

Supplementary Information

The redox driven Na⁺-pumping mechanism in *Vibrio cholerae* NADH-quinone oxidoreductase relies on dynamic conformational changes

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Supplementary Fig. 1: Data statistics and resolutions of the **WT_{red}** dataset.

Supplementary Fig. 2: Data statistics and resolutions of the **WT_{red}/-Na⁺** dataset.

Supplementary Fig. 3: Data statistics and resolutions of the **WT_{red}+ KR** dataset.

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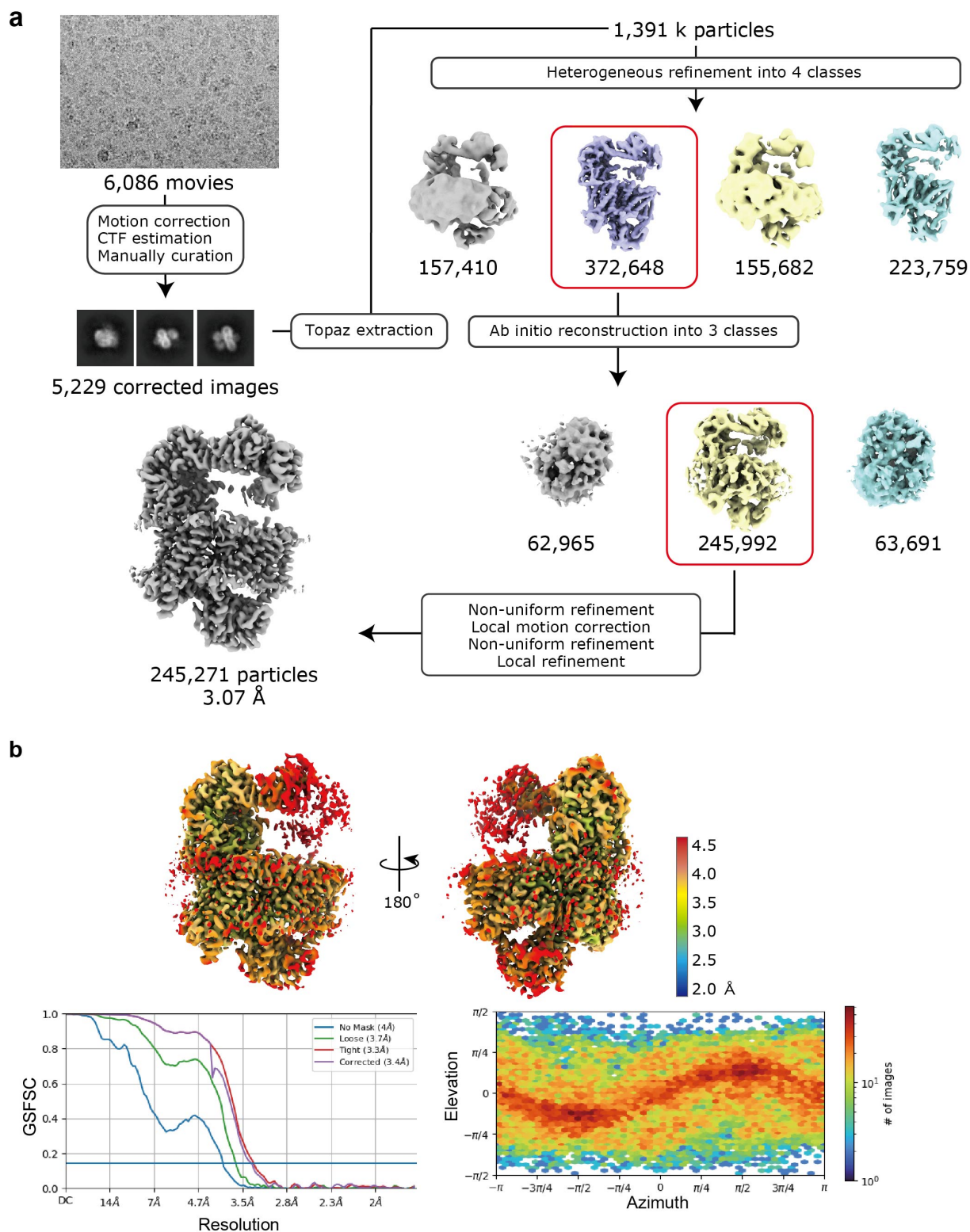
Supplementary Fig. 25: Water spatial density from conventional MD (cMD1).

Supplementary Table 1: Statistics of cryo-EM data, refinement, and validation of Na⁺-NQR.

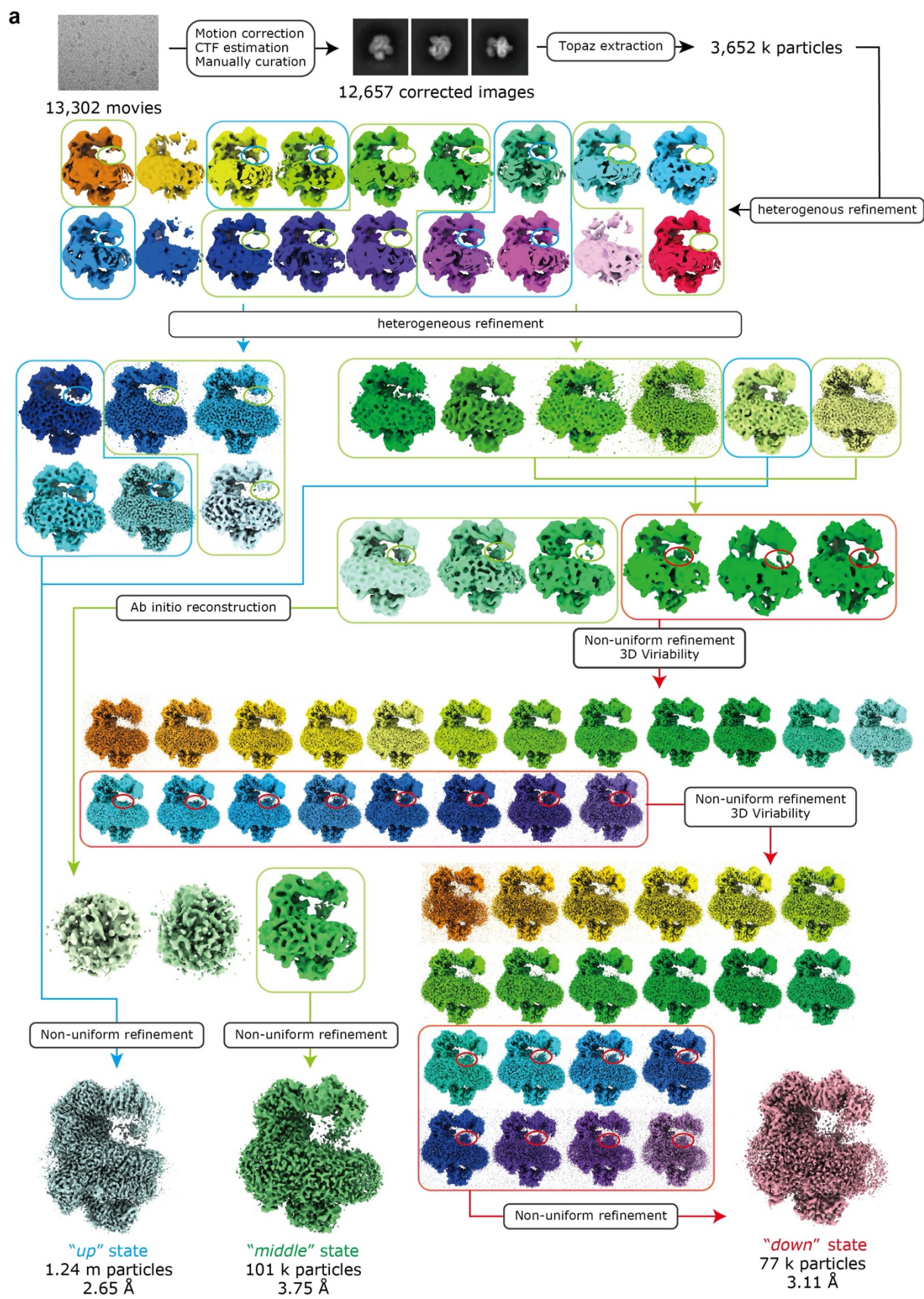
Supplementary Table 2: List of atomistic MD simulations.

Supplementary Table 3: Na⁺ binding site by MD simulation.

Supplementary Table 4: MD simulation checklist.

