Stoichiometry of GluA1/GluA2 heteromer of AMPA receptor

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Introduction

AMPA receptors are one of the ionotropic glutamate receptors in neurons whose structural flexibility is important for synaptic plasticity. AMPA receptors are made of four structural domains namely N-terminal domain, ligand-binding domain, transmembrane domain and C-terminal domain [1]. They are tetrameric and made of four main subunits GluA1, GluA2, GluA3, GluA4 and several auxiliary subunits such as γ2, CNIH, TARP etc. It has been observed that AMPA receptors are mostly heteromeric (GluA1-GluA2/GluA2-GluA3) in brain hippocampus, dorsal striatum and prefrontal cortex and GluA1-GluA2 heteromers are one of the predominant AMPA receptor heteromers in brain [2]. Studies have been conducted to decipher the stoichiometry of heteromers and they suggest that there is a 2:2 stoichiometry preference for GluA1/GluA2 heteromer but none of them has a conclusive reason behind this preference[3-5]. Now a day's single-molecule imaging is widely used to study receptors at the molecular level. Hence in this research, the assembly pattern of GluA1/GluA2 heteromers in frog oocytes will be studied using a previously established single-molecule imaging method [6].

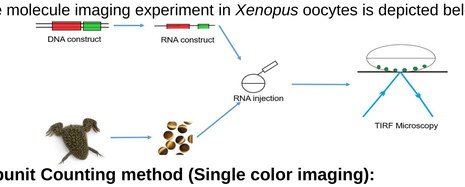
Objective

Some studies suggest that GluA1/GluA2 heteromer assembly controls the conductance and calcium permeability, but it is still not clear if any structural domain is responsible for this assembly with a 2:2 preference or if it is a random process. It is reported that GluA2 show lower conductivity than other AMPA receptor subunits (GluA1, 3, 4) because there is an RNA editing site (glutamine to arginine) in the transmembrane re-entrant loop of GluA2 subunit of AMPA receptor that makes the subunit calcium impermeable. Therefore, it may be possible that the RNA editing site in the transmembrane region affects the heteromeric assembly of GluA1/GluA2.

So, the aim of this research is to understand **how does the transmembrane domain containing the RNA editing site affect the GluA1/GluA2 heteromer assembly?**

Materials and Methods

We have used *Xenopus* oocytes for studying receptor stoichiometry in vivo. A simplified diagram of Single molecule imaging experiment in *Xenopus* oocytes is depicted below:



M1. Subunit Counting method (Single color imaging):

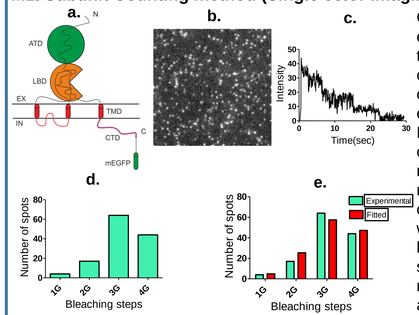


Fig M1 a. A simplified diagram of AMPA receptor, **b.** Observed single molecules **c**. Example of bleaching step profile from a spot, **d.** Observed number of spots depending on number of bleaching steps, **e.** Fitted observation with a tetrameric model

GluA1 is known to form tetramer when expressed alone. So GluA1 was tagged with mEGFP (Fig: M1a) and the construct was expressed in Xenopus oocytes. A typical experiment showed expression of single molecules (Fig: M1b) and most of the spots showed clear bleaching steps (Fig: M1c). The number of bleaching steps of mEGFP molecules from single spots were counted. It was observed that there were high number of spots with 4 bleaching steps. As earlier reports suggests that there is a fraction of GFP not being fluorescent [6], there were also spots with 3, 2 and 1 bleaching steps (Fig: M1d). The observation was then fitted with a tetrameric model (Fig: M1e) which indeed suggests that GluA1 assemble in tetramers.

Materials and Methods

M2. Subunit Counting method (Dual color imaging):

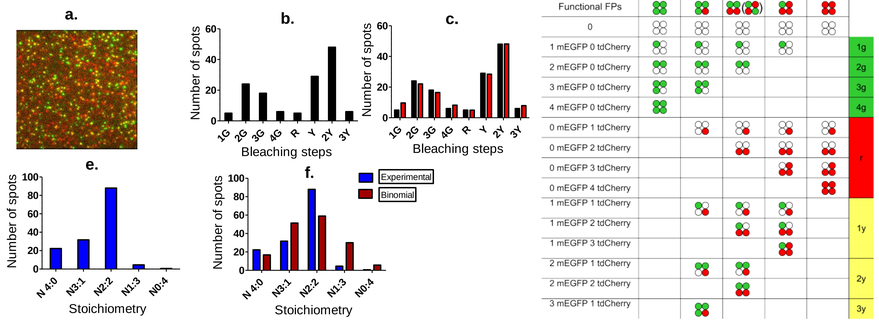


Fig M2 a. Example of dual color imaging **b**. Observed mEGFP bleaching steps from green and yellow spots **c.** Fitted experimental data **d**. Relation between observed bleaching steps and stoichiometry **e.** Reconstructed stoichiometry from observed data **f.** Fitted stoichiometry with a binomial model

To understand the assembly of GluA1/GluA2 heteromer, GluA1 and GluA2 were genetically fused with mEGFP and tdCherry respectively. A typical observation showed green, red and co-localized yellow spots (Fig: M2a). Then the bleaching steps of mEGFP were counted from green and yellow spots (Fig: M2b) (tdCherry bleaches very fast so it's bleaching steps were not counted but both mEGFP and tdCherry were used to understand the stoichiometry as depicted in Fig: M2d). The experimental observations were fitted and the stoichiometry of GluA1/GluA2 heteromer was reconstructed by least square fitting (Fig: M2c , M2e). Finally, the result was fitted with a binomial model where probability of GluA1 being present in a heteromeric assembly and total number of spots were free parameters (Fig: M2f).

