA receptors containing an amino terminal domain (ATD), a ligand-binding domain (LBD), and a transmembrane domain (TMD) [1].

Structural studies of NMDA receptors has been considered challenging since they are obligatory heterotetramers composed of large transmembrane subunit proteins, namely GluN1 and GluN2 subunits. Toward obtaining the crystal structure, we extensively tested various protein constructs and expression methods and found that the best yield of properly assembled heterotetrameric GluN1a/GluN2B NMDA receptors can be obtained by truncating the carboxy terminal domain (CTD) (hence called GluN1a/2B$_{cryst}$) and by using a combination of *Sf*9 insect cells with a recombinant baculovirus under the Hsp70 promoter from *Drosophila melanogaster*.

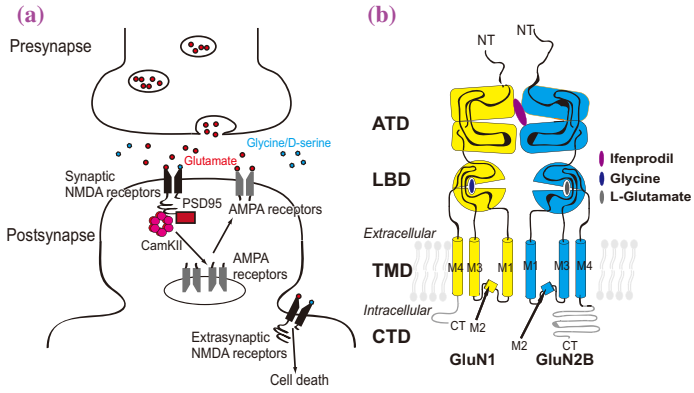

Fig. 1. NMDA receptors **(a)** NMDA receptors are critical for neuronal signaling involved in brain development and function. Binding of glutamate and glycine or D-serine opens NMDA receptor ion channels and facilitate high calcium influx. **(b)** NMDA receptors are heterotetrameric ion channels composed of two subunits, GluN1 and GluN2 and in some cases GluN3. Each subunit is composed of ATD, LBD, TMD, and CTD.

The GluN1a/2B$_{cryst}$ construct was crystallized in the presence of the GluN1 agonist, glycine, the GluN2 agonist, glutamate, and an ATD-binding allosteric inhibitor, ifenprodil. The structure was initially solved at 5.7 Å by molecular replacement using the structures of the ATD [2] and LBD [3] as search probes. To improve the diffraction power of these crystals, we stabilized the heterotetrameric subunit interactions by forming inter-subunit disulfide cross-links at the extracellular and juxtamembrane regions based on the 5.7 Å structure above. This construct manipulations improved the diffraction limit to beyond 4 Å, which resulted in electron density sufficient to build most of the GluN1a/2B NMDA receptor including the entire extracellular domains, TMD, linkers between ATD and LBD and between LBD and TMD.

The GluN1a/GluN2B NMDA receptor bound to glycine, L-glutamate, and ifenprodil is shaped like a "hot-air balloon" where a balloon and a basket correspond to the entire extracellular domains and the TMD, respectively. The structure has a clear boundary between the layers of LBD and TMD while ATD and LBD appear as a single unit. Both ATD and LBD form two GluN1a-GluN2B heterodimers that are assembled as GluN1-GluN2-GluN1-GluN2 (1-2-1-2) heterotetramer where the two-fold symmetry axis runs across the center of the molecule through the interface between the two GluN2B ATDs to the middle of the ion channel pore (Fig. 2). The overall shape of GluN1a/GluN2B NMDA receptor is highly distinct from that of homotetrameric GluA2 AMPA receptor, another member of the iGluR family, which has the overall "Y" shape [4]. This difference in the overall architecture is attributed to the fact that ATD and LBD pack tightly through numbers of well-defined inter-domain interfaces in GluN1a/GluN2B NMDA receptors whereas ATD and LBD interact minimally in GluA2 AMPA receptors. This surprisingly "compact" architecture of GluN1a/GluN2B NMDA receptor stems from the inter-subunit and inter-domain interactions over large surface areas that are unique to NMDA receptors.