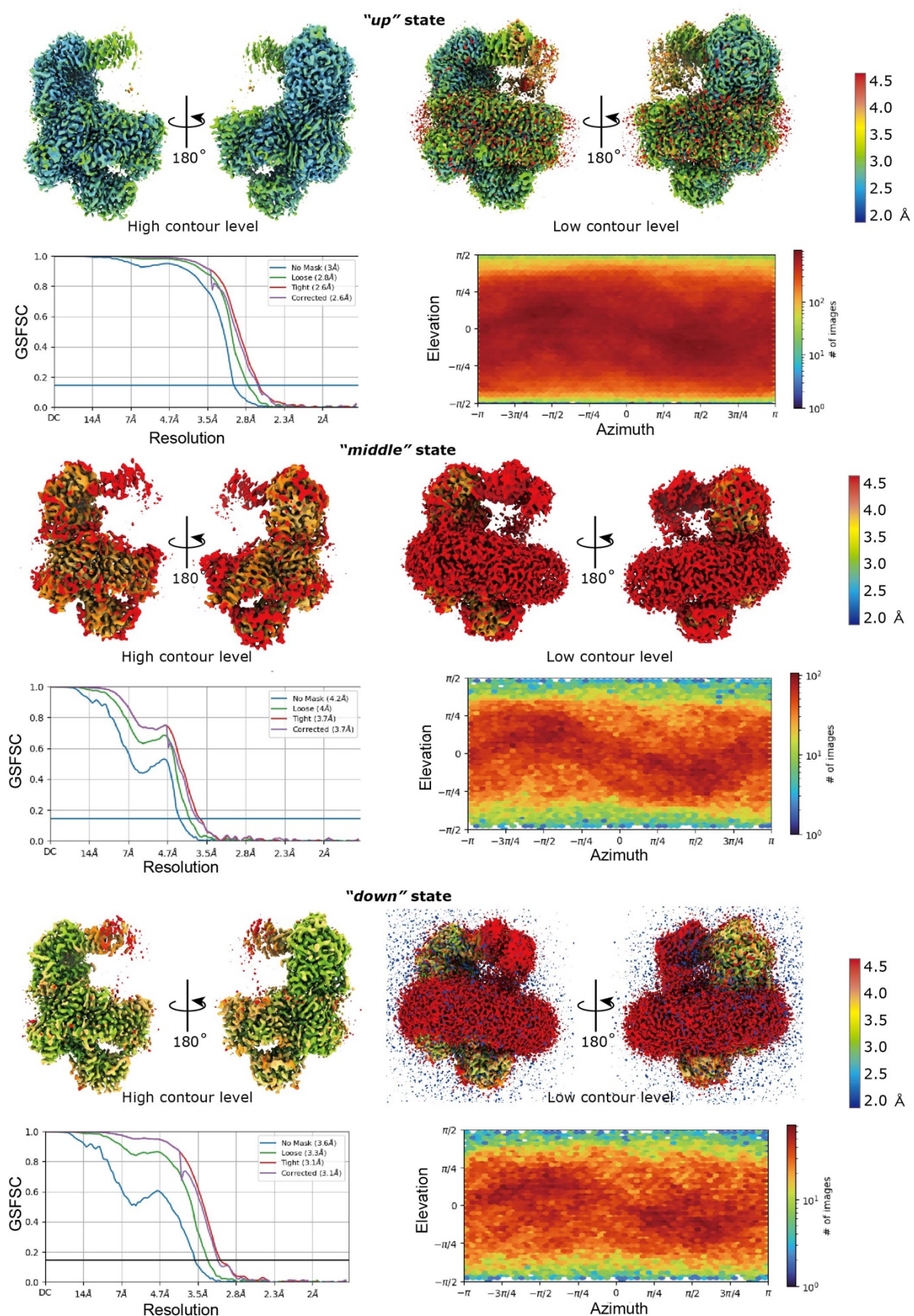


Supplementary Fig. 1: Data statistics and resolutions of the WT_{red} dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.



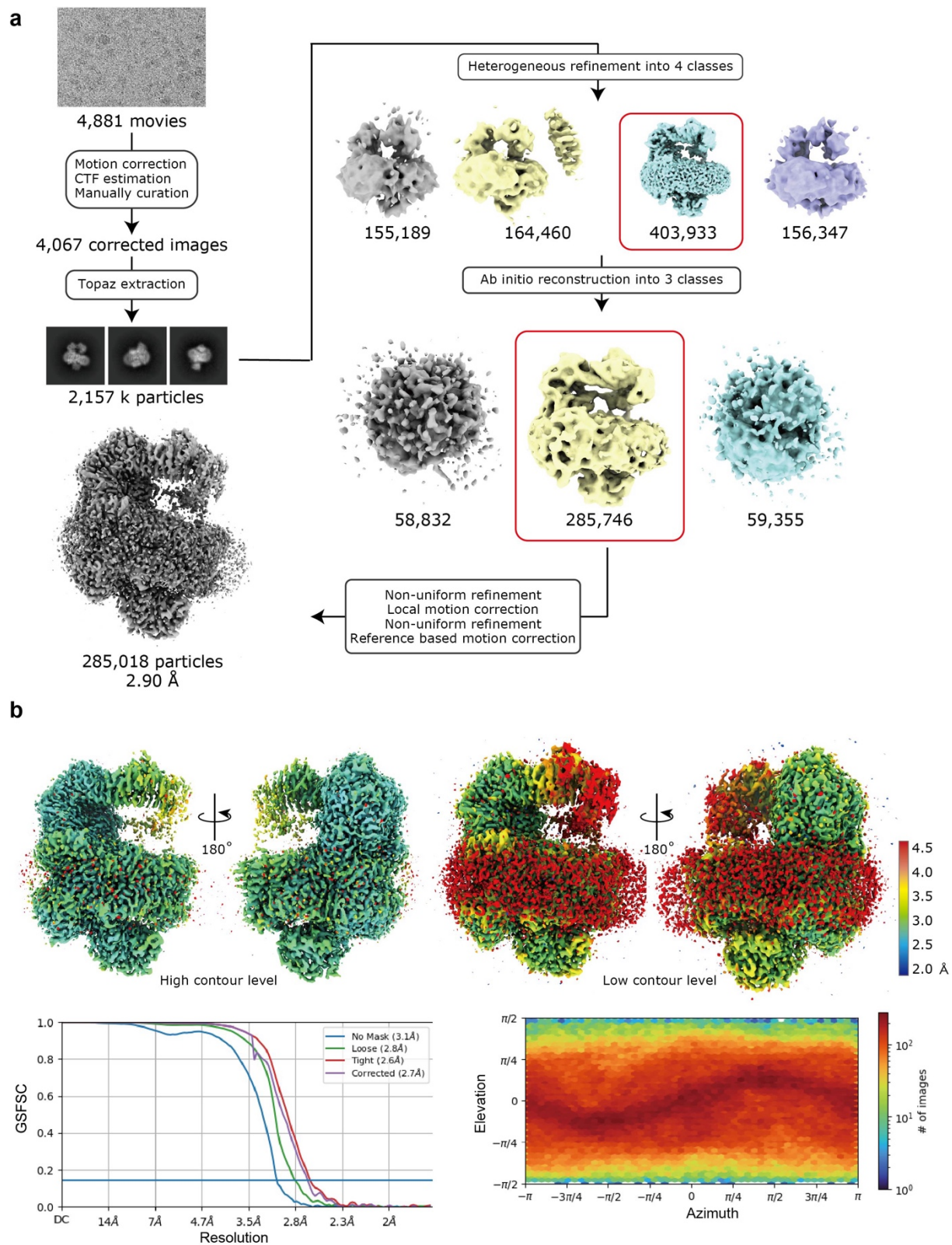
Supplementary Fig. 2 (continued)

b



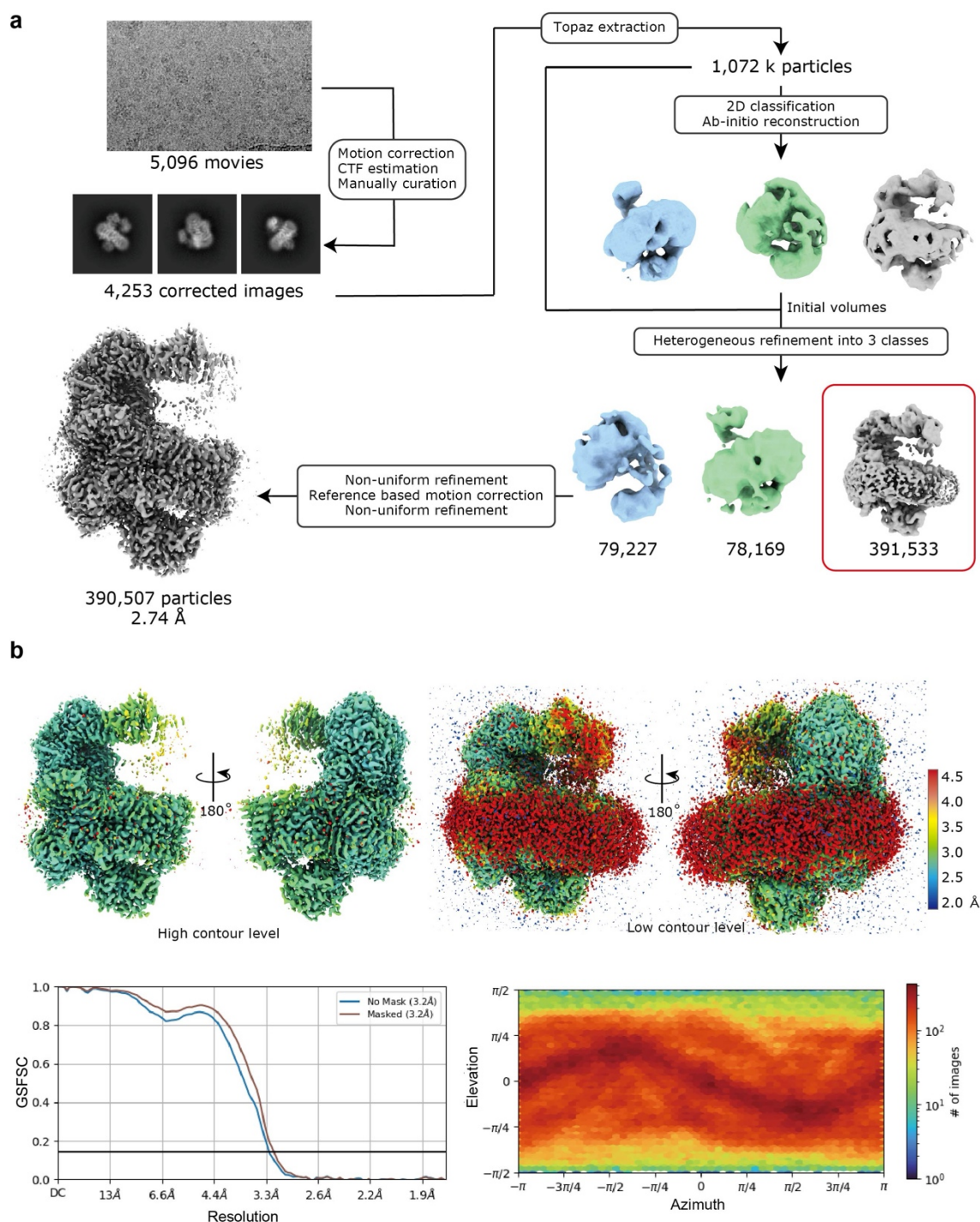
Supplementary Fig. 2 (continued)

Supplementary Fig. 2: Data statistics and resolutions of the WT_{red}/-Na⁺ dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.



