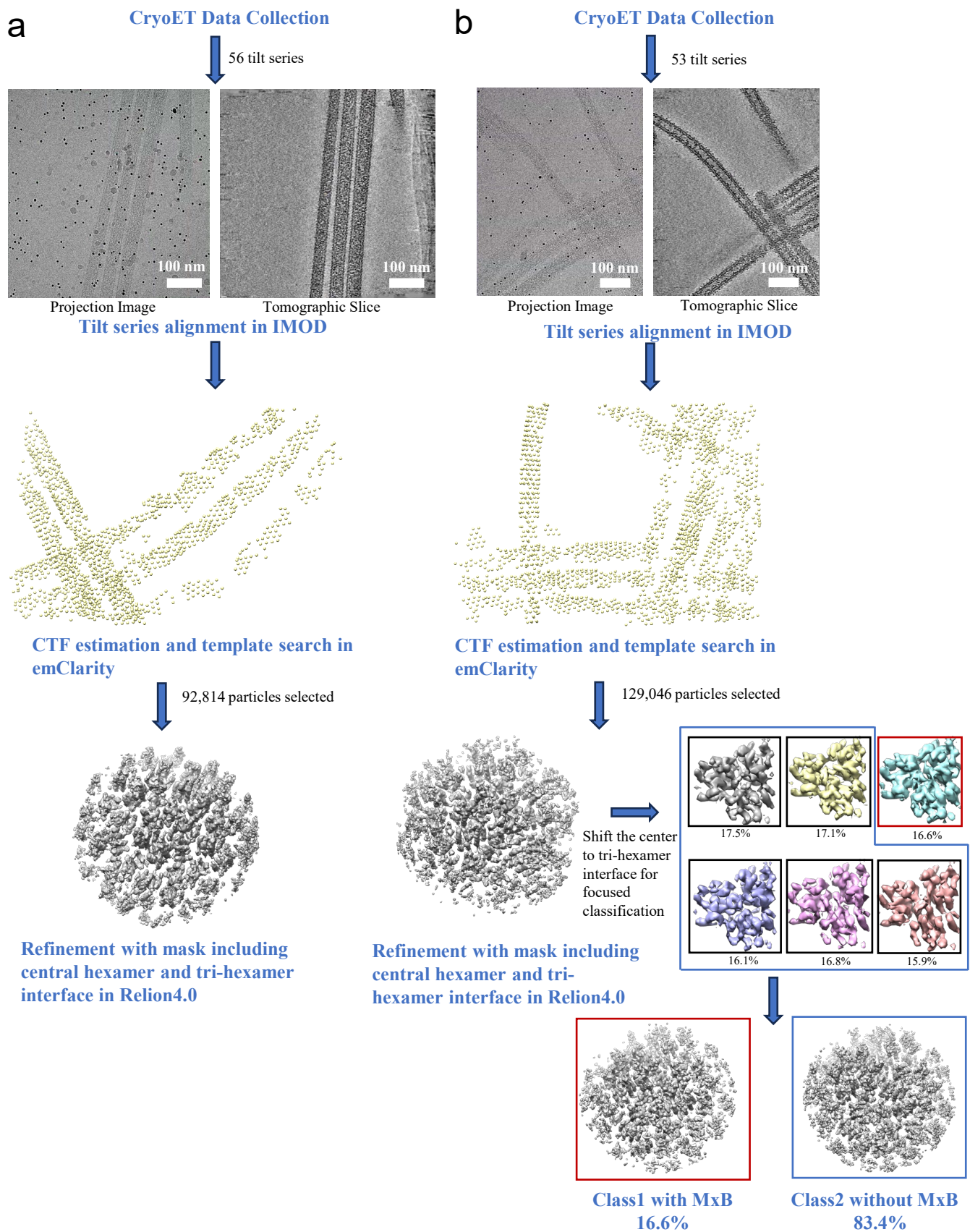


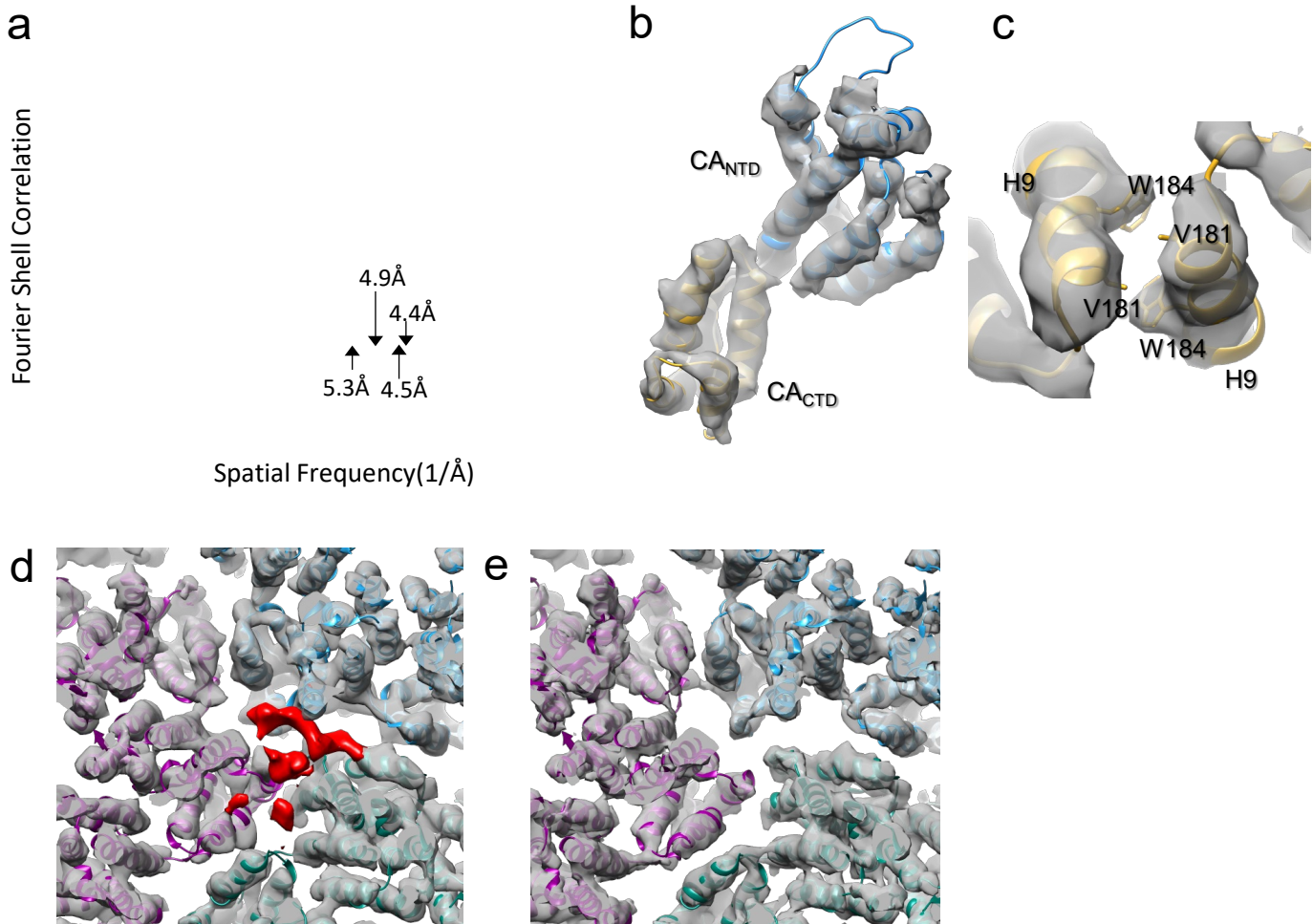
MxB N-Terminus Adopts a Stable α -Helix to Engage the HIV-1 Capsid Trimer Interface

Yanan Zhu^{1,2,3†}, Juan S. Rey^{4†}, Juan Shen^{1†}, Juan R. Perilla^{4*}, Peijun Zhang^{1,5,6*}