The TMD of GluN1a/GluN2B NMDA receptor forms the heterotetrameric ion channel with pseudo four-fold symmetry. The ion channel of GluN1a/GluN2B NMDA receptor is similar to the closed state of GluA2 AMPA receptors and KcsA bacterial potassium channels. In this plausible allosterically inhibited state, the ion channel is closed to the similar extent to the

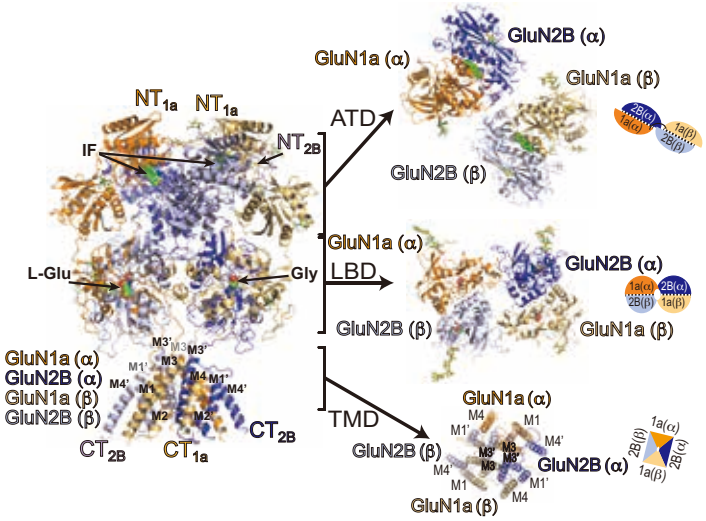

Fig. 2. The first crystal structure of heterotetrameric NMDA receptors. We crystallized the GluN1/GluN2B NMDA receptors without CTD. The heterotetramers are formed as a dimer of heterodimer in the GluN1-GluN2B-GluN1-GluN2B arrangement in each domain layer. Interestingly, dimer pairs are swapped between ATD and LBD.

closed channels of AMPA receptor and KcsA. One of the key functions of NMDA receptors is their high permeation of calcium ions, which plays the major role in neuronal plasticity as well as excitotoxicity. The crystal structure complexed with holmium, a lanthanide known to recognize calcium binding sites in many biological macromolecules, shows the binding in between the LBD-TMD linkers of GluN1 right above the center of the ion channel (Fig. 3). A set of acidic residues in GluN1 (DRPEER motif) located in this region has been shown previously to be a critical part of high calcium flux characteristic of NMDA receptors [5]. Thus, the lanthanide binding site along with the previous electrophysiological study further confirms the physiological relevance of the current crystal structure. However, despite extensive efforts, the regions of TMD that determines voltage-dependent Mg^{2+} block and Ca^{2+} permeation was not clearly resolved in this crystal structure. Structure-based understanding of ion selectivity and flux regulation is thus a question that remains to be addressed.

The crystal structure of GluN1a-GluN2B NMDA receptors marks the first crystal structure of a heteromeric ion channel. The structure will serve as a template for designing experiments that further address complex function of NMDA receptors. Finally, the defined subunit interfaces should serve as an invaluable blueprint for design of therapeutic compounds.

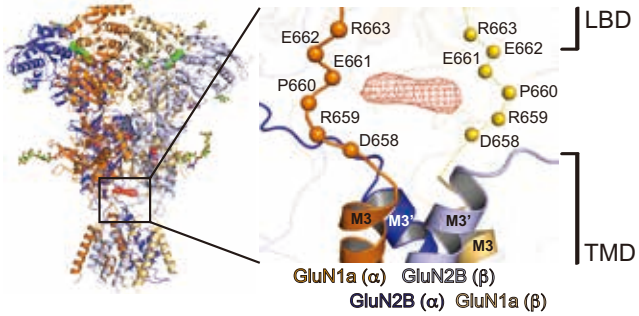

Fig. 3. Plausible calcium binding site at the juxtamembrane site. Here anomalous difference Fourier map obtained from diffraction data of crystals soaked against holmium is shown. Hollmium has been previously shown to bind calcium binding site.

Erkan Karakas and Hiro Furukawa*

Cold Spring Harbor Lab., WM Keck Structural Biology Laboratory, USA

*E-mail: furukawa@cshl.edu

References

[1] *E. Karakas and H. Furukawa: Science* ***344*** *(2014) 992.*
[2] E. Karakas *et al.*: Nature **475** (2011) 249.
[3] A. Jespersen *et al.*: Neuron **81** (2014) 366.
[4] A.I. Sobolevsky *et al.*: Nature **462** (2009) 745.
[5] J. Watanabe *et al.*: J. Neurosci. **22** (2002) 10209.