

Supplementary Information

for

Cryo-EM structures of human ABCB7 reveal the molecular basis of mitochondrial matrix heme export

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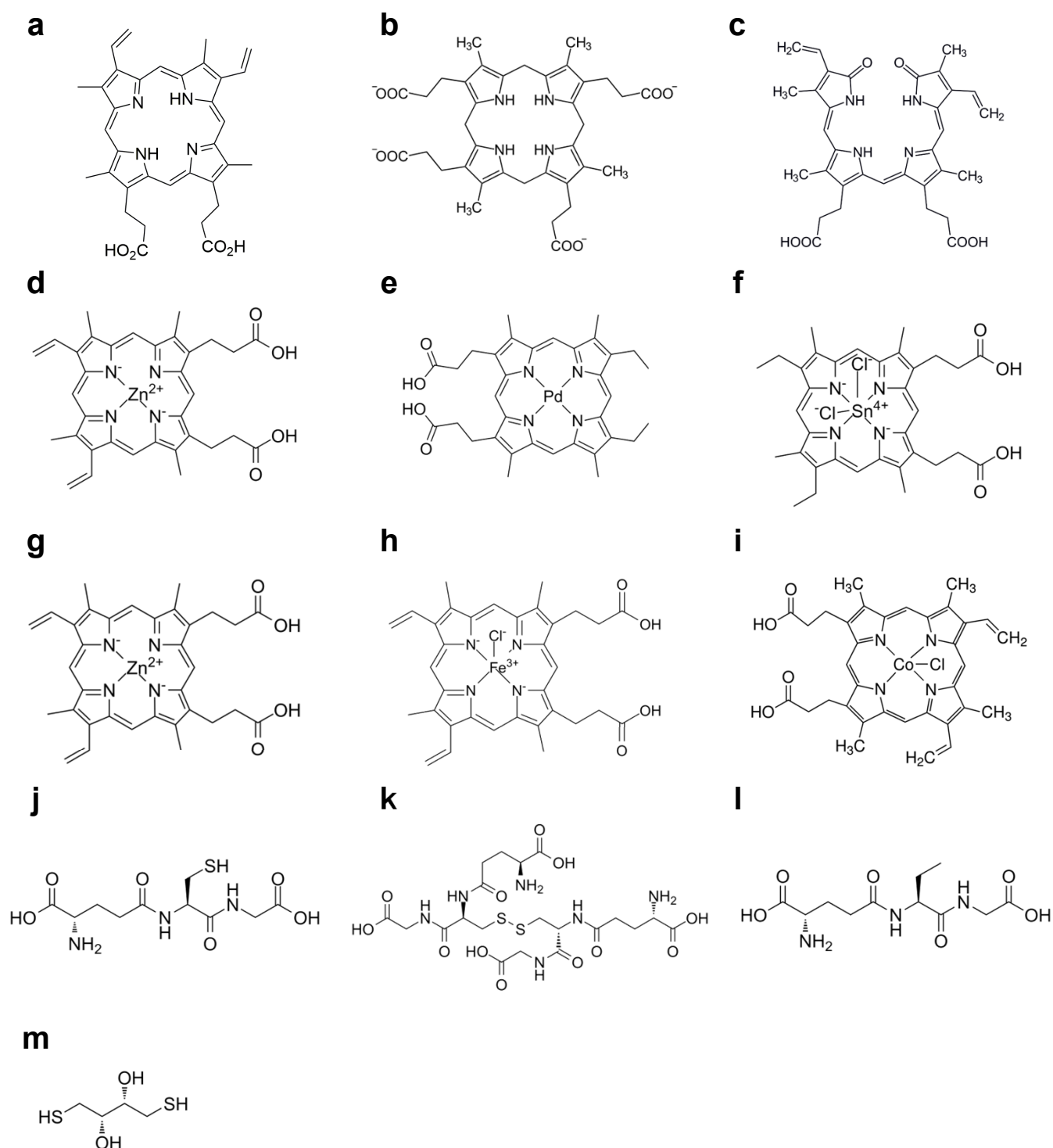
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hABCB7			
hABCB6	-----MVTVGNYCEAEGPVGPAWMQDGLSPCFFFT		30
hABCB10			
hABCB7	-----		
hABCB6	LVPSTRMALGTLALVLALPCRRRERPAGADSLSWGAGPRISPYVLQLLLATLQAALPLAG		90
hABCB10	-----		
hABCB7	-----MALLAMHSWRWAAAAAFEKRRHSAIL		27
hABCB6	LAGRVGTARGAPLPSYLLLASVLES LAGACGLWLLVVERS QARQRLAMGIWIKFRHSPGL		150
hABCB10	-----MRGPPAWPLRLLEPPSPAEPGRLLPVACVWAAASRVPGSLSPFTGLRPARLWGA		54
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hABCB7	IRPLVSVSGSGPQWRPHQLGALGTARAYQIPESLKSITWQRLGKGNSGQFLDAAKALQVW		87
hABCB6	LLLWTVAF AENLALVSWNSPQWWWARADLGQQVQFSLWVLRVVS GGLFVLGLWAPGLR		210
hABCB10	GPALLWGVGAARRWRS GCRGGPGASRGVLGLARLLGLWARGPGSCRCGAFAGPGAPRLP		114
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hABCB7	PLIEKRTCWHG--HAGGGLHTDPKEGLKDVDTRKIIKAMLSYVWPKD--RPDLRARVAIS		143
hABCB6	PQSYTLQVHEEDQDVERSQVRSAAQQSTWRDFGRKLRLLSGYLWPRG--SPALQLVVLIC		268
hABCB10	RARFPGGPAAA WAGDEAWRRGPAAPP GDKGRLRPAAAGLPEAR KLLGLAYPERRRLAAA		174
	. . : : :		
	TM1		
hABCB7	LGFLGGAKAMNIVVPFMFKYAVDSL NQMSGNMLNLS DAPNTVATMATAVLIGYGVSRAGA		203
hABCB6	LGLMGLERALNVLVPIFYRNIVNLLTEKAP---WNSLAWTVTSYVFLKFLQGGGTG--ST		323
hABCB10	VGFLTMS SVISMSAPFFLGKIIDVIYTNPT-----VDYSDNLTRLCLGLSAVFLCG		225
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	TM2 ICH1		
hABCB7	AFFNEVRNAVFGKVAQNSIRRIAKNVFLHLHNLDLGFHL SRQTGALSKAIDRGTRGISFV		263
hABCB6	GFVSNLRTFLWIRVQQFTSRREVELLIFSHLHEL SLRWHLGRRTGEVLRIADRGTS SVTGL		383
hABCB10	AAANAI R VYLMQTS GQRIVNRLRTSLFSSILRQEVAFFDKTRTGELINRLSSD TALLGRS		285
	. . :* : * .*: :* : . : : : ** : . . .*		
	TM3 TM4		
hABCB7	LSALVFNLLPIMFEVMLVSGVLYYKCGAQFALVTLGTLGTYTAFTVAVTRWRTRFRIEMN		323
hABCB6	LSYLVFNVIPTLADIIIGIIYFSMFFNAWFG LIVFLCMSLYLTLTIVVTEWRTKFRRAMN		443
hABCB10	VTENLSDGLRAGA QASVGISMMFFVS-PNLATFVLSVVPVSIIAVIYGRYLRLTKVTQ		344
	: : : : : : . . . : : : : : : : :		
	ICH2 TM5		
hABCB7	KADNDAGNAAIDSL LNYETVKYFN NERYEAQRYDGFLKTYETASLKSTSTLAMLNFGQSA		383
hABCB6	TQENATRARA VDSL NFETVKY YNAESYEVERYREAI IKYQGLEWKSSASLVLLNQTQNL		503
hABCB10	DSL A QATQLAEERIGNVRTVRAFGKEMTEIEKYASKVDHVMQLARKEAFARAGFFGATGL		404
	: * : : * .** : . * * : :* : * . : : .		
	TM6		
hABCB7	IFSVGLTAIMVLASQGIVAGTLTVGDLVMVNGLLFQLSLPLNFLGTVYRETRQALIDMNT		443
hABCB6	VIGLGLLAGSLLCAYFVTEQKLQVG DYVLF GTYIIQLY MPLNWF GTYYRMIQTNFIDMEN		563
hABCB10	SGNLIVLSVLYKGGLLMGSAHMTVGELSSFLMYAFWVGISIGGLSSFYSELMKGLGAGGR		464
	. : : : . : : ** : . : : : : : * :		

