

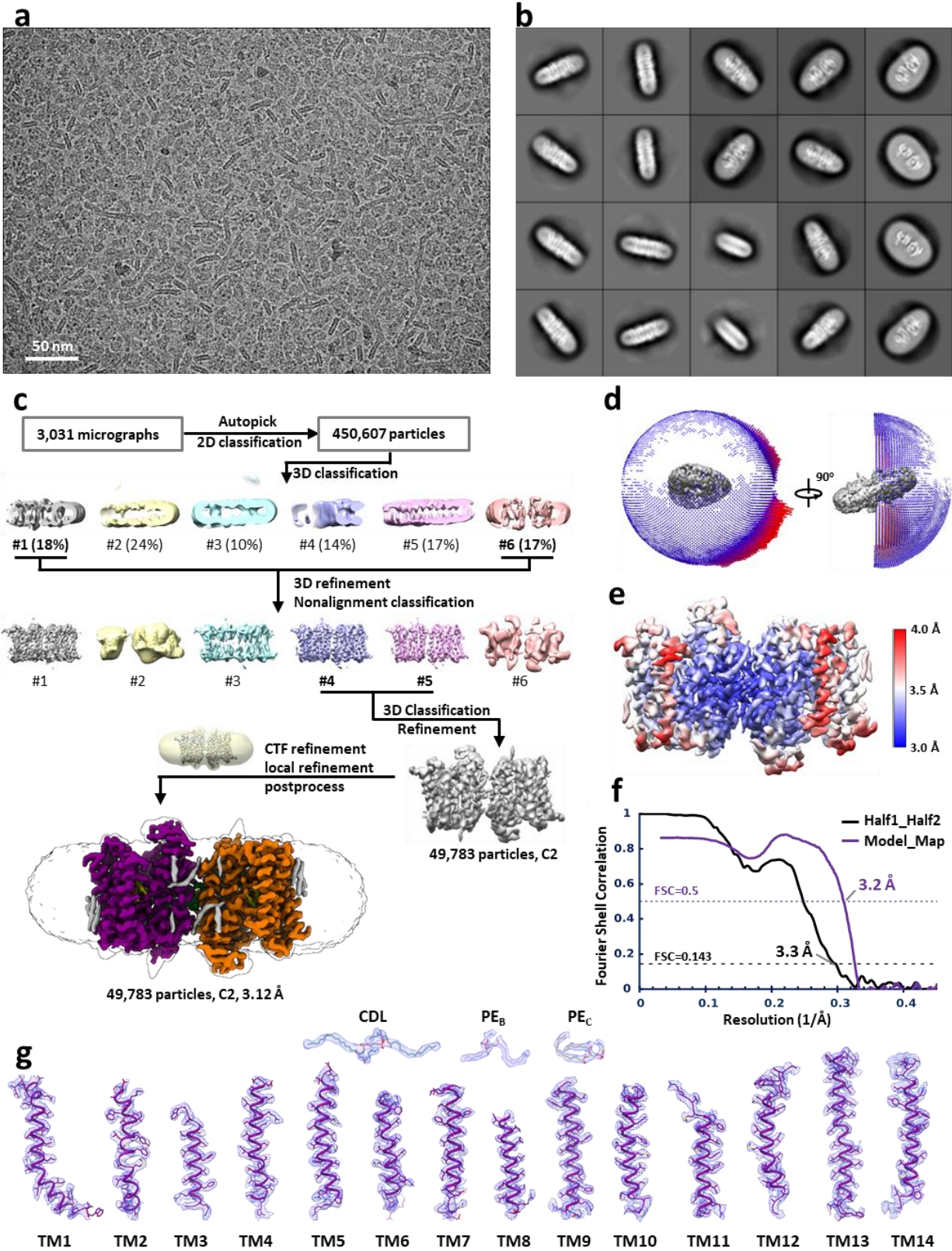
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2 **Supplementary Fig. 1 | Characterization and structural analysis of EfpA.** a, Phylogenetic tree of EfpA
 3 homologues. *M. tuberculosis* and other pathogenic species of mycobacterium are highlighted with black
 4 star and empty stars, respectively. b, Size-exclusion chromatography profile of *MtEfpA* with C-terminal

5 GFP tag after solubilized by LMNG-CHS. Source data are provided as a Source Data file. **c**, SDS-PAGE with
6 GFP fluorescence scan (left) and Coomassie stain (right). Source data are provided as a Source Data file.
7 **d**, Flow cytometry assay of *MtEfpA* topology on HEK plasma membrane. HEK_{emp}, HEK293 cells without
8 *MtEfpA*-GFP expressed. HEK_{MtE}, HEK293 cells with *MtEfpA*-GFP overexpressed. S-HEK_{emp}/S-HEK_{MtE}, cells
9 incubated with APC anti-GFP antibody without permeabilization. Intra-HEK_{emp}/Intra-HEK_{MtE}, cells
10 incubated with APC anti-GFP antibody after permeabilization. The oval on the right is represent the
11 plasma membrane of cells. Green represents GFP signal, and red represents APC signal. Source data are
12 provided as a Source Data file. **e**, Lipidomics and small-molecule analysis of dimeric *MtEfpA*-LMNGCHS
13 and monomeric *MtEfpA*-DDMCHS using negative mode (top) and positive mode (bottom). One biological
14 repeat was performed for this experiment. Source data are provided as a Source Data file. **f**, Electrostatic
15 surface and hydrophobic surface of *MtEfpA*. **g**, Dimeric interface of *MtEfpA* dimer.

