Autodisplay of the NMDA receptor GluN1 and GluN2A ligand binding domains as a platform for binding assays

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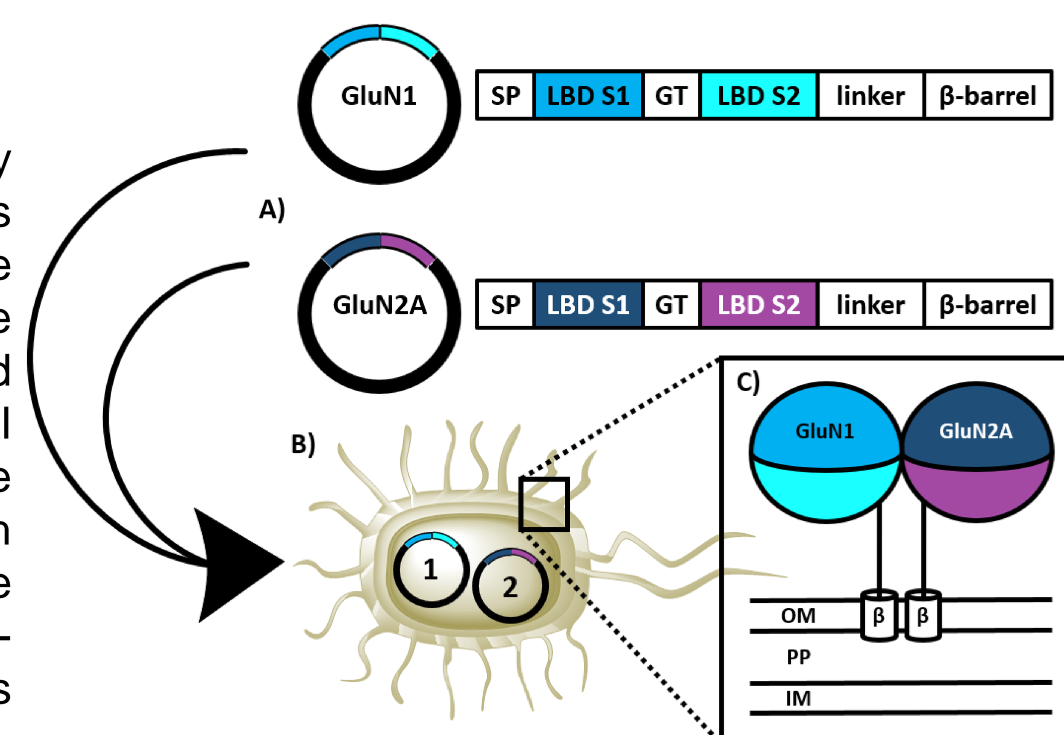
Background

