

Figure S1

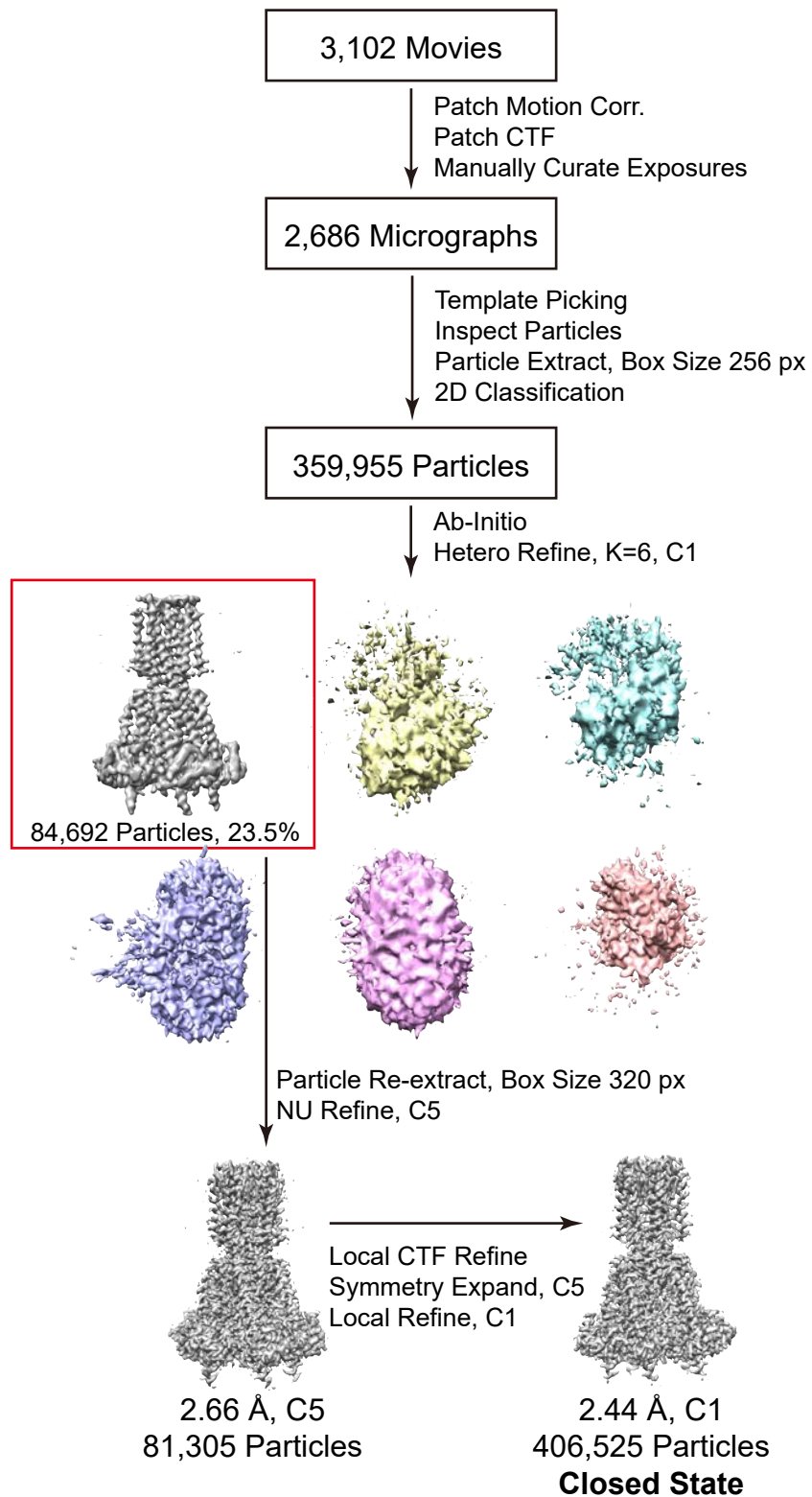


Figure S2

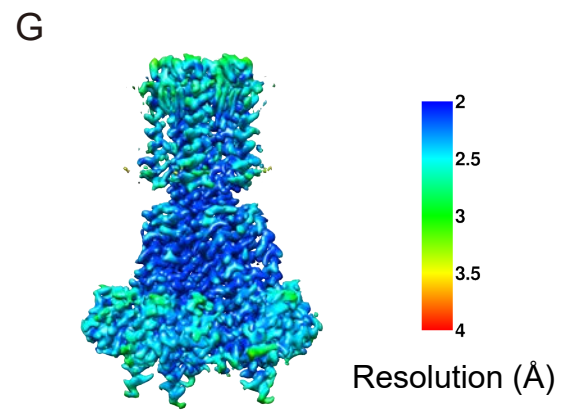
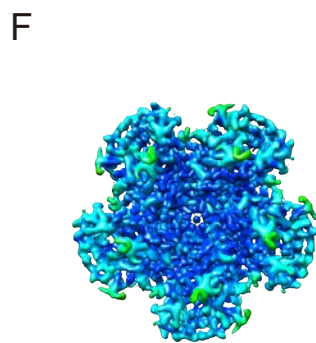
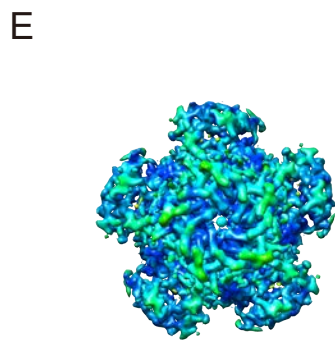
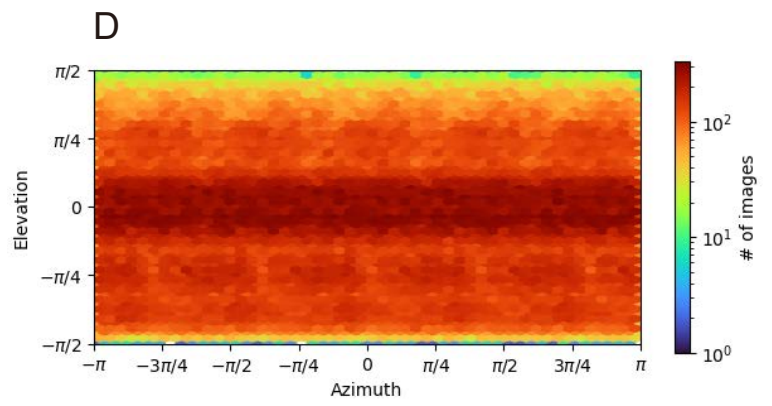
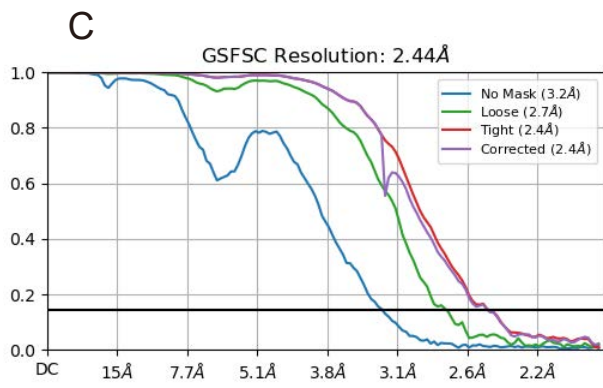
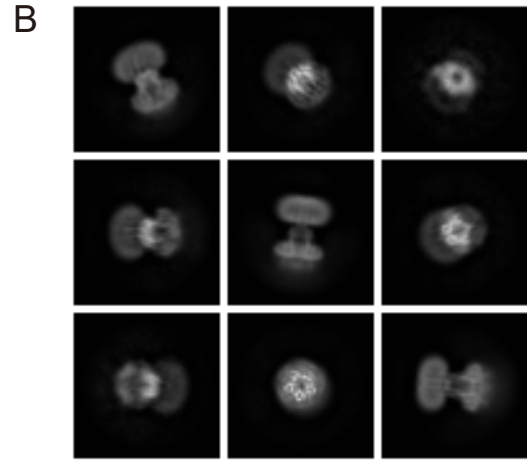
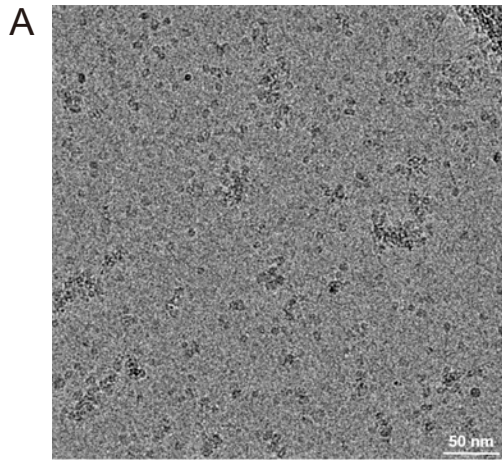