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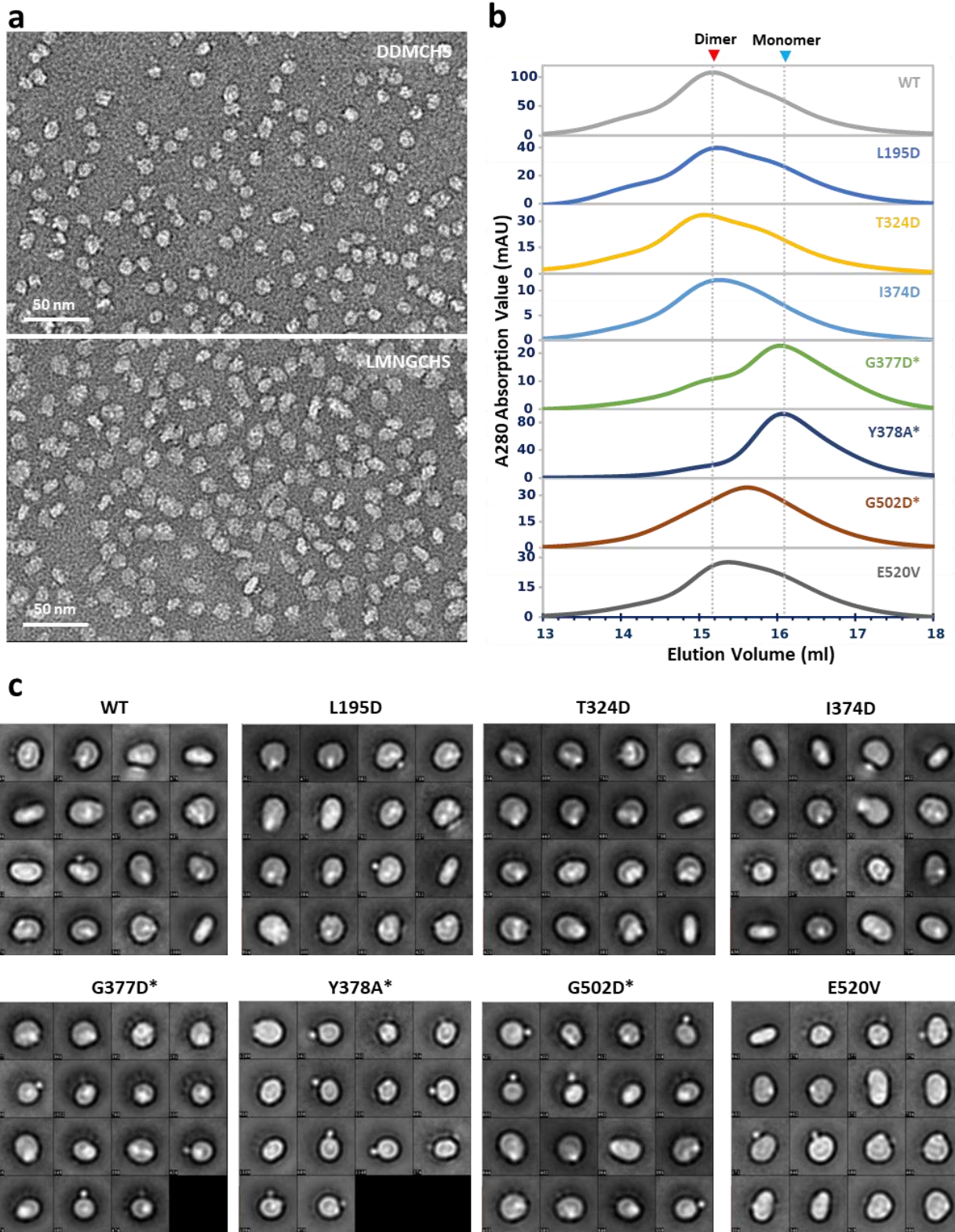
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19 **Supplementary Fig. 2 | Cryo-EM reconstruction of dimeric *MtEfpA*.** **a**, Representative cryo-EM
20 micrograph of *MtEfpA*. **b**, 2D class averages of *MtEfpA* with a box size of 266 Å. **c**, Flowchart for processing
21 cryo-EM data of *MtEfpA*. **d**, Particle angle distribution for the final map of dimeric *MtEfpA*. **e**, Local
22 resolution of the final cryo-EM map. **f**, Gold standard Fourier shell correlation (FSC) curve for the final