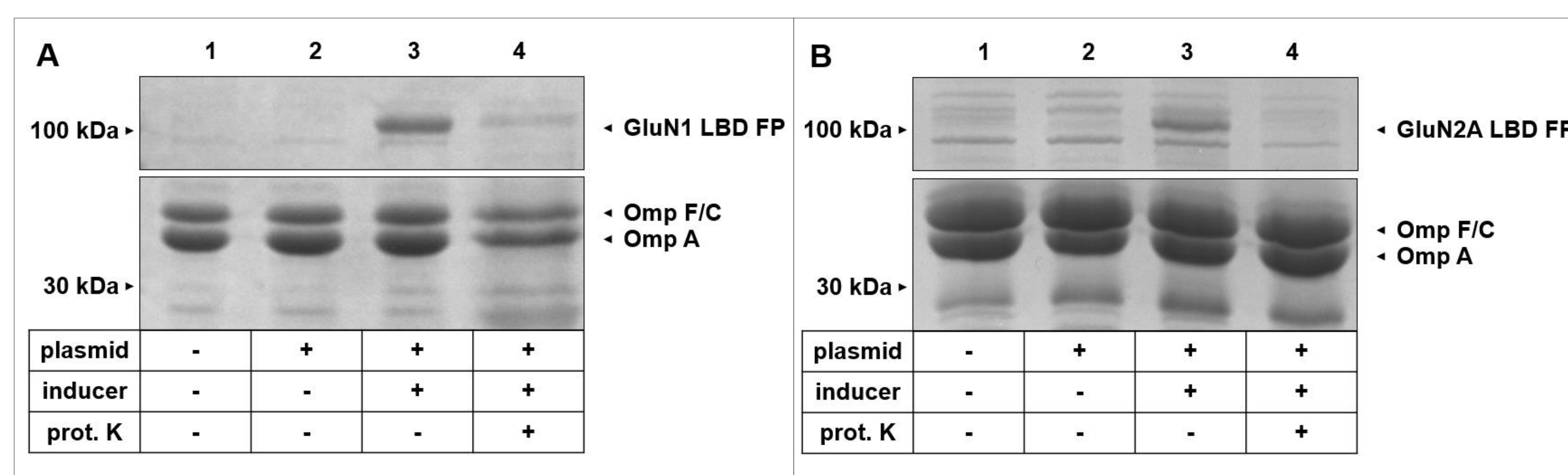
NMDA receptor ligand binding domains (LBD) of GluN1 and GluN2A subunits were surface displayed on *E. coli* by inserting the gene fragments of LBDs into pMATE autodisplay plasmids. The genetic sequence encoding the LBDs was inserted between the signal peptide (SP) and the translocation domain (linker and β-barrel). The SP directs the nascent fusion protein through the inner membrane (IM) to the periplasm (PP) and is cleaved off afterwards. The translocation domain anchors the fusion protein via the β-barrel domain into the outer membrane (OM) and translocates the linker and the adjacent protein of interest through the β-barrel pore onto the surface of the bacterial cell. **(A)** Plasmids were constructed enabling the surface display of either the GluN1 or the GluN2A LBD. The bilobed LBDs consisting of S1 and S2 lobes normally are segregated by transmembrane domains in the full-length receptor subunits. For the bacterial surface display of LBDs the transmembrane domains were discarded and the S1 and S2 lobes connected by a short dipeptide glycine-threonine (GT) linker. **(B)** Co-display was performed by co-transformation of *E. coli* with two plasmids. **(C)** Schematic representation of co-display of GluN1 and GluN2A LBDs on the surface of *E. coli* and formation of LBD heterodimers.