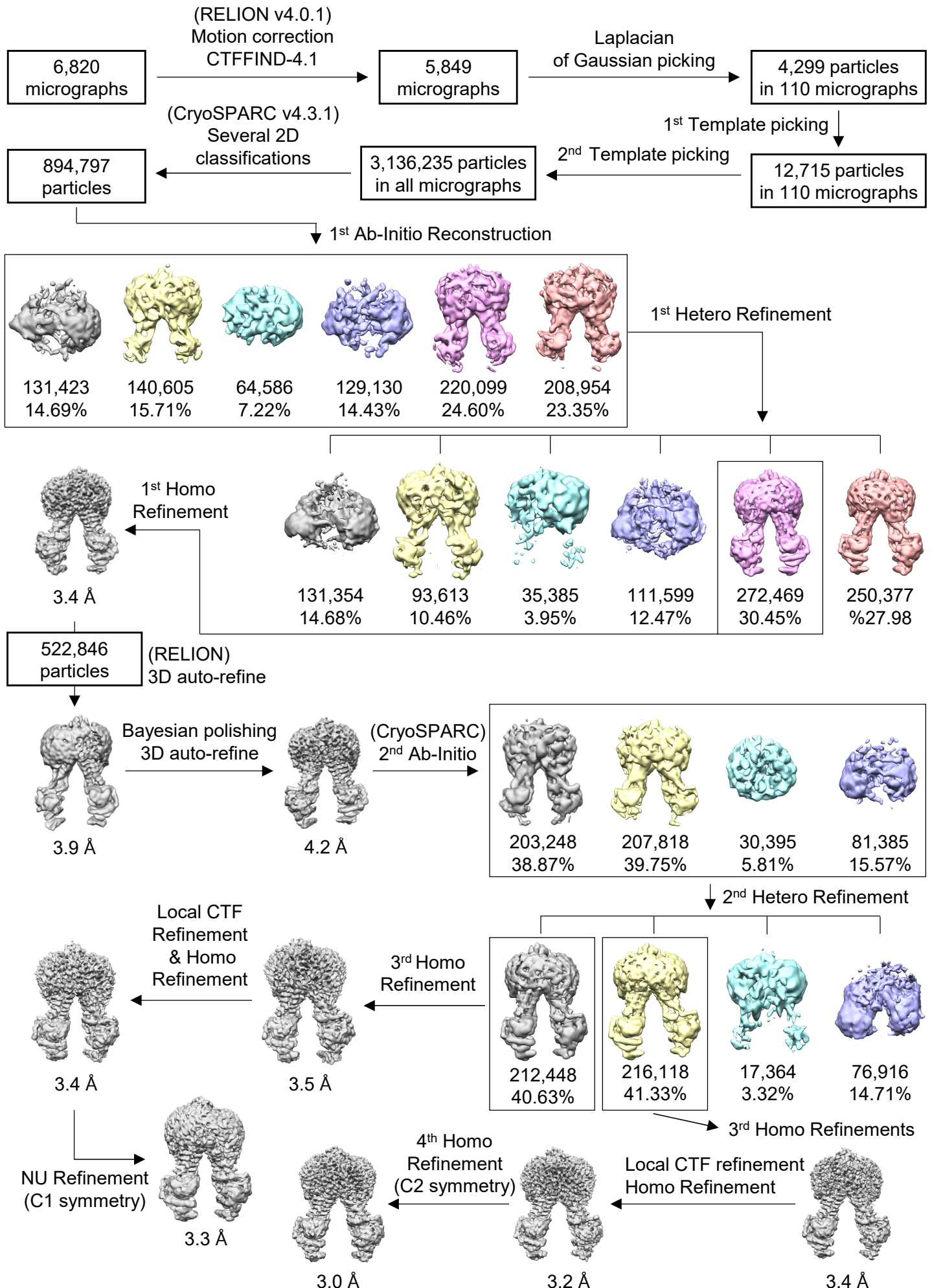
Supplementary Figure 1. Sequence alignment of human ABCB6, ABCB7 and ABCB10. The secondary structure (above) refers to human ABCB7. Conserved NBD motifs are indicated in yellow (Walker A), red (Signature motif), and green (Walker B).

			NBD	
hABCB7	LFTLLKVD	TQIKDKVMASPLQITPQTATVAFDNVHFEYIE--GQKVL	SGISFEVPAGKKV	501
hABCB6	MFDLLKEE	TEVKDLPAGAGPLRFQ--KGRIEFENVHESYAD--GRETLQ	DVSFTVMPGQTL	619
hABCB10	LWELLERE	PKLPFNEG	VILNEKS-FQGALEFKNVHFAYPARPEVP	523
	:. **:	:.: . . . : *.**** *	:...*: : .*.	
		Walker A		
hABCB7	AIV	GGSGSGKST	IVRLLFRFYEPQKGSIIYLAGQNIQDVSLES	561
hABCB6	ALV	GPSGAGKST	ILRLLFRFYDISSGCIRIDQDISQVTQASLRSHIGVVPQ	679
hABCB10	ALV	GPSGSGKST	TVLSLLLRLYPDASGTISLDGHDIRQLNPVWLR	583
	*** **:	*****: **:*:*: . * * : *:*: :. ** :*. *.*:.*: ** :		
			Signature motif	
hABCB7	IYYNLLYGN---	ISASPEEVYAVAKLAGLHDAILRMPHGYDTQVGERGLK	LSGGEKQORVA	618
hABCB6	IADNIRYGR---	VTAGNDEVEAAAQAAGIHDAIMAFPEGYRTQVGERGLK	LSGGEKQORVA	736
hABCB10	IAENIAYGADDP	SSVTAEIQRVAEVANAVAFIRNFPQGFNTVVGEKGV	LSGGQKQORIA	643
	* *:	** :. :*: .*: *. * :*. *: * *****: *****:*		
		Walker B		
hABCB7	IARAILKDPP	VILYDEATSS	LDSITEETILGAMKDVVKHRTSIFIAHRLSTVVD	678
hABCB6	IARTILKAPGI	ILLDEATSAL	DTSNERRAIQASLAKVCANRTTIVVAHRLSTVV	796
hABCB10	IARALLKNPKI	LLLDEATSAL	DAENEYLVQEALDRLMDGRTVLVIAHRLSTIK	703
	:.* *	:.* **:.* : : : ** :.:*****: :*: : *		
hABCB7	LDQGKVAERG	THHGLLANPHSIYSEM	WHTQSSRVQNHDPKWEAKKENISKEEERKKLQE	738
hABCB6	IKDGCIVERGR	HEALLS-RGGVYADMWQLQQGQEETS	EDTKPQTMER-----	842
hABCB10	LDQGKITEY	GKHEELLSKPNGIYRKL	MNKQSFISA-----	738
	:.:* :.* * *. **:	.:* : : *		
hABCB7	EIVNSVKGCG	NCSC	752	
hABCB6	-----			
hABCB10	-----			

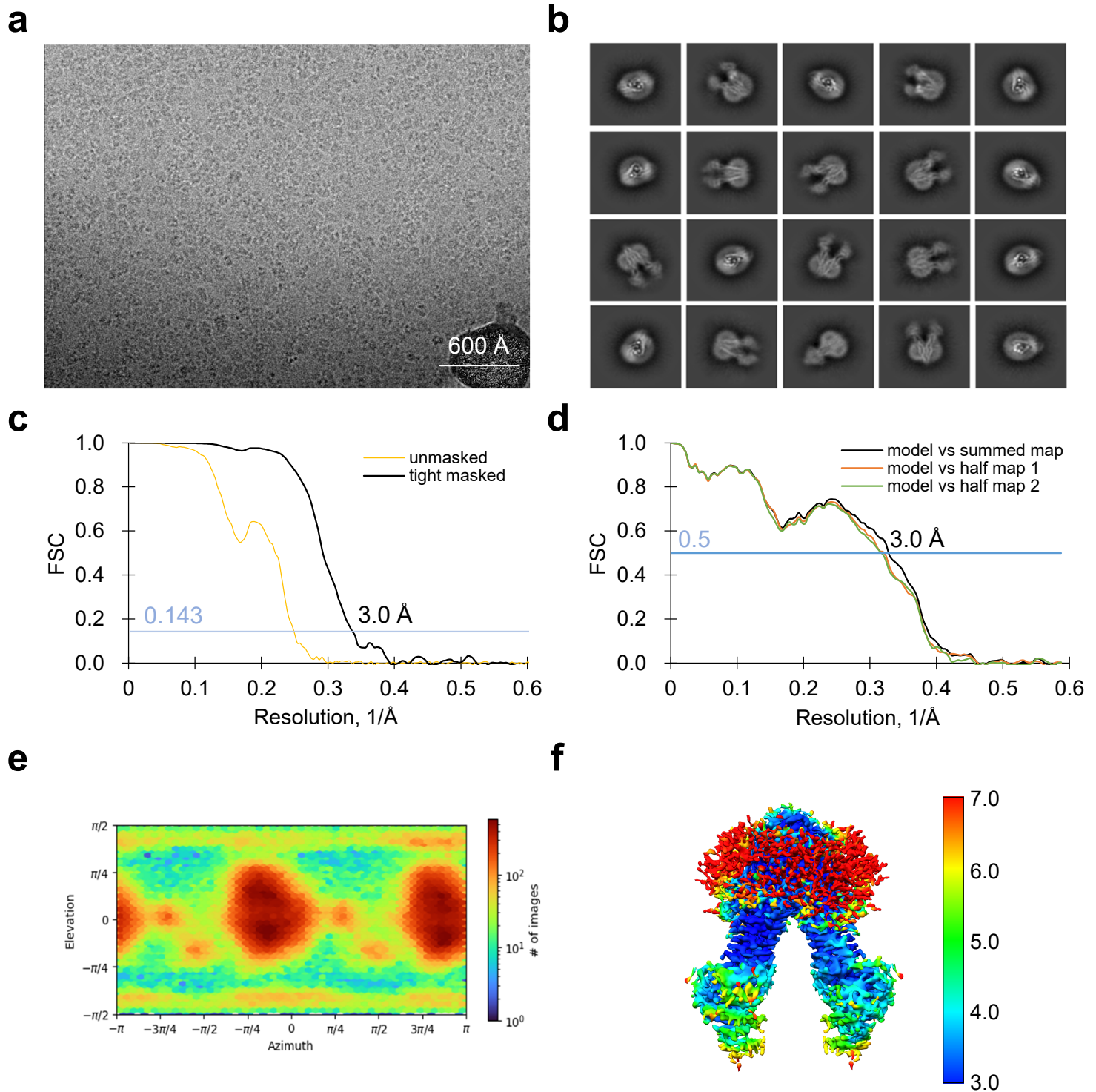
Supplementary Figure 1 (continued).