23 reconstruction. Half map #1 vs. half map #2 of *MtEfpA* with masked shown in black. Model vs. refined
24 map with masked shown in purple. **g**, Close-up view of all structure elements fitted in densities with
25 contour level at 5σ (blue).

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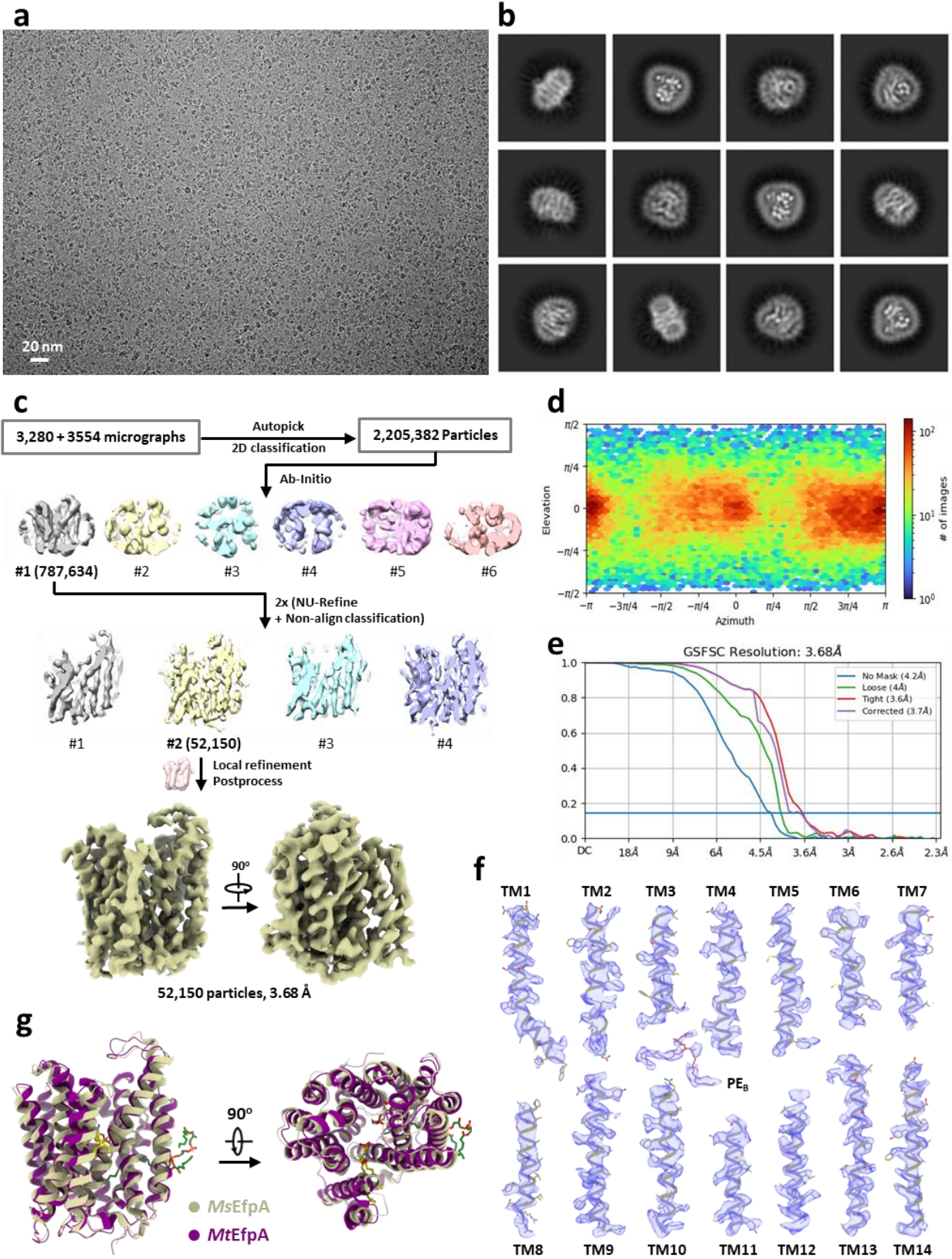
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29 **Supplementary Fig. 3 | Negative stain analysis of *MtEfpA* and mutants.** **a**, Representative micrographs
 30 of monomeric *MtEfpA* purified with DDMCHS (upper) and dimeric *MtEfpA* purified with LMNGCHS