Figure S3

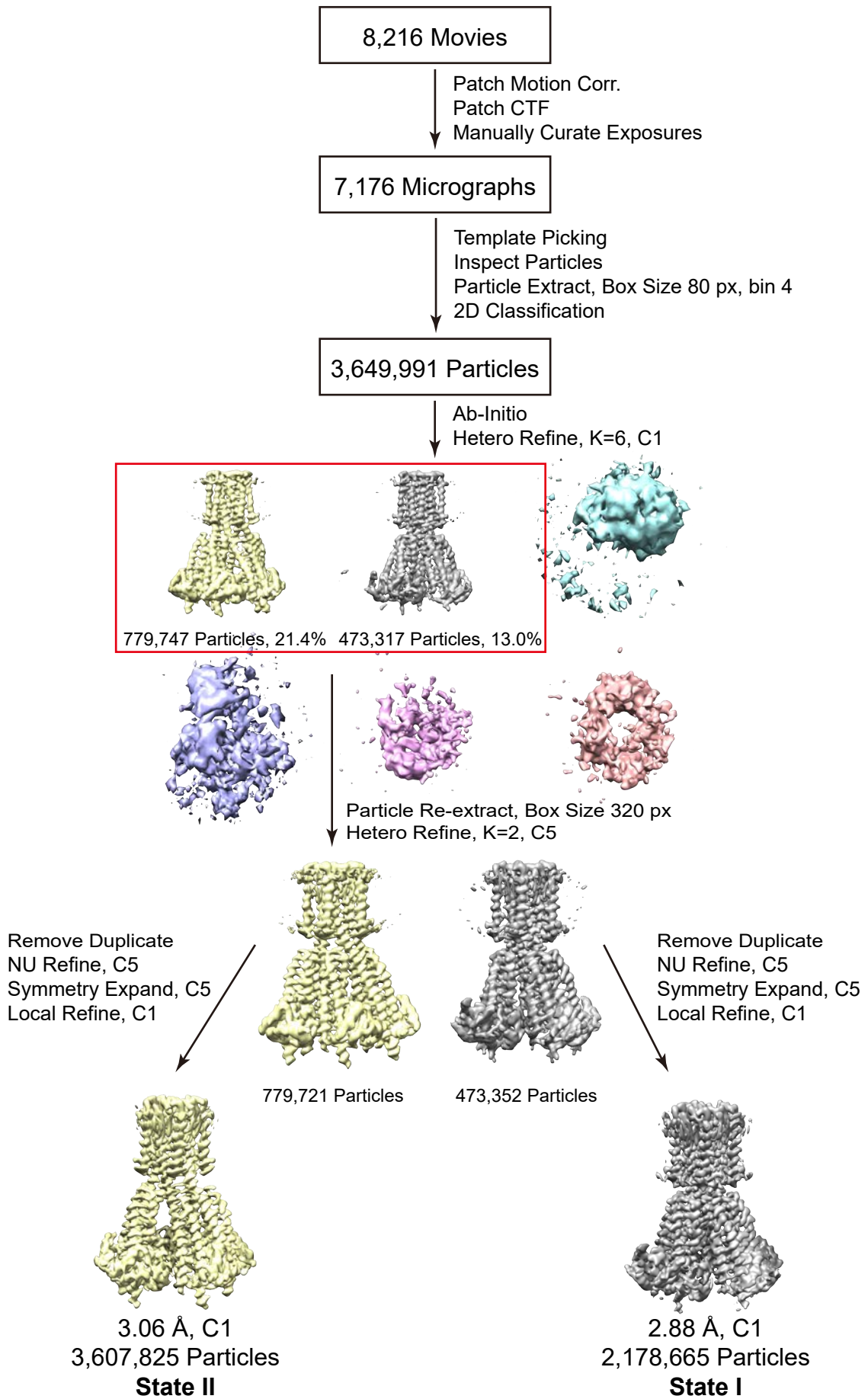


Figure S4

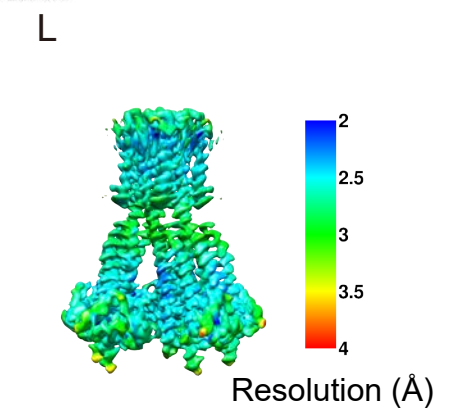
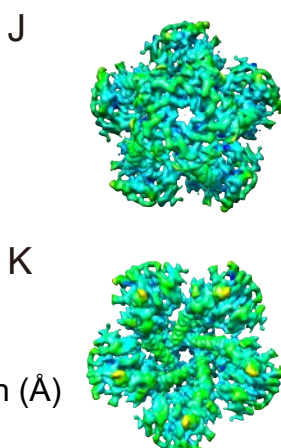
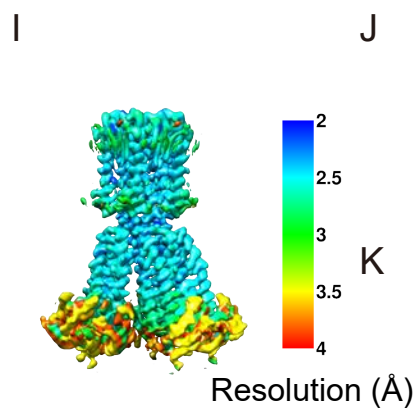
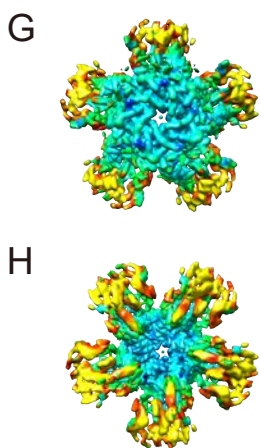