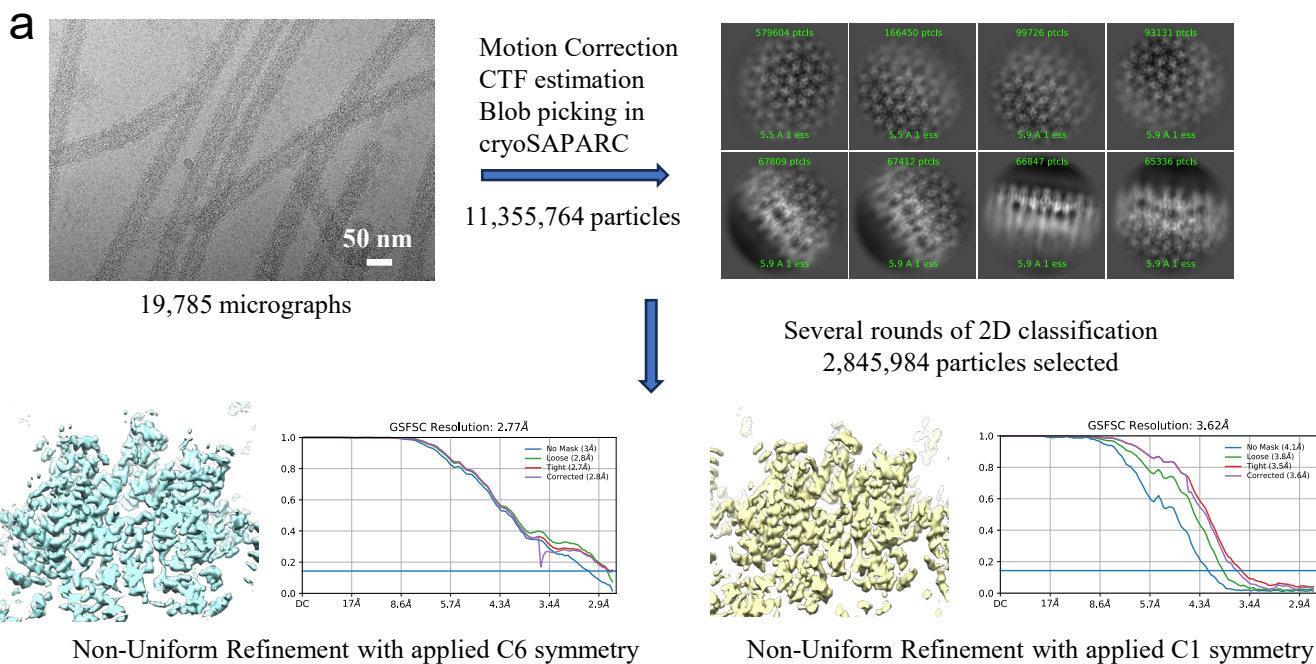
Supplementary figures and tables



Supplementary Figure 1 | CryoET STA data processing workflow. (a) workflow for the CA-NC tubes. (b) workflow for the CA-NC/MxB₁₋₃₅ complexes.