31 (bottom). **b**, Size exclusion chromatography (SEC) profile of *MtEfpA* wide type and mutants purified with
32 LMNGCHS. The mutants forming monomers were marked with stars. Source data are provided as a Source
33 Data file. **c**, Negative stain 2D averages of *MtEfpA* wild type (WT) and mutants purified with LMNGCHS.

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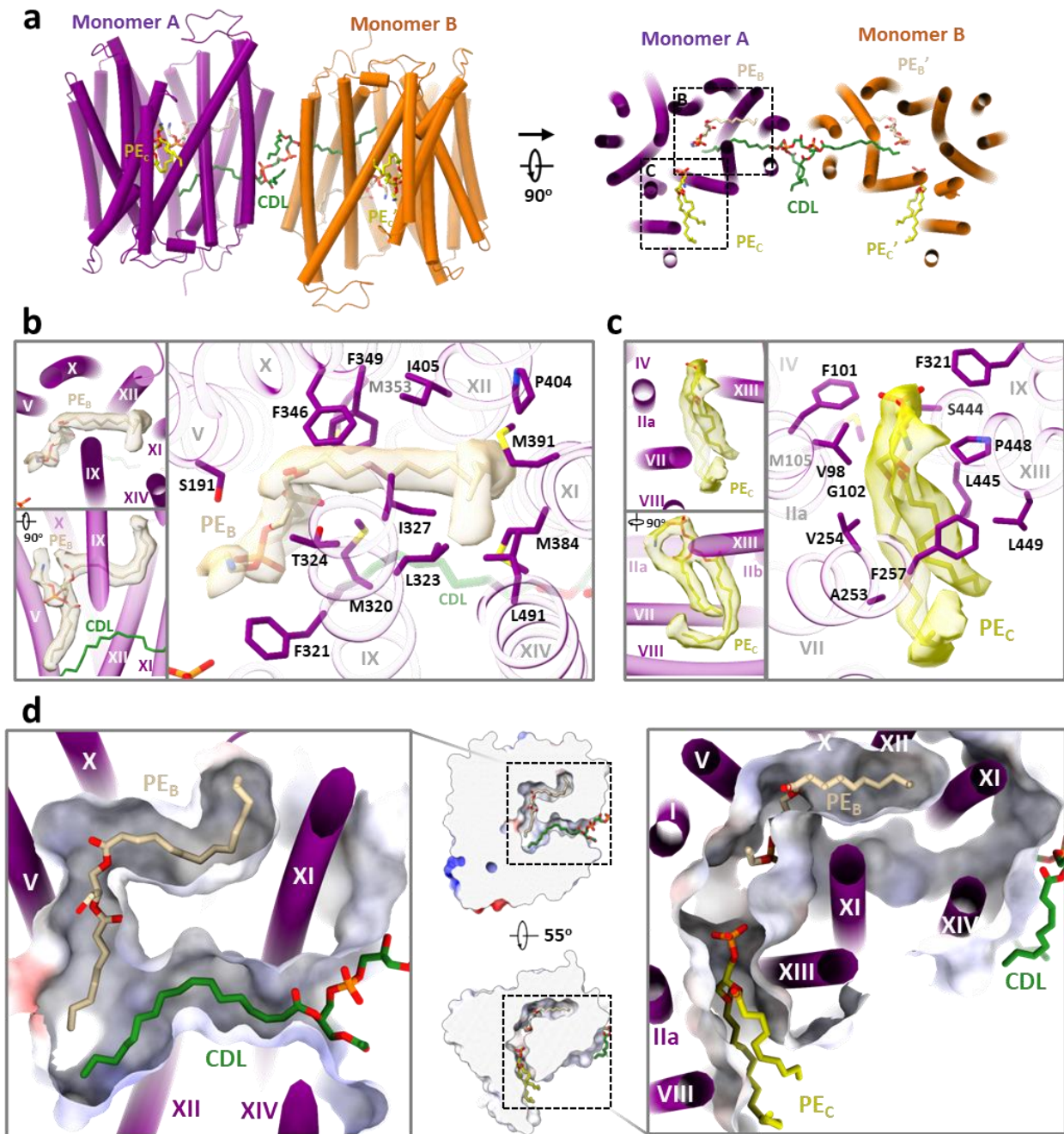
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37 **Supplementary Fig. 4 | Cryo-EM reconstruction of monomeric MsEfpA.** **a**, Representative cryo-EM
38 micrograph of MsEfpA. **b**, 2D class averages of MsEfpA with a box size of 180.4 Å. **c**, Flowchart for
39 processing cryo-EM data of MsEfpA. **d**, Particle angle distribution for the final map of monomeric MsEfpA.
40 **e**, Gold standard Fourier shell correlation (FSC) curve for the final reconstruction. **f**, Close-up view of all
41 helices (olive) fitted in densities with contour level at 5σ (blue) and a molecular of PE (brown) fitted into
42 the density at lipid-binding site B. **g**, Superimposing the model of MsEfpA (olive) to MtEfpA (purple).