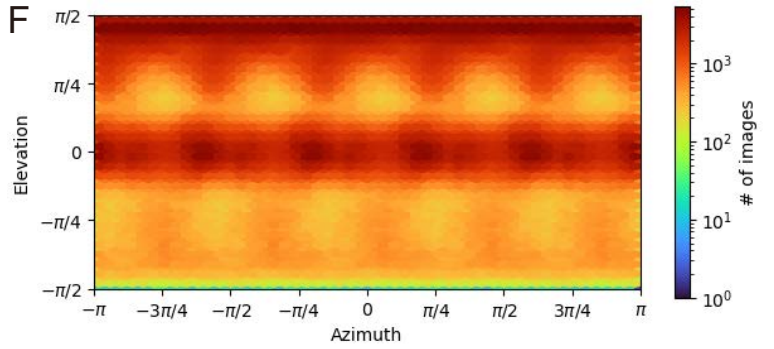
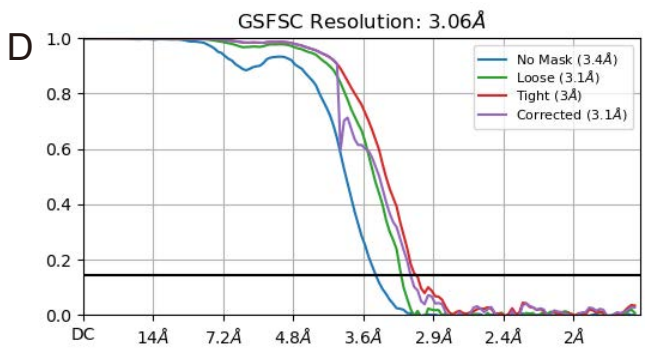
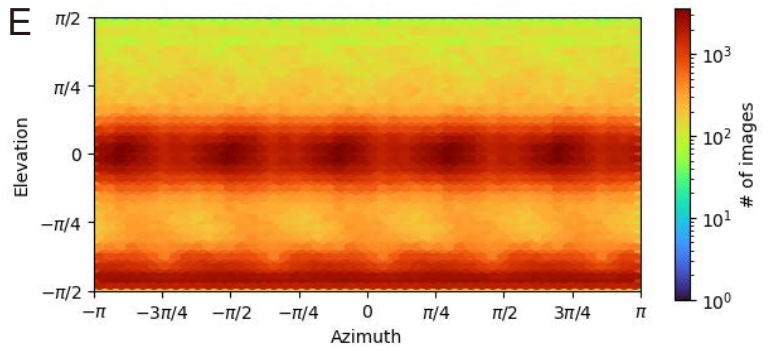
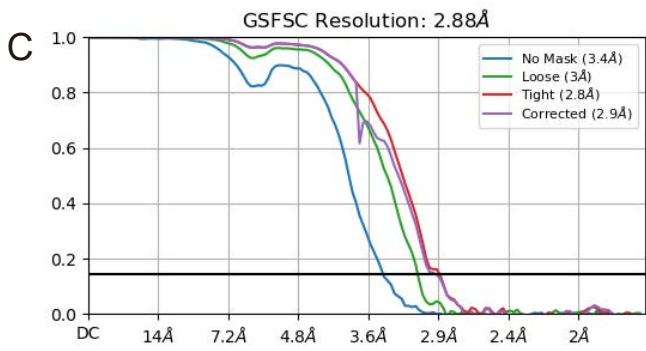
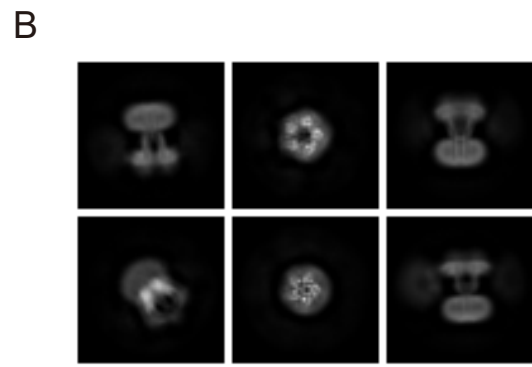
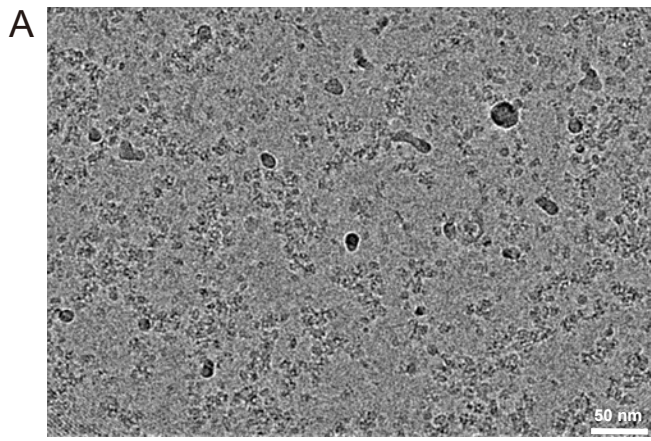