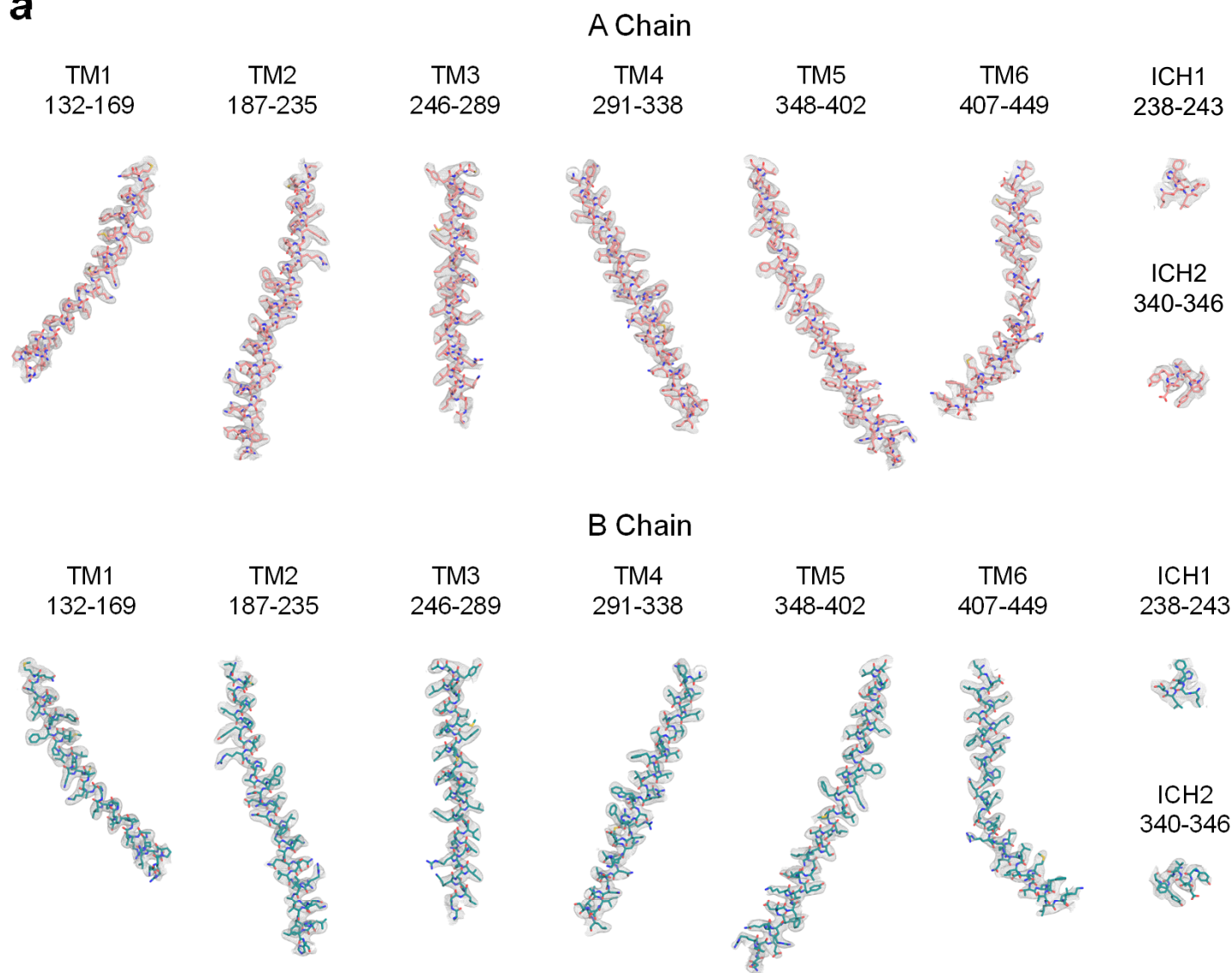
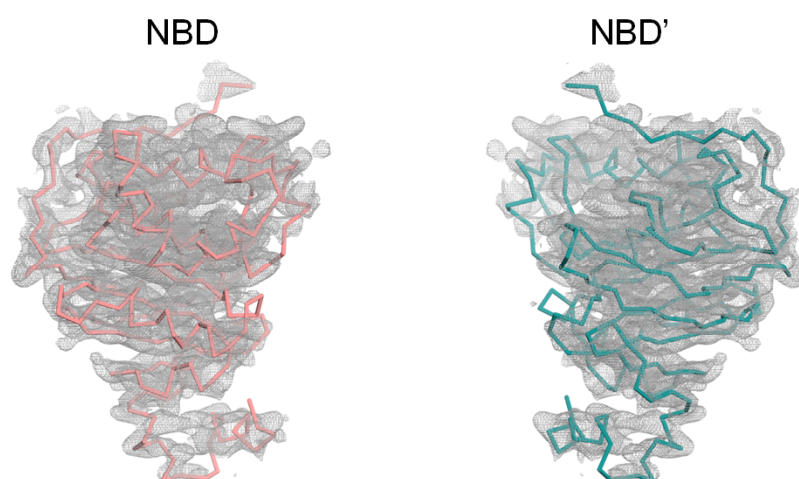
Supplementary Figure 2. Chemical structures of the potential ABCB7 substrates tested in this study. (a) PPIX (Protoporphyrin IX). (b) CPIII (Coproporphyrinogen III). (c) BV (Biliverdin). (d) ZnMP (Zinc(II) Mesoporphyrin IX). (e) PdMP (Pd(II) Mesoporphyrin IX). (f) SnMP (Tin Mesoporphyrin IX). (g) ZnPP (Zinc protoporphyrin). (h) Hemin (Iron (III) Protoporphyrin IX chloride). (i) CoPP (Cobalt (III) Protoporphyrin IX chloride). (j) GSH (Reduced Glutathione). (k) GSSG (Oxidised Glutathione). (l) OPT (Ophthalmic acid). (m) DTT (Dithiothreitol).



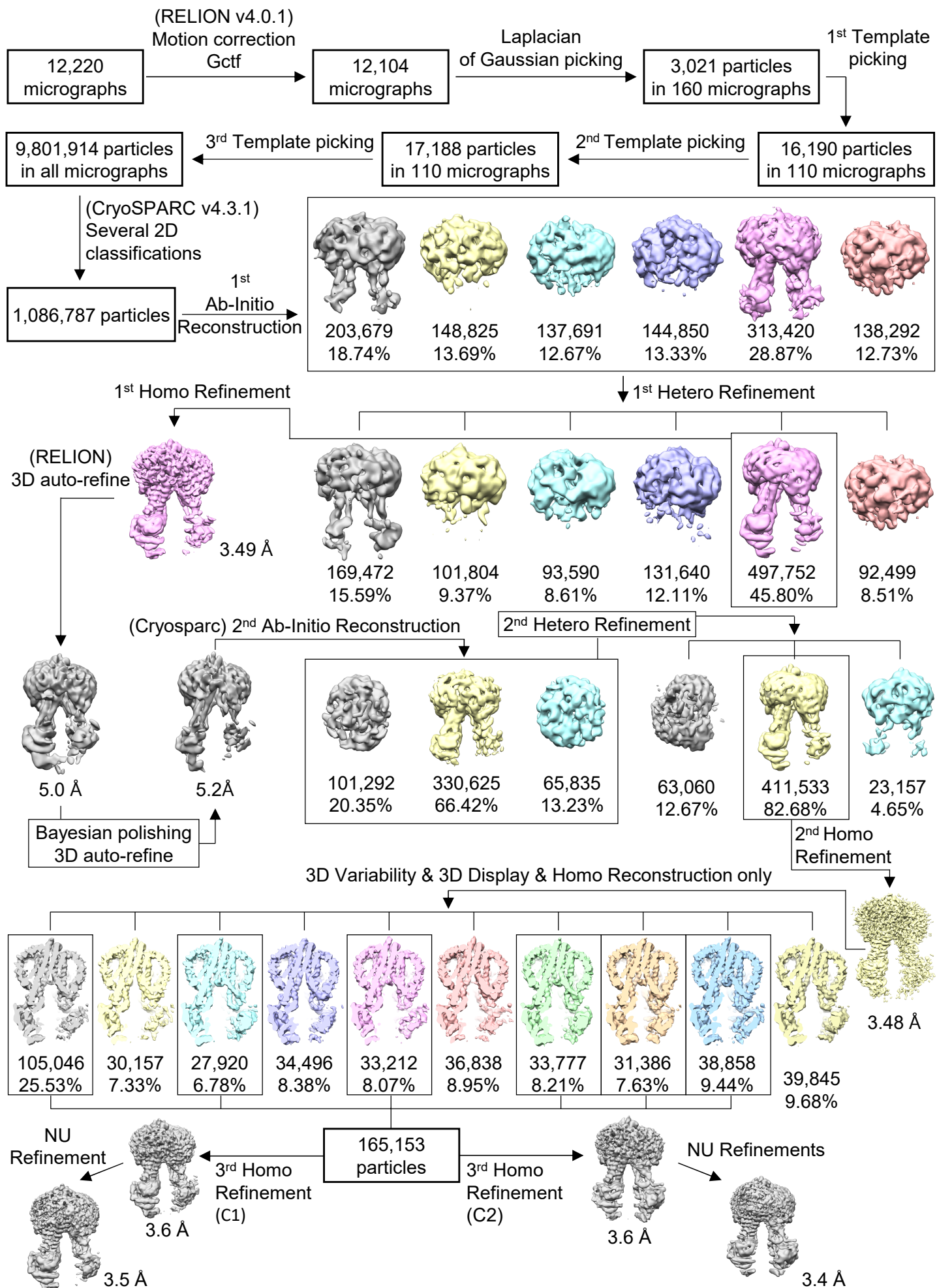
Supplementary Figure 3. Cryo-EM data-processing workflow for apo ABCB7. The main steps of cryo-EM data processing are shown.



