Study Conformational Changes in NMDA Receptors Using smFRET

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Introduction

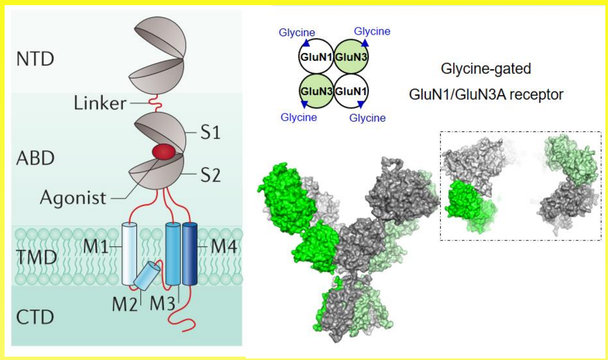


Figure : Left: NMDAR subunit topology diagram. Right: Structure of the GluN1/GluN3A receptor

Provided by researcher Shu-Jia Zhu.

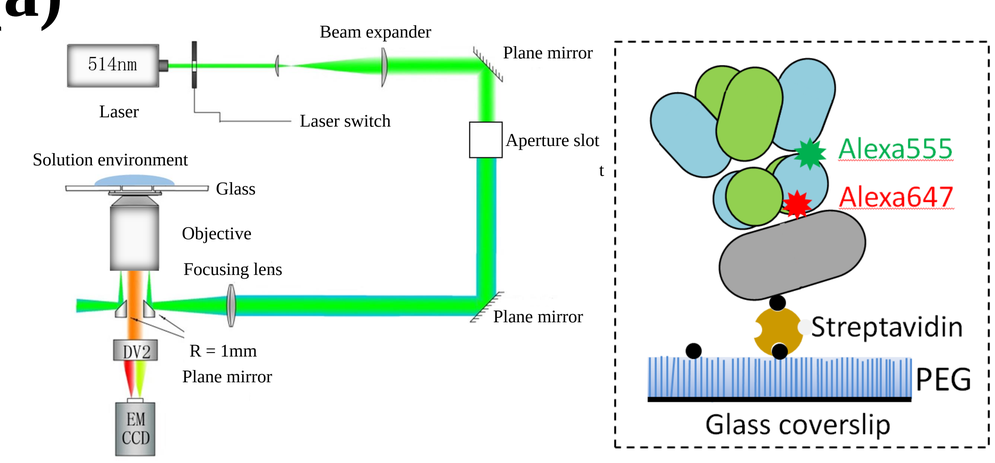
Neurons are the basic unit of the central nervous system. Synapses are the basic structure for the transmission of chemical signals between neurons. The normal function of ion channels located in the presynaptic membrane and the postsynaptic membrane is the basis for ensuring the orderliness of neurons.

NMDA receptors (N-methyl-D-aspartic acid Receptors, NMDARs) are cationic ligand-gated channels located at synapses. They play an important role in synaptic plasticity, and their function is considered to be the basis of brain memory and learning. The non-classical GluN1/GluN3A receptor is different from other glutamate excitatory receptors in that it is activated by Glycine. But so far, its gating mechanism is unknown.

Here, we use single molecule fuorescence resonance energy transfer (smFRET) to explore the dynamic gating mechanism of GluN1/GluN3A receptor in apo, open, and desensitized state.

Experimental Setup

(a)



(b)