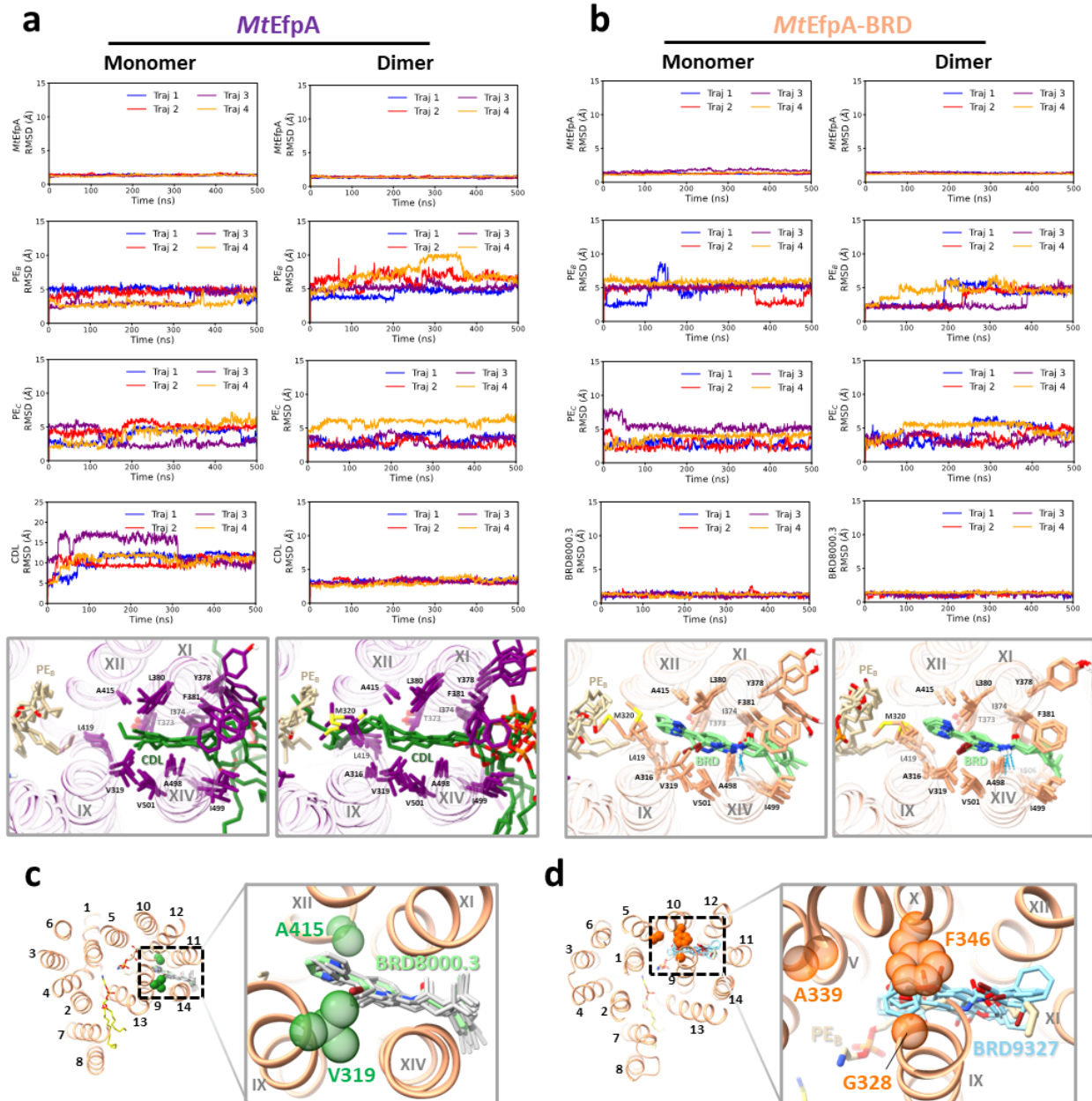
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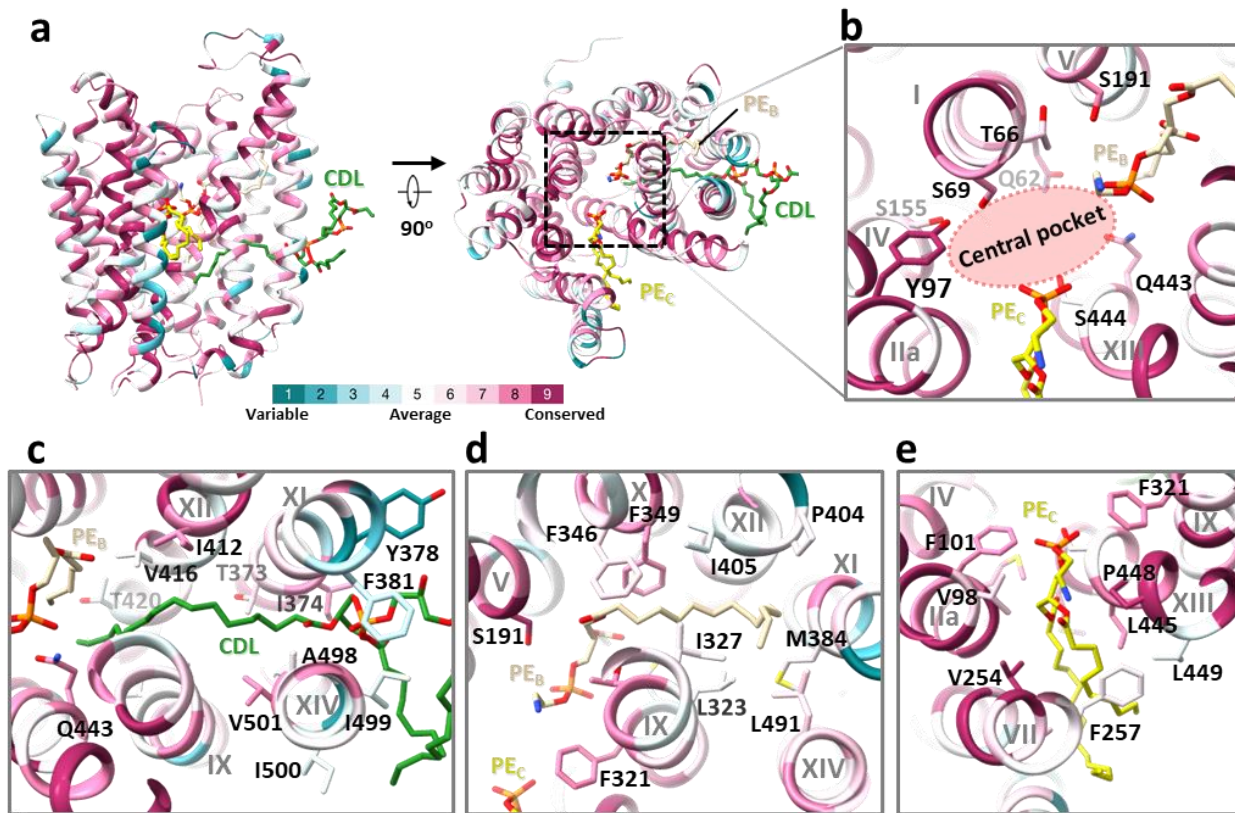
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46 **Supplementary Fig. 5 | Lipids binding channel in *MtEfpA*.** **a**, The cartoon model of *MtEfpA* (purple and
 47 orange) binding with cardiolipin (green) and two PE (wheat and yellow) from side view (left) and top view
 48 (right). **b**, Superimposition of phosphatidylethanolamine (PE_B) and the density at site B at a contour level
 49 of 6σ, presented in top view (left upper), side view (left bottom), and the *MtEfpA* residues interacting with
 50 PE_B within 4 Å (right). **c**, The density at site C (6σ) fitted with the model of phosphatidylethanolamine (PE_C)
 51 showed in top view (left upper), side view (left bottom), and the residues of *MtEfpA* interacting with PE_C
 52 within 4 Å (right). **d**, Cross-sectional views of lipids binding cavities in *MtEfpA*. Left, the cavities of
 53 cardiolipin (CDL, green) and phosphatidylethanolamine (PE_B, wheat). Right, the cavities of
 54 phosphatidylethanolamines (PE_B and PE_C).



