Supplementary Information

Trapping of spermine, Kukoamine A, and polyamine toxin blockers in GluK2 kainate receptor channels

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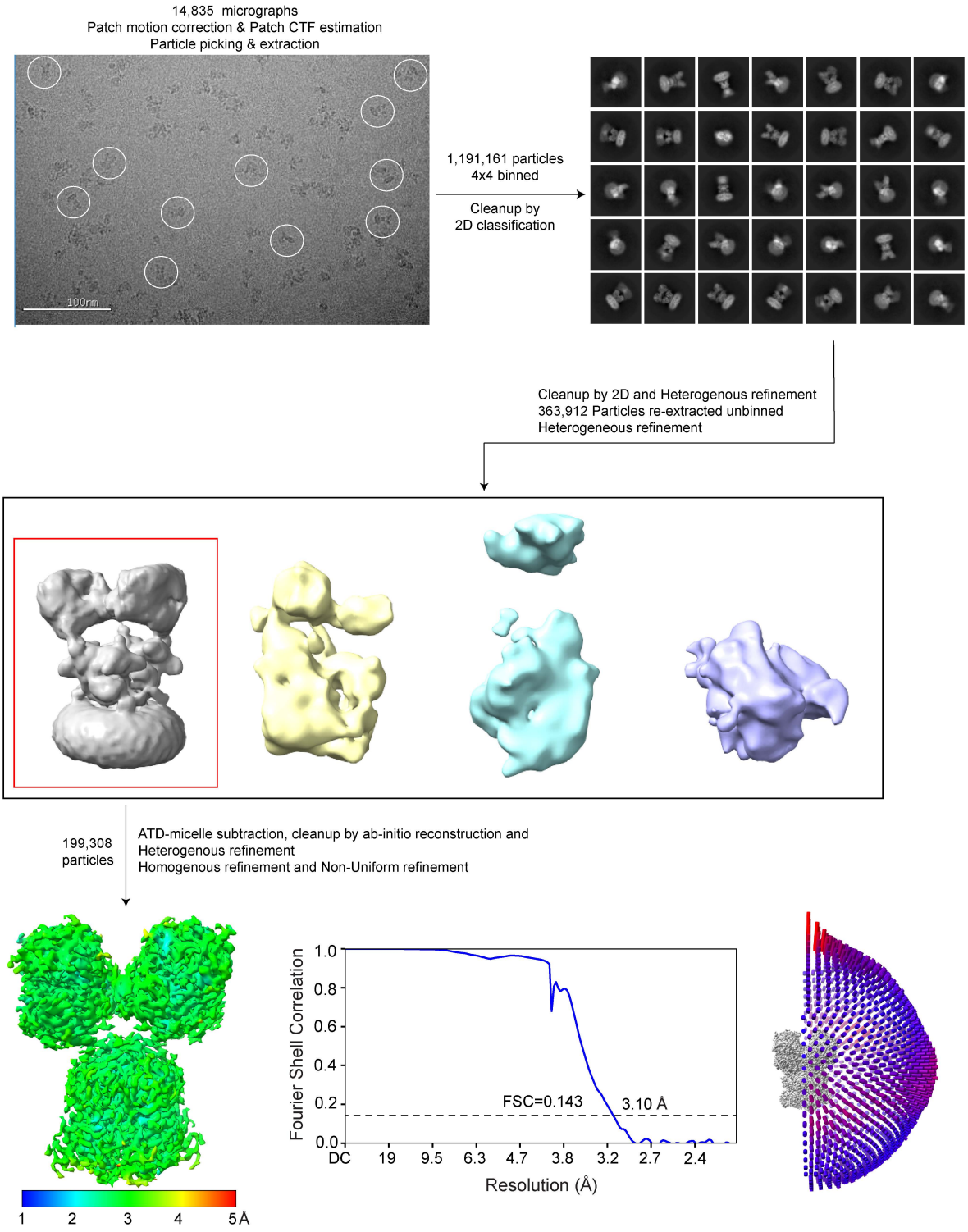
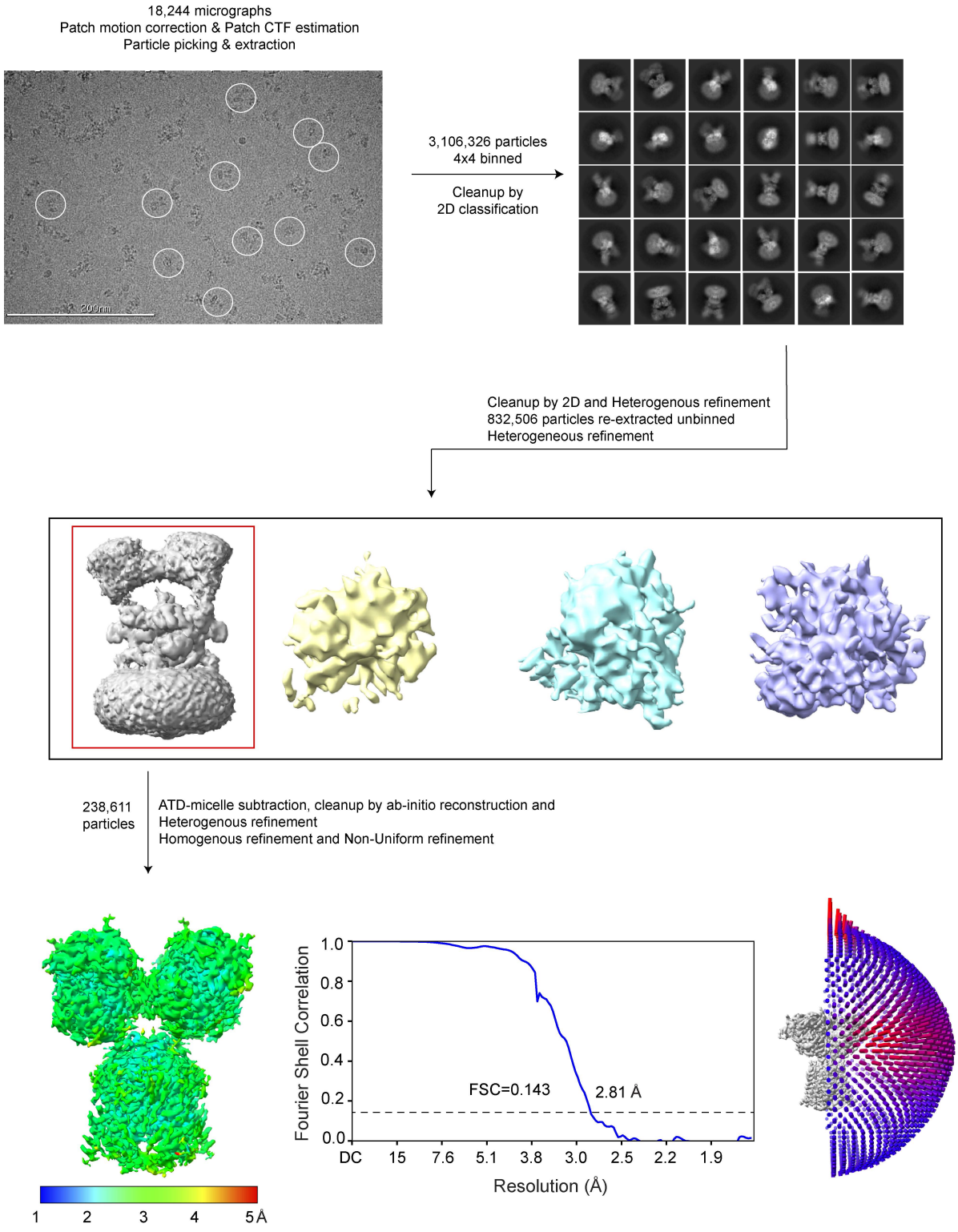
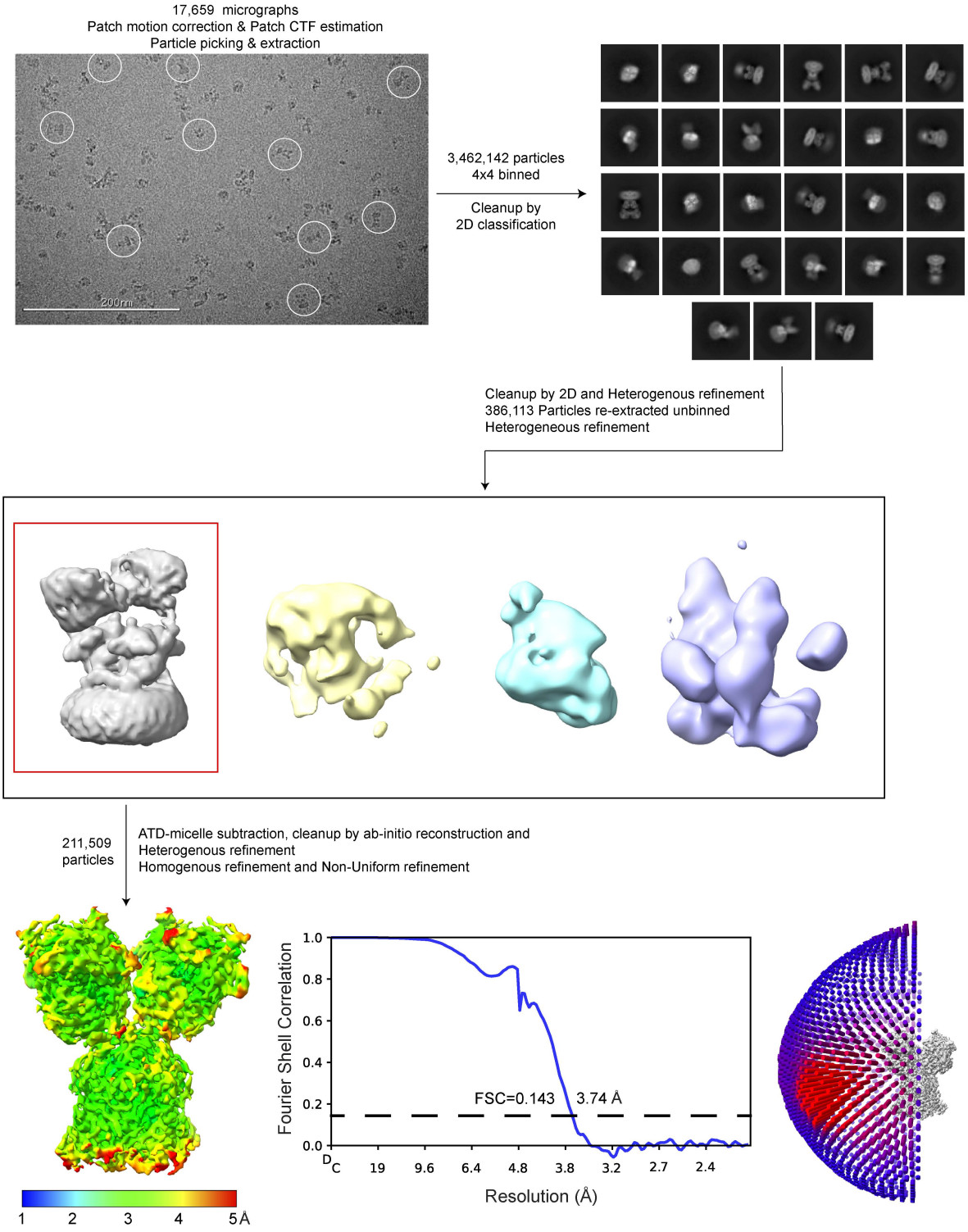