Figure S5

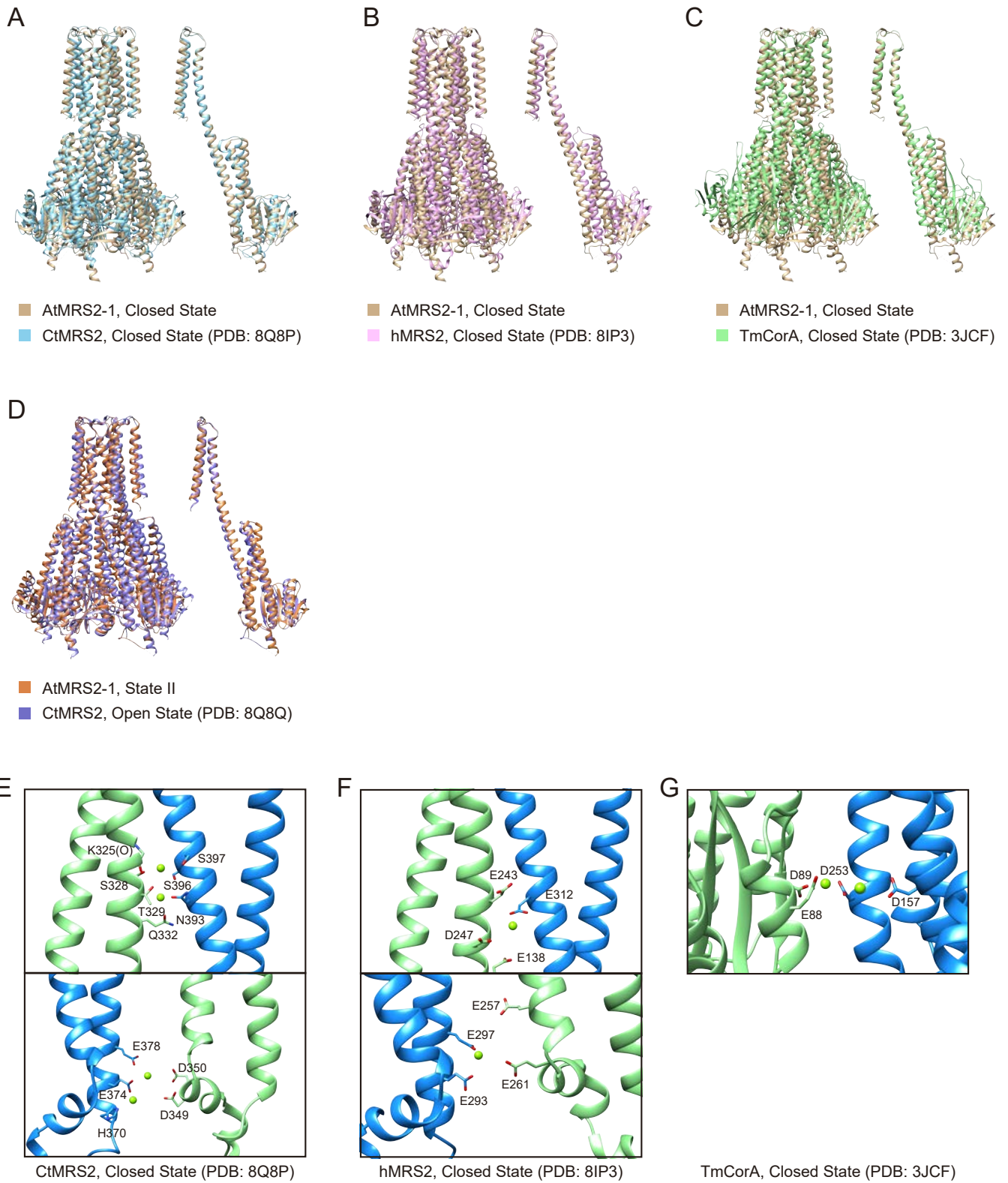


Figure S6

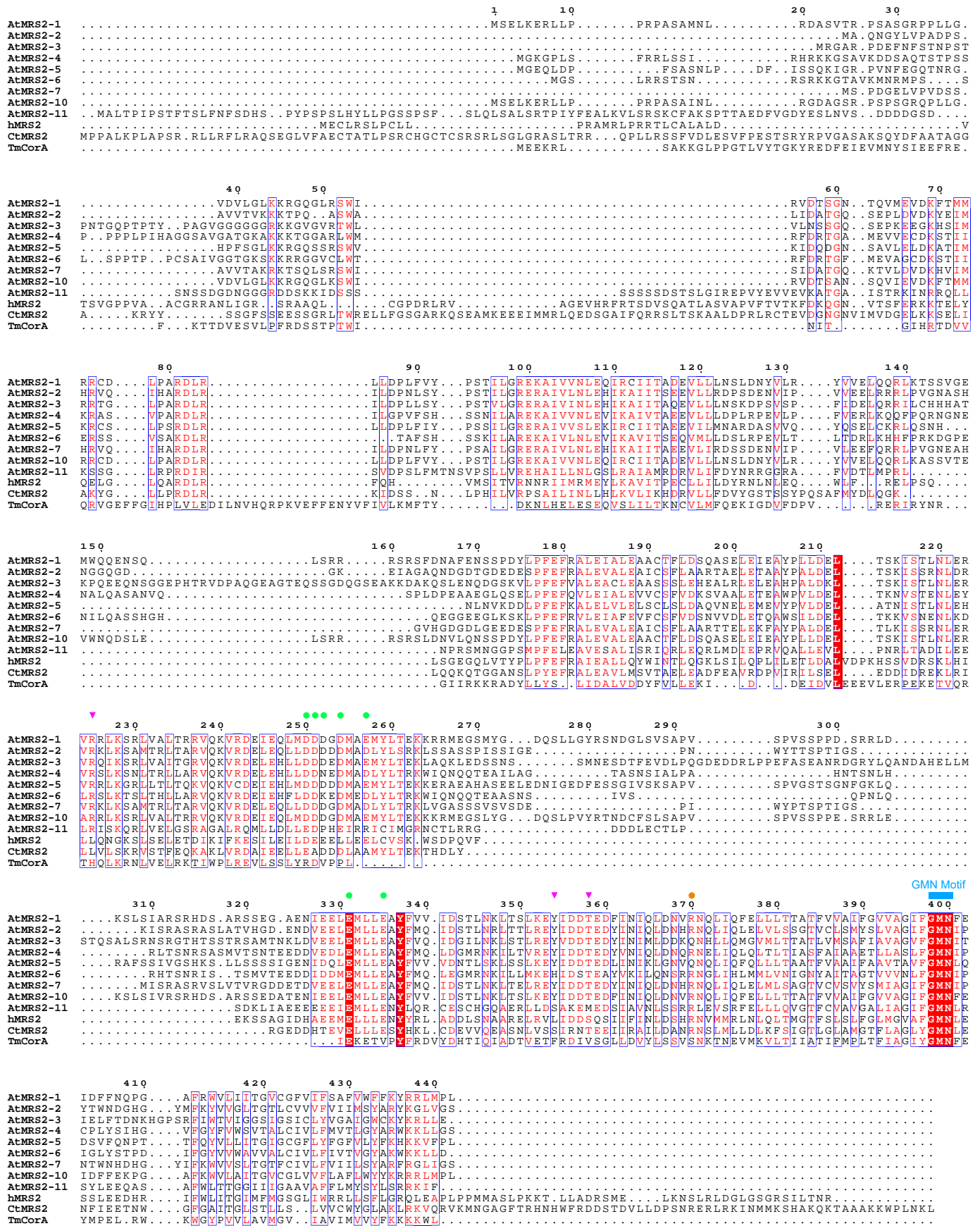


Figure S7

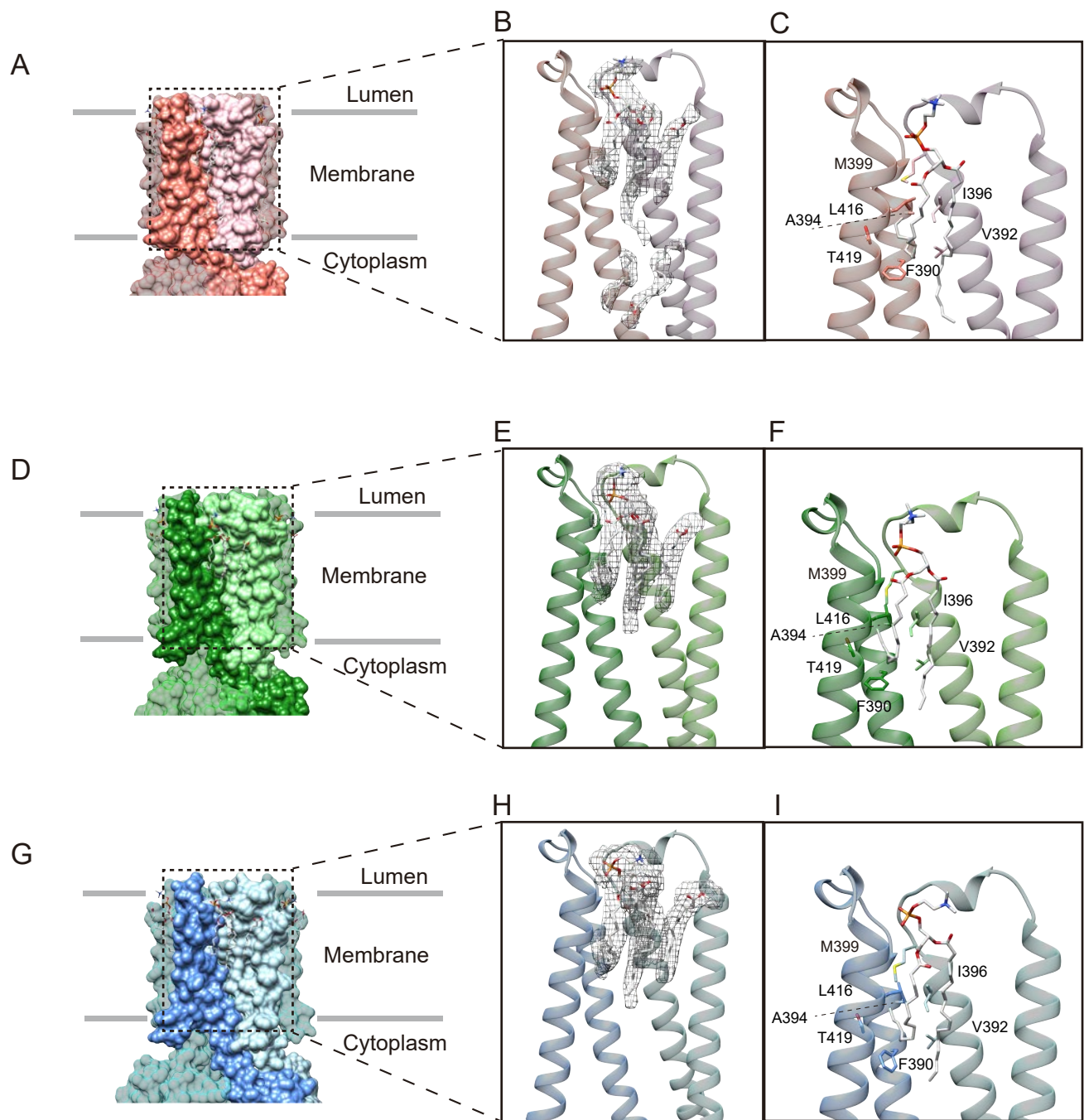