Supplementary Figure 2 | CryoET STA analysis of MxB₁₋₃₅ and CA-NC tube complexes. Fourier Shell Correlation (FSC) plot. (a) Fourier Shell Correlation (FSC) plot of cryoET STA density maps from CA-NC tubes (blue), CA-NC tubes in complex with MxB₁₋₃₅ (grey) and a subtomogram class with MxB₁₋₃₅ bound to the CA trimer interface (red). The resolution is indicated at the FSC value of 0.143. (b) The density map of one CA monomer from CA-NC tubes in complex with MxB₁₋₃₅ superimposed with the atomic model (PDB:4XFX) with CA_{NTD} (blue) and CA_{CTD} (orange) fitted separately. (c) A close-up view of the CA dimer interface from CA-NC tube in complex with MxB₁₋₃₅, displaying the interaction between W184 and V181. (d-e) Comparison of trimer interfaces of two 3D classes from MxB₁₋₃₅ and CA-NC tube complexes superimposed with the model (PDB 9SLY). Class1 (d) distinguishes from Class2 (e) by additional densities (segmented in red). Both maps were presented at similar contour level.

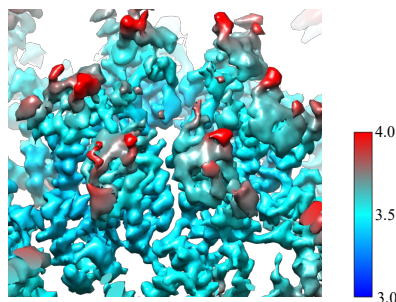
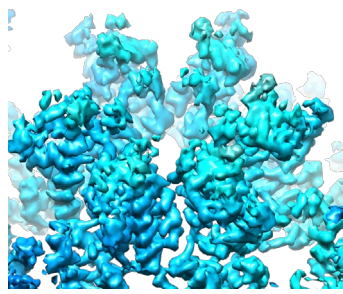


b

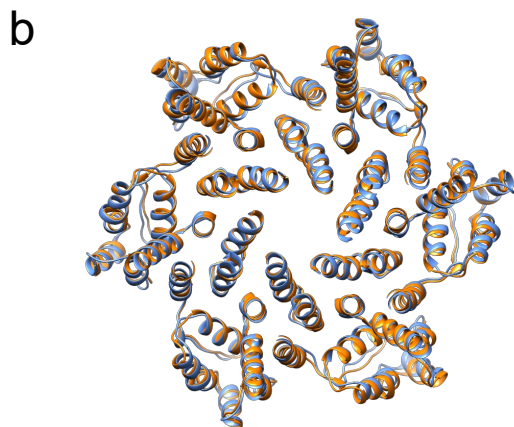
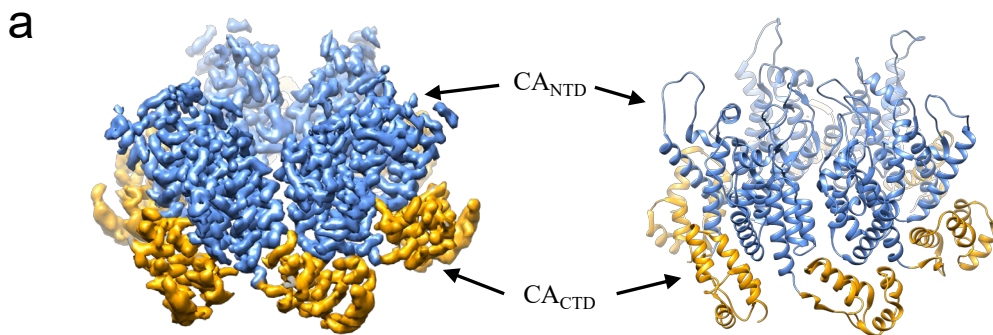
Viewing Direction Distribution (C6)

Viewing Direction Distribution (C1)