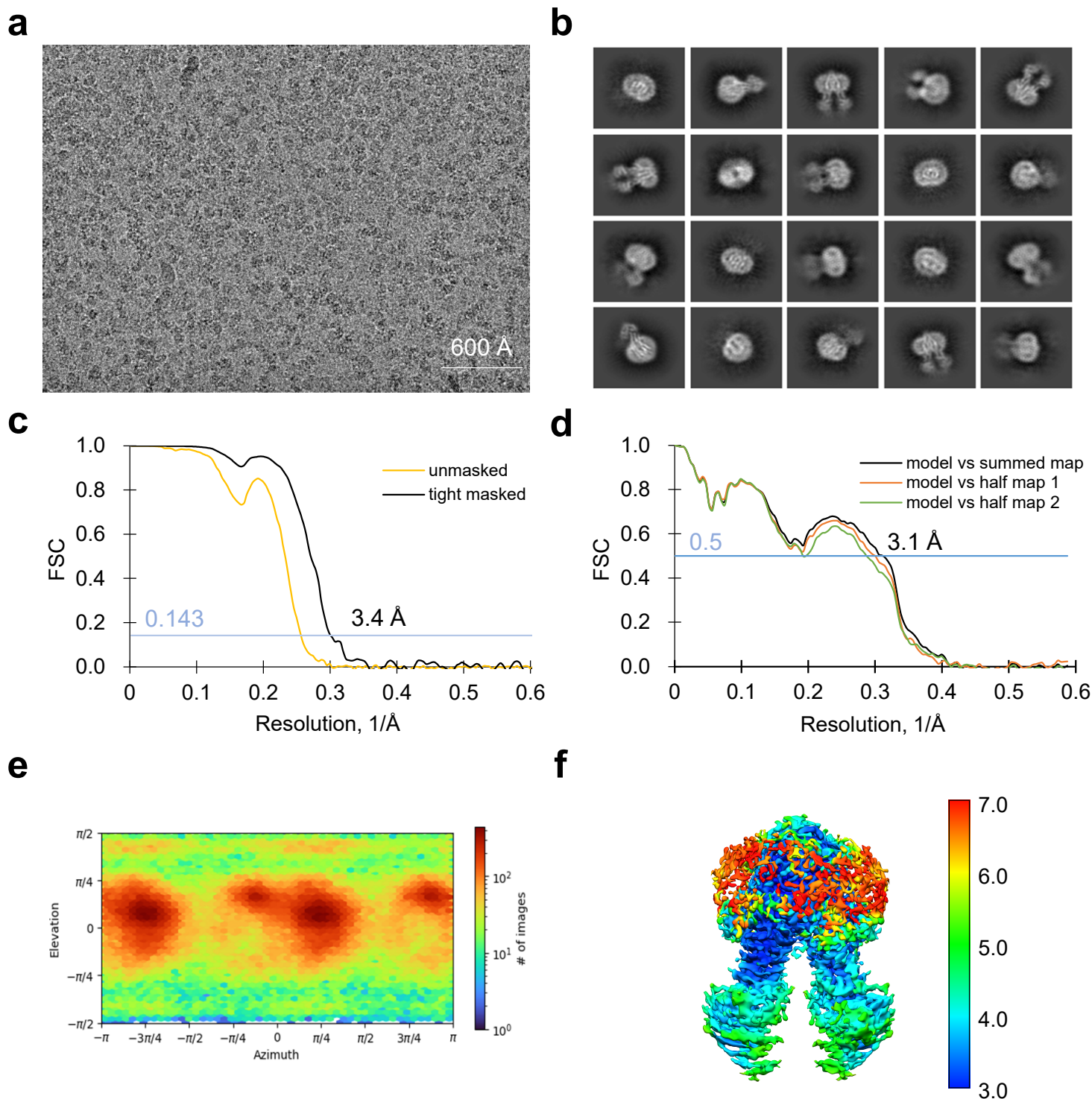
Supplementary Figure 4. Cryo-EM data and model quality analysis of apo ABCB7. (a) Representative micrograph. (b) Selected 2D class averages. (c) Gold-standard Fourier Shell correlation (FSC) curve. The resolution (blue line) was estimated based on an FSC cut-off of 0.143. (d) FSC curves calculated between the refined structure and the half map used for refinement (orange), the other half map (green) and the summed map (black). (e) Particle distribution plot for the final 3D reconstruction. (f) Density map colored by local resolution estimation from 3 Å (blue) to 7 Å (red).

a**b**

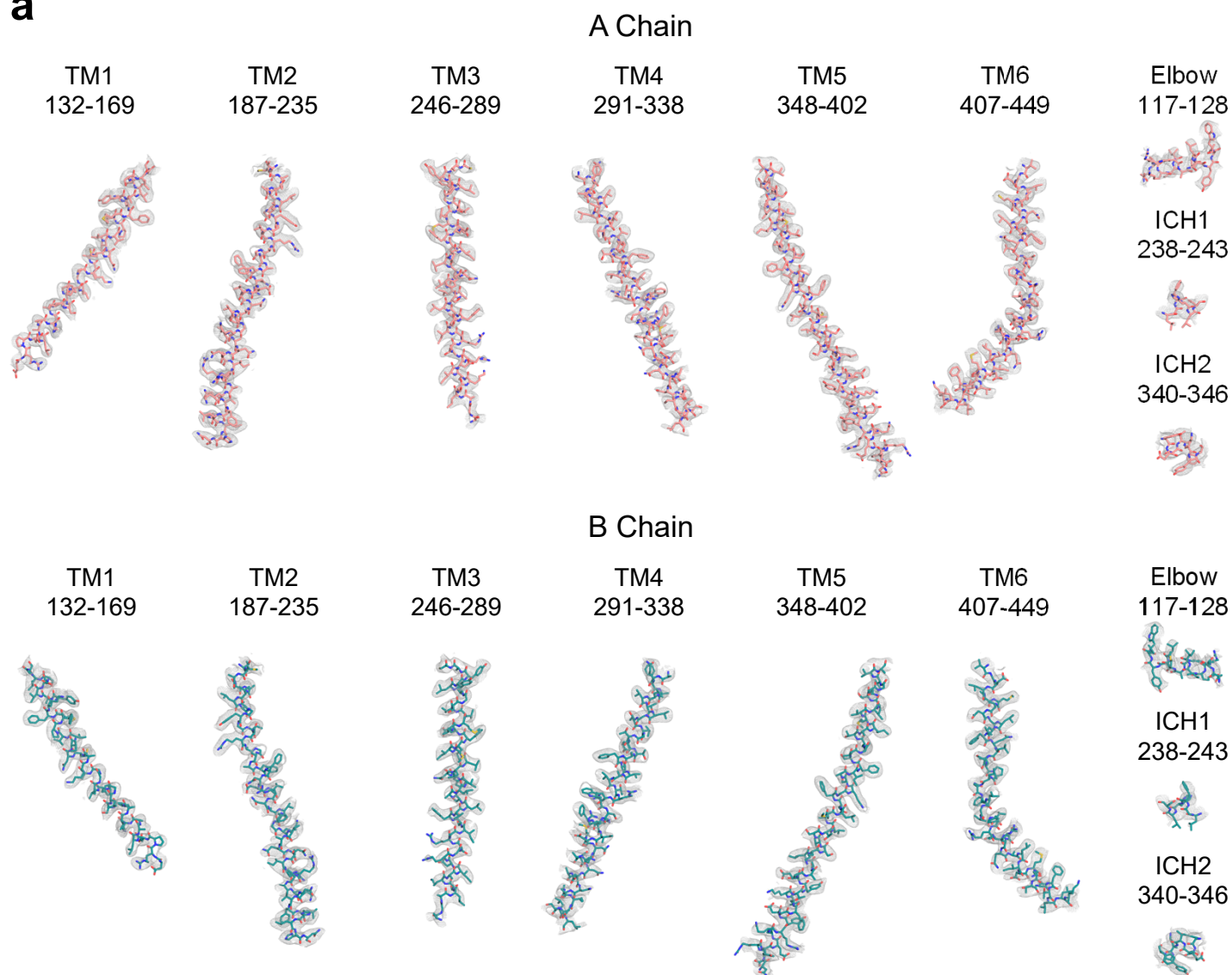
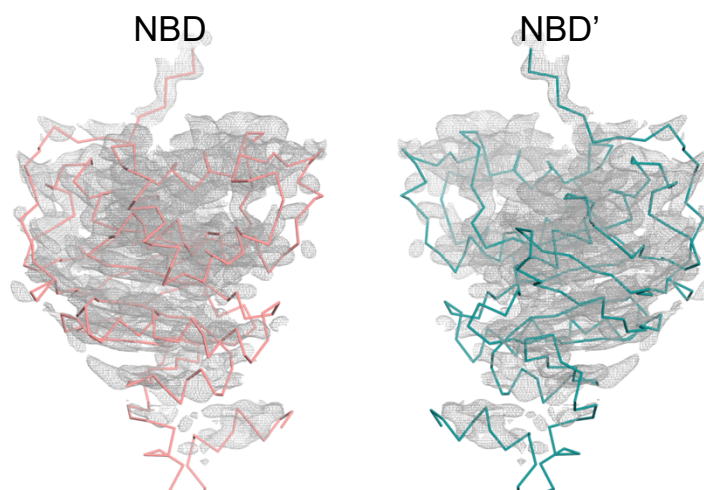
Supplementary Figure 5. Cryo-EM density maps of apo ABCB7. (a) The amino acid residues of each TM helix are shown. The EM density (grey mesh) is contoured at the 4 σ level. (b) The cryo-EM density of the NBD region.



