

Statistics of the cryo-EM data collection and structural refinement

	Agonists (Gly/Glu)	Agonists/modulator (Gly/Glu/Ro25-6891)	Antagonists (DCKA/D-APV)
Data collection			
Microscopy	Krios (UCLA)	Krios I (Janelia)	Krios I (Janelia)
Voltage (kV)	300	300	300
Defocus range (μm)	-2.5 — -4.0	-1.5 — -3.5	-1.5 — -3.0
Pixel size (\AA)	1.07	1.35	1.35
Dose rate ($\text{e}^-/\text{pixel/s}$)	8.7	10.2	10.5
Total exposure time (s)	8	15	9.6
Total frames per movie	40	50	32
Structural analysis			
Micrographs	3347	2371	5523
Initial particle processed	185009	186539	393513
Particles after 2D class average	172379	134678	374728
Particles refined	116968	87851	
Resolution (full-length, unmasked)	8.0 \AA	8.8 \AA	
Resolution (full-length, masked)	7.0 \AA	7.5 \AA	
Resolution (ECD, masked)	6.7 \AA	7.0 \AA	
			Particles refined Resolution (masked)
			Class 1 (36907) 9.25 \AA
			Class 2 (38007) 13.5 \AA
			Class 3 (31605) 14.1 \AA
			Class 4 (36878) 13.5 \AA
			Class 5 (32608) 14.1 \AA
			Class 6 (18309) 15.4 \AA