Figure S8

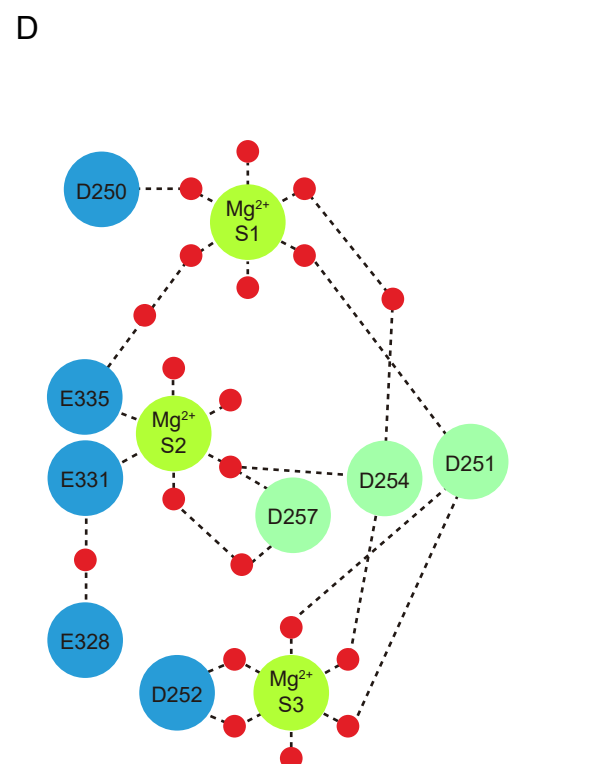
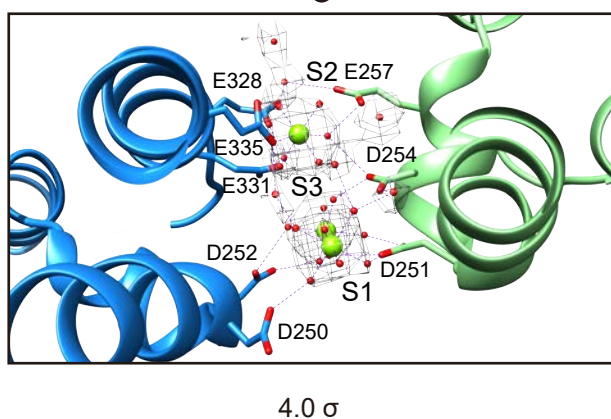
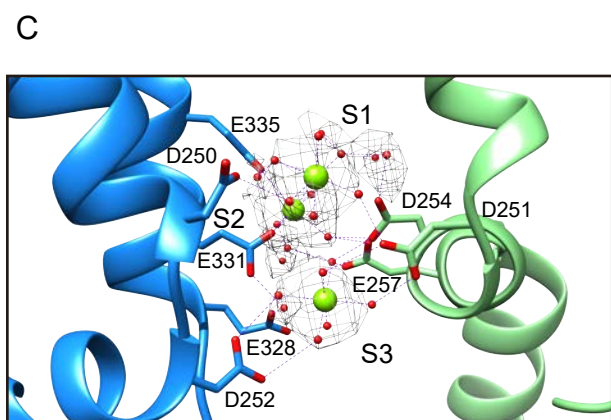