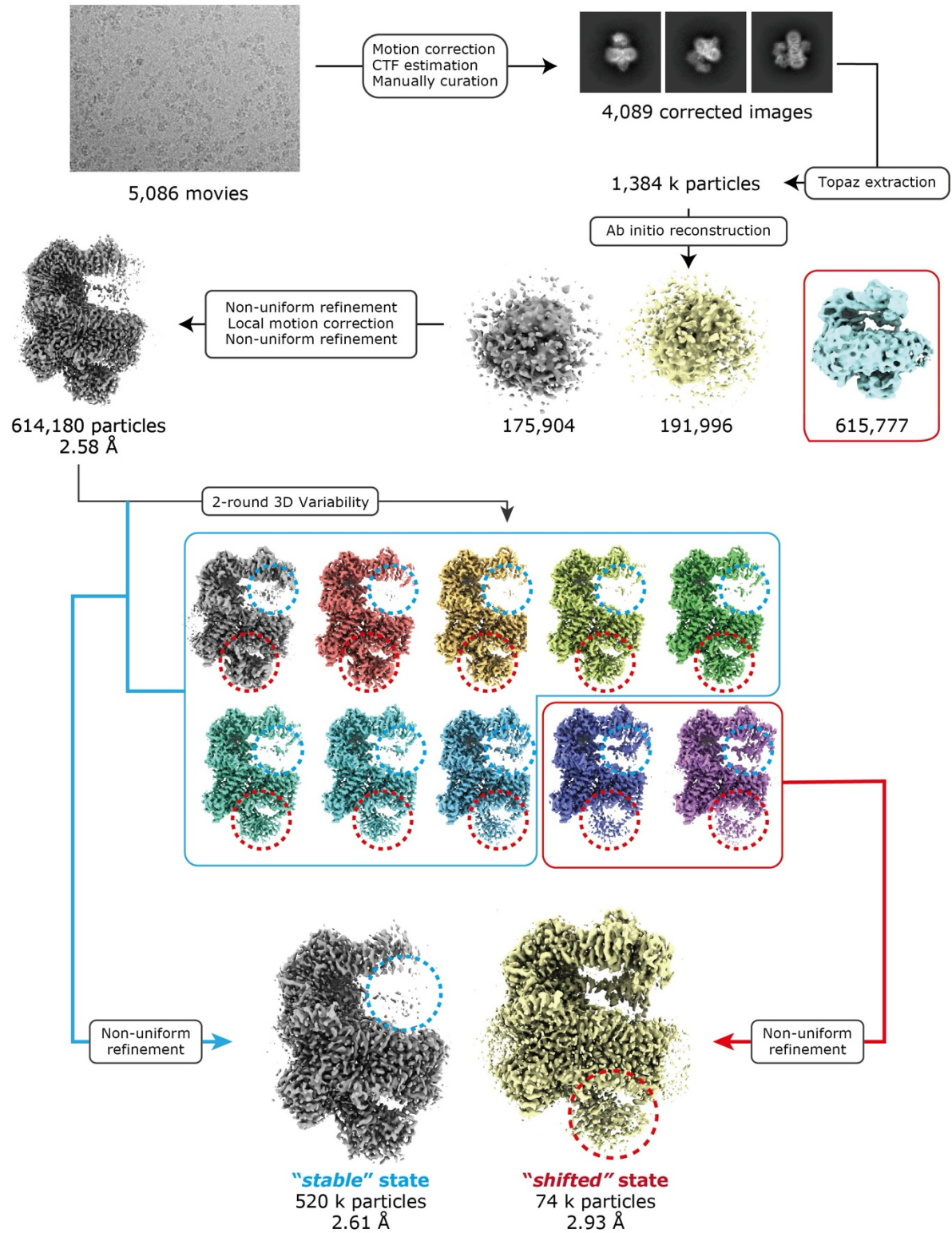
Supplementary Fig. 3 (continued)

Supplementary Fig. 3: Data statistics and resolutions of the WT_{red} + korormicin (KR) dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.

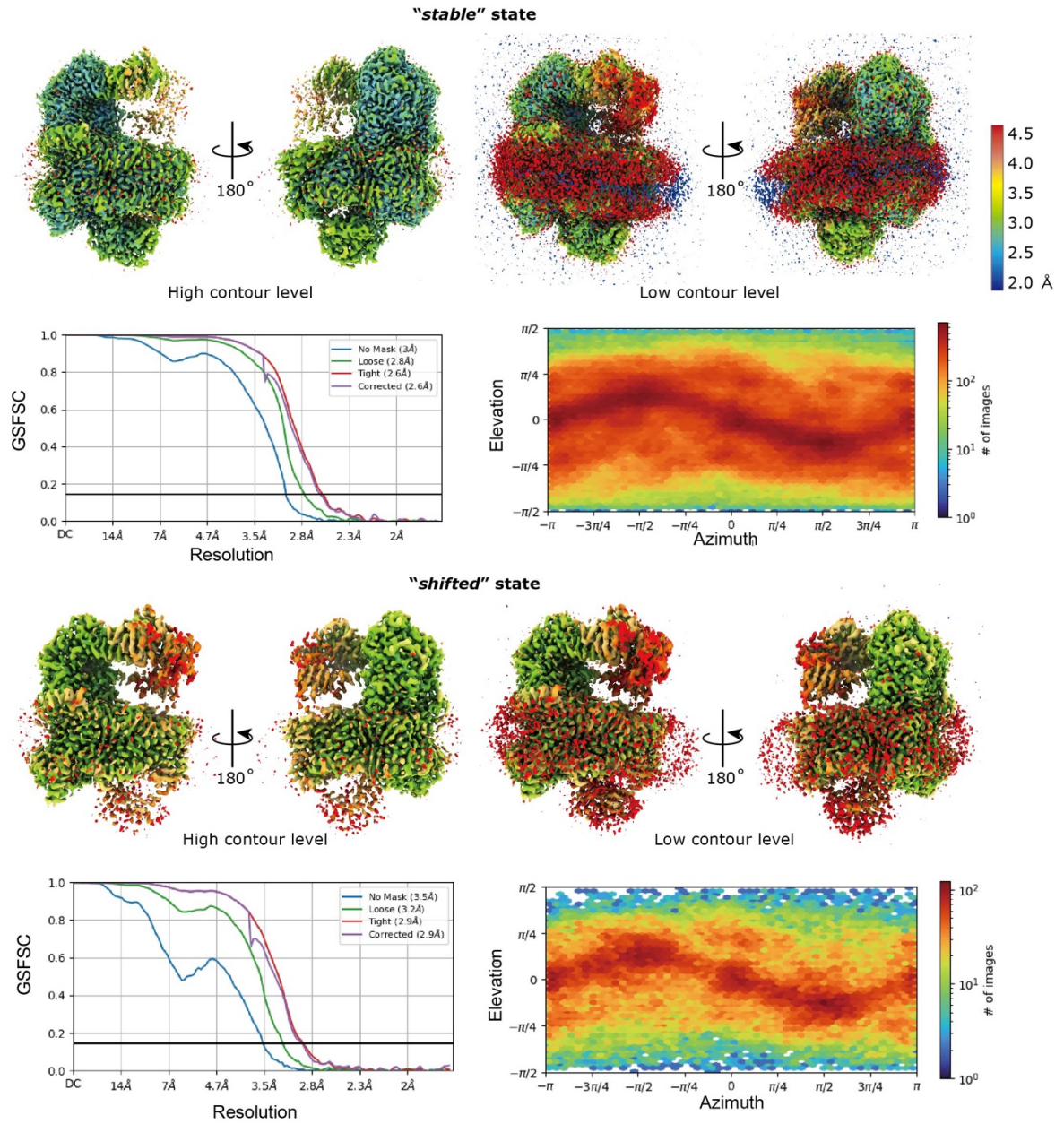


Supplementary Fig. 4: Data statistics and resolutions of the WT_{red} + aurachin D-42 (AD-42) dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.

