

Table S1. Statistics of CryoEM Data Collection, Classification, and Refinement for the Dimeric GluN1/GluN2A at pH 7.4. Related to Figures 1, 2 and 4

	DiNMDAR	DiNMDAR	DiNMDAR	DiNMDAR
[Glutamate] (mM)	1.0	1.0	1.0	1.0
[Glycine] (mM)	1.0	1.0	1.0	1.0
[EDTA] (mM)	1.0	0	0	3.0
[ZnCl ₂] (μM)	0	1.0	1,000	1,000
pH	7.4	7.4	7.4	7.4
Antagonist Used for Expression	Memantine	Memantine	Memantine	Memantine
Data Collection/Processing				
Microscope	Krios (Janelia)	Krios (OHSU)	Krios (OHSU)	Krios (OHSU)
Voltage (kV)	300	300	300	300
Defocus Range (μm)	-1.3 – -2.5	-1.3 – -2.6	-1.2 – -2.4	-1.2 – -2.2
Exposure time (s)	10	22	22	22
Dose rate (e/Å ² /s)	5.5	2.5	2.4	2.4
Pixel Size (Å)	1.32	1.71	1.71	1.71
Number of Frames	50	80 – 96	88	88
Micrographs	2,667	1,653	1,068	714
Particles Processed (Reference Free)	695,027	491,476	248,810	286,226
Particles Processed (Template-Based)	1,256,436	950,166	468,703	427,834
Combined Particles after 2D Cleanup	181,664	270,711	105,778	84,260
3D Classification				
Class 1 Name	2 Knuckle Sym	2 Knuckle Asym	Extended 2	2 Knuckle Sym
Refined Particles	88,979	52,489	23,049	47,313
Masked Resolution	6.23 Å	7.14 Å	8.21 Å	5.13 Å
Class 2 Name		1 Knuckle	Splayed Open	
Refined Particles		64,045	9,129	
Masked Resolution		6.08 Å	8.93 Å	
Class 3 Name		Extended	Super Splayed	
Refined Particles		47,299	6,081	
Masked Resolution		6.84 Å	16.5 Å	

Table S2. Statistics of CryoEM Data Collection, Classification, and Refinement for the Dimeric GluN1/GluN2A at pH 8.0 and pH 6.1. Related to Figures 2 and 4

	DiNMDAR	DiNMDAR	DiNMDAR
[Glutamate] (mM)	1.0	1.0	1.0
[Glycine] (mM)	1.0	1.0	1.0
[EDTA] (mM)	0.1	0	0
[ZnCl ₂] (μM)	0	1.0	1.0
pH	8.0	8.0	6.1
Antagonist Used for Expression	MK-801	MK-801	Memantine
<i>Data Collection/Processing</i>			
Microscope	Krios (OHSU)	Krios (OHSU)	Krios (OHSU)
Voltage (kV)	300	300	300
Defocus Range (μm)	-1.0 – -2.5	-1.0 – -2.5	-0.8 – -2.6
Exposure time (s)	20	20	22
Dose rate (e/Å ² /s)	2.6	2.6	2.4
Pixel Size (Å)	1.71	1.71	1.71
Number of Frames	80	80	88
Micrographs	1,841	1,605	2,163
Particles Processed (Reference Free)	466,631	618,512	1,228,202
Particles Processed (Template-Based)			1,284,550
Combined Particles after 2D Cleanup	183,139	314,419	326,255
<i>3D Classification</i>			
Class 1 Name	2 Knuckle Sym	2 Knuckle Sym	1 Knuckle
Refined Particles	84,793	104,520	94,934
Masked Resolution	6.88 Å	7.51 Å	5.97 Å
Class 2 Name			Extended
Refined Particles			86,310
Masked Resolution			6.31 Å
Class 3 Name			Super Splayed
Refined Particles			10,238
Masked Resolution			12.7 Å

Table S3. Statistics of CryoEM Data Collection, Classification, and Refinement for the Triheteromeric GluN1/GluN2A/GluN2A* at pH 7.4. Related to Figures 2 and 3

	TriNMDAR	TriNMDAR
[Glutamate] (mM)	1.0	1.0
[Glycine] (mM)	1.0	1.0
[EDTA] (mM)	1.0	0
[ZnCl ₂] (μM)	0	1.0
pH	7.4	7.4
Antagonist Used for Expression	Memantine	Memantine
<i>Data Collection/Processing</i>		
Microscope	Krios (OHSU)	Krios (OHSU)
Voltage (kV)	300	300
Defocus Range (μm)	-1.2 – -2.4	-1.1 – -2.6
Exposure time (s)	22	22
Dose rate (e ⁻ /Å ² /s)	2.4	2.4
Pixel Size (Å)	1.71	1.71
Number of Frames	88	88
Micrographs	690	1,891
Particles Processed (Reference Free)	275,599	720,692
Particles Processed (Template-Based)	416,121	1,523,115
Combined Particles after 2D Cleanup	52,342	244,286
<i>3D Classification</i>		
Class 1 Name	2 Knuckle Sym	2 Knuckle Sym
Refined Particles	33,349	58,461
Masked Resolution	5.38 Å	6.20 Å
Class 2 Name		2 Knuckle Asym
Refined Particles		68,275
Masked Resolution		5.30 Å (ECD 4.71 Å)
Class 3 Name		1 Knuckle
Refined Particles		32,232
Masked Resolution		7.46 Å
Class 4 Name		Extended
Refined Particles		40,881
Masked Resolution		6.99