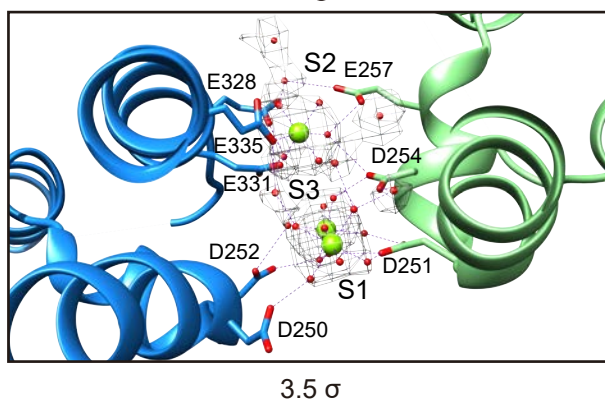
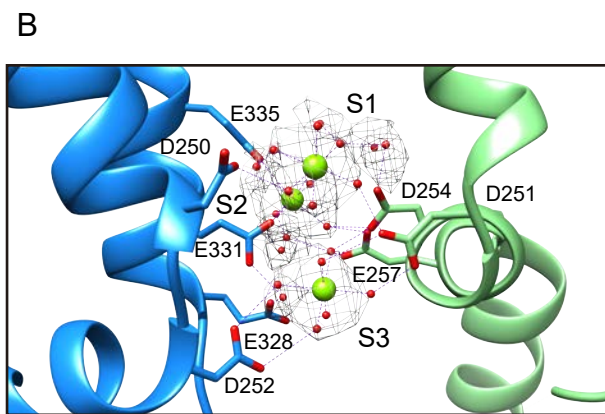
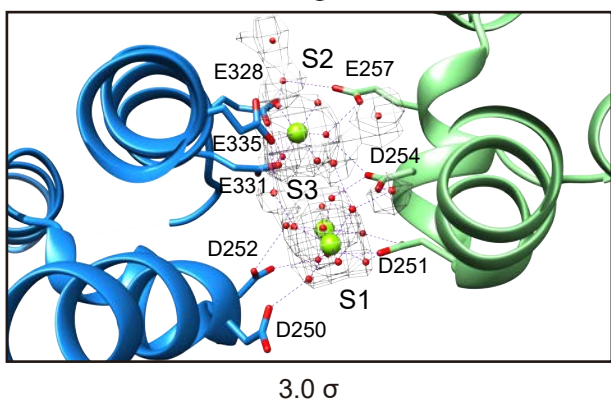
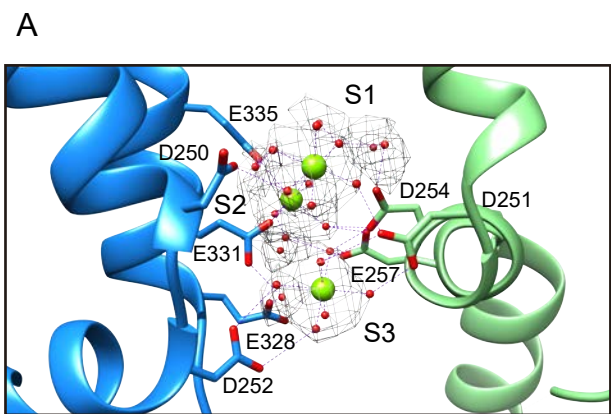


