SUPPORTING INFORMATION

Crystal structures of potent dimeric positive allosteric modulators at the ligand-binding domain of the GluA2 receptor

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MATERIALS AND METHODS

TDPAM01 and TDPAM02 were synthesized as previously reported.[1] GluA2-LBD flop as well as the N775S (flip) and L504Y-N775S (predimer) mutants were expressed and purified as previously described.[2,3] The proteins were crystallized with TDPAM01 and TDPAM02 using the hanging drop vapor diffusion method with drop size of 1 μL protein-ligand solution plus 1 μL crystallization solution. The volume of the reservoir crystallization solution was 500 μL.

GluA2-LBD N775S (flip) with Glutamate and TDPAM01. A suspension of 20 mM TDPAM01 in buffer containing 5 mM L-glutamate, 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA and 10% DMSO was mixed using shaking in the cold room overnight. Then, the TDPAM01 suspension was added to the protein solution (in 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA), resulting in a concentration of 5 mg/mL GluA2-LBD flip and 5 mM TDPAM01 suspension. The protein−ligand suspension was left at 6 °C for 4 days with periodic shaking. Crystallization of the complex was performed at 6 °C. The crystal used for data collection was obtained using a reservoir solution consisting of 15% PEG4000, 0.1 M ammonium sulfate and 0.1 M phosphate citrate, pH 4.5.

GluA2-LBD L504Y-N775S (predimer) with Glutamate and TDPAM01. A suspension of 20 mM TDPAM01 in buffer containing 5 mM L-glutamate, 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA and 10% DMSO was mixed using shaking in the cold room overnight. Then, the TDPAM01 suspension was added to the protein solution (in 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA), resulting in a concentration of 5 mg/mL GluA2-LBD predimer and 5 mM TDPAM01 suspension. The protein−ligand suspension was left at 6 °C for 4 days with periodic shaking. Crystallization of the complex was performed at 6 °C. The crystal used for data collection was obtained using a reservoir solution consisting of 15% PEG4000, 0.1 M zinc acetate and 0.1 M sodium acetate, pH 5.5.

GluA2-LBD flop with TDPAM02. TDPAM02 was dissolved in 100% DMSO and added to the protein solution (in 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA), resulting in a concentration of 6 mg/mL GluA2-LBD flop, 4 mM TDPAM02 and 20% DMSO. The protein−ligand suspension was left at 6 °C for 1 day with periodic shaking. Crystallization of the complex was performed at room temperature. The crystal used for data collection was obtained using a reservoir solution consisting of 24% PEG4000, 0.3 M lithium sulfate and 0.1 M phosphate citrate, pH 4.5.

GluA2-LBD N775S (flip) with Glutamate and TDPAM02. TDPAM02 was dissolved in 100% DMSO and added to the protein solution (in 1 mM L-glutamate, 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA), resulting in a concentration of 5 mg/mL GluA2-LBD flip, 4 mM TDPAM02 and 20% DMSO. The protein−ligand suspension was left at 6 °C for 1 day with periodic shaking. Crystallization of the complex was performed at room temperature. The crystal used for data collection was obtained using a reservoir solution consisting of 24% PEG4000, 0.3 M lithium sulfate and 0.1 M phosphate citrate, pH 4.5.

GluA2-LBD L504Y-N775S (predimer) with Glutamate and TDPAM02. TDPAM02 was dissolved in 100% DMSO and added to the protein solution (in 10 mM HEPES (pH 7.0), 20 mM NaCl, 1 mM EDTA), resulting in a concentration of 6.5 mg/mL GluA2-LBD predimer, 4 mM TDPAM02 and 20% DMSO. The protein−ligand suspension was left at 6 °C for 1 day with periodic shaking. Crystallization of the complex was performed at room temperature. The crystal used for data collection was obtained using a reservoir solution consisting of 20% PEG4000, 0.3 M lithium sulfate, 0.1 M phosphate citrate, pH 4.5. L-glutamate was clearly visible in the orthosteric binding site and must be a left over from the purification as previously seen.[2]