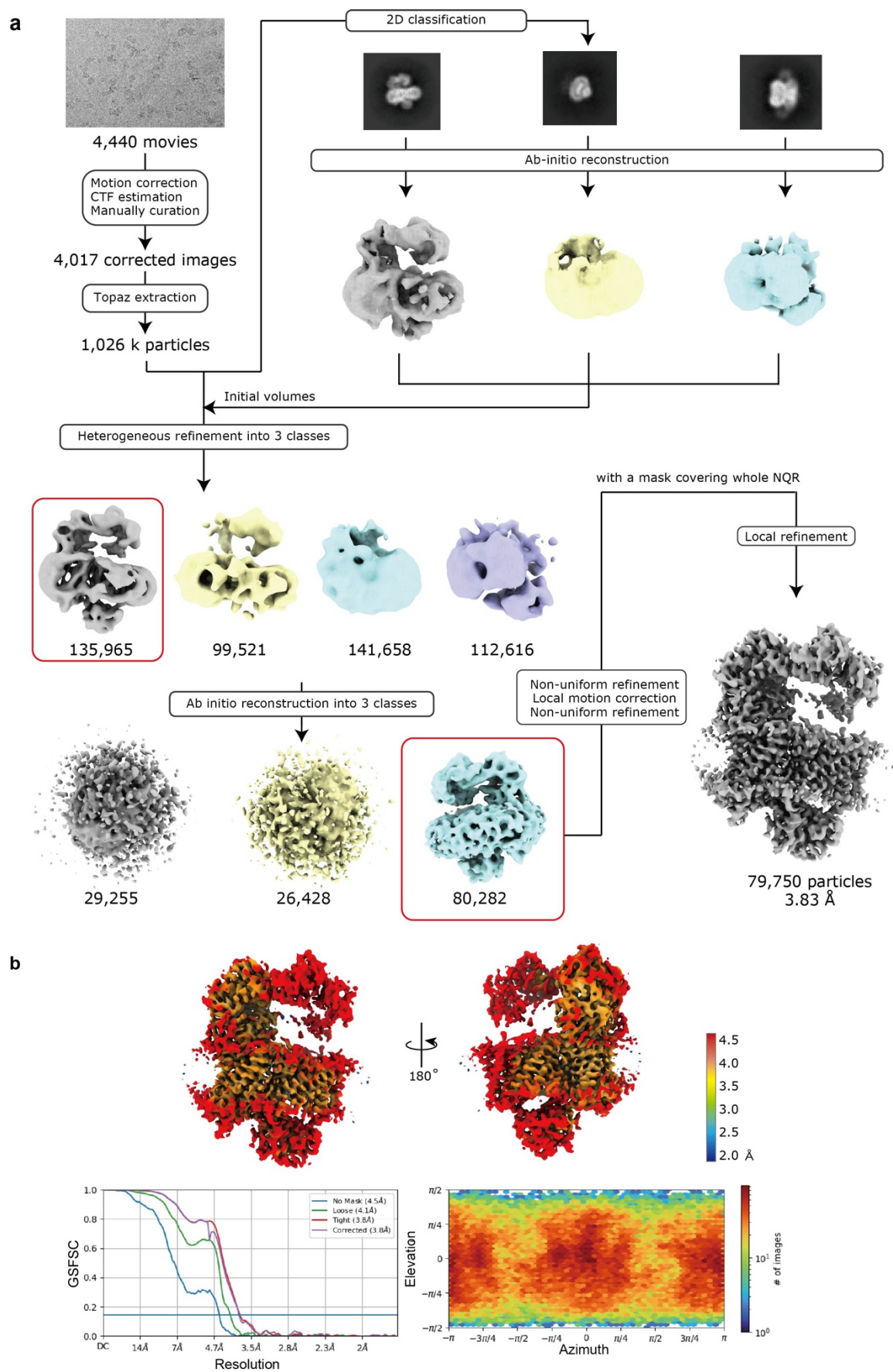
a



Supplementary Fig. 5 (continued)

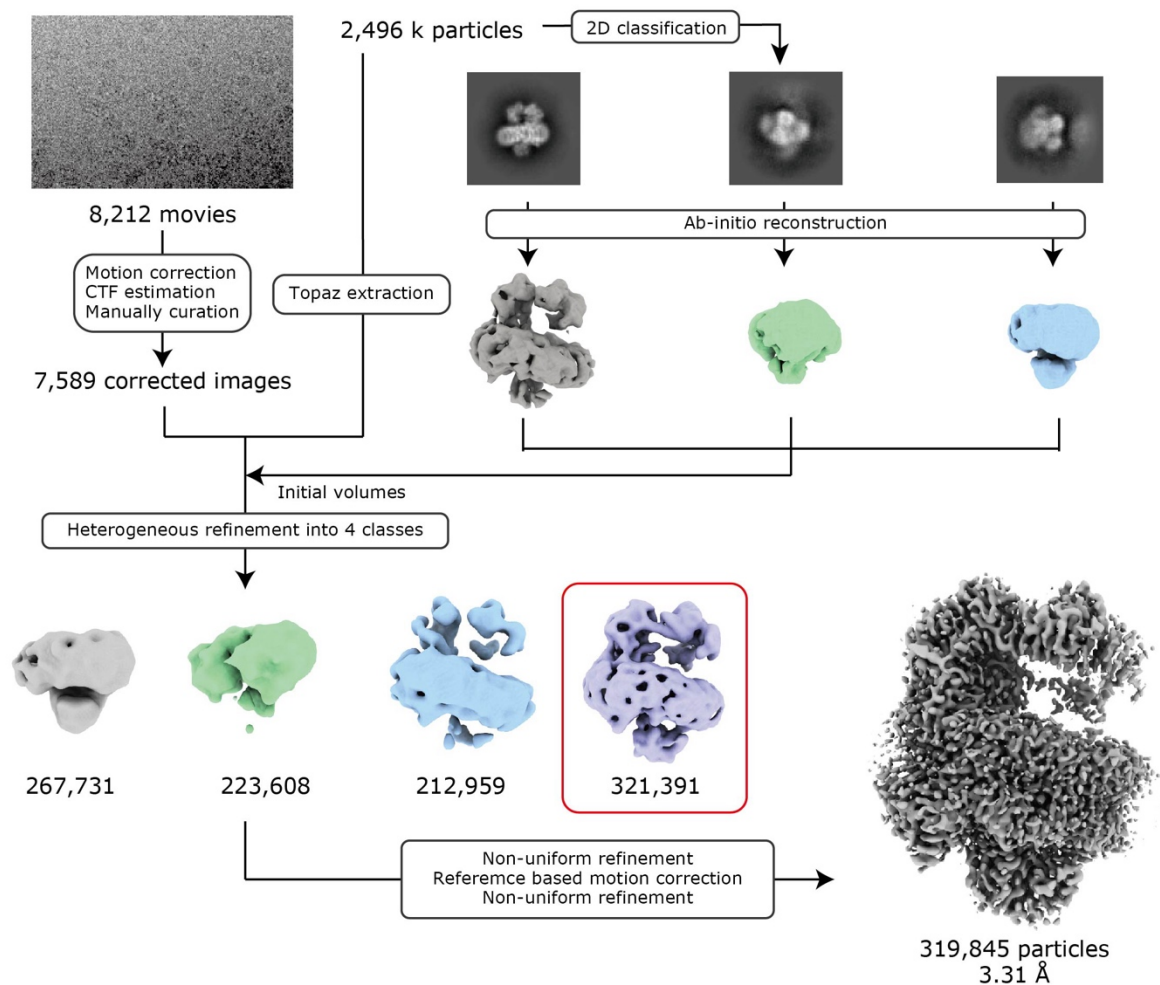
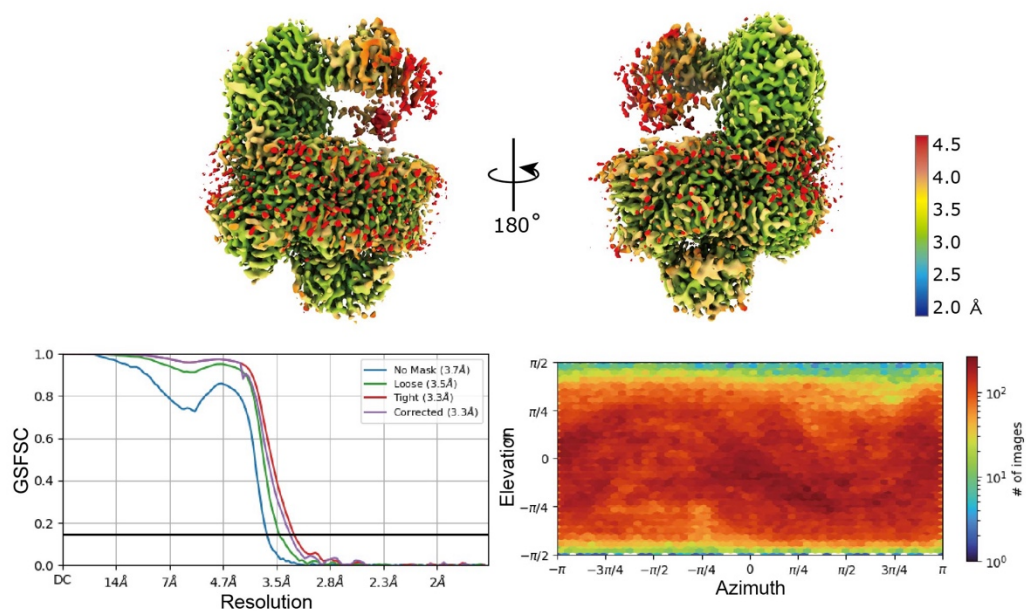
b

Supplementary Fig. 5: Data statistics and resolutions of the NqrB-G141A_{red} +KR dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. ***b*,** Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.