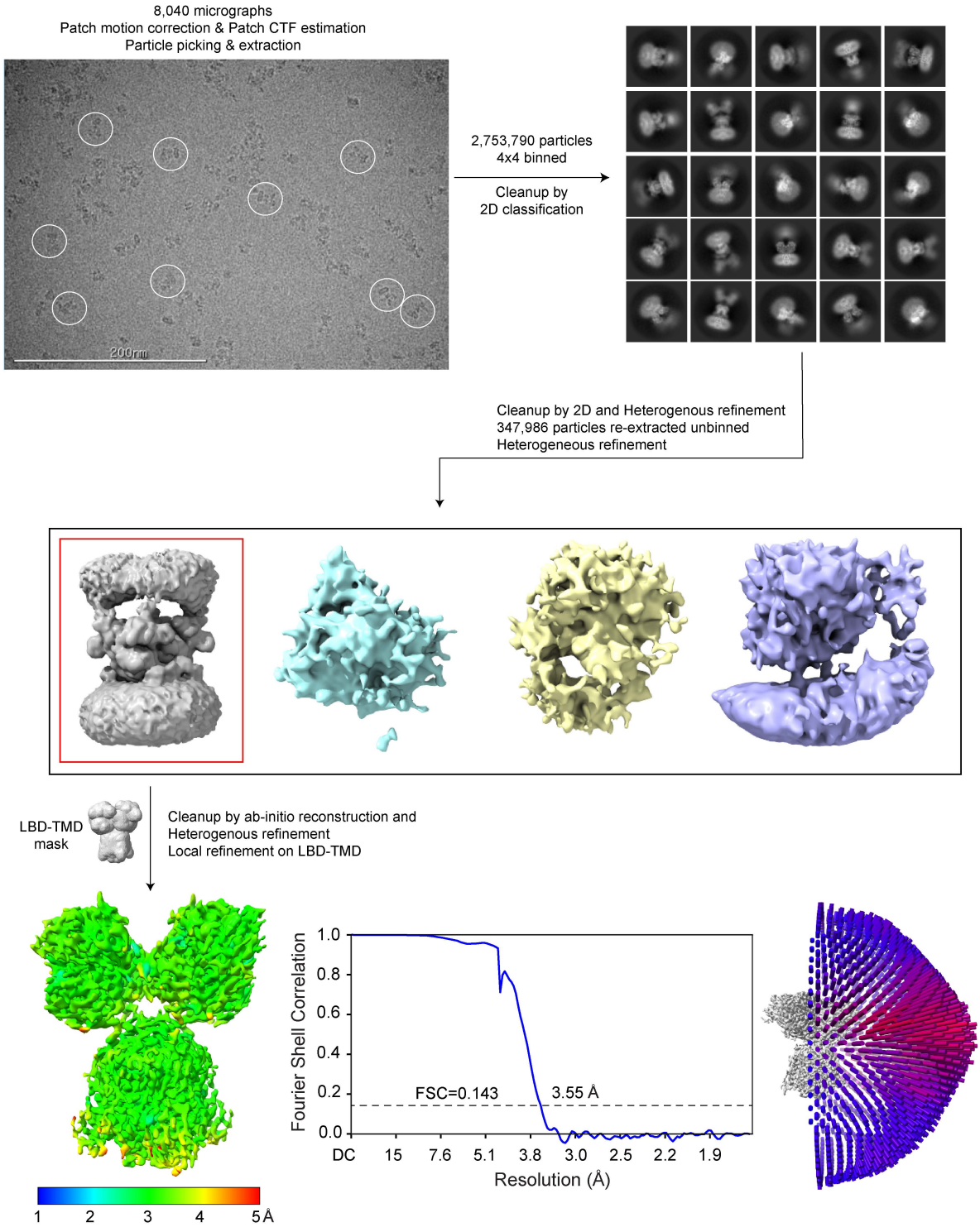
Supplementary Fig. 1. Overview of cryo-EM for GluK2$_{NpTx8}$. On the top, 3D reconstruction workflow, with a representative micrograph and 2D class averages. At the bottom, the local resolution presented as coloring of the GluK2$_{NpTx8}$ map, FSC curve, and Euler angle distribution of particles contributing to the final reconstruction, with larger red cylinders representing orientations comprising more particles.