c

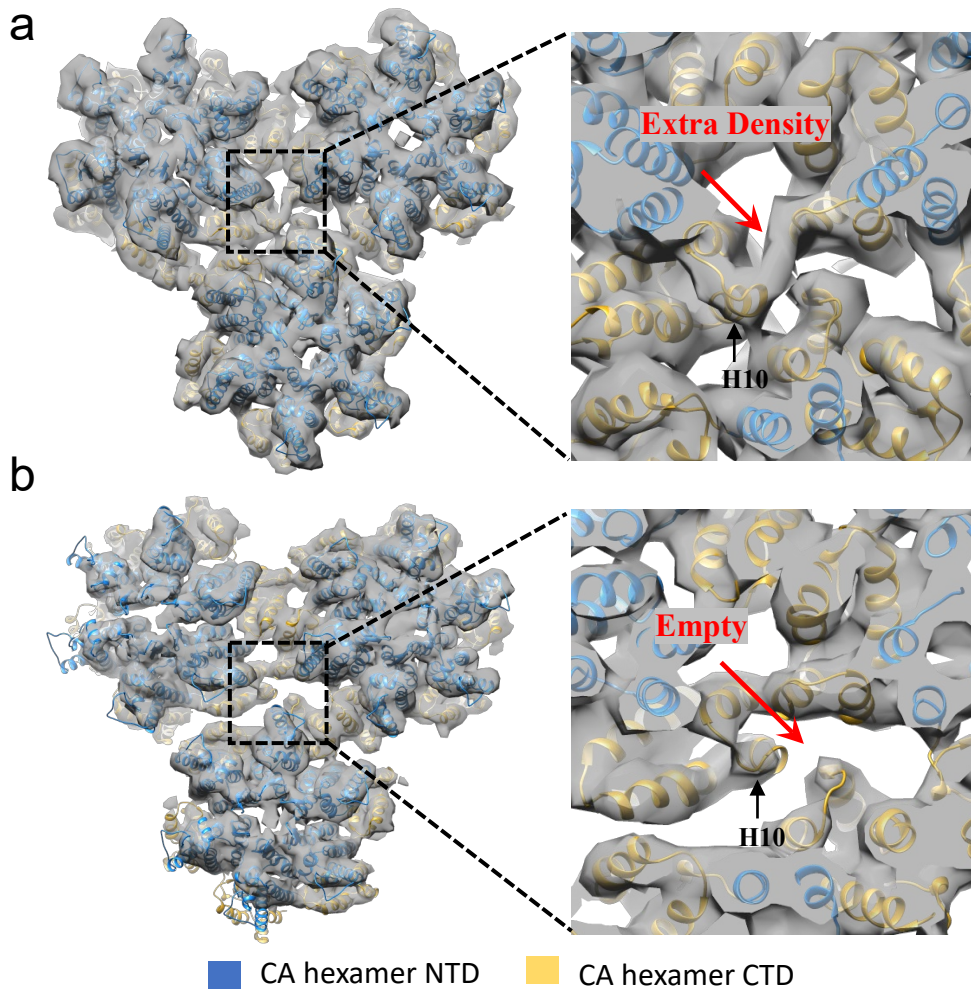


Supplementary Figure 3 | CryoEM SPA data processing workflow. (a) workflow for SPA data processing. (b) View direction distribution with applied C6 symmetry (left) and C1 symmetry (right). (c) Local resolution estimation with applied C6 symmetry (left) and C1 symmetry (right).

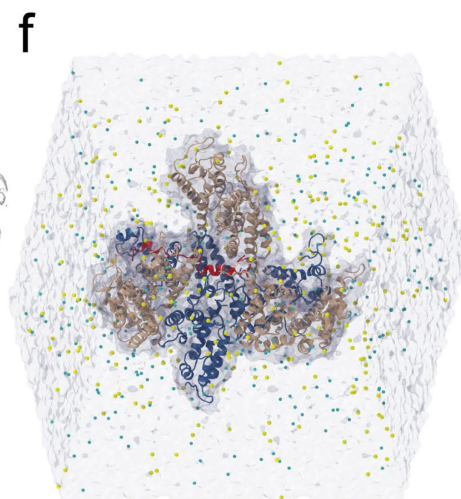
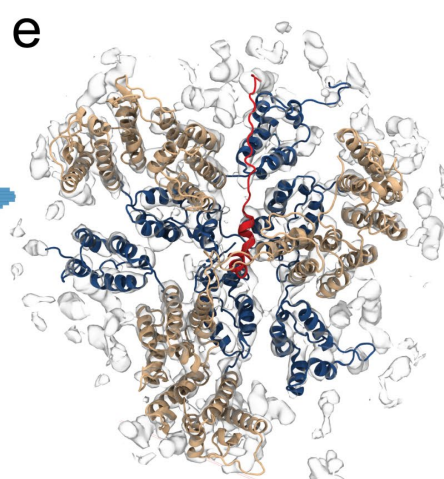
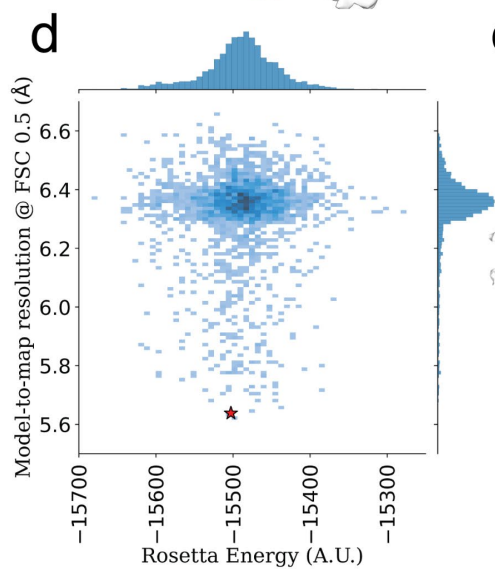
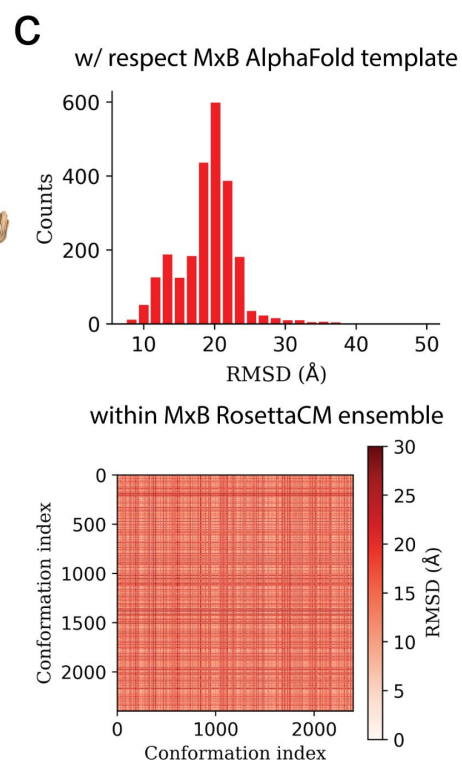
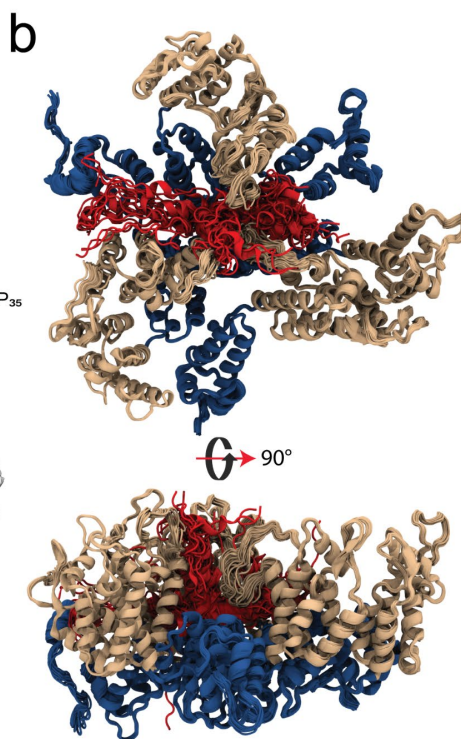
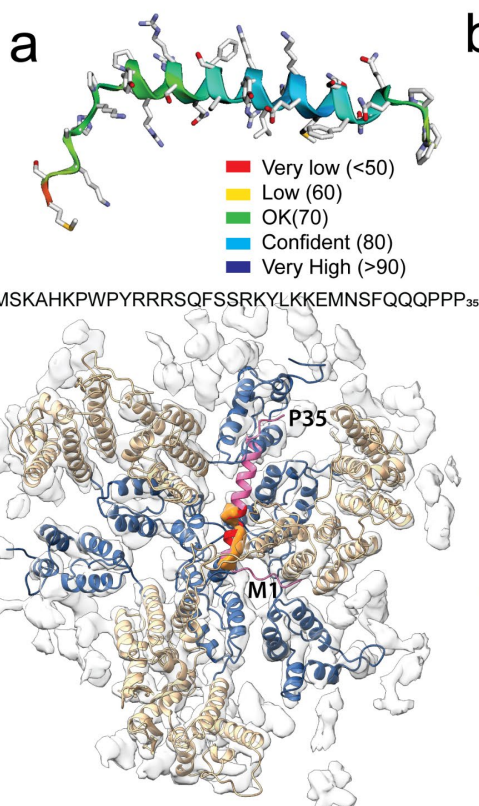