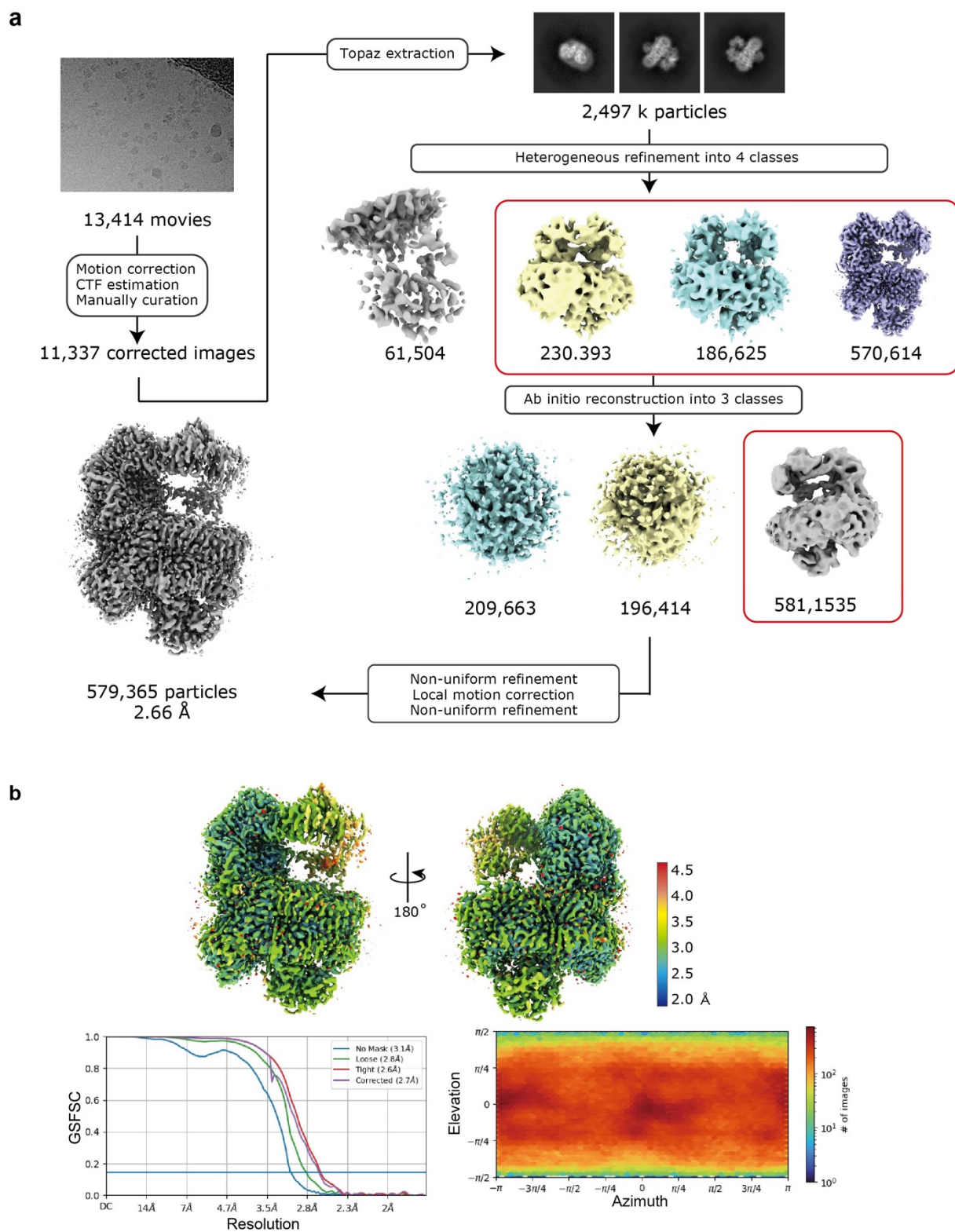


Supplementary Fig. 6 (continued)

Supplementary Fig. 6: Data statistics and resolutions of the NqrB-T236Y_{ox} dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.

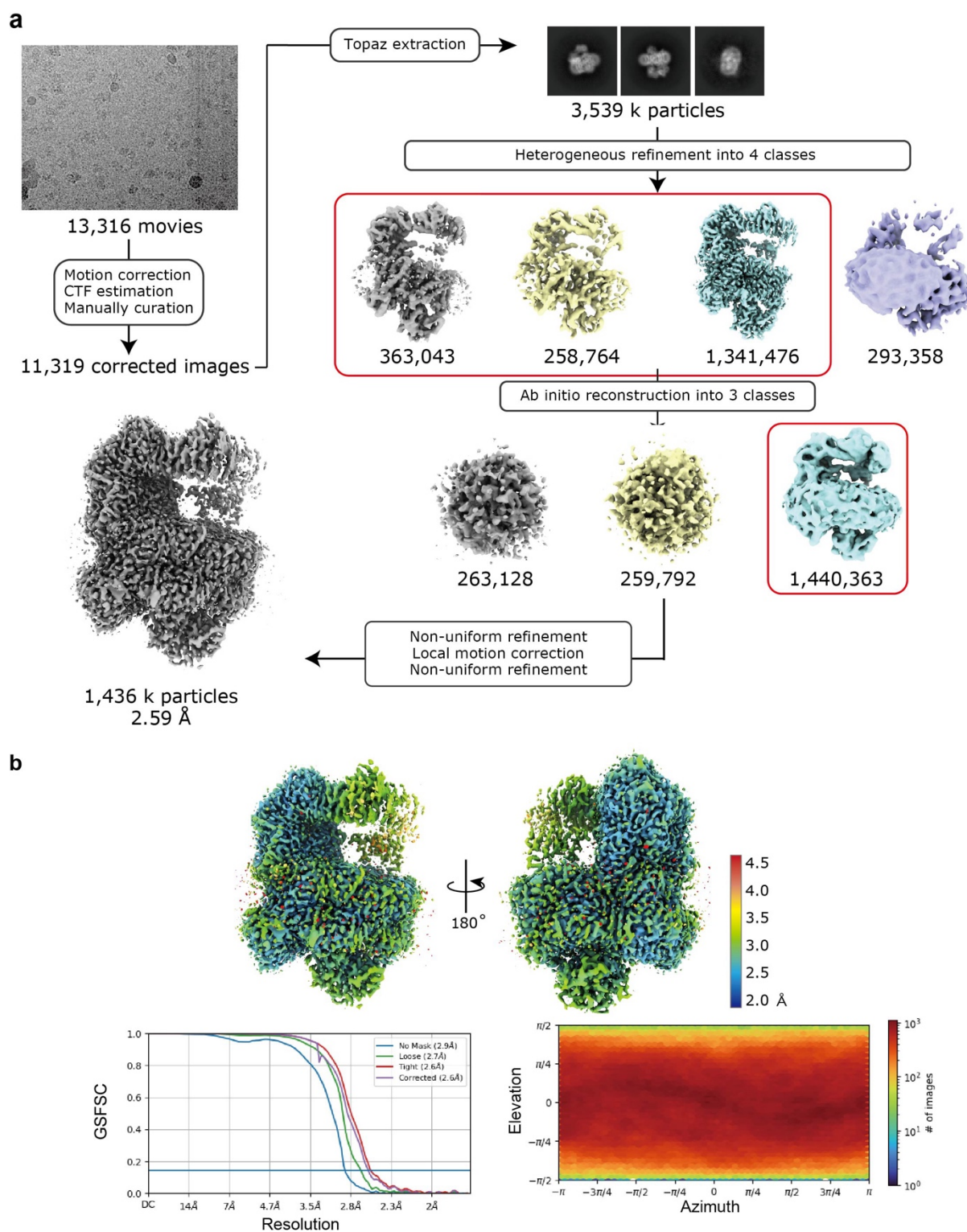
a**b****Supplementary Fig. 7 (continued)**

Supplementary Fig. 7: Data statistics and resolutions of the NqrB-T236Y_{red} dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.

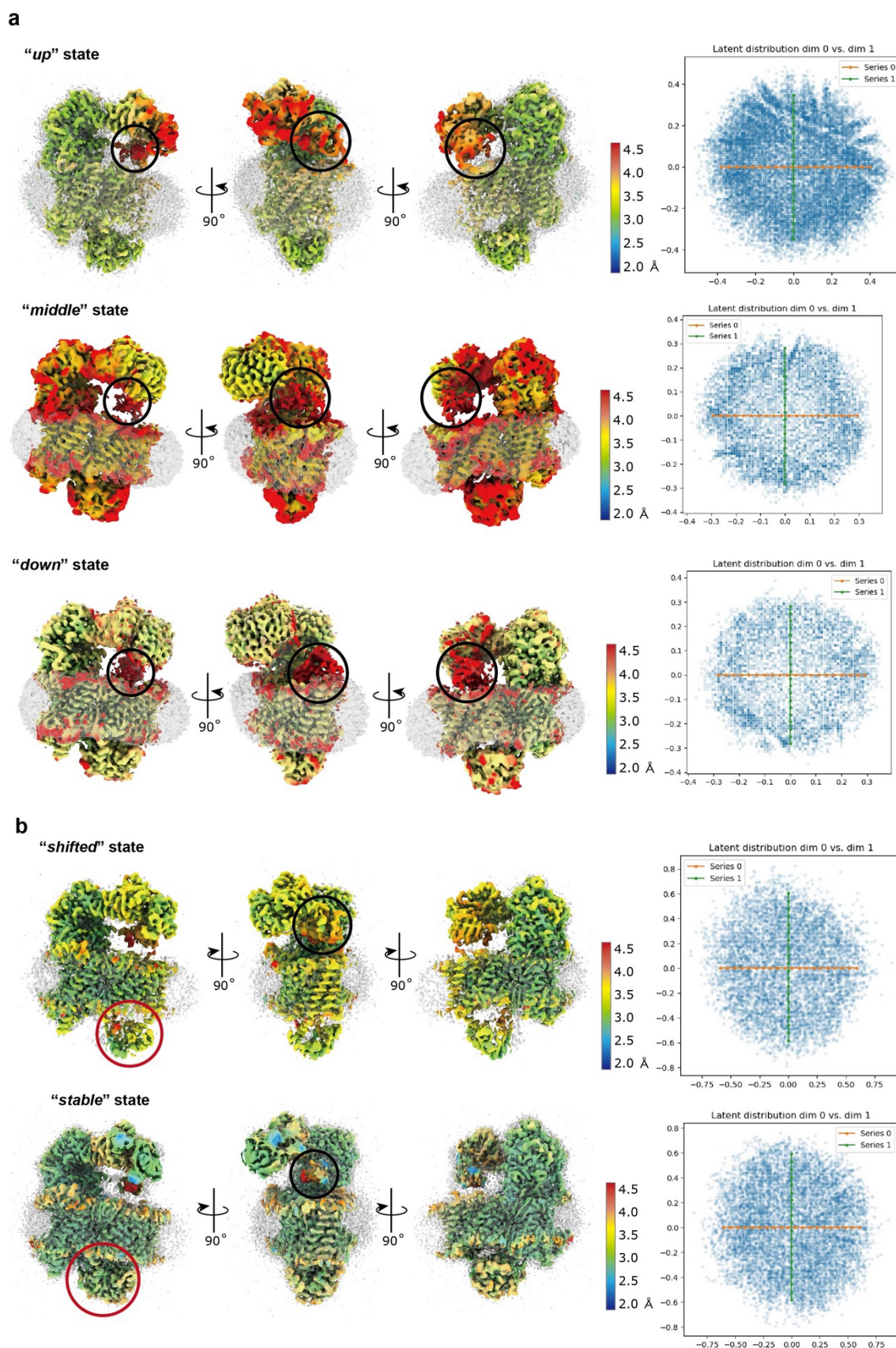


Supplementary Fig. 8 (continued)

Supplementary Fig. 8: Data statistics and resolutions of the NqrC-T225Y_{ox} dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.