Supplementary Fig. 2. Overview of cryo-EM for GluK2$_{PhTx74}$. On the top, 3D reconstruction workflow, with a representative micrograph and 2D class averages. At the bottom, the local resolution presented as coloring of the GluK2$_{PhTx74}$ map, FSC curve, and Euler angle distribution of particles contributing to the final reconstruction, with larger red cylinders representing orientations comprising more particles.



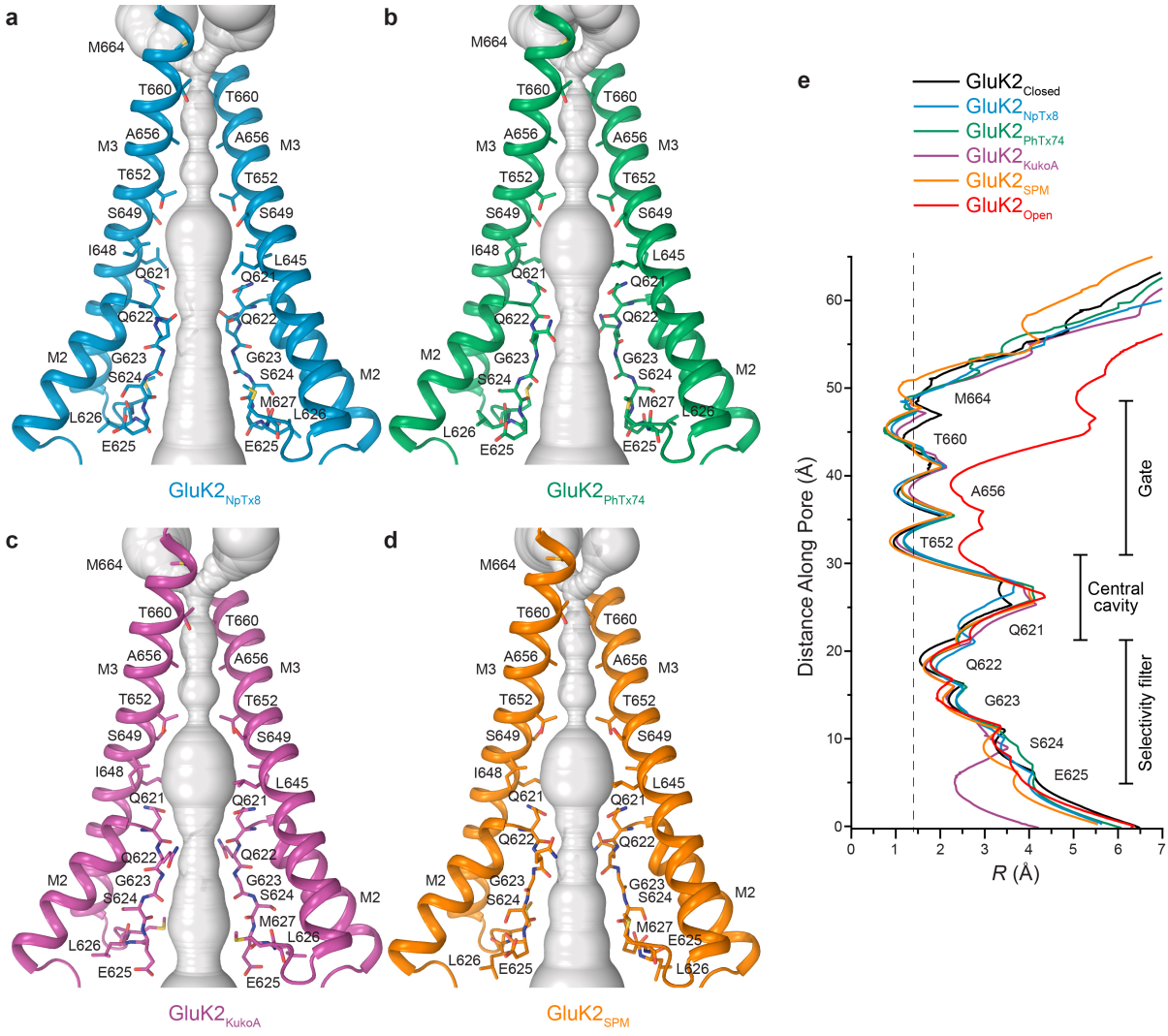
Supplementary Fig. 3. Overview of cryo-EM for GluK2$_{KukoA}$. On the top, 3D reconstruction workflow, with a representative micrograph and 2D class averages. At the bottom, the local resolution presented as