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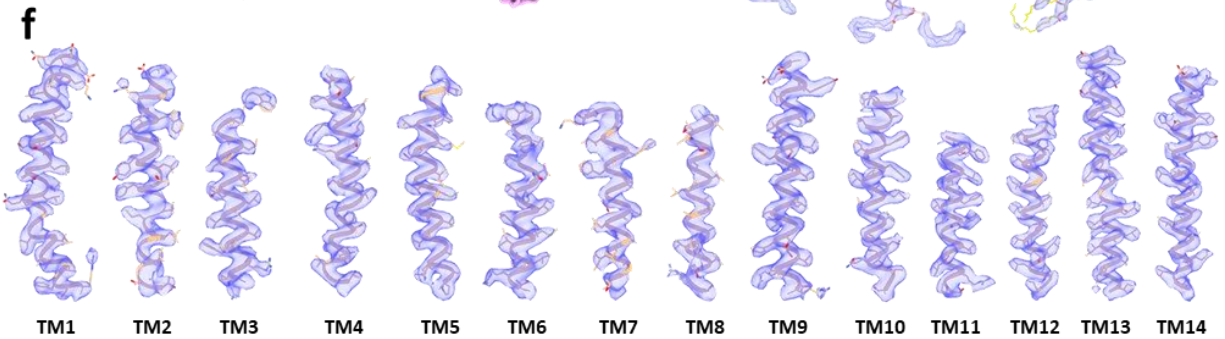
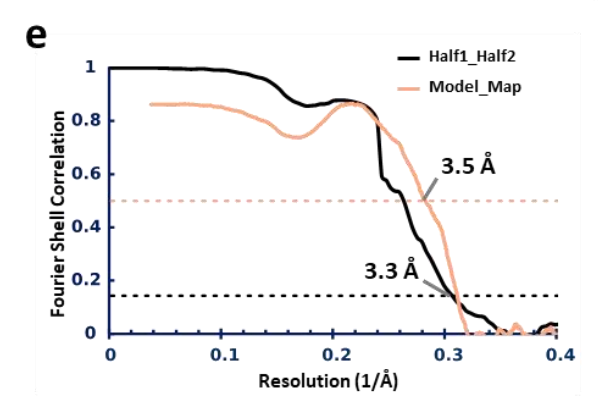
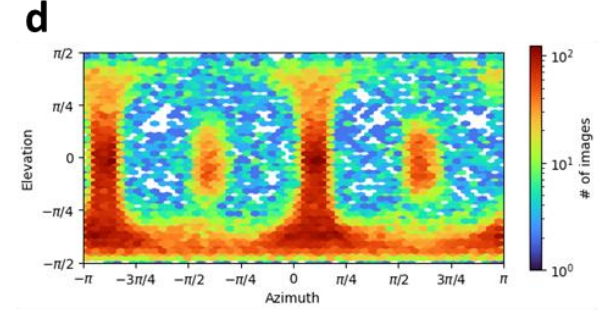
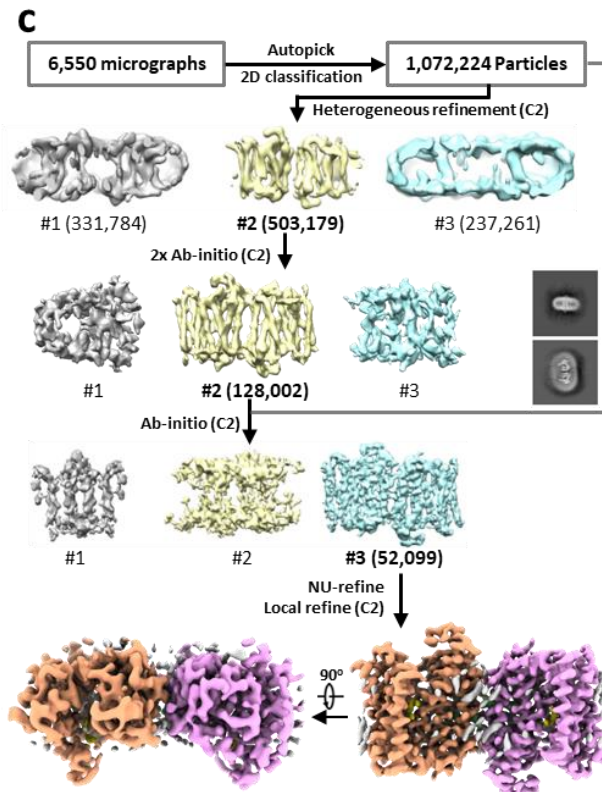
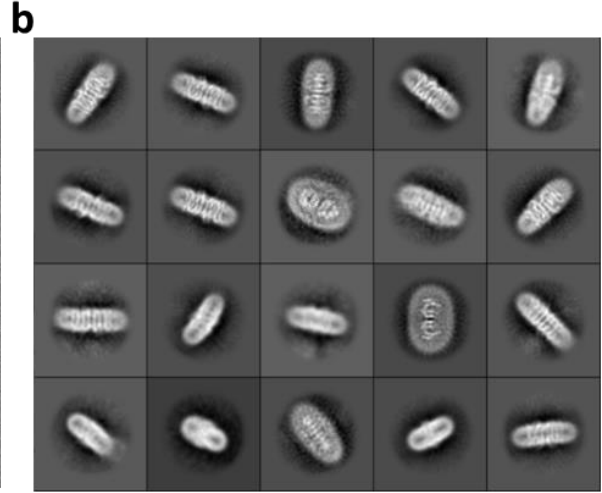
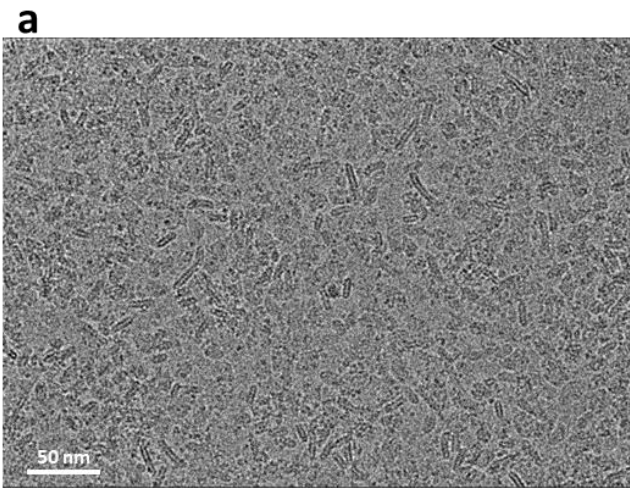
56 **Supplementary Fig. 6 | Molecular dynamics simulation analysis of ligands in *MtEfpA* and Docking of**
 57 **BRD-9327. a**, Molecular dynamics simulation analysis for *MtEfpA* in monomer or dimer and RMSD of three
 58 lipids. The bottom images present the interaction between *MtEfpA* and cardiolipin from the final frame
 59 of four trajectories. **b**, Molecular dynamics simulation analysis for *MtEfpA*-BRD8000.3 in monomer or
 60 dimer and RMSD of BRD-8000.3 and two lipids. The bottom images depict the interaction between *MtEfpA*
 61 and BRD8000.3 from the final frame of four trajectories. The hydrogen bond is represented by a blue
 62 dashed line. **c**, The configuration of BRD-8000.3 in one of the trajectories extracted from every 50 ns of
 63 simulation (gray) superimposed with the solved structure of *MtEfpA*-BRD and the location of resistant
 64 mutations to BRD-8000.3 (green). **d**, The location of resistant mutations (orange) to BRD-9327 (light blue)
 65 on *MtEfpA* and the top 5 docking results of BRD-9327 at lipid-binding site B.