Figure S9

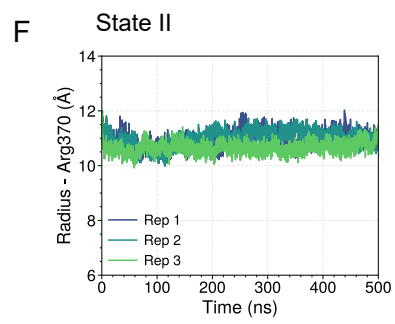
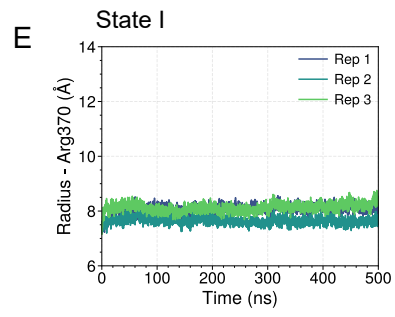
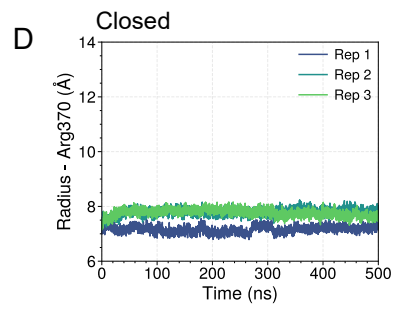
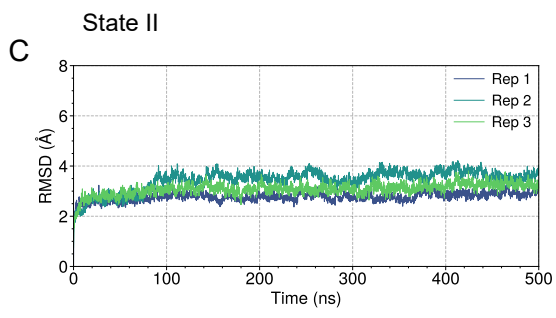
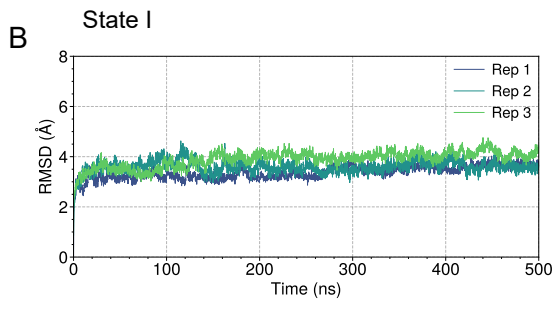
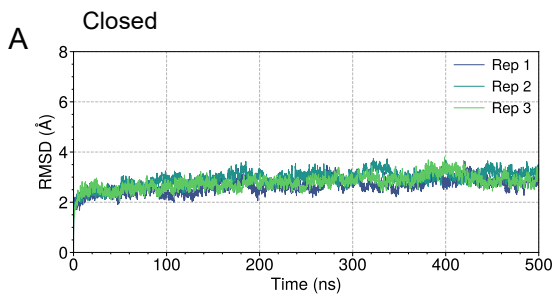