coloring of the GluK2$_{KukoA}$ map, FSC curve, and Euler angle distribution of particles contributing to the final reconstruction, with larger red cylinders representing orientations comprising more particles.



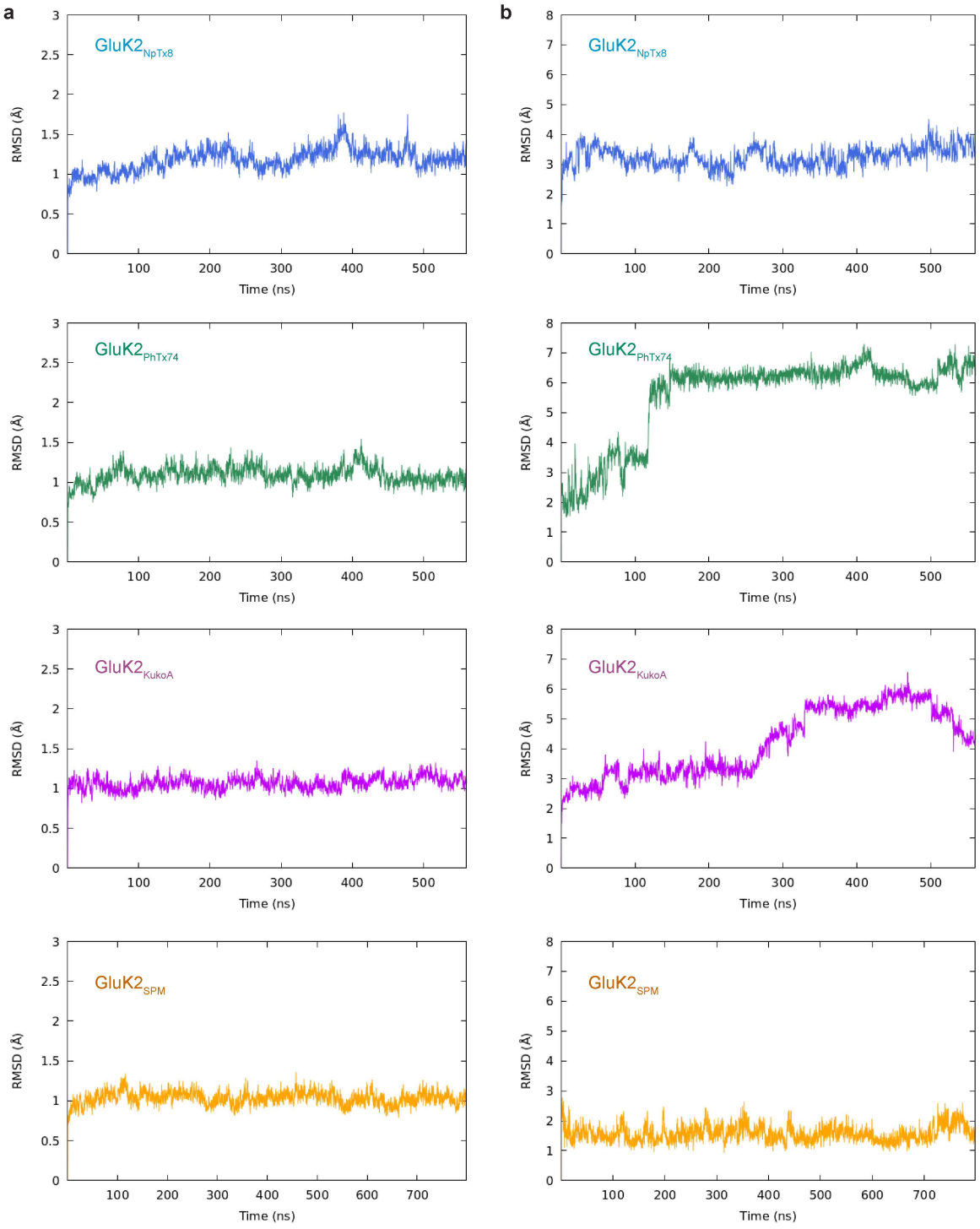
Supplementary Fig. 4. Overview of cryo-EM for GluK2$_{SPM}$. On the top, 3D reconstruction workflow, with a representative micrograph and 2D class averages. At the bottom, the local resolution presented as coloring of the GluK2$_{SPM}$ map, FSC curve, and Euler angle distribution of particles contributing to the final reconstruction, with larger red cylinders representing orientations comprising more particles.