The crystals were cryo-protected using reservoir solution containing at least 20% glycerol before flash-cooling in liquid nitrogen. X-ray diffraction data were collected at BioMAX, MAX IV, Lund, Sweden (Table S1). The data were processed with XDS[4] and scaled using SCALA in CCP4.[5] The structures were solved with molecular replacement using PHASER in CCP4. For the GluA2-LBD N775S and GluA2-LBD L504Y-N775S structures with TDPAM01, the structure of GluA2-LBD L504Y-N775S with BPAM121 (PDB code 5O9A, chain A) was used as search model in PHASER. For the three structures with TDPAM02, GluA2-LBD flop was phased using the apo structure of GluA2-LBD flop (PDB code 1FTO, chain A) and the two mutant structures using the structure of GluA2-LBD flop with L-glutamate (PDB code 1FTJ, chain A). The structures were refined with AUTOBUILD in PHENIX.[6] The program MAESTRO [version 10.1.013, Schrödinger, LLC, New York, NY, 2015] was used to generate coordinate files for TDPAM01 and TDPAM02. The energy minimization and conformational search were performed in water. The modulator parameter files for refinements in PHENIX were obtained using eLBOW,[7] keeping the geometry obtained from MAESTRO. The structures were manually adjusted in COOT[8] and refined in PHENIX with individual isotropic B-factors, NCS, TLS and riding H atoms. GluA2-LBD domain closures were calculated using the DynDom server[9] relative to the apo structure of GluA2-LBD flop with TDPAM02 (chain A). Figure 1 was made using ChemDraw [version 16.0, PerkinElmer Informatics,

Inc.]. Figures 2-5 and S1-2 were prepared in PyMOL [version 2.0.3, The PyMOL Molecular Graphics Systems, V.S., LLC].

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Table S1. Crystal Data, Data Collection, and Refinement Statistics

	TDPAM02:flop	TDPAM01:flip	TDPAM02:flip	TDPAM01:predimer	TDPAM02:predimer
PDB-code	6HCA	6HCB	6HCC	6HCH	6HC9
space group	$P2_12_12_1$	$P2_12_12$	$P2_12_12$	$P2_12_12$	$P2_12_12$
mols in the AU unit[a]	2	2	2	3	2
unit cell *a*, *b*, *c* (Å)	62.0, 91.4, 98.6	97.4, 122.0, 47.2	98.0, 122.0, 47.4	114.6, 163.6, 47.5	97.7, 121.9, 47.4
resolution range (Å)[b]	29.58-1.88 (1.98-1.88)	48.72-1.90 (2.00-1.90)	29.74-1.62 (1.70-1.62)	49.24-1.60 (1.62-1.60)	121.94-2.40 (2.53-2.40)
unique observations[b]	46,079 (6,528)	45,663 (6,551)	72,996 (10,454)	118,901 (17,175)	22,932 (3,267)
average multiplicity[b]	7.5 (7.4)	13.3 (12.5)	7.5 (7.5)	13.3 (13.4)	7.5 (6.3)
completeness (%)[b]	99.7 (98.4)	100 (100)	99.4 (98.9)	100 (100)	100 (99.8)
R_{merge}[b]	0.11 (0.72)	0.13 (0.66)	0.10 (0.63)	0.10 (0.85)	0.28 (0.92)
mean (I)/σ(I)[b]	10.7 (2.4)	10.8 (4.0)	11.0 (2.2)	13.6 (3.3)	4.9 (1.6)
mean CC(1/2)[b]	1.00 (0.80)	1.00 (0.91)	1.00 (0.88)	1.00 (0.87)	0.98 (0.73)
R-value, R_{free} (%)	17.1/20.8	16.9/19.2	15.3/18.2	15.5/17.8	20.8/23.9
rmsd from ideal bond lengths (Å)/angles (deg)	0.007/0.88	0.005/0.76	0.008/1.05	0.008/1.06	0.002/0.58

[a]AU refers to asymmetric unit.

[b]Values within parentheses refer to the highest resolution shell.

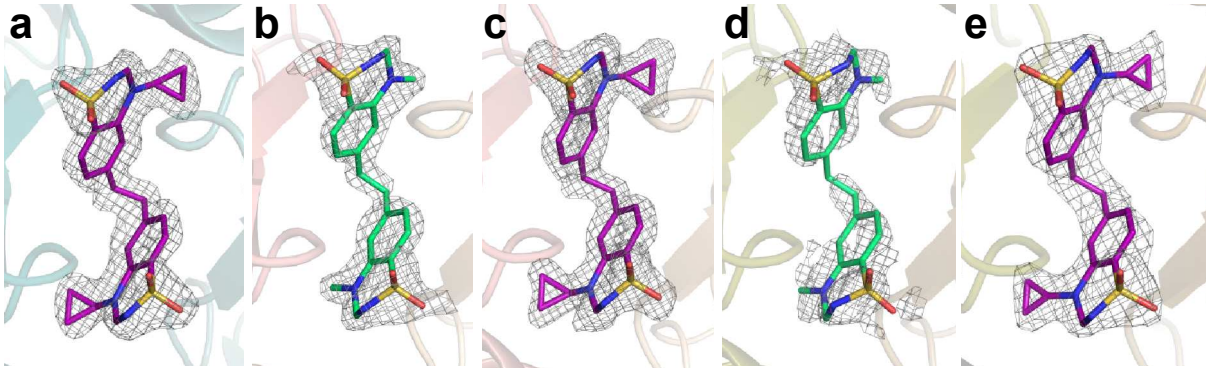

Figure S1. 2Fo−Fc omit electron density maps contoured at 1σ and carved at 1.6 Å around the modulators. (a) GluA2-LBD flop with TDPAM02. (b) GluA2-LBD N775S (flip) with L-glutamate and TDPAM01. (c) GluA2-LBD N775S (flip) with L-glutamate and TDPAM02. (d) GluA2-LBD L504Y-N775S (predimer) with L-glutamate and TDPAM01. (e) GluA2-LBD L504Y-N775S (predimer) with L-glutamate and TDPAM02. TDPAM01 is shown in green and TDPAM02 in purple.