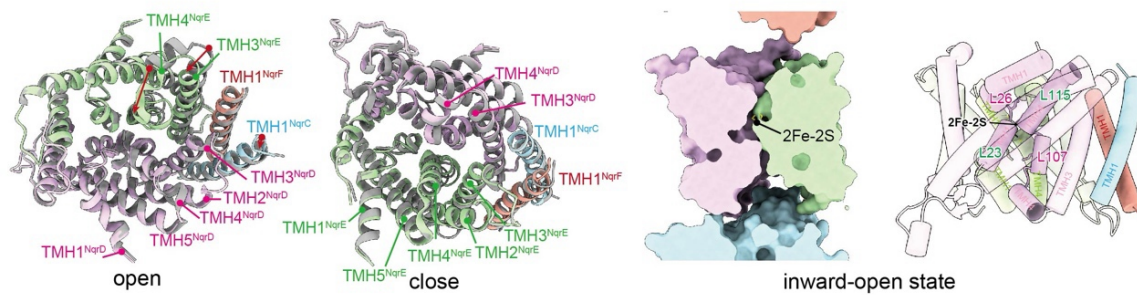
Supplementary Fig. 9: Data statistics and resolutions of the NqrC-T225Y_{red} dataset. *a*, Workflow for single particle analysis with cryoSPARC. The classes surrounded by red squares were used in the next step. *b*, Summary of the local resolution of the obtained map. The upper panels show the local resolution maps; the lower left panels show the golden standard Fourier shell correlation (GSFSC) curves, and the bottom right panels show 2D distribution histograms.



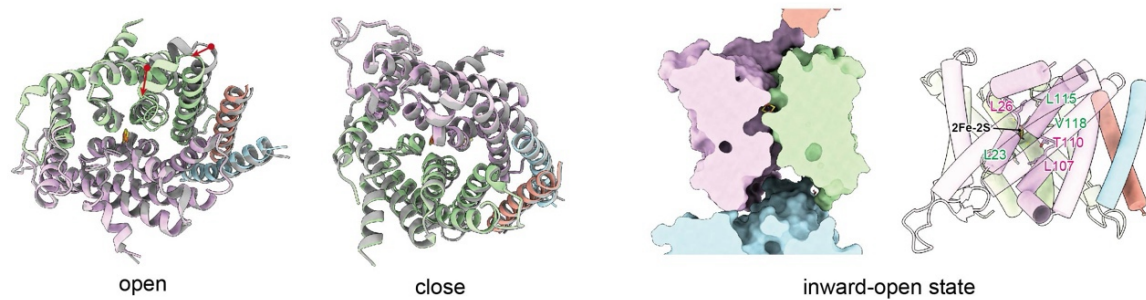
Supplementary Fig. 10 (continued)

Supplementary Fig. 10: 3D Flex analysis of the complete Na⁺-NQR. The *left* panel shows a canonical density map from 3DFlex reconstruction colored by local resolution. The *black circle* and *red circle* indicate the ferredoxin-like domain of NqrF and the hydrophilic domain of NqrC, respectively. The *right* panel shows the latent distribution of particles produced by 3DFlex, with the orange line traversing the two major conformations. *a*, WT_{red}/– Na⁺. *b*, NqrB-G141A_{red} + KR.

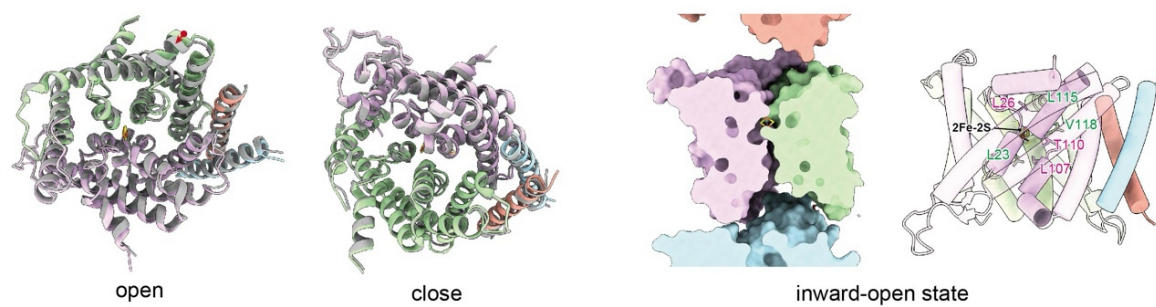
a WT_{red}



b “up” state of WT_{red} / -Na⁺

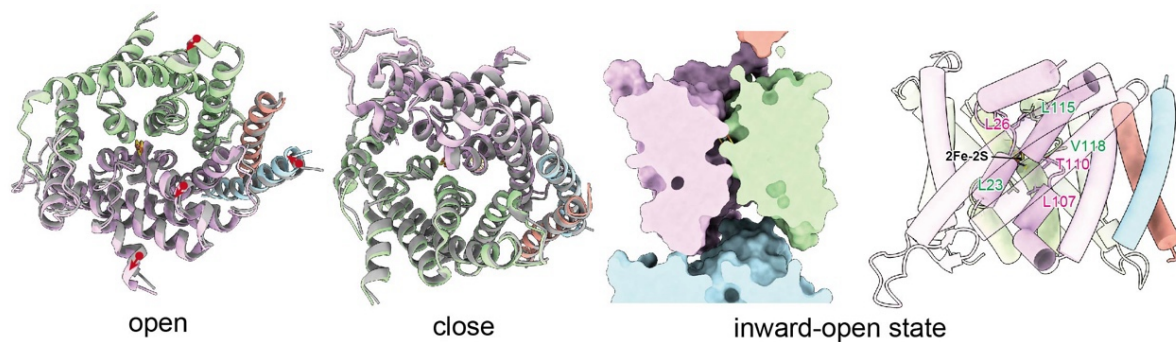


c “middle” state of WT_{red} / -Na⁺

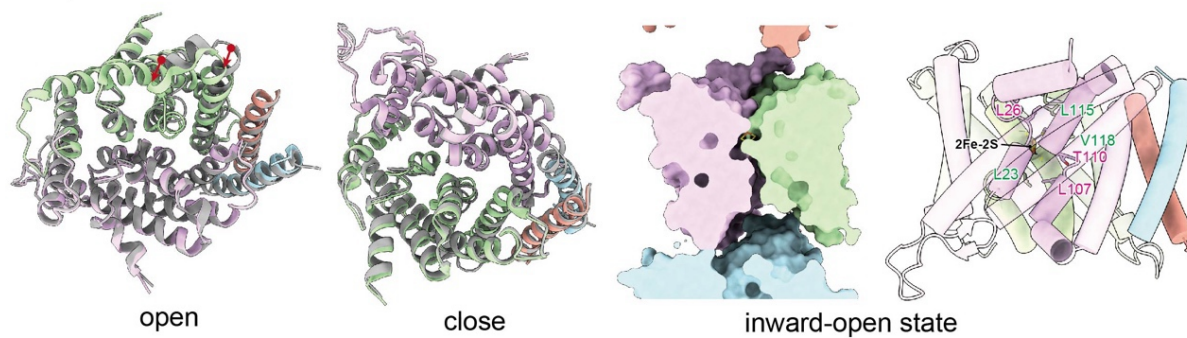


Supplementary Fig. 11 (continued)