M3. Chimera constructs:

In order to understand the role of RNA editing site in assembly of AMPA receptor subunits, two chimera constructs were cloned by swapping the transmembrane domains of GluA1 and GluA2 subunit (e.g. GluA121 and GluA212).

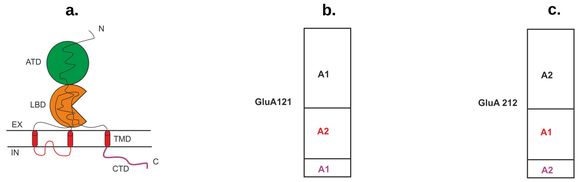


Fig M3 a. Simplified diagram of AMPA receptor. **b**. Simplified diagram of GluA121 chimera **c.** Simplified diagram of GluA212 chimera

Results and Analysis

R4. Assembly of chimera constructs:

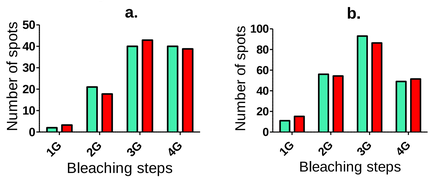


Fig R4: Assembly pattern of **a.** GluA121 homomers **b**. GluA212 homomers

GluA121-mEGFP and GluA212-mEGFP were expressed alone. The results were fitted with the method of subunit counting for single color imaging (M1) and the results showed that the chimera structures assemble in tetramers (Fig: R4a , R4b).

Question:

How do GluA121 and GluA212 chimera assemble when co-expressed with GluA1 or GluA2? Do they assemble randomly or show any preference for 2:2 stoichiometry?

Results and Analysis

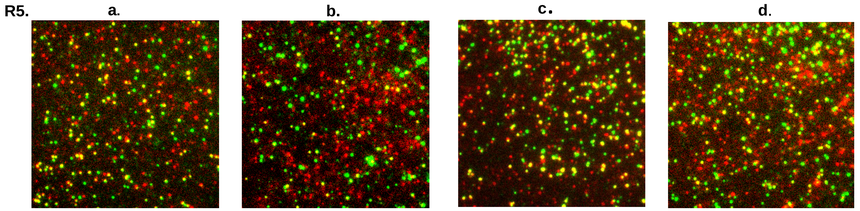


Fig R5: Observed single molecules when **a.** GluA121+GluA1 **b**. GluA121+GluA2 **c.** GluA212+GluA1 **d.** GluA212+GluA2 are co-expressed

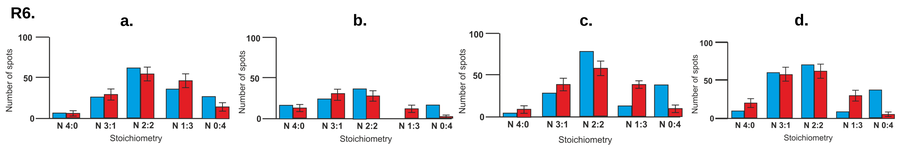


Fig R6: Binomially fitted and simulated reconstructed stoichiometry of **a.** GluA121 + GluA1 **b.** GluA121 + GluA2 **c.** GluA212 + GluA1 **d.** GluA212 + GluA2

GluA121-mEGFP and GluA212-mEGFP were expressed with both GluA1-tdCherry and GluA2-tdCherry. Movies were recorded from different batches of oocytes to understand the assembly. Example of movies are shown in Fig: R5 (a-d). The observations were fitted as stated in the method M2. The binomial data was further simulated 1000 times to get a clear understanding (Fig: R6 (a-d)). The mean values (red bars) of the simulations were plotted with experimental observations (blue bars). The error bar on the simulated data represents the standard deviation.

Discussion

Our study shows that the transmembrane domain is not changing the assembly behavior of the homomeric structures but the 2:2 preference of GluA1/GluA2 heteromers is getting disturbed. The chimera structures assembled randomly with both GluA1 and GluA2 subunits. The transmembrane domain is not the sole responsible domain for the preference as otherwise we would have expected 2:2 preference when GluA121+GluA1 (or GluA212+GluA2) were expressed. Also, we would have expected 2:2 preference when GluA121+GluA2 (or GluA212+GluA1) were expressed if the assembly was independent of transmembrane domain involvement. So, it is a possibility that the transmembrane domain is negatively regulating the assembly of heteromers.

Conclusion and Outlook

- The chimera structures can form homo-tetramers.
- The chimera constructs randomly assemble with GluA1 or GluA2.
- Besides the transmembrane domain, there might be other factors also that regulate the 2:2 stoichiometry of GluA1/GluA2 heteromers.
- In future, intra-subunit interaction study of AMPA receptors can provide further insight into this assembly process.

References.

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