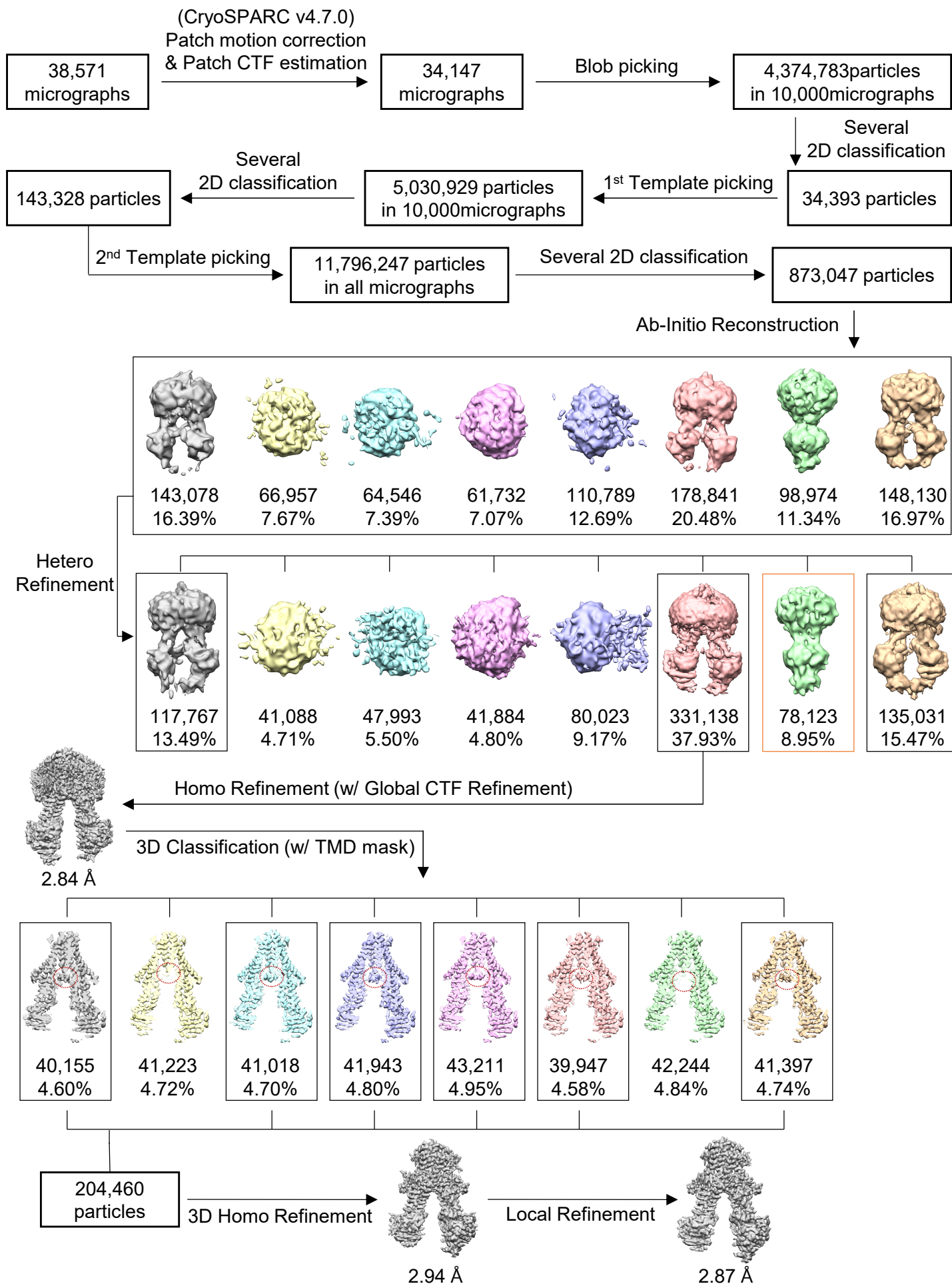
Supplementary Figure 6. The cryo-EM data-processing workflow for CoPP:GSH-bound ABCB7. The main steps of the cryo-EM data processing pipeline for the substrate-bound sample are shown.



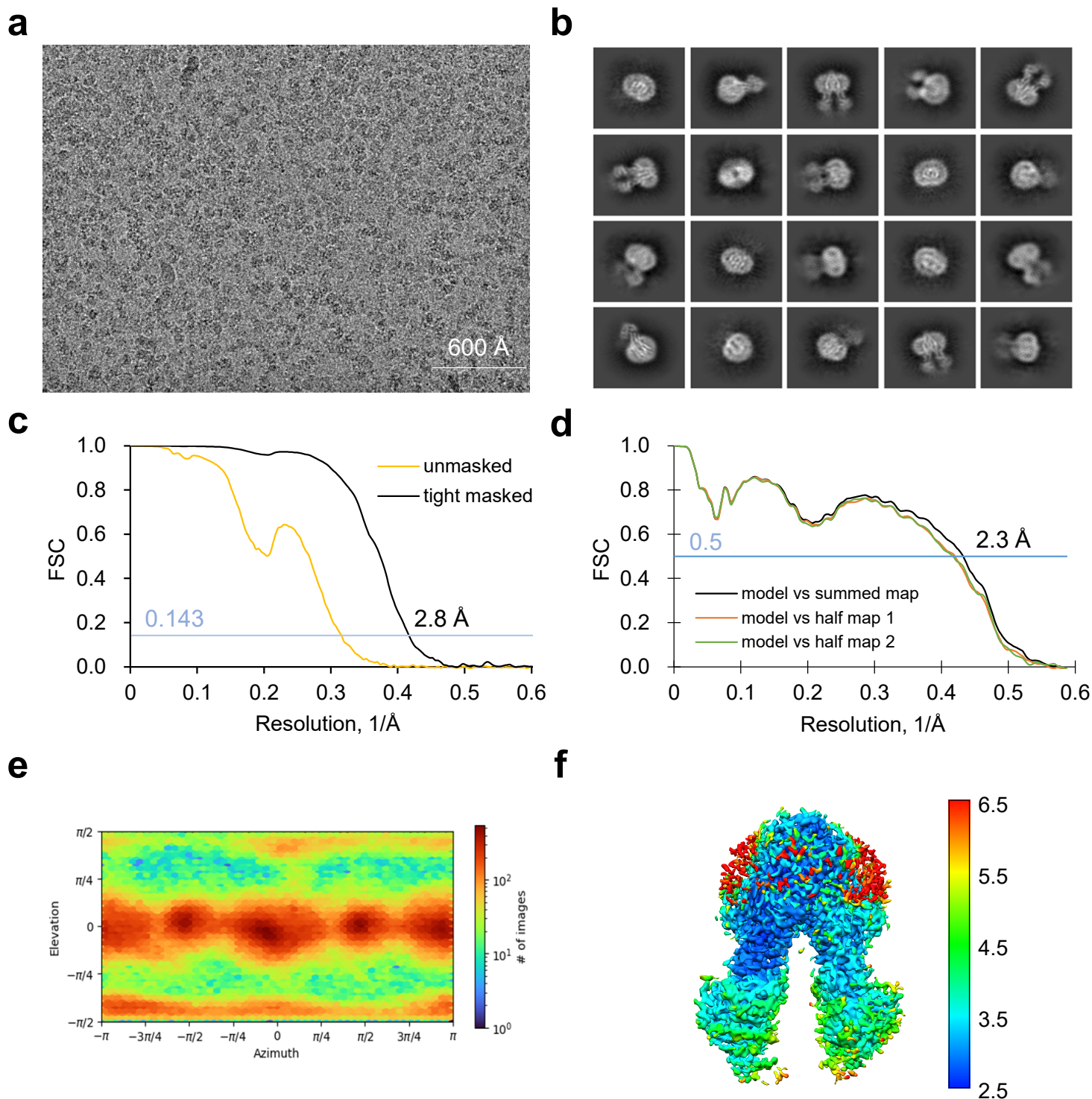
Supplementary Figure 7. Cryo-EM data and model quality analysis of CoPP:GSH-bound ABCB7. (a) Representative micrograph. (b) Selected 2D class averages. (c) Gold-standard Fourier Shell correlation (FSC) curve. The resolution estimation (blue line) was calculated based on an FSC cut-off of 0.143. (d) FSC curves calculated between the refined structure and the half map used for refinement (orange), the other half map (green) and the summed map (black). (e) Particle distribution plot for the final 3D reconstruction. (f) Density map colored by local resolution estimation from 3 Å (blue) to 7 Å (red).

a**b**

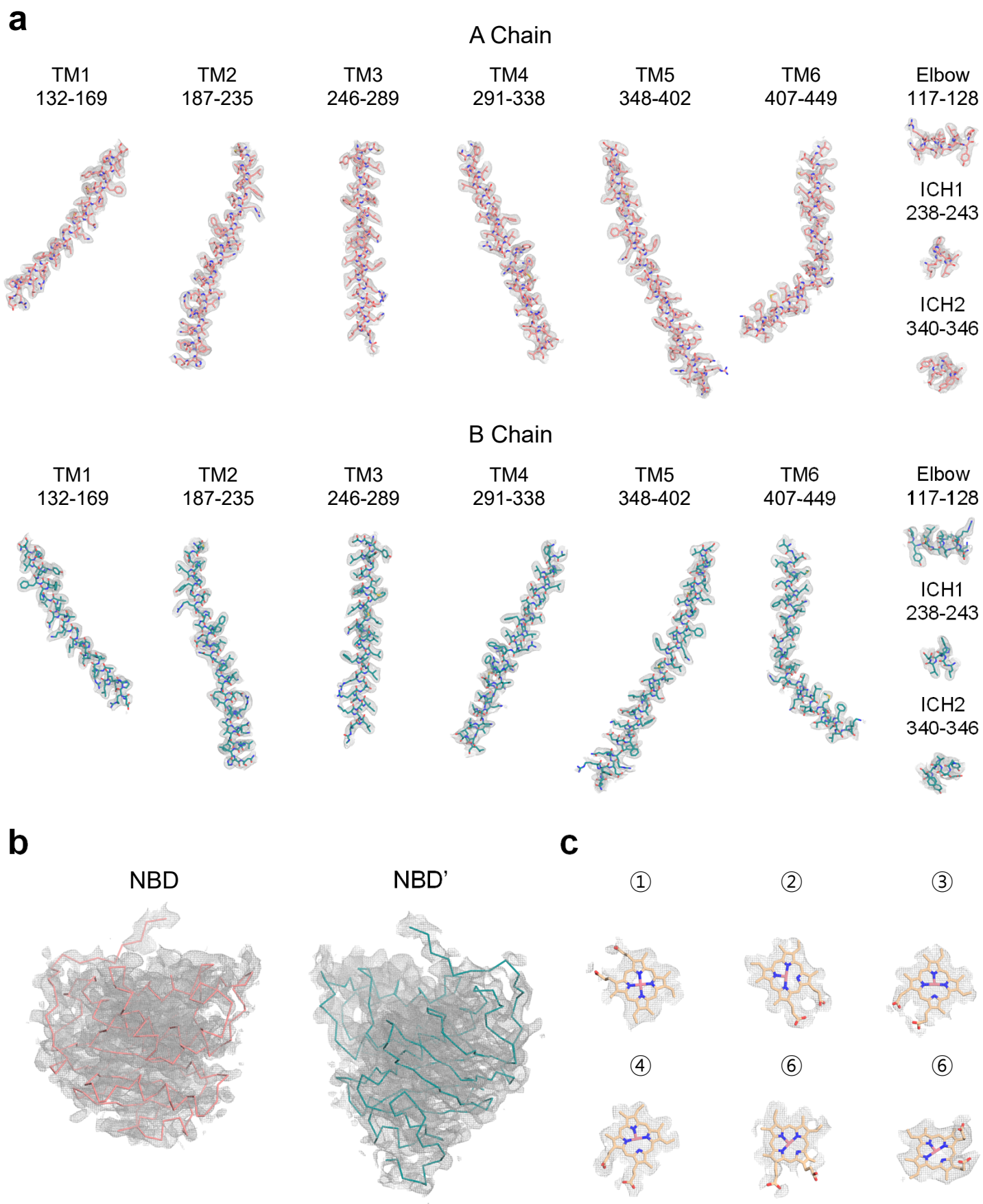
Supplementary Figure 8. Cryo-EM density maps of CoPP:GSH-bound ABCB7. (a) The amino acid residues of each TM helix are shown. The EM density (grey mesh) is contoured at 4 σ level. (b) The cryo-EM density of the NBD region.