Figure S10

	249-258				330-336				224-226		353-359		
Q9S9N4_AtMRS2-1	MDD	DG	DMA	EM	LEMLLEA	VR	R	VR	R	EY	IDD	TE	
Q9FLG2_AtMRS2-2	LDD	DD	DMA	DL	LEMLLEA	VR	K	VR	K	EY	IDD	TE	
Q9LJN2_AtMRS2-3	LDD	DE	DMA	EM	LEMLLEA	VR	Q	VR	Q	EY	VDD	TE	
Q93ZD7_AtMRS2-4	LDD	NE	DMA	DL	LEMLLEA	VR	S	VR	S	EY	IDD	TE	
Q9ZPR4_AtMRS2-5	MDD	DD	DMA	EM	LEMLLEA	VR	R	VR	R	EY	IDD	TE	
Q1PE39_AtMRS2-6	LDD	KE	DMED	L	MEMLLEA	LR	S	LR	S	EH	ID	STE	
Q304A0_AtMRS2-7	LDD	DG	DMA	DL	LEMLLEA	VR	K	VR	K	EY	IDD	TE	
Q9SAH0_AtMRS2-10	MDD	DG	DMA	EM	LEMLLEA	AR	R	AR	R	EY	IDD	TE	
Q058N4_AtMRS2-11	LED	PH	EIRRI	IEMLEN	LR	I	LR	I	DS	AK	EME		
Q9AUK4_OsMRS2-A	LDD	PH	EIRRI	IEMLEN	LR	L	LR	L	DS	ARE	ME		
Q67UQ7_OsMRS2-B	MDD	DG	DMA	EM	LEMLLEA	VR	R	VR	R	EY	IDD	TE	
Q0JBZ6_OsMRS2-C	MDD	DG	DMA	EM	LEMLLEA	VR	R	VR	R	EY	IDD	TE	
Q7XQQ1_OsMRS2-D	LDD	DM	DMA	AL	LEILLES	VR	Q	VR	Q	EY	VDD	TE	
Q8S1N1_OsMRS2-E	LDD	DM	DMSEM	LEMLLEA	VR	N	VR	N	EY	ADD	TE		
Q8L4S2_OsMRS2-F	LDD	EM	DMA	EM	LEMLLEA	VR	Q	VR	Q	EY	VDD	TE	
A3BV82_OsMRS2-G	LDD	NE	DMA	HL	LEMLLEA	VR	T	VR	T	EY	IDD	TE	
Q10S25_OsMRS2-H	LDD	NE	AMAHL	LDMLLEA	VW	S	VW	S	QY	IV	DTE		
Q10D38_OsMRS2-I	LDD	DD	DMA	DL	LEMLLEA	VR	K	VR	K	EY	IDD	TE	
A0A317Y4T9_ZmMRS2-A	LDD	PH	EIRRI	IEMLEN	LR	L	LR	L	NS	ARE	ME		
A0A3L6DDP3_ZmMRS2-B_1	MDD	DG	DMA	EM	LEMLLEA	AR	R	AR	R	EY	IDD	TE	
A0A3L6G7L2_ZmMRS2-D	LD	ADV	DMA	AM	LENLLEA	EY	VDD	TE
A0A3L6FS80_ZmMRS2-E	LDD	DV	DMA	AM	LEILLEA	VR	Q	VR	Q	EY	VDD	TE	
A0A3L6DNR6_ZmMRS2-F_0	LDD	EM	DMA	EM	LEMLLEA	VR	Q	VR	Q	EY	VDD	TE	
A0A8J8XSX0_ZmMRS2-G_0	LDD	NE	DMEHL	LEMLLEA	VR	N	VR	N	EY	IDD	TE		
A0A8J8XLQ5_ZmMRS2-G_1	LDD	NE	DMEHL	LEMLLEA	VR	S	VR	S	EY	IDD	TE		
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A0A317Y479_ZmMRS2-I	LDD	DD	DMA	DL	LEMLLEA	VR	I	VR	I	EY	IDD	TE	
G0S186_CtMRS2	LE	ADD	DLA	AM	VELLLES	LL	V	LL	V	SS	IR	NTE	
Q9HD23_hMRS2	LDE	EEL	LLE	EL	MELLEN	LL	Q	LL	Q	VL	IDD	SQ	
Q9WZ3_TmCorA	IDD	EI	DVL	EE	IEKETVP	IR	Y	IR	Y	TF	R	DIVS	

Figure S11

Table S1. Cryo-EM data collection, refinement and validation statistics

	AtMRS2-1 Mg ²⁺ -bound closed (EMDB: EMD-69740) (PDB: 24PF)	AtMRS2-1 State I, Preopen (EMDB: EMD-69741) (PDB: 24PG)	AtMRS2-1 State II, Open (EMDB: EMD-69742) (PDB: 24PH)
Data collection and processing			
Magnification	130,000×	81,000×	81,000×
Voltage (kV)	300	300	300
Electron exposure (e-/Å ²)	40	50	50
Defocus range (μm)	-1.0 to -1.5	-1.0 to -2.2	-1.0 to -2.2
Pixel size (Å)	0.959	0.894	0.894
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	3,029,468	9,823,207	9,823,207
Final particle images (no.)	406,525	2,178,665	3,607,825
Map resolution (Å)	2.44	2.88	3.06
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	2.166 to 27.694	1.933 to 7.242	1.933 to 7.241
Refinement			
Initial model used (PDB code)	AlphaFold2	AlphaFold2	AlphaFold2
Model resolution (Å)	2.44	2.88	3.06
FSC threshold	0.143	0.143	0.143
Model resolution range (Å)	2.166 to 27.694	1.933 to 7.242	1.933 to 7.241
Map sharpening <i>B</i> factor (Å ²)	-63.3	-120.6	-132.9
Model composition			
Non-hydrogen atoms	14,010	10,986	11,851
Protein residues	1,570	1,480	1,525
Ligands	POPC:20, Mg:16, Cl:1	POPC:15, Mg:1	POPC:15, Mg:1
<i>B</i> factors (Å ²)			
Protein	98.21	132.27	137.41
Ligand	121.16	101.09	125.71
Water	98.36		
R.m.s. deviations			
Bond lengths (Å)	0.005	0.004	0.004
Bond angles (°)	0.479	0.639	0.567
Validation			
MolProbity score	1.44	1.73	1.42
Clashscore	3.40	5.40	7.71
Poor rotamers (%)	2.56	3.83	0.09
Ramachandran plot			
Favored (%)	98.51	98.55	98.13
Allowed (%)	1.49	1.45	1.87
Disallowed (%)	0	0	0

Cryo-EM Structure	Box Size (nm × nm × nm)	System Composition	Trajectories
Mg ²⁺ -bound closed State	13.7×13.7×21.0	(404288 atoms) Protein 1 POPC 513 H ₂ O 100973 Mg ²⁺ 16, K ⁺ 385, Cl ⁻ 364	500 ns * 3
State I	13.2×13.2×20.3	(361453 atoms) Protein 1 POPC 510 H ₂ O 86919 Mg ²⁺ 1, K ⁺ 288, Cl ⁻ 236	500 ns * 3
State II	13.2×13.2×21.6	(388212 atoms) Protein 1 POPC 510 H ₂ O 95795 Mg ²⁺ 1, K ⁺ 315, Cl ⁻ 259	500 ns * 3

Table S2. Summary of molecular dynamics simulation systems and trajectories