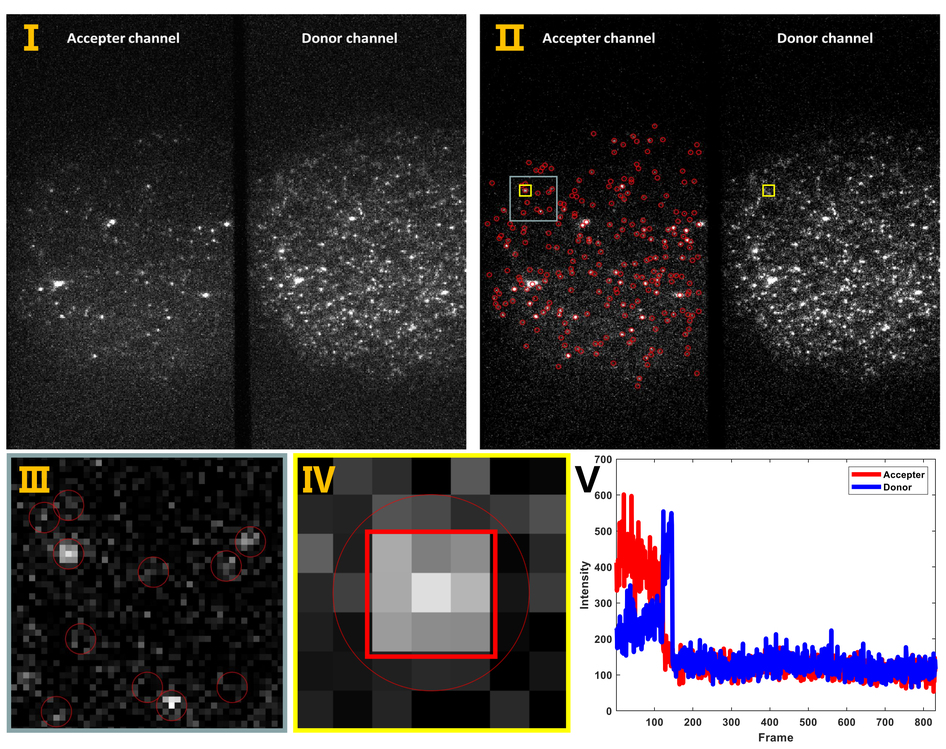


Figure (a) : Setup of total internal reflection fluorescence microscopy (TIRFM) in our lab and protein label、attachment strategy.
Figure (b) : I :TIRFM raw data II~V :Our post processing to get two channel intensity trace of every valid bright point.

Results

➢ Conformation changes in GluN1-LBD after the addition of glutamate

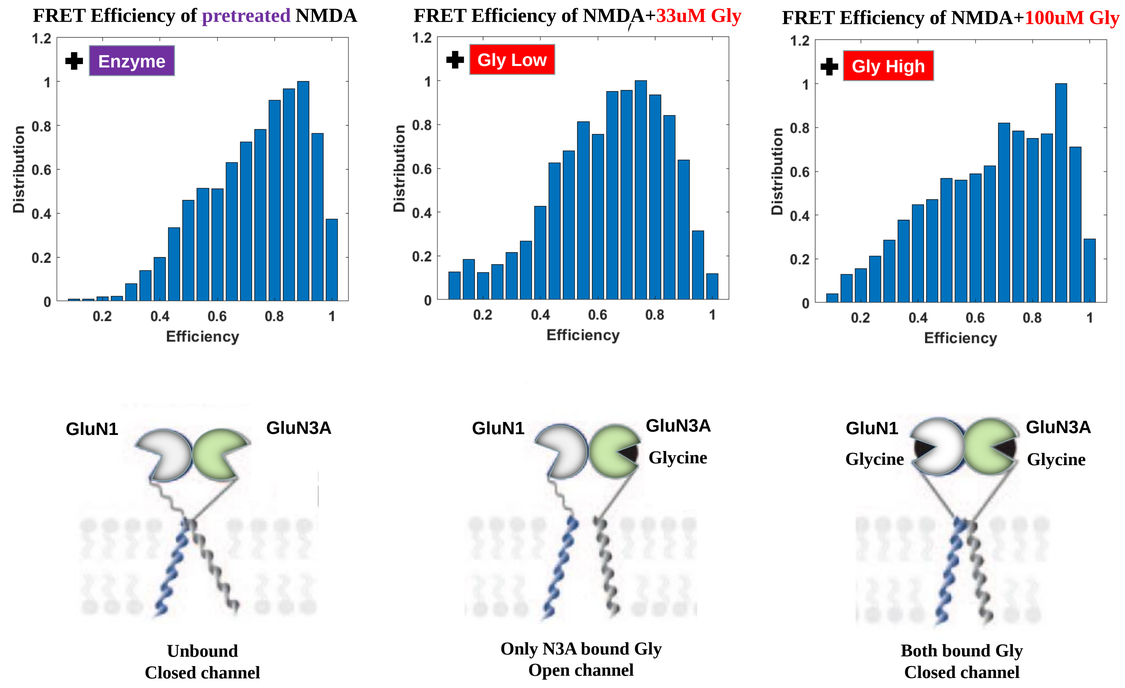


Figure : FRET efficiency changes in GluN1-LBD. Left: after the addition of enzyme with glycine as substrate(Closed channel). Middle: after the addition of 1uM glycine(Open channel). Right: after the addition of 100uM glycine(Closed channel).