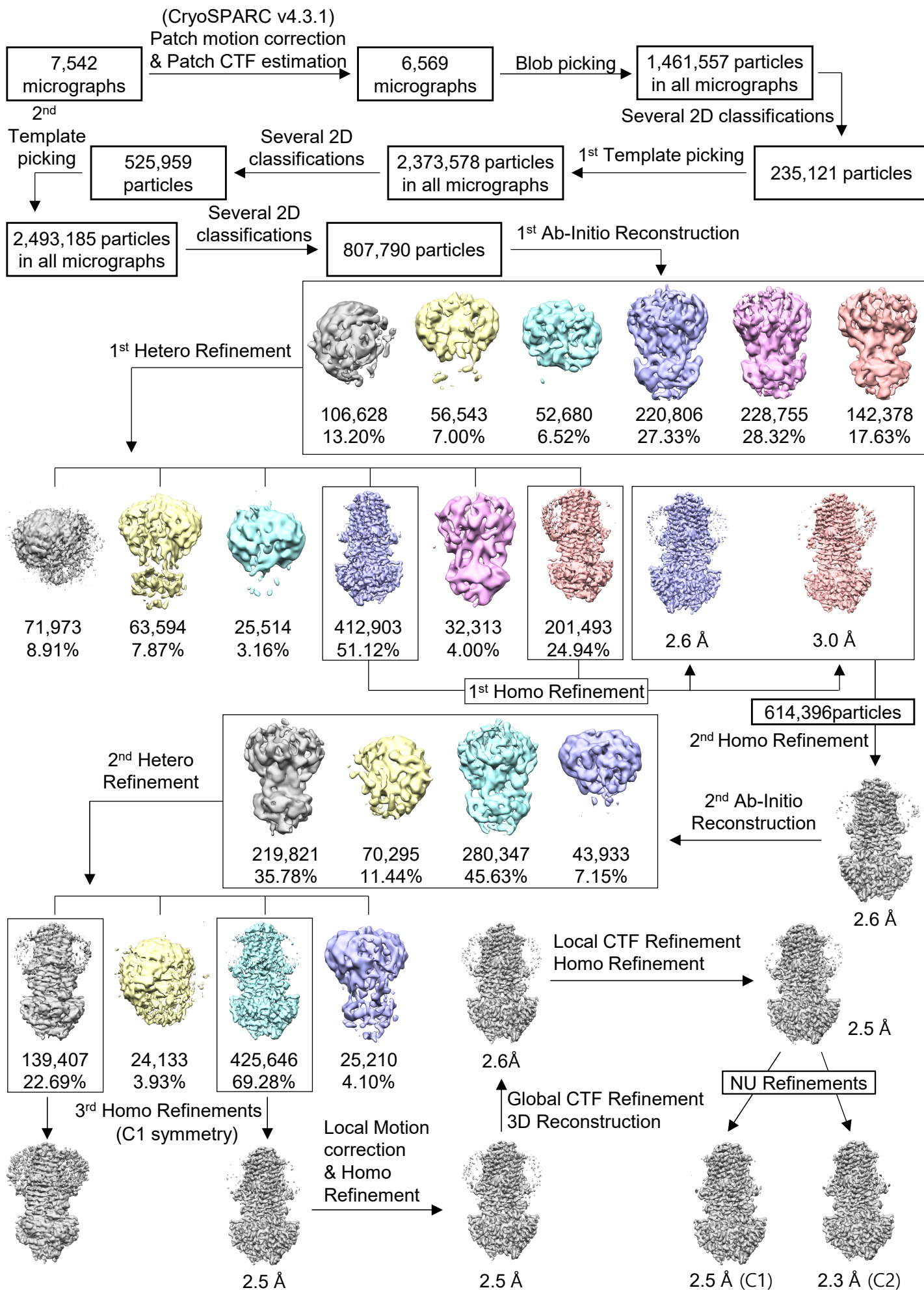
Supplementary Figure 9. Cryo-EM data-processing workflow for CoPP:GSH/ADP·VO₄-bound ABCB7. The main steps of the cryo-EM data processing pipeline for the CoPP:GSH/ADP·VO₄-bound sample are shown. The 3D class corresponding to the inward-facing and occluded state is highlighted by black and red boxes, respectively.



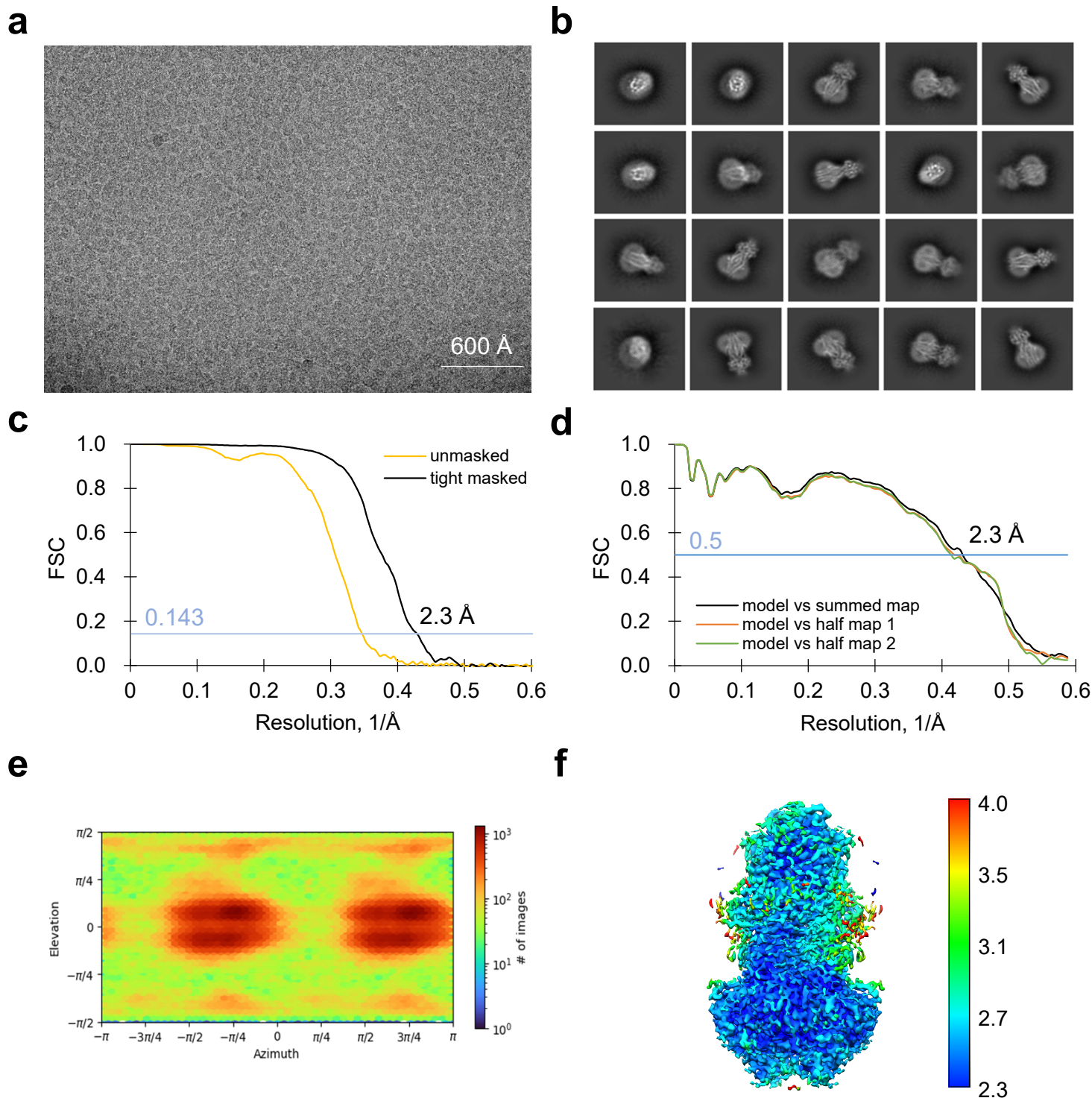
Supplementary Figure 10. Cryo-EM data and model quality analysis of CoPP:GSH/ADP·VO₄-bound ABCB7. (a) Representative micrograph. (b) Selected 2D class averages. (c) Gold-standard Fourier Shell correlation (FSC) curve. The resolution (blue line) was estimated based on an FSC cut-off of 0.143. (d) FSC curves calculated between the refined structure and the half map used for refinement (orange), the other half map (green) and the summed map (black). (e) Particle distribution plot for the final 3D reconstruction. (f) Density map colored by local resolution estimation from 2.5 Å (blue) to 6.5 Å (red).