Supplementary Fig. 5. Cryo-EM density. a, Fragments of the GluK2$_{PhTx74}$ TMD with the structural model shown as a ribbon and sticks and the corresponding cryo-EM density as a blue mesh. **b-c,** Representative GluK2$_{PhTx74}$ density for the positive allosteric modulator BPAM (**b**), lipids cholesterol and phosphatidylcholine (PC), and N546-linked carbohydrate (**c**), with the molecular models shown in sticks (yellow). **d-e,** Closeup view of the blocker binding site in GluK2$_{NpTx8}$, GluK2$_{PhTx74}$, GluK2$_{KukoA}$ and GluK2$_{SPM}$, with density for the channel blockers and the surrounding protein shown as red and blue mesh, respectively. The protein model is shown in yellow. One fitted blocker

molecule (**d**) is shown in green, while its three equivalent poses obtained via 90-degree rotations around the axis of local 4-fold rotational symmetry (**e**) are shown in grey. Only two subunits (A and C) are shown for the protein, with the other two subunits (B and D) removed for clarity. **f,** Intracellular view of the same region as shown in **e** but with all four protein subunits present.



Supplementary Fig. 6. Closed conformation of the ion channel pore. a-c, Pore-forming domains M2 and M3 in GluK2$_{NpTx8}$ (**a**), GluK2$_{PhTx74}$ (**b**), GluK2$_{KukoA}$ (**c**) and GluK2$_{SPM}$ (**d**), with the residues lining pore shown as sticks. Only two (A and C) of four subunits are shown, with the front and back subunits (B and D) omitted for clarity. The pore profile is shown as a space-filling model (grey). **e**, Pore radius for GluK2$_{Closed}$ (black; PDB ID: 8FWS), GluK2$_{NpTx8}$ (blue), GluK2$_{PhTx74}$ (green), GluK2$_{KukoA}$ (violet), GluK2$_{SPM}$ (orange) and GluK2$_{Open}$ (red; PDB ID: 9B35) calculated using HOLE. The vertical dashed line denotes the radius of a water molecule, 1.4 Å.



Supplementary Fig. 7. Protein and blocker stability during MD simulations. a, Root-mean square deviation (RMSD) from the initial structures in the production runs for $GluK2_{NpTx8}$ (blue), $GluK2_{PhTx74}$ (green), $GluK2_{KukoA}$ (violet) and $GluK2_{SPM}$ (orange) systems. For each system, Cα RMSDs of the M1-4 TMD segments are shown. **b**, RMSDs of the blocker molecules NpTx-8 (blue), PhTx-74 (green), KukoA (violet) and SPM (orange), from the initial conformations in the production runs of corresponding systems. The alignment was done by the TMD helices and the heavy-atom RMSDs of the blockers in each system are shown.