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67 **Supplementary Fig. 7 | Conservation analysis of EfpA homologous in *Mycobacterium* at the hydrophilic**
 68 **central pocket and three lipid-binding sites. a,** Overall structure of *MtEfpA*-lipids colored by conservation
 69 of 61 EfpA homologues from different species of *Mycobacterium*. **b-e,** Zoomed-in view of residue
 70 conservation at the hydrophilic central pocket (**b**) and three lipid-binding sites (**c-e**).

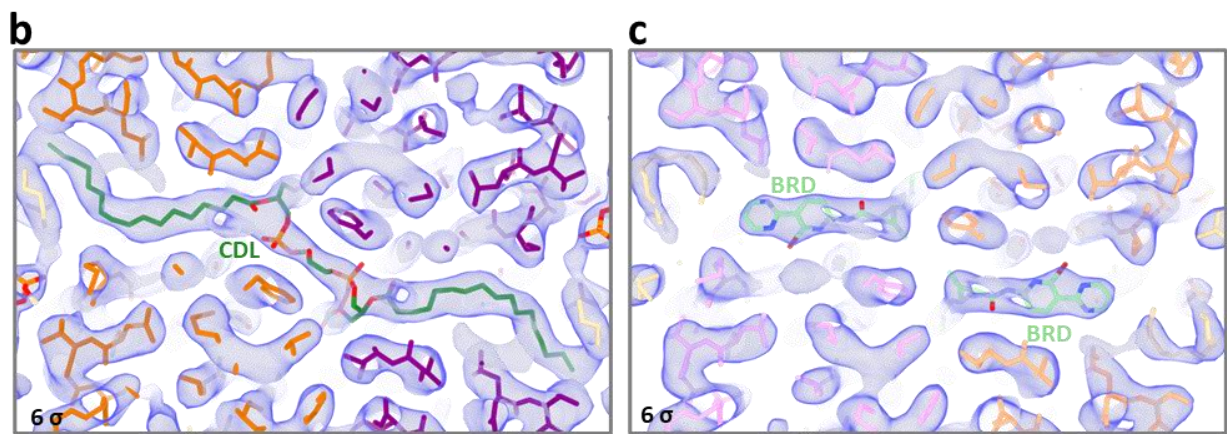
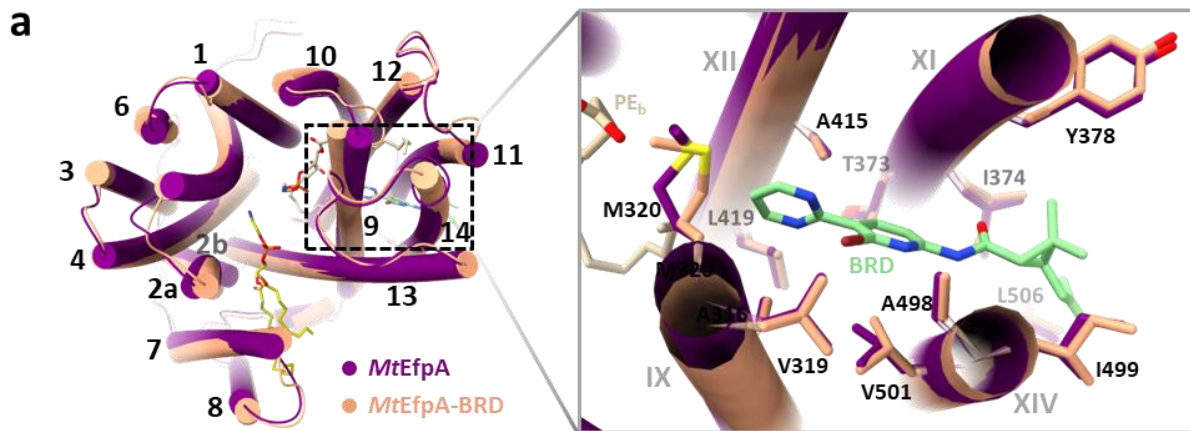
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73 **Supplementary Fig. 8 | Cryo-EM reconstruction of dimeric *MtEfpA*-BRD8000.3.** **a**, Representative cryo-
74 EM micrograph of *MtEfpA* complexed with BRD-8000.3. **b**, 2D class averages of *MtEfpA* with a box size of
75 252 Å. **c**, Flowchart for processing cryo-EM data of *MtEfpA*-BRD8000.3. **d**, Particle angle distribution for
76 the final map of dimeric *MtEfpA*-BRD8000.3. **e**, Gold standard Fourier shell correlation (FSC) curve for the
77 final reconstruction of *MtEfpA*-BRD8000.3. **f**, Close-up view of all structural elements of *MtEfpA*-
78 BRD8000.3 fitted in densities with contour level at 5σ (blue).

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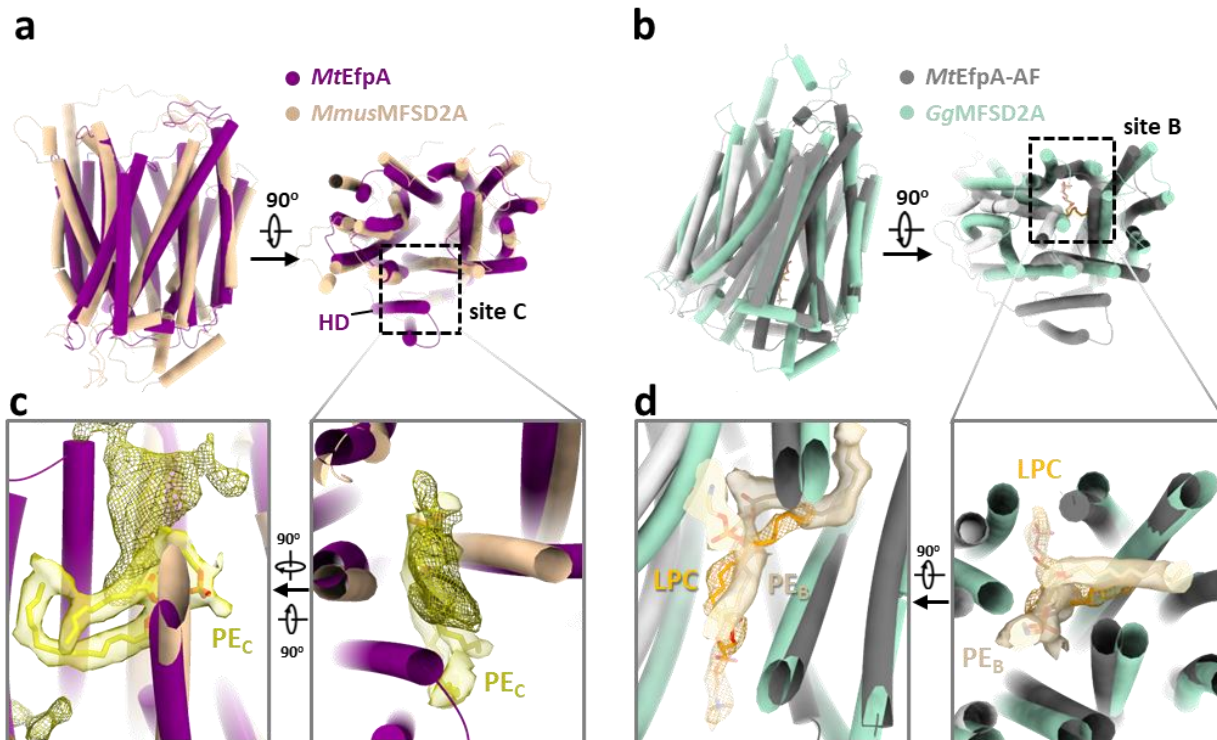
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82 **Supplementary Fig. 9 | Structural comparison of *MtEfpA* binding with cardiolipin and BRD-8000.3. a,**
 83 **Superimposing structure of *MtEfpA* (purple) and *MtEfpA*-BRD (orange) at binding site A. b, c, Density of**
 84 **cardiolipin (dark green) in *MtEfpA* (b) and BRD-8000.3 (light green) in *MtEfpA*-BRD (c) with a contour level**
 85 **at 6σ.**