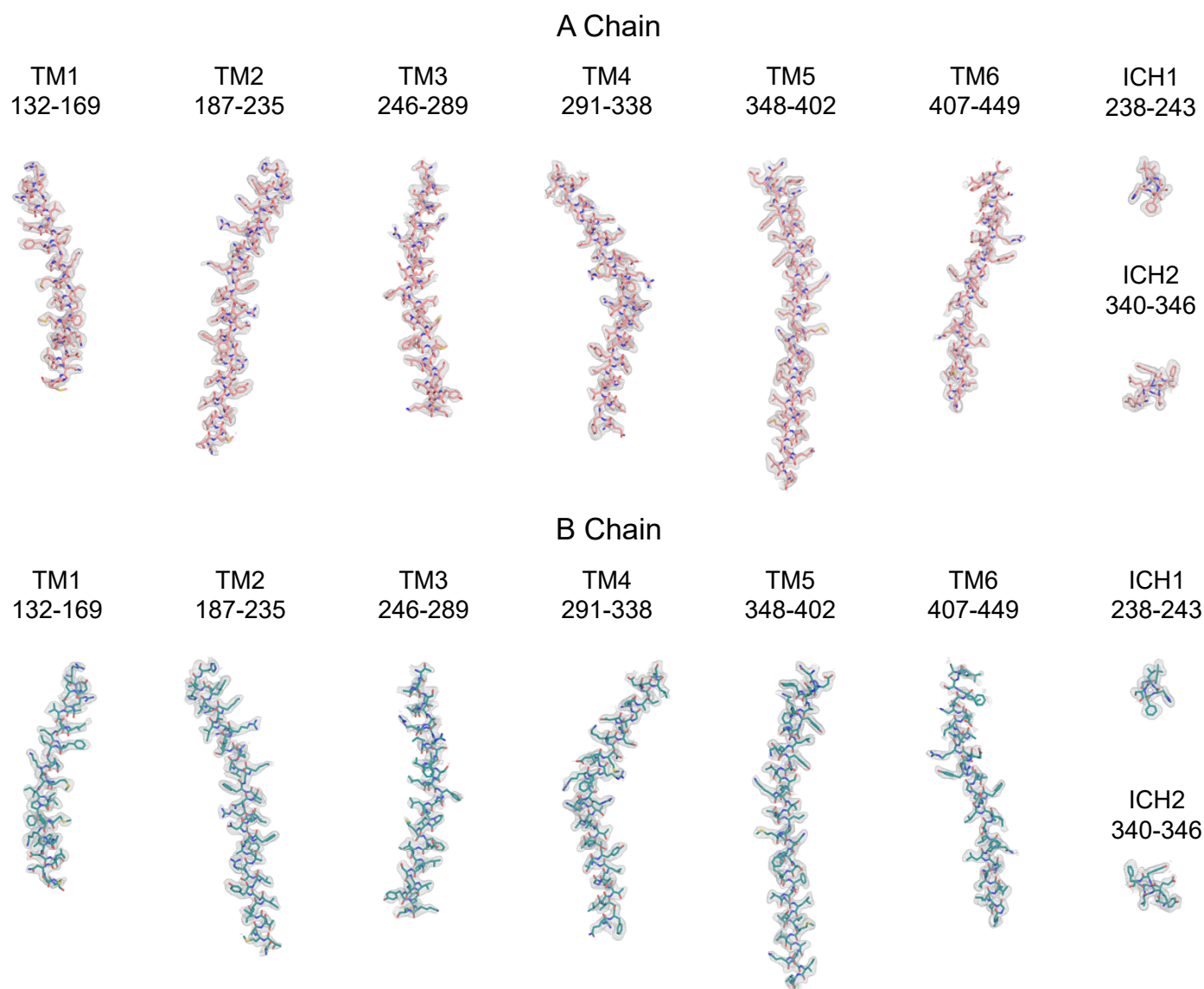
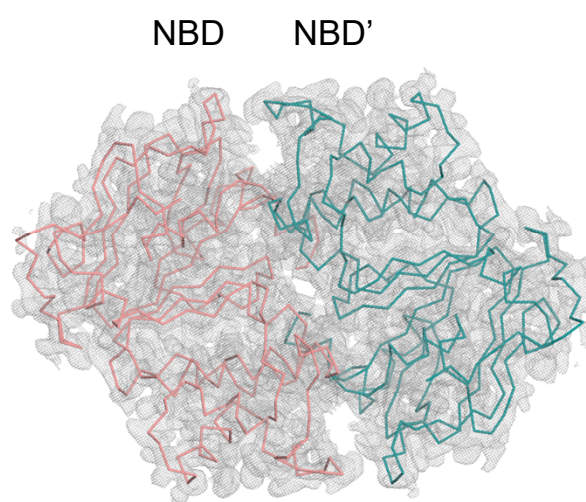
Supplementary Figure 11. Cryo-EM density maps of CoPP:GSH/ADP·VO₄-bound ABCB7. (a) The amino acid residues of each TM helix are shown. The EM density (grey mesh) is contoured at 4 σ level. (b, c) Cryo-EM densities of the NBD region (b), and the CoPP molecules (c) corresponding to those shown in Fig. 4. The densities are contoured at 4 σ and 3 σ levels, respectively.



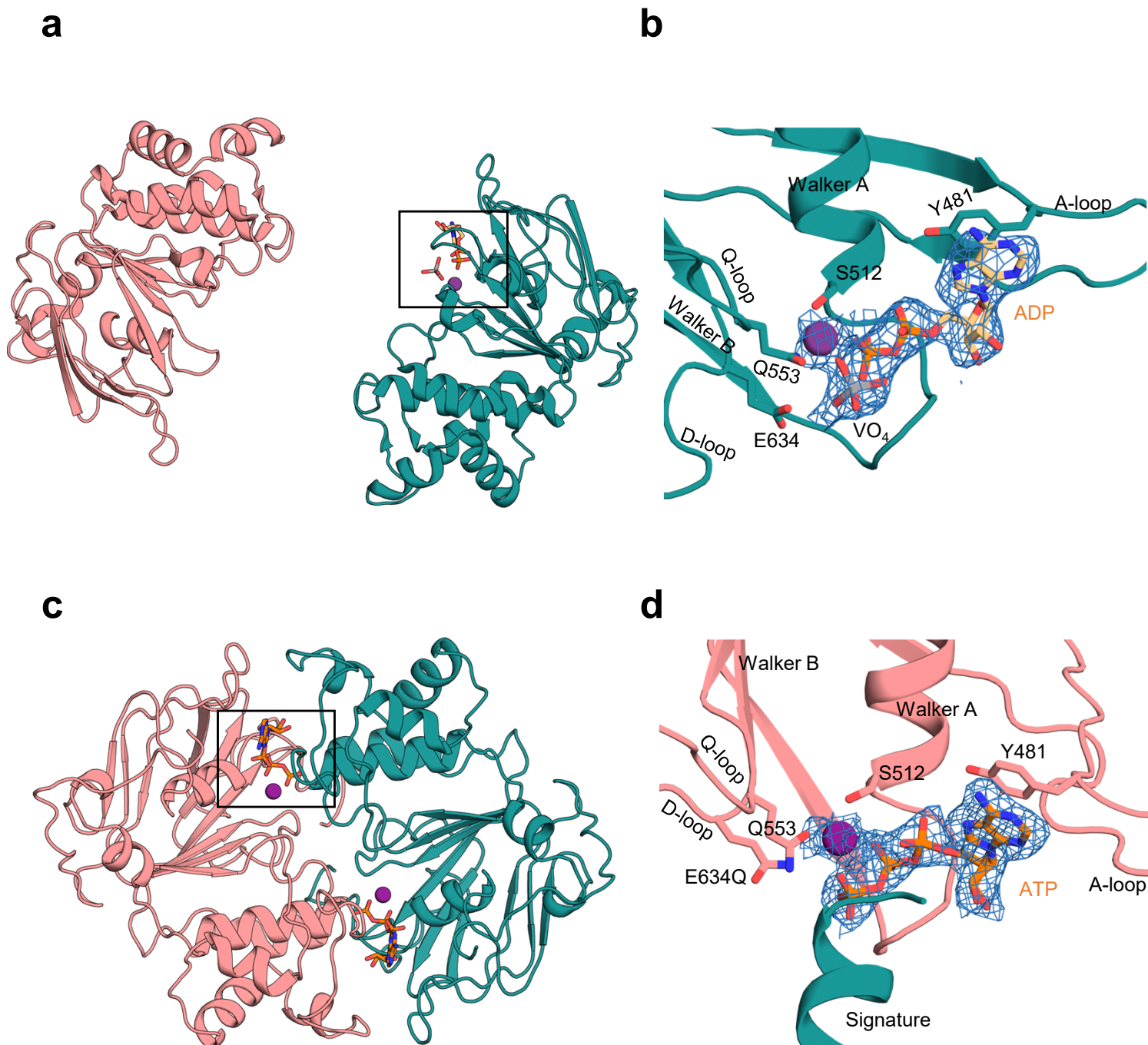
Supplementary Figure 12. Cryo-EM data-processing workflow for ATP-bound ABCB7 (E634Q) in the occluded state. The main steps of the cryo-EM data processing pipeline for the ATP-bound sample are shown.