Supplementary Table 1. Heavy-atom contact frequencies between the channel blockers and key protein residues during the simulations. Contacts that lasted for less than 60% of the simulation are not shown.

Simulation System	GluK2 Subunit	Contact Frequency (%)									
		Q621	Q622	G623	S624	E625	M627	L645	I648	S649	T652
$GluK2_{SPM}$	A	99.70	98.07	-	62.14	89.16	-	-	-	-	-
	B	98.67	90.19	-	-	-	-	-	-	-	-
	C	98.18	84.63	-	-	-	-	-	-	-	-
	D	91.83	88.22	-	62.56	69.22	-	-	-	-	-
$GluK2_{KukoA}$-1	A	95.72	91.08	-	60.10	-	-	-	-	70.45	-
	B	97.36	-	-	-	-	-	-	63.05	-	89.20
	C	100.00	78.81	-	-	62.73	-	-	-	-	-
	D	99.84	96.72	-	72.93	72.41	67.37	-	60.66	-	-
$GluK2_{KukoA}$-2	A	100.00	96.81	-	80.07	-	-	-	84.95	-	-
	B	95.38	-	-	-	-	-	-	-	-	-
	C	98.19	98.38	-	-	-	-	-	75.64	-	87.13
	D	99.81	73.33	97.75	100.00	-	99.94	83.76	68.96	-	79.89
$GluK2_{NpTx8}$	A	84.36	-	-	-	-	-	82.50	65.52	-	-
	B	96.54	90.36	-	-	-	-	-	-	-	-
	C	100.00	98.34	94.82	-	-	-	-	83.84	94.77	60.70
	D	97.84	99.80	-	-	-	-	-	-	-	83.04
$GluK2_{PhTx74}$	A	79.95	-	-	-	-	-	61.45	73.57	92.25	78.82
	B	99.95	63.86	-	-	-	-	66.30	95.07	-	98.20
	C	99.11	-	-	-	-	-	-	69.45	80.27	99.73
	D	98.93	-	-	-	-	-	-	-	-	74.75

Supplementary Table 2. List of hydrogen bond interactions between the channel blockers and the binding site residues. Duty fraction refers to the percentage of frames in which a given hydrogen bond is present in the trajectory. Hydrogen bonds that persist less than 20% of the trajectory are not listed. Single asterisk (*) indicates the alternating hydrogen bond atoms within the same residue. Double asterisk (**) indicates the alternating hydrogen bonds among the subunits.

Simulation System	GluK2 Subunit	Hydrogen bonds (Acceptor-Donor)	Duty Fraction (%)
$GluK2_{SPM}$	A	**Q621:O-----SPM:N5 **Q622:O-----SPM:N10 *E625:OE1/2-----SPM:N14 **Q621:OE1-----SPM:N1	89.38 47.93 100.00 57.27
	B	**Q621:O-----SPM:N5 **Q621:OE1-----SPM:N1 *E625:OE1/2-----SPM:N14	67.77 39.58 26.02
	C	**Q621:O-----SPM:N5 *E625:OE1/2-----SPM:N14 **Q621:OE1-----SPM:N1 **Q622:O-----SPM:N10	23.73 31.85 21.3 25.70
	D	**Q621:O-----SPM:N5 *E625:OE1/2-----SPM:N14 **Q622:O-----SPM:N10 **Q621:OE1-----SPM:N1 S624:O-----SPM:N14	44.97 100.00 44.32 34.09 22.49
$GluK2_{KukoA}$-1	A	**Q622:O-----KUK:N15 Q621:OE1-----KUK:N24 **Q621:O-----KUK:N20	52.2 45.04 38.90
	B	T652:OG1-----KUK:O35 **Q621:O-----KUK:N20	74.00 20.01
	C	**Q621:O-----KUK:N20 KUK:O26-----Q621:NE2 **Q622:O-----KUK:N15	72.44 95.40 43.11
	D	**Q621:O-----KUK:N20 **Q622:O-----KUK:N15	83.36 64.04
$GluK2_{KukoA}$-2	A	Q622:O-----KUK:O37 *Q621:OE1/NE2-----KUK:O36	93.38 99.07
	B	**Q622:O-----KUK:N11	35.35
	C	**Q621:O-----KUK:N15 KUK:O26-----T652:OG1 KUK:O10-----S624:N **Q621:OE1-----KUK:N20	34.54 66.46 31.54 40.23
	D	**Q622:O-----KUK:N11 **Q621:O-----KUK:N15 *Q621:OE1/NE2-----KUK:O35 **Q621:OE1-----KUK:N20 *KUK:O35/36-----Q621:NE2 *KUK:O37/38-----S624:N	56.53 59.90 35.11 50.47 98.87 50.66
$GluK2_{NpTx8}$	A	Q621:OE1-----NTX:N6 S624:O-----NTX:N2 *E625:OE1/2-----NTX:N1 Q622:OE1-----NTX:N3 Q621:O-----NTX:N4	63.28 26.65 56.25 29.90 61.17
	B	**Q622:O-----NTX:N3 *E625:OE1/2-----NTX:N1 NTX:O4-----Q621:NE2 NTX:O3-----T652:OG1	63.24 53.93 53.02 23.09
	C	S649:OG-----NTX:N8 Q621:OE1-----NTX:N8	56.23 37.38
	D	*Q622:OE1/NE2-----NTX:N4 **Q622:O-----NTX:N3	97.73 25.38
$GluK2_{PhTx74}$	A	**Q622:O-----PTX:N4 PTX:O1-----T652:OG1	56.02 22.94
	B	Q621:OE1/NE2-----PTX:O2 **Q622:O-----PTX:N4 Q621:O-----PTX:N3 PTX:O1-----T652:OG1 PTX:O2-----Q621:NE2	100.00 37.36 20.43 81.84 36.64
	C	**Q621:O-----PTX:N3 PTX:O3-----T652:OG1 **Q622:O-----PTX:N4	22.82 57.02 22.13
	D	**Q621:O-----PTX:N3	78.68