➢ Conformation changes in GluN1-LBD after the addition of glutamate and CGP

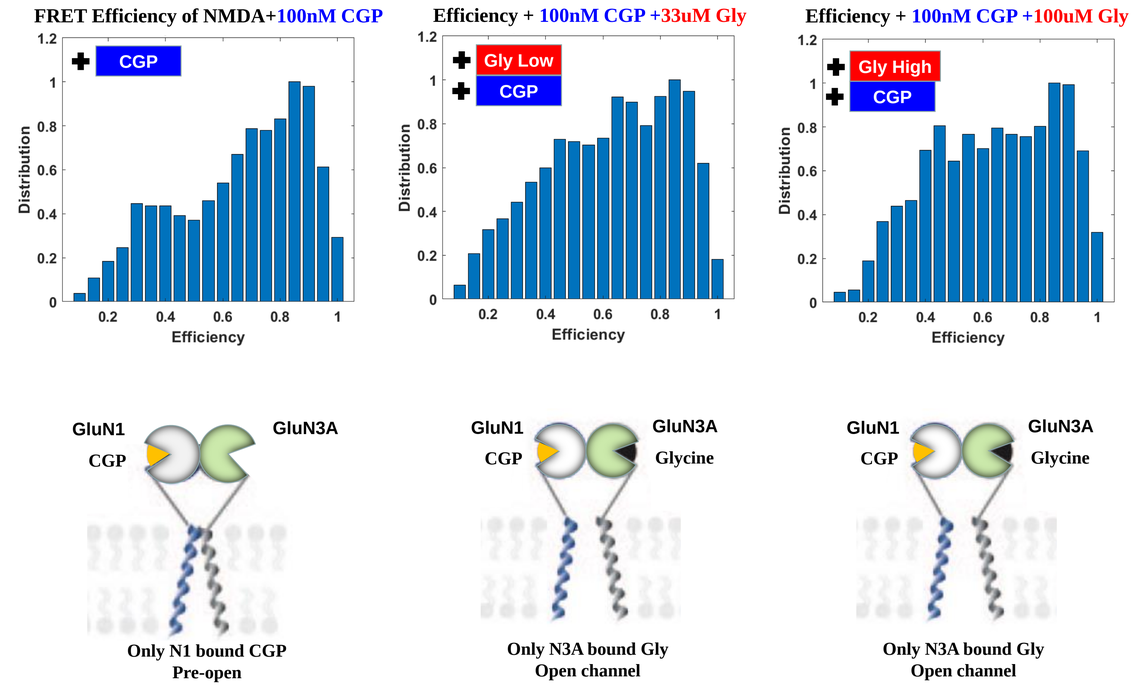


Figure : FRET efficiency changes in GluN1-LBD. Left: after the addition of 100nM CGP bound with GluN1(Pre open). Middle: after the addition of 100nM CGP and 33uM glycine(Open channel). Right: after the addition of 100nM CGP and 100uM glycine(Open channel).

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Conclusions

1. We have obtained that the GluN1/GluN3A receptor ’s channel will open under low glycine concentration and will close under high glycine concentration. which is due to the different roles played by GluN1 and GluN3A subunits in receptor gating mechanism
2. With the addition of competitive anti-caking agent CGP bounded specifically to the GluN1 subunit, the GluN1/GluN3A receptor can maintain channel open even under high glycine concentration , which is consistent with the gating mechanism we expected.
3. GluN1/GluN3A receptor ’s channel will open and close multipliable times when glycine concentration increase, means that there are more details worth studying.