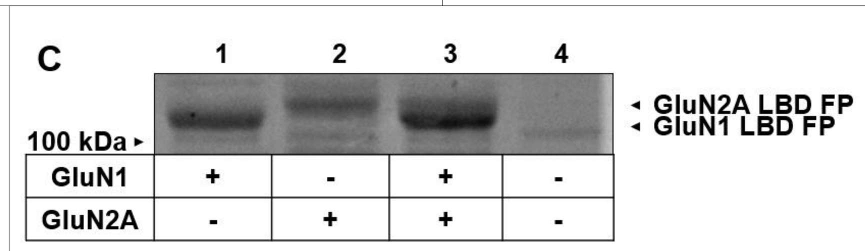


Results

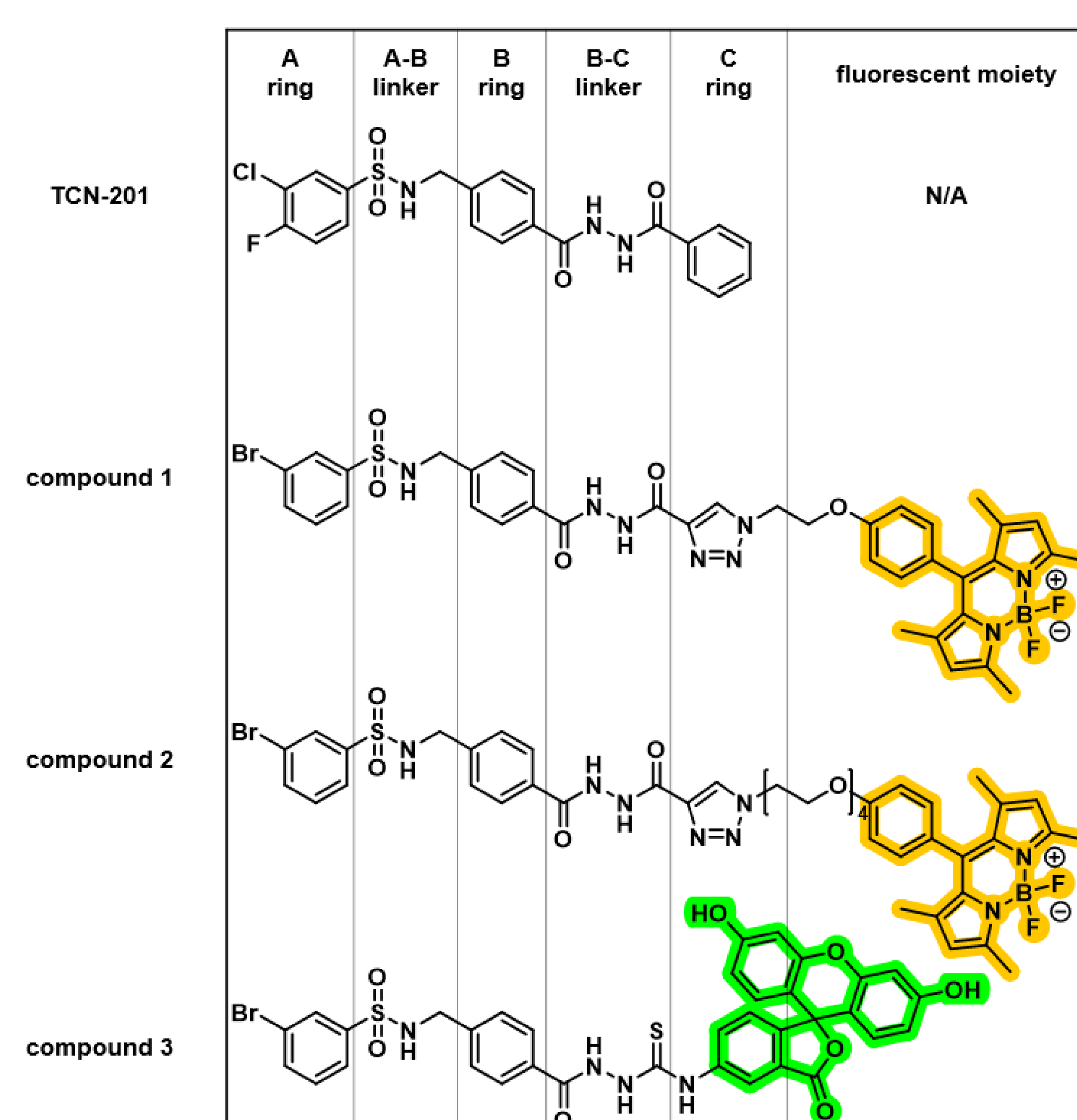
surface display and co-display of GluN1 and GluN2A LBDs

The surface display of individual GluN1 **(A)** and GluN2A **(B)** LBDs was confirmed by SDS PAGE analysis of outer membrane protein isolates. Additional protein bands corresponding to the LBD autotransporter fusion proteins (LBD FP) appear only in samples with cells harbouring the respective plasmid and additionally supplied with an inducer (A and B, lanes 3). When the samples were additionally treated with membrane-impermeable proteinase K (prot. K) before outer membrane protein isolation a strong diminishment of the LBD FP bands was observed. This confirms that the LBD FPs were located within the outer membrane, ranging into the extracellular medium. Omp A, F and C served as loading- and membrane integrity control. After confirming surface display for both LBDs individually, both plasmids were used to co-transform *E. coli* enabling co-display of both GluN1 and GluN2A LBDs **(C)**.