RMSD=0.846 Å

Supplementary Figure 4 | CryoEM SPA structure of CA hexamer in MxB₁₋₃₅-bound CA-NC tubes. (a) CryoEM map (left) and model (right) of CA hexamer from MxB₁₋₃₅-bound CA-NC tubes with CA NTD colored blue and CA CTD colored yellow. (b) Comparison of CA hexamer from MxB₁₋₃₅-bound CA-NC tubes by SPA (orange) and STA (blue).



Supplementary Figure 5 | CryoET STA density maps of CA-NC tubes and in complex with MxB₁₋₃₅. (a) Density map of CANC tube with MxB₁₋₃₅ and extra density shown on the trimer interface. (b) Density map of CANC tube without MxB₁₋₃₅.



Supplementary Figure 6 | Computational modeling of the MxB₁₋₃₅–CA complex. (a) MxB₁₋₃₅ AlphaFold2 prediction colored by predicted local distance difference test (pLDDT) score (top) and the predicted structure rigid-body fitted into the STA density at the CA trimer interface (bottom). MxB₁₋₃₅, CA-NTD and CA-CTD are colored in pink, gold, and blue, respectively. MxB residues 11-18, used as a template for RosettaCM density-guided modeling, are colored in red, and corresponding STA MxB density is colored in orange.

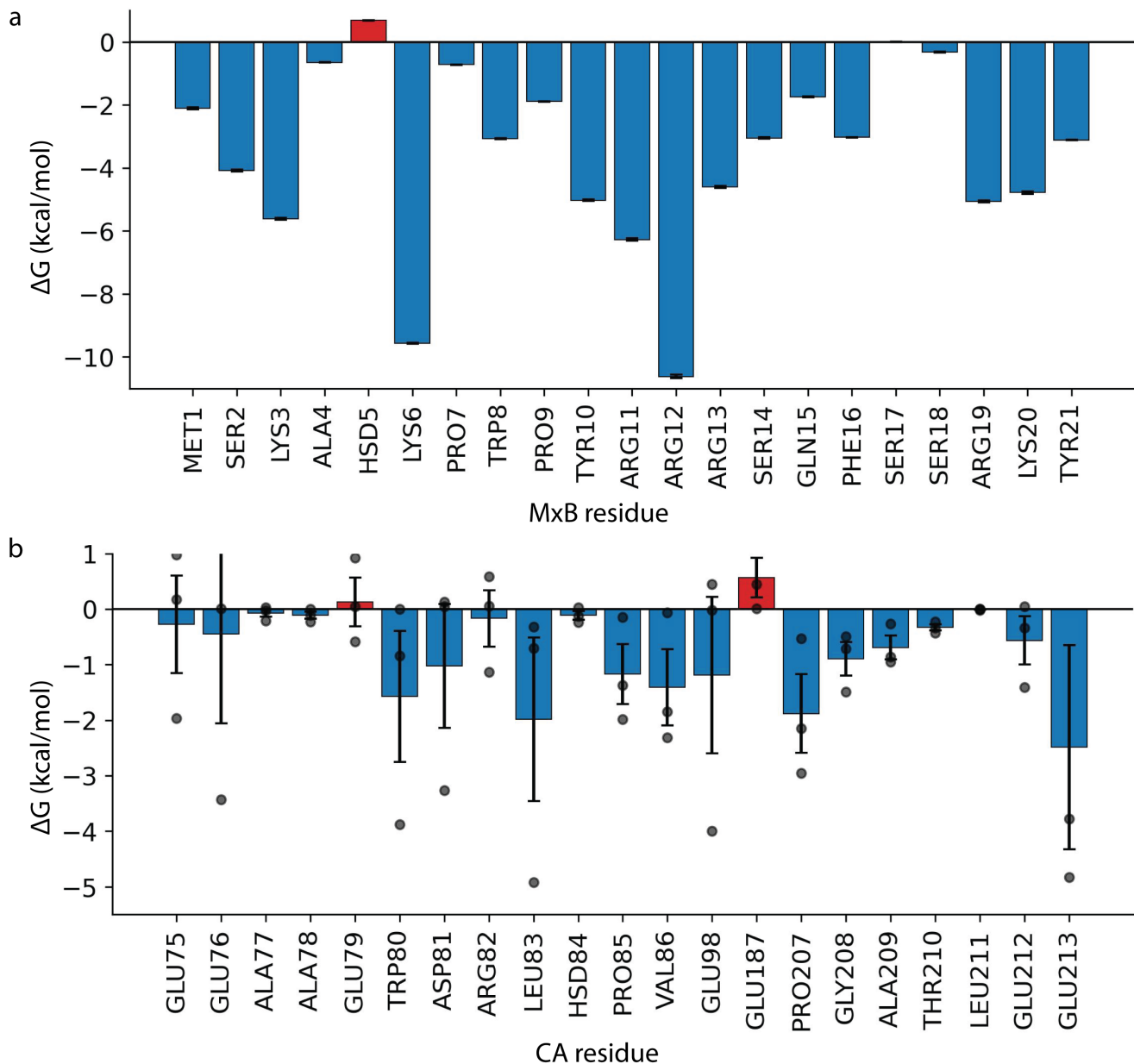
(b) Ensemble of models generated by RosettaCM using the MxB₁₋₃₅ AlphaFold2 prediction as template. 2,500 independent models were generated; 50 representative models are shown in cartoon representation. MxB, CA-CTD and CA-CTD are colored in red, gold and blue, respectively.