Supplementary Table 3. List of hydrophobic interactions between the channel blockers and the hydrophobic side chains of the binding site residues. Duty fraction refers to the percentage of frames in which a given hydrophobic contact is present in the trajectory. Contacts that persist less than 20% of the trajectory are not listed.

Simulation System	GluK2 Subunit	Hydrophobic Contacts	Duty Fraction (%)
$GluK2_{SPM}$	A	-	-
	B	-	-
	C	-	-
	D	-	-
$GluK2_{KukoA}$-1	A	KUK:C34-----I648:CG2	29.70
	B	-	-
	C	KUK:C28-----I648:CG2	33.10
	D	KUK:C27-----I648:CD1	47.00
$GluK2_{KukoA}$-2	A	KUK:C31-----I648:CG2 KUK:C30-----I648:CG2 KUK:C32-----I648:CG2 KUK:C30-----T652:CG2	36.50 30.90 25.70 20.10
	B	-	-
	C	KUK:C23-----I648:CG1 KUK:C23-----I648:CG2	34.80 25.90
	D	KUK:C4-----M627:CE KUK:C3-----M627:CE KUK:C2-----M627:CG KUK:C3-----M627:CG KUK:C5-----M627:CE KUK:C2-----M627:CE KUK:C6-----M627:CE KUK:C1-----M627:CE KUK:C3-----G623:C KUK:C33-----L645:CD1 KUK:C33-----I648:C	97.40 91.40 81.60 81.10 80.00 49.90 38.10 24.70 23.30 23.00 22.20
$GluK2_{NpTx8}$	A	NTX:C18-----L645:CD1 NTX:C19-----L645:CD1 NTX:C18-----I648:CG2	47.40 29.20 35.40
	B	NTX:C21-----L645:CD1 NTX:C20-----I648:CG2	33.60 29.80
	C	NTX:C23-----I648:CG2 NTX:C22-----I648:CG2 NTX:C24-----I648:CG2 NTX:C21-----I648:CG2 NTX:C28-----T652:CG2	61.10 34.40 27.90 22.70 25.50
	D	-	-
$GluK2_{PhTx74}$	A	PTX:C1-----L645:CD1 PTX:C3-----I648:CG2 PTX:C1-----I648:CG2 NTX:C4-----T652:CG2	43.00 31.80 23.90 20.20
	B	PTX:C12-----I648:CG2 PTX:C11-----I648:CG2 PTX:C7-----I648:CG2 PTX:C9-----L645:CD1 PTX:C12-----I648:CG1 PTX:C10-----I648:CG2 PTX:C9-----I648:CG2 PTX:C8-----I648:CG2	72.80 65.70 44.00 35.90 32.70 32.00 24.80 24.20
	C	PTX:C9-----I648:CG2 PTX:C15-----L645:CD1 PTX:C17-----L645:CD1	47.10 27.60 27.20
	D	-	-