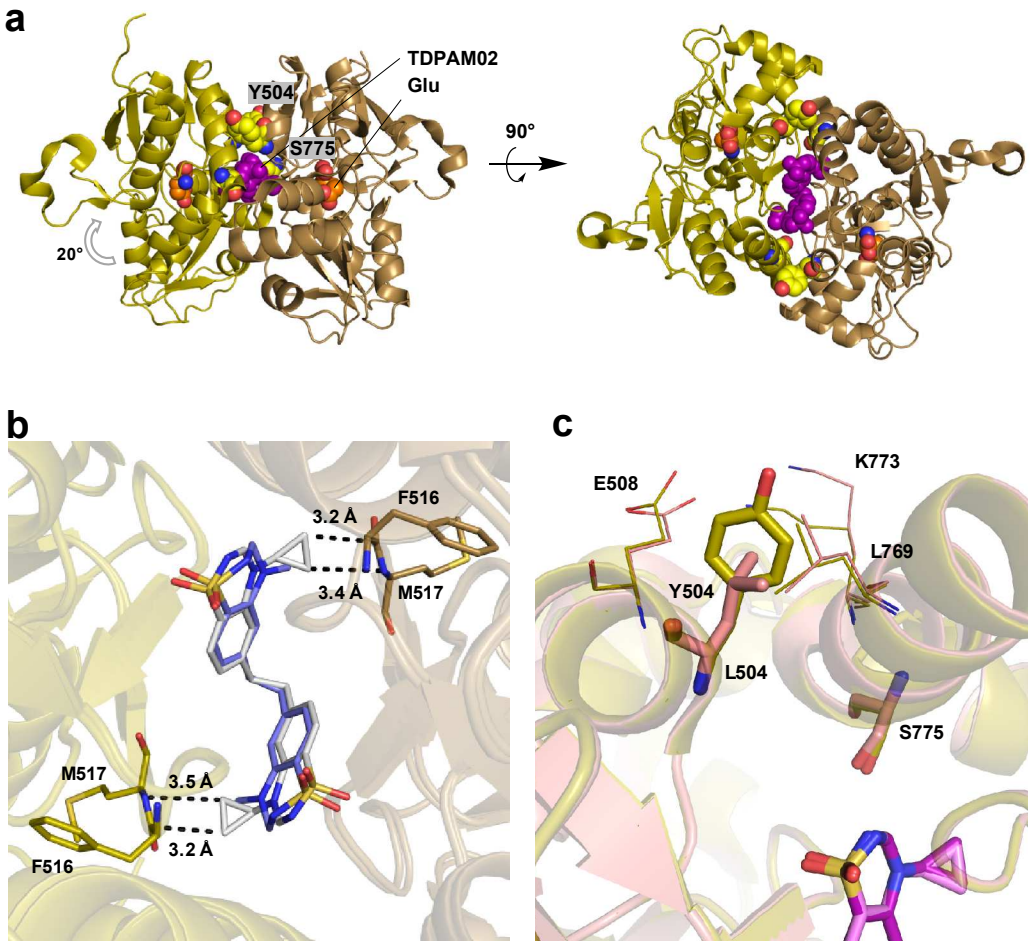

Figure S2. Dimeric structure of GluA2-LBD predimer with TDPAM01 and TDPAM02. (a) Left: side-view of the TDPAM02 bound GluA2-LBD predimer in cartoon representation (olive for chain A and sand for chain B). L-glutamate is shown as orange spheres, TDPAM02 as purple spheres and Tyr504 and Ser775 as yellow spheres. Right: a 90° horizontally rotated view (top view). (b) Comparison of the binding mode of TDPAM01 (blue) and TDPAM02 (grey) in the GluA2-LBD predimer. Phe516 and Met517 are shown in sticks representation. Distances from the N-cyclopropyl group of TDPAM02 to the amide bond between Phe516 and Met517 are shown as stippled black lines. (c) Zoom on L504Y mutation site in structure with TDPAM02. The structure was overlaid with GluA2-LBD flip without this mutation (shown in salmon). Residues within 4 Å of Leu504/Tyr504 with side chains pointing towards these residues are shown as lines.