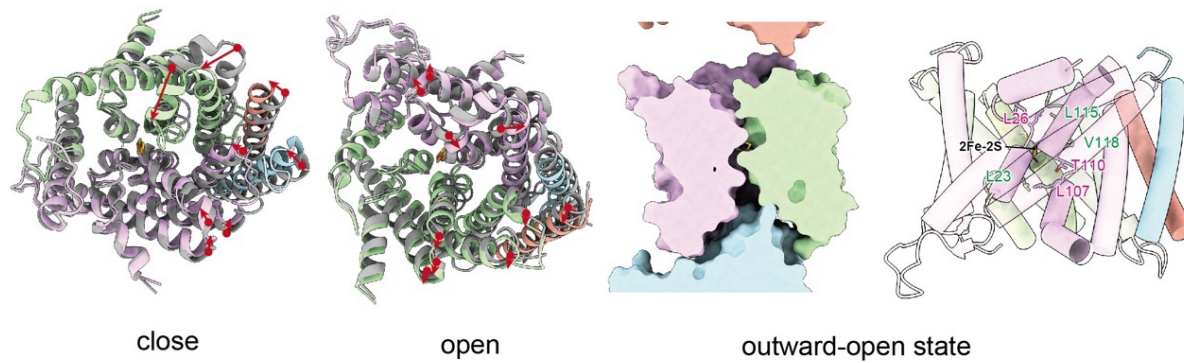
d NqrB-T236Y_{ox}



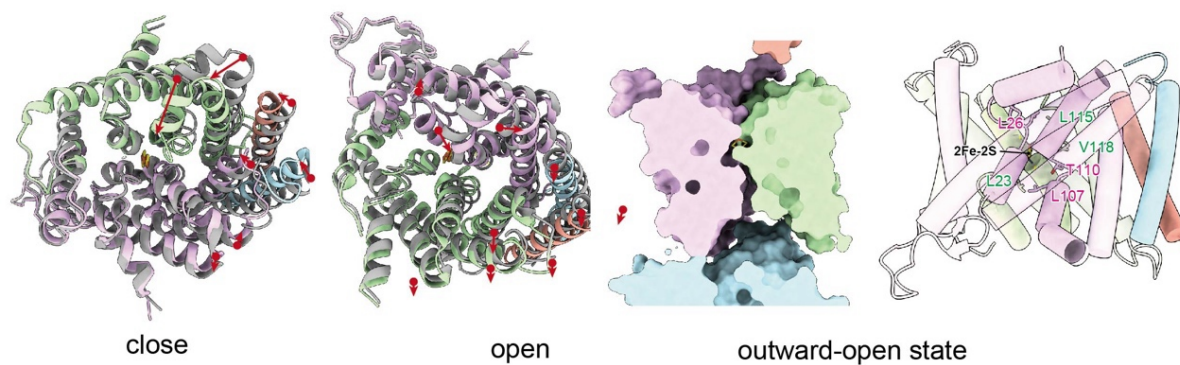
e NqrB-T236Y_{red}



f NqrC-T225Y_{ox}

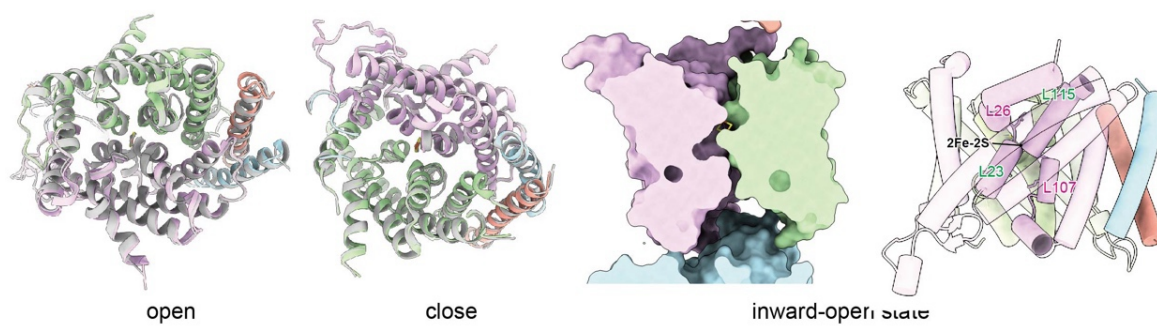


g NqrC-T225Y_{red}

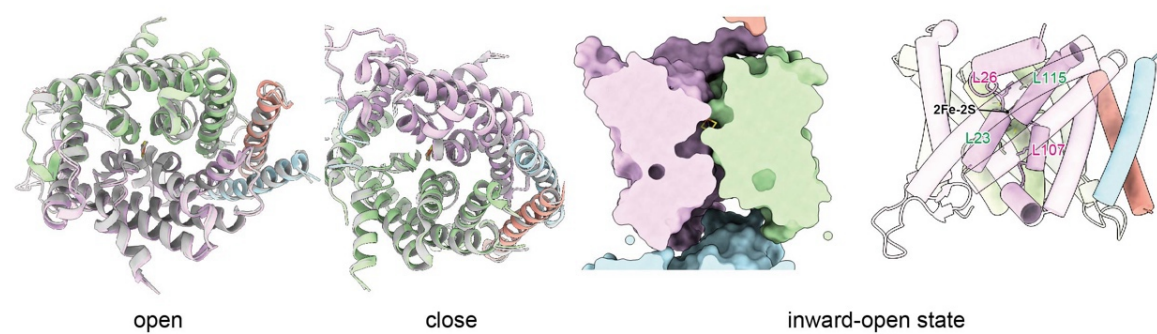


Supplementary Fig. 11 (continued)

h WT_{red} + KR

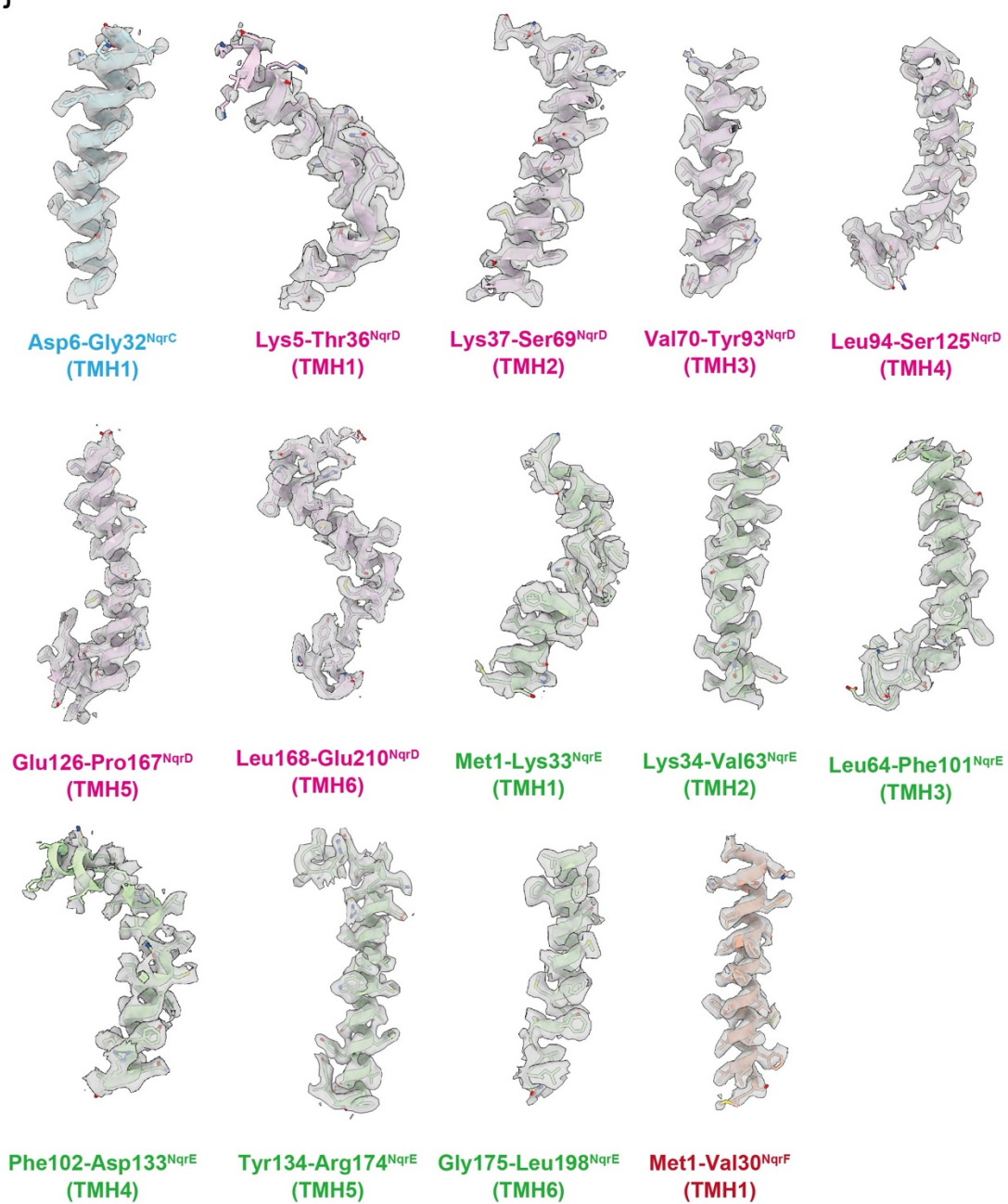


i WT_{red} + AD

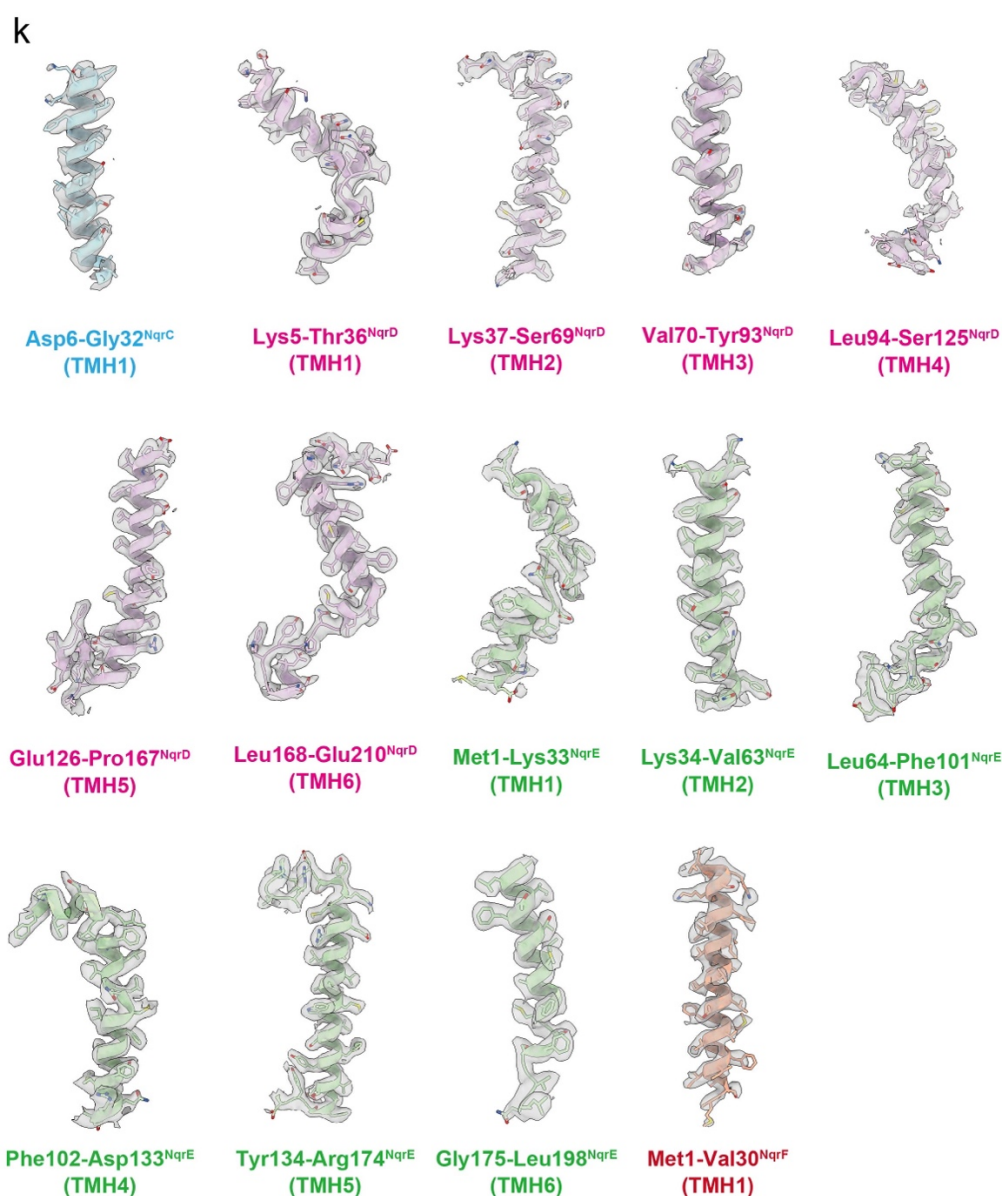


Supplementary Fig. 11 (continued)