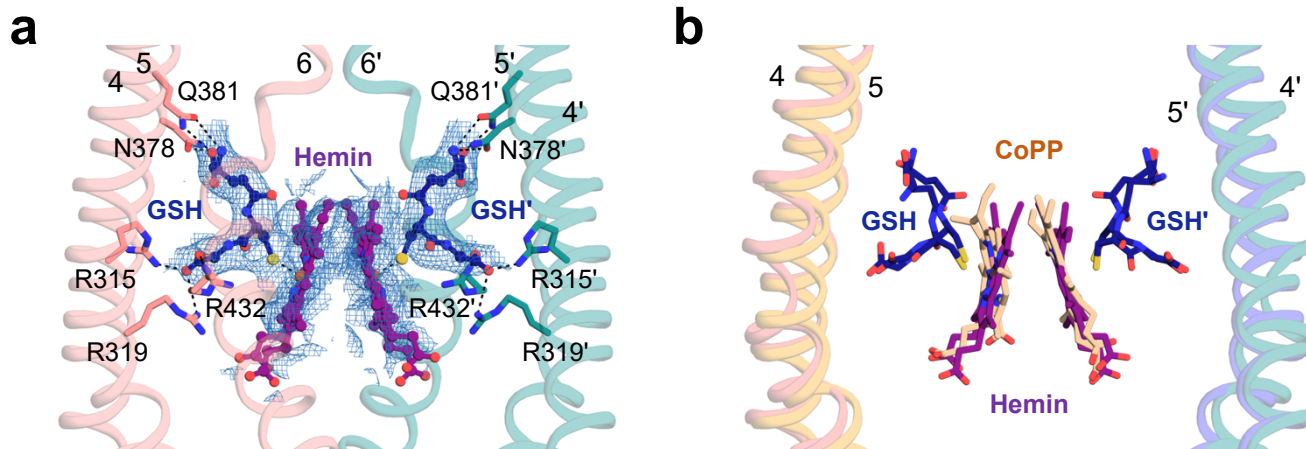
Supplementary Figure 13. Cryo-EM data and model quality analysis of ATP-bound ABCB7 (E634Q) in the occluded state. (a) Representative micrograph. (b) Selected 2D class averages. (c) Gold-standard Fourier Shell correlation (FSC) curve. The resolution estimation (blue line) was calculated based on an FSC cut-off of 0.143. (d) FSC curves calculated between the refined structure and the half map used for refinement (orange), the other half map (green) and the summed map (black). (e) Particle distribution plot for the final 3D reconstruction. (f) Density map colored by local resolution estimation from 2.3 Å (blue) to 4.0 Å (red).

a**b**

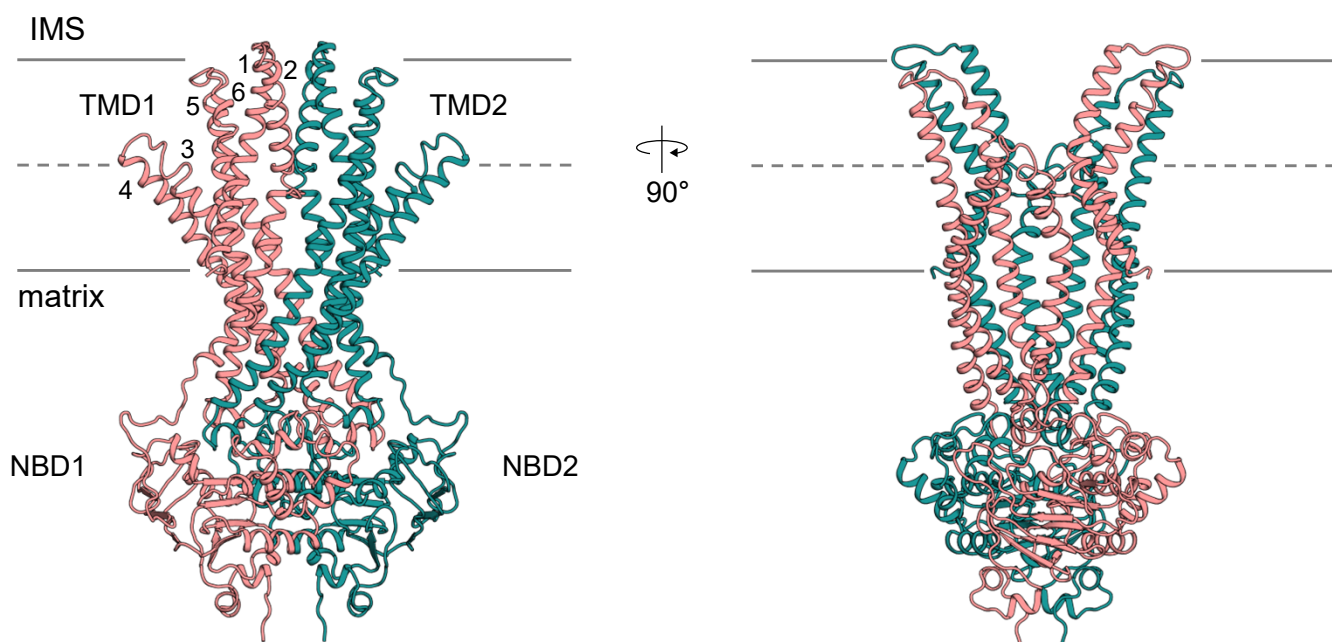
Supplementary Figure 14. Cryo-EM density maps of ATP-bound ABCB7 (E634Q) in the occluded state. (a) The amino acid residues of each TM helix are shown. The EM density (grey mesh) is contoured at 4 σ level. (b) Cryo-EM density of the NBD region.



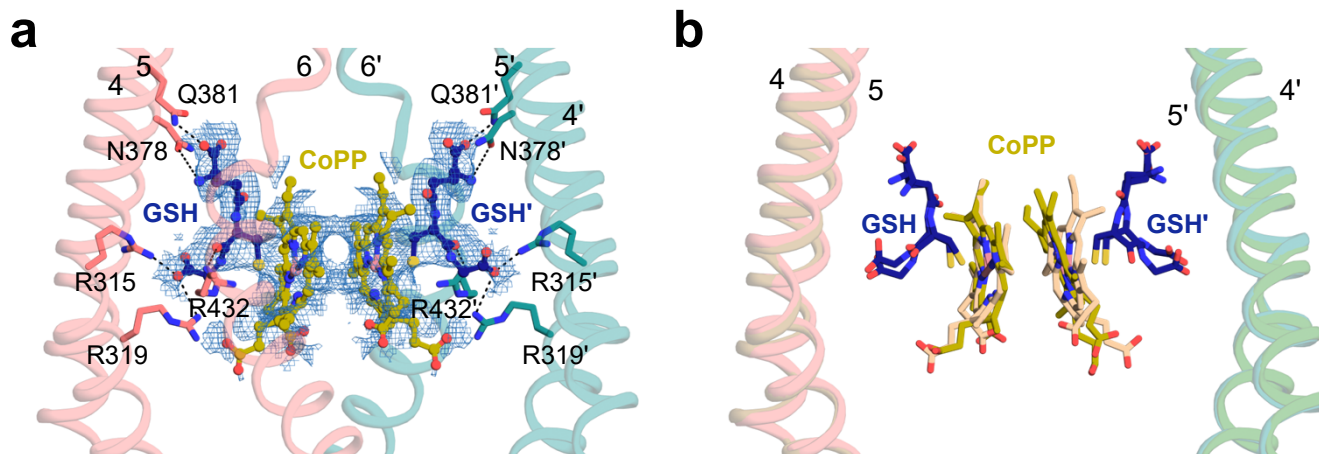
Supplementary Figure 15. The NBD structures of ADP·VO₄-bound and ATP-bound human ABCB7. (a) Overall NBD structure of ADP·VO₄-bound ABCB7 (wild-type) in a post-hydrolysis conformation. The two NBDs are colored pink and teal. The bound ADP·VO₄ and Mg²⁺ are shown as sticks and spheres, respectively. (b) Close-up view of the ADP·VO₄ binding site. The EM density (blue mesh) of the ADP·VO₄ and Mg²⁺ are shown at the 5 σ level. (c) Overall NBD structure of ATP-bound ABCB7 (E634Q) in the occluded state. The bound ATP and Mg²⁺ are shown as sticks and spheres, respectively. (d) Close-up view of the ATP binding site in the occluded state. The EM density (blue mesh) of Mg²⁺/ATP is shown at the 5 σ level.



Supplementary Figure 16. Structural comparison of the CoPP:GSH and hemin:GSH complexes bound to ABCB7. (a) Close-up view of the hemin:GSH-binding site. Cryo-EM density maps of bound hemin and GSH are shown as meshes contoured at 4σ . (b) Structural comparison of the CoPP:GSH-bound and hemin:GSH-bound ABCB7 complexes.



Supplementary Figure 17. Homology model of outward-facing ABCB7 generated by MODELLER. The overall structure of outward-facing ABCB7 showing the predicted TMD arrangement and NBD dimerization.



Supplementary Figure 18. Structural comparison of ABCB7 bound to CoPP at low concentrations. (a) Close-up view of the substrate-binding cavity in the presence of 0.1 mM CoPP and 1 mM GSH. The EM density maps for bound CoPP and GSH are contoured at 6 σ and 4 σ , respectively. (b) Structural comparison of ABCB7 complexes prepared with 1 mM and 0.1 mM CoPP (both in the presence of 1 mM GSH).

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics

	Apo EMDB-69144 PDB ID 23PH	CoPP:GSH /ADP·VO₄ EMDB-69143 PDB ID 23PG	ATP-bound EMDB-69145 PDB ID 23PI
Data collection and processing			
Microscope	Titan Krios G4 with XFEG	Titan Krios G4 with h CFEG	Titan Krios G4 with XFEG
Camera	K3	Falcon 4i	K3
Energy filter	BioQuantum	Selectris X	BioQuantum
Magnification	130,000	165,000	130,000
Voltage (kV)	300	300	300
Total movies (no.)	6,820	38,571	7,542
Electron exposure (e ⁻ /Å ²)	50	50	50
Defocus range (μm)	-0.8 ~ -2.2	-0.8 ~ -2.0	-0.8 ~ -2.0
Pixel size (Å)	0.673	0.760	0.673
Symmetry imposed	C2	C1	C2
Initial particle images (no.)	3,136,235	11,796,247	2,493,185
Final particle images (no.)	216,118	204,460	425,646
Map resolution (Å)	3.0	2.8	2.3
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	3.0 ~ 7.0	2.8 ~ 6.5	2.3 ~ 4.0
Refinement			
Initial model used (PDB code)	7VGF	7VGF	ABCB10
Model resolution (Å)	3.0.	2.8	2.3
Map sharpening <i>B</i> factor (Å ²)	116.5	83.9	83.3
Model composition			
Non-hydrogen atoms			
Protein residues	8,580	8,544	8,616
Ligands	70	679	326
<i>B</i> factors (Å ²)			
Protein	137.2	69.6	43.6
Ligand	108.5	73.4	56.8
R.m.s. deviations			
Bond lengths (Å)	0.004	0.005	0.003
Bond angles (°)	0.896	0.712	0.596
Validation			
MolProbity score	1.3	1.8	1.5
Clashscore	2.5	5.7	3.8
Poor rotamers (%)	0.3	2.0	2.5
Ramachandran plot			
Favored (%)	96.3	96.6	98.2
Allowed (%)	3.7	3.4	1.8
Disallowed (%)	0.0	0.0	0.0