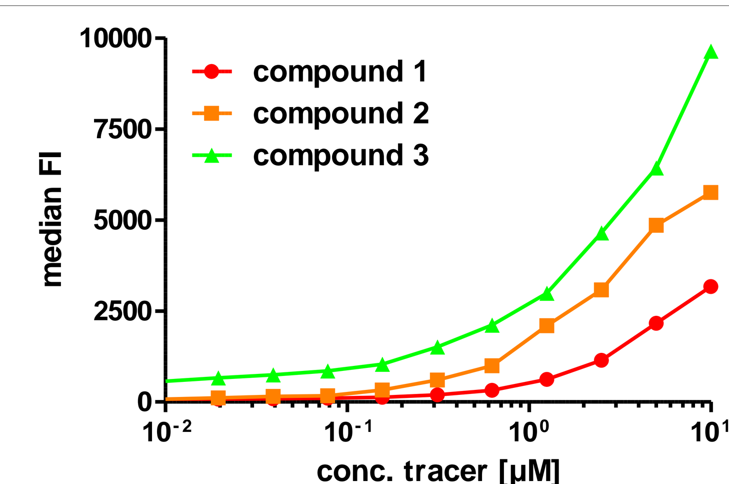


fluorescently labeled tracer ligands



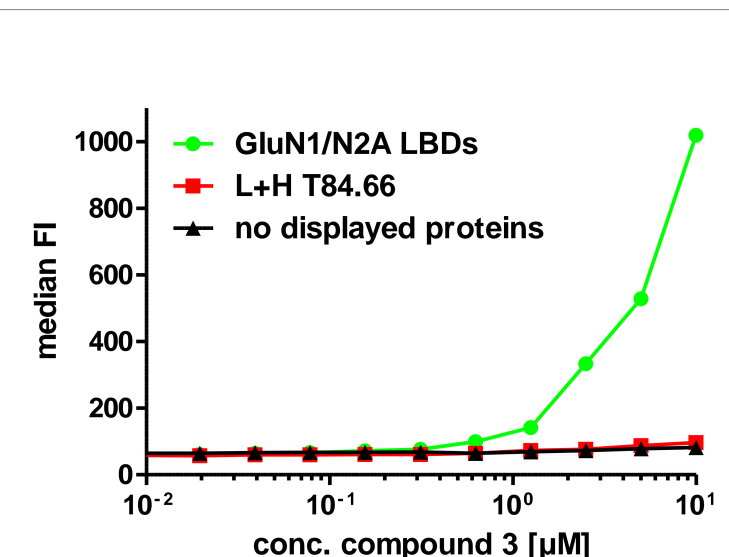
Based on the crystal structure of TCN-201-bound LBD heterodimer we proposed, that the C ring allows for derivatization, as this residue is slightly protruding out of the binding pocket. Three fluorescent derivatives were synthesized utilizing BODIPY- (compound 1 and 2) or fluorescein-based (compound 3) fluorescent moieties. Fluorophore coupling was either by linking to the C ring (compound 1 and 2) or by directly integrating an aromatic moiety of the fluorophore as C ring substitution (compound 3).

The binding of fluorescently labeled compounds to LBD co-displaying cells was investigated by flow cytometry. For all three compounds concentration dependent increase of median fluorescence intensity (FI) of the cells was observed. Under these conditions compound 3 (green) showed the best detection efficiency of the investigated compounds.



Binding specificity of compound 3 was probed by quantifying binding to cells displaying an unrelated dimeric protein (light and heavy chains of T84.66 antibody). Under these conditions the off-target binding (red) exceeded the binding to LBD co-displaying cells (green) and the surface display conditions were adapted to overcome this problem.

Promoter strength was altered by exchanging L-arabinose inducible pBAD promoter with L-rhamnose inducible rhaBAD promoter for both investigated plasmid combinations. Compound 3 binding to various cells was repeatedly quantified. The results after employing surface display optimization suggested that the previously observed off-target binding occurred not due to the properties of compound 3 itself.



Conclusion

- **LBDs of NMDA receptor GluN1 and GluN2A subunits were individually and co-displayed on the surface of *E. coli***
- **three fluorescent tracer ligands based on TCN-201 scaffold were synthesized**
- **fluorescent ligands were confirmed to bind to cells co-displaying GluN1 and GluN2A LBDs in a concentration dependent manner**
- **surface display was optimized to reduce off-target binding of compound 3 to a negligible level**

References

[1] Jose J. and Meyer T.F.: Microbiol. Mol. Biol. Rev. **2007, 71:600-619**
[2] Furukawa H. et al.: Nature. **2005, 438:185-192**
[3] Traynelis S.F. et al.: Pharmacol. Rev. **2010, 62:405-496**
[4] Yi F. et al.: Neuron. **2016, 91:1316-1329**

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