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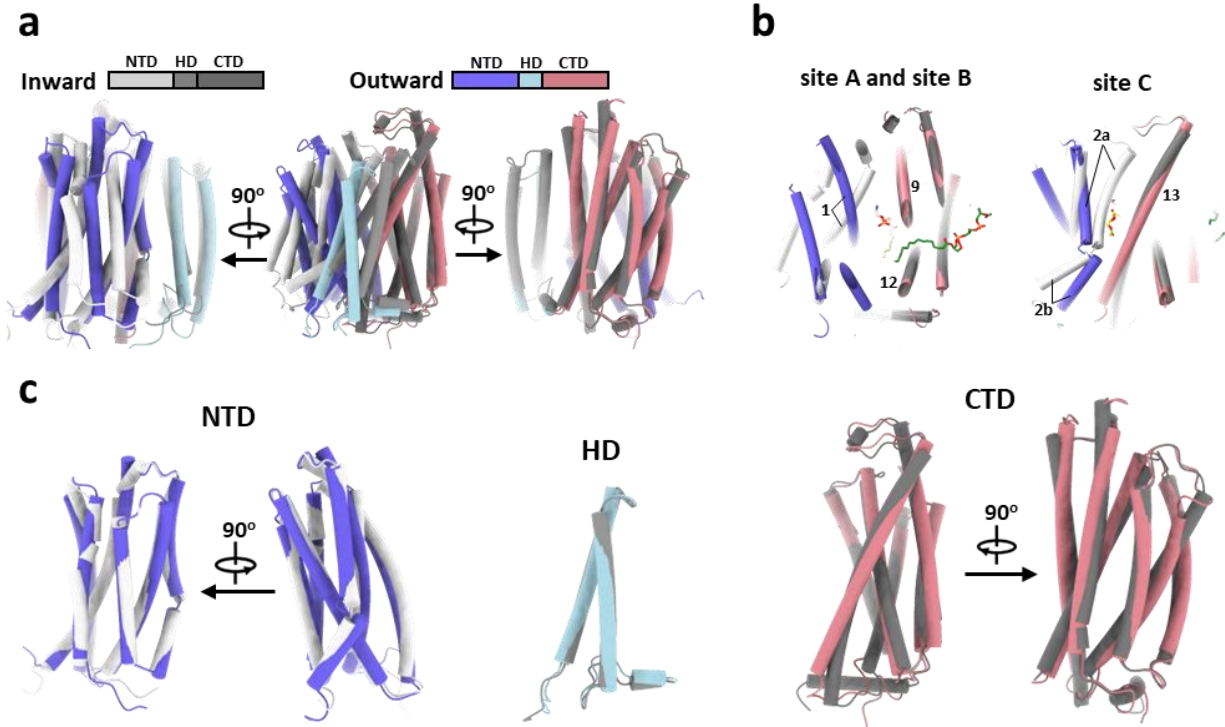


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88 **Supplementary Fig. 10 | Comparison of the structures of *MtEfpA* to the structures of MFSD2A.** **a,**
 89 Superimposing the outward-open structure of *MtEfpA* (purple) with the outward-open structure of
 90 lysophospholipid transporter MFSD2A from *Mus musculus* (*MmusMFSD2A*, light orange. PDB ID: 7N98).
 91 **b,** Superimposing the inward structure of *MtEfpA*-AF (gray) with the inward structure of MFSD2A from
 92 *Gallus gallus* (*GgMFSD2A*, blue-green. PDB ID: 7MJS). **c,** Zoomed-in view of lipid binding site C shows the
 93 lipids density in *MtEfpA* (yellow) and *MmusMFSD2A* (mesh, EMD-24252). **d,** Zoomed-in view of lipid
 94 binding site B shows the lipids density in *MtEfpA* (wheat) and the density of lysophosphatidylcholine (LPC,
 95 orange, EMD-23883) in *GgMFSD2A*.

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99 **Supplementary Fig. 11 | Inward and outward state of *MtEfpA*.** **a**, Superimposing AlphaFold-predicted
 100 inward-state of *MtEfpA* (*MtEfpA*-AF, lightgray-gray-darkgray) with the outward state of *MtEfpA* (blue-
 101 lightblue-lightpink). **b**, The lipid binding site A/B (left) and site C (right) after superimposing. **c**, Alignment
 102 of NTD (left), HD (mid) and CTD (right) from *MtEfpA* and *MtEfpA*-AF separately.

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	<i>MtEfpA</i> (EMD-37641) (PDB 8WM5)	<i>MtEfpA-BRD</i> (EMD-42204) (PDB 8UFD)	<i>MsEfpA</i> (EMD-42205) (PDB 8UFE)	
Data collection and processing				
Magnification	45,000	29,000	36,000	130,000
Voltage (kV)	200	300	200	300
Electron exposure (e-/ Å ²)	54	49	44	70
Defocus range (µm)	1.2-2.5	1.2-2.5	1.2-2.5	1.2-2.2
Pixel size (Å)	0.87	0.788	1.1	0.6632
Final Micrographs processed (no.)	3,031	6,550	3,280	3,554
Initial particle images (no.)	2,424,800	2,014,853	6,546,828	3,142,841
Final particle images (no.)	49,783	52,099	52,150	
Symmetry imposed	C2	C2	C1	
Map resolution (Å)	3.12	3.26	3.68	
FSC threshold	0.143	0.143	0.143	
Refinement				
Model resolution (Å)	3.2	3.5	4.1	
FSC threshold	0.5	0.5	0.5	
Model composition				
Non-hydrogen atoms	7179	7164	3449	
Protein residues	950	950	470	
Ligands	5	6	1	
<i>B</i> factors (Å ²)				
Protein	55.14	101.10	114.57	
Ligand	55.79	102.03	105.33	
R.m.s. deviations				
Bond lengths (Å)	0.009	0.030	0.003	
Bond angles (°)	0.924	1.462	0.640	
Validation				
MolProbity score	1.88	1.90	2.19	
Clashscore	12.86	18.45	12.83	
Poor rotamers (%)	0.00			
Ramachandran plot				
Favored (%)	96.19	97.25	94.44	
Allowed (%)	3.81	2.75	5.34	
Disallowed (%)	0.00	0.00	0.21	