(c) Backbone atom root-mean-square deviation (RMSD) of MxB₁₋₃₅ conformations generated by RosettaCM relative to the AlphaFold2 template (top) and between models within the RosettaCM ensemble (bottom).

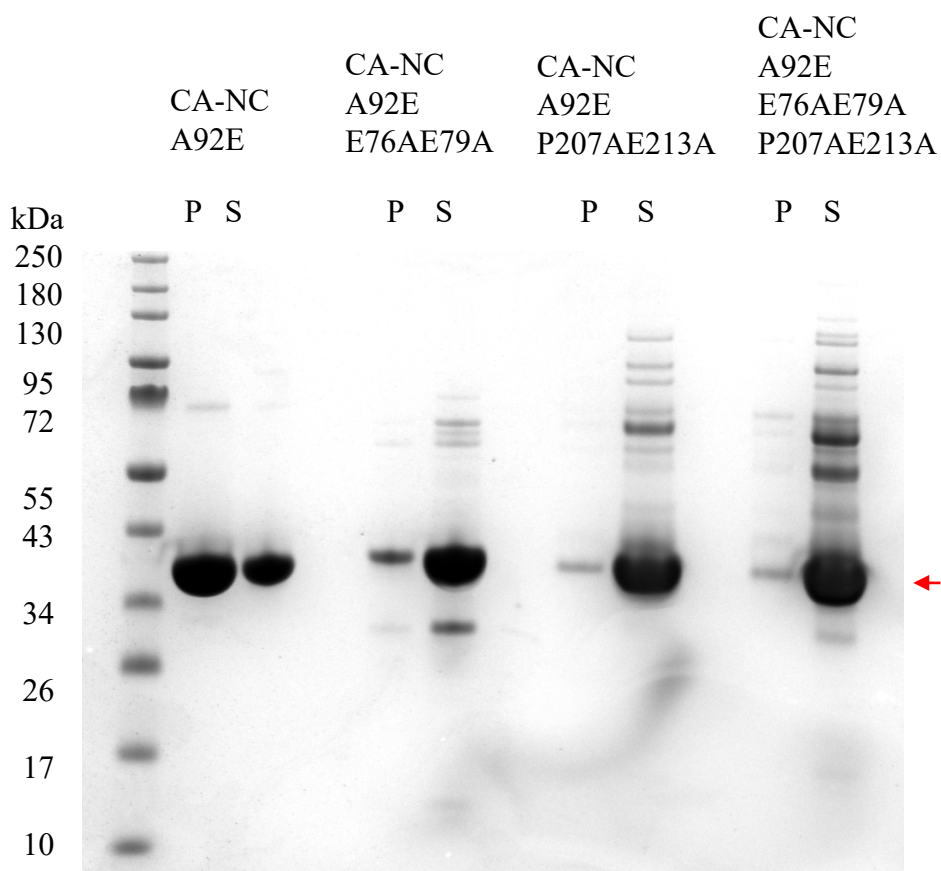
(d) Distribution of model-to-map FSC resolution and Rosetta all-atom energy scores for the 2,500 RosettaCM MxB₁₋₃₅/CA models. The model selected as the starting structure for MD simulations, exhibiting the highest resolution and lowest Rosetta energy, is indicated with a red star in the joint plot.

(e) Best scoring MxB₁₋₃₅/CA trimer-of-dimers model.