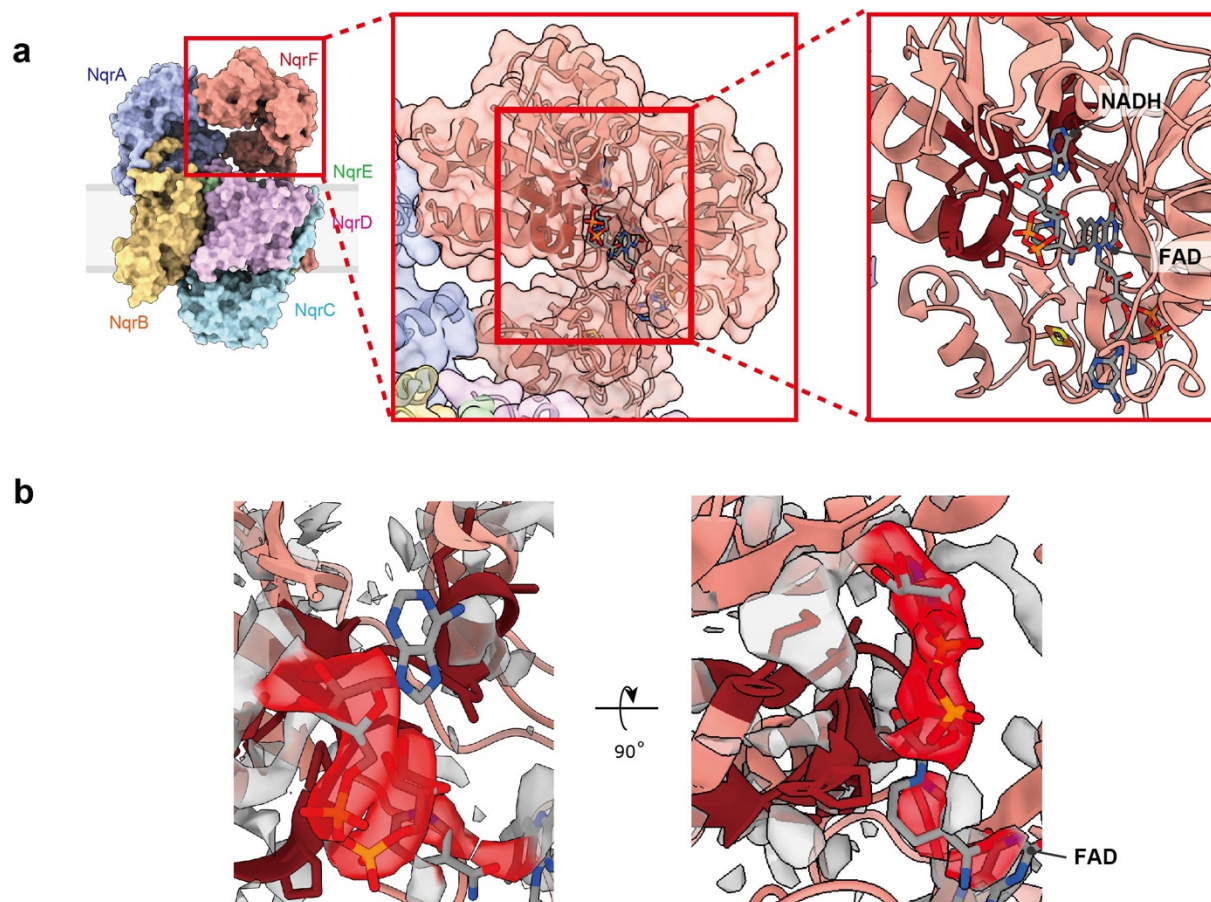
j



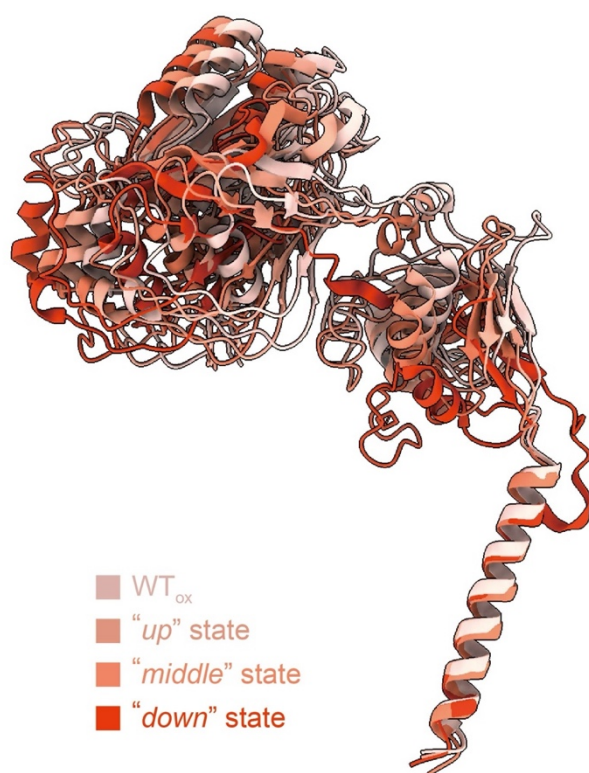
Supplementary Fig. 11 (continued)



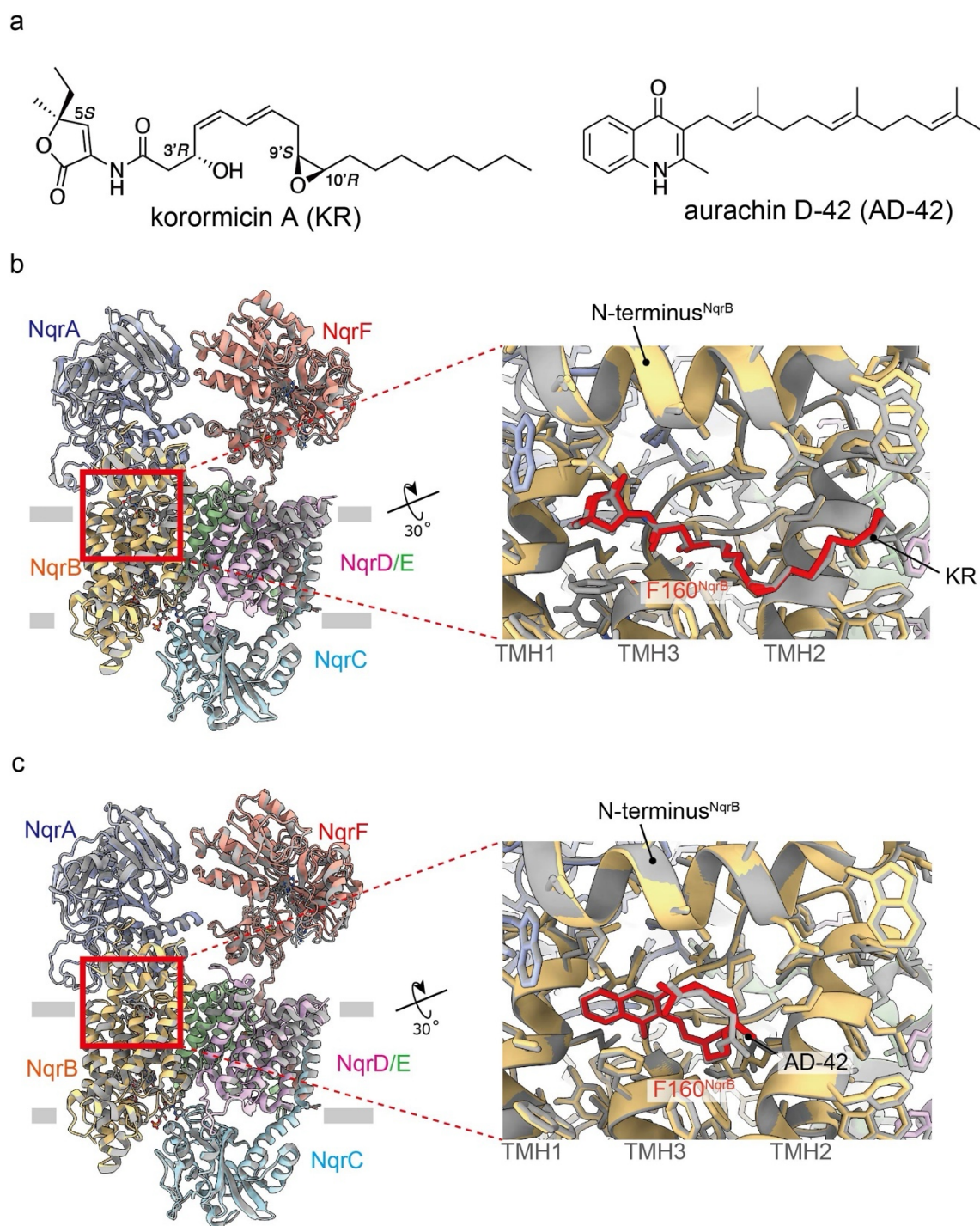
Supplementary Fig. 11: Shifts in the conformation of the TMHs in NqrCDEF. The TMHs architecture of NqrCDEF. The *left* and *middle* panels show cytoplasmic views and periplasmic views, respectively. To help visualize the transition, the WT_{ox} structure is superimposed in *gray*. The *right* panel shows the sliced view. *a*: WT_{red