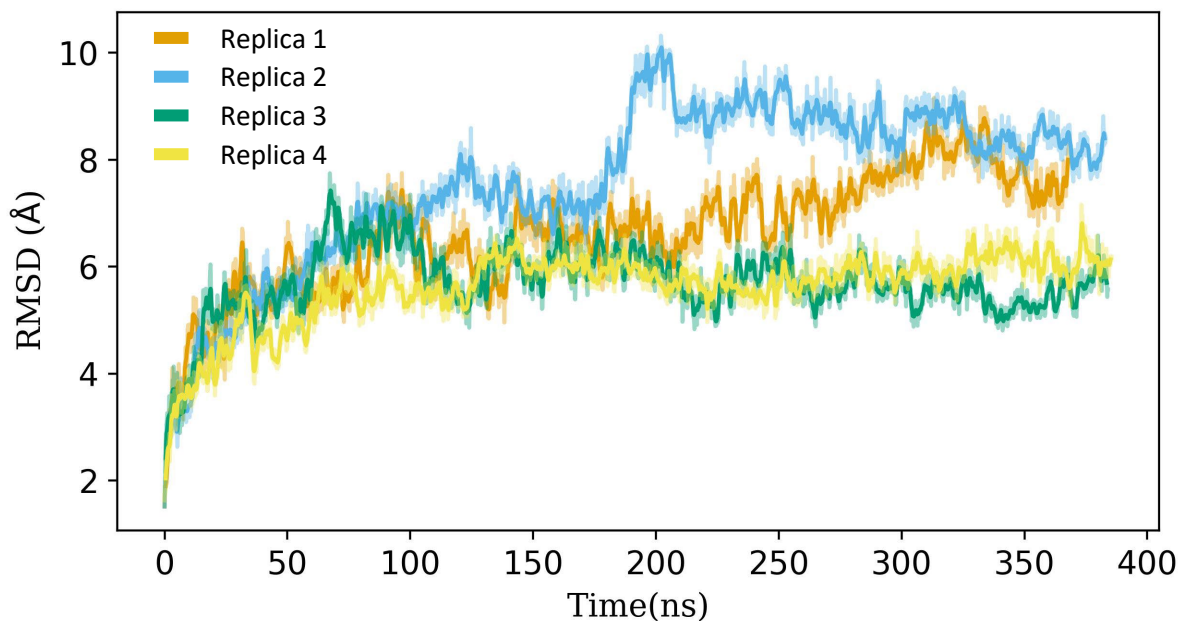
(f) Ionized and solvated MxB₁₋₃₅/CA complex used for MD simulations. MxB₁₋₃₅, CA-NTD and CA-CTD are colored in red, gold and blue, respectively. Sodium and chloride ions are colored in yellow and cyan, respectively, water molecules are represented as a transparent blue molecular surface.



Supplementary Figure 7 | MM/GBSA-derived per-residue energetic contributions to MxB₁₋₃₅ binding. (a) Energetic contribution of MxB₁₋₃₅ residues that interact with CA during the MD simulations. Bar heights represent the mean binding free-energy contribution, and error bars indicate the SEM. Favorable energetic contributions ($\Delta G < 0$) are colored in blue while unfavorable energetic contributions ($\Delta G > 0$) are shown in red. (b) Energetic contributions of CA residues that interact with MxB during the MD simulations. For each CA residue, the bar height represents the average binding free-energy contribution across the three equivalent CA chains in the trimer-of-dimer assembly, and error bars indicate the standard error of the mean (SEM). Individual datapoints for each chain are shown as black circles. Favorable energetic contributions ($\Delta G < 0$) are colored in blue, while unfavorable energetic contributions ($\Delta G > 0$) are shown in red.



Supplementary Figure 8 | SDS PAGE analysis of pellet/supernatant assembly assays of CA-NC mutants. From left to right: pellet (P) and supernatant (S) fractions of CA-NC A92E, CA-NC A92E E76A/E79A, CA-NC A92E P207A/E213A, and CA-NC A92E E76A/E79A/P207A/E213A following in vitro assembly. The red arrow indicates the CA-NC protein bands.



Supplementary Figure 9 | Root-mean-square deviation (RMSD) of the CA- MxB₁₋₃₅ complex during MD simulations. Backbone RMSD trace of the CA trimer-of-dimers is shown for each MD simulation replica. The 1ns windowed average is shown as a solid line, and the raw RMSD trace is shown with 50% transparency.

Supplementary Table 1 | CryoET data collection and structure determination of CA-NC/MxB₁₋₃₅ complex

Data acquisition				
Samples	CA hexamer from CA-NC tube only	CA hexamer from CA-NC/MxB ₁₋₃₅	CA tri-hexamer Class1 of CA-NC/MxB ₁₋₃₅	CA tri-hexamer Class2 of CA-NC/MxB ₁₋₃₅
Microscope	Titan Krios	Titan Krios		
Voltage (kV)	300	300		
Detector	Falcon 4i with SelectrisX	Gatan Quantum K3 Direct Electron Detector		
Energy-filter	Yes	Yes		
Slit width (eV)	10	20		
Super-resolution mode	No	No		
Å/pixel	1.501	1.34		
Defocus range (µm)	-2.5 to -6	-2.5 to -6		
Defocus increment (µm)	0.3	0.3		
Acquisition scheme	Dose-Symmetric, -60/60, 3° step, group 3	Dose-Symmetric, -60/60, 3° step, group 3		
Total Dose (electrons/Å ²)	123	123		
Number of Frames	10	10		
Number of Tomograms	56	53		
Structure determination				
No. of subtomograms	92,814	129,046	21,392	107,654
Resolution (Å)	4.9	4.4	5.3	4.5
Symmetry	C1	C1	C1	C1
b-factor applied	-50	-50	-50	-50
Data deposited	EMD-54042	EMD-54043	EMD-54044	EMD-55015

Supplementary Table 2. CryoEM data collection and structure determination CA hexamer from MxB₁₋₃₅-bound-CA-NC tubes.

Data collection and processing	
Microscope	Titan Krios
Magnification	64,000
Voltage	300kV
Electron dose	40e ⁻ /Å ²
Detector	Gatan Quantum K3
Defocus Range	-0.8 to -2.5 μm
Pixel Size	1.34 Å
Symmetry	C6
Particle numbers	2,845,984
Map resolution(Å)	2.77
FSC threshold	0.143
Local resolution range(Å)	2.5-3.5
Refinement	
Initial Model used (PDB code)	PDB:9I8I
Model resolution(Å)	3.45
FSC threshold	0.5
Map sharpening <i>B</i> factor(Å ²)	-120
Model composition	
Non-hydrogen atoms	10350
Residues	1326
R.m.s.d deviations	
Bond length(Å)	0.004
Bond angles(°)	0.984
Validation	
MolProbity score	2.31
Clashscore	20.52
Ramachandran Plot	
Favored(%)	94.98
Allowed(%)	5.02
Outliers(%)	0.00
Data deposition	EMD-55019 PDB:9SLY

Supplementary Table 3 | Top 3 MxB₁₋₃₅ and CA contacts per residue by occupancy throughout 1.6μs MD simulation

No.	MxB residue	Top 1 contact	Top 2 contact	Top 3 contact
1	MET1	GLU98 (66.60%)	HIS120 (49.57%)	ILE124 (33.45%)
2	SER2	PRO85 (68.76%)	GLU98 (62.96%)	MET96 (28.80%)
3	LYS3	TRP80 (99.68%)	LEU83 (98.96%)	GLU98 (95.15%)
4	ALA4	LEU83 (51.10%)	VAL86 (43.31%)	PRO85 (32.89%)
5	HIS5	VAL86 (46.00%)	LEU83 (41.25%)	PRO85 (14.68%)
6	LYS6	GLU79 (99.68%)	GLU76 (98.46%)	LEU83 (84.79%)
7	PRO7	GLY208 (24.34%)	PRO207 (24.32%)	GLU187 (11.00%)
8	TRP8	VAL86 (81.59%)	PRO85 (54.74%)	LEU83 (53.94%)
9	PRO9	LEU83 (92.64)	VAL86 (70.64%)	GLU79 (39.39%)
10	TYR10	ALA209 (67.60%)	GLU213 (61.89%)	LEU205 (51.39%)
11	ARG11	PRO207 (74.15%)	THR210 (71.83%)	GLU213 (54.21%)
12	ARG12	LEU205 (98.42%)	GLU213 (84.06%)	PRO207 (63.84%)
13	ARG13	GLU213 (61.21%)	VAL86 (61.17%)	HIS87 (41.17%)
14	SER14	ARG82 (84.14%)	GLU79 (61.17%)	HIS87 (41.17%)
15	GLN15	THR210 (56.10%)	GLY208 (55.47%)	GLU75 (39.43%)
16	PHE16	GLU213 (79.88%)	GLU212 (63.74%)	THR216 (62.06%)
17	SER17	ARG82 (74.33%)	HIS87 (67.74%)	VAL86 (27.50%)
18	SER18	ARG82 (94.15%)	THR110 (45.54%)	GLU75 (27.34%)
19	ARG19	GLY208 (72.78%)	THR210 (55.00%)	GLU187 (46.97%)
20	LYS20	GLU75 (72.34%)	GLU212 (46.47%)	LYS140 (45.74%)
21	TYR21	GLN176 (82.71%)	ARG143 (76.08%)	LYS140 (65.93%)
22	LEU22	THR110 (73.97%)	SER178 (65.41%)	THR108 (58.11%)
23	LYS23	LEU136 (79.18%)	GLU76 (68.38%)	GLU79 (63.85%)
24	LYS24	GLU113 (55.77%)	GLN179 (34.64%)	THR108 (23.51%)
25	GLU25	GLN112 (65.63%)	LEU136 (63.53%)	GLU79 (48.21%)
26	MET26	ARG132 (40.83%)	ILE129 (20.36%)	GLU113 (19.51%)
27	ASN27	GLN112 (46.90%)	GLN114 (28.23%)	ARG132 (27.40%)
28	SER28	TRP80 (25.06%)	HIS84 (23.13%)	GLN112 (12.02%)
29	PHE29	ILE129 (60.13%)	PRO125 (54.76%)	ILE124 (53.55%)
30	GLN30	ILE124 (24.15%)	PRO125 (15.46%)	HIS120 (11.63%)
31	GLN31	ARG132 (37.00%)	GLU128 (21.75%)	PRO125 (20.89%)
32	GLN32	PRO122 (8.62%)	ARG132 (8.07%)	ARG100 (7.77%)
33	PRO33	ILE135 (25.51%)	ARG132 (20.61%)	SER41 (12.65%)
34	PRO34	ARG132 (29.97%)	ILE135 (29.94%)	LEU136 (28.66%)
35	PRO35	GLN179 (17.50%)	ALA88 (7.25%)	VAL86 (7.19%)

Supplementary Table 4 | Summary of the CA-MxB₁₋₃₅ complex system built for MD simulations.

System description	Dimensions (Å)	Total number of atoms	Total number of water molecules	Salt concentration (mM NaCl)	Simulation length (ns)	No. replicas
CA trimer-of-dimers in complex with MxB ₁₋₃₅ NPT equilibration/production.	160.4 × 163.9 × 117.7	312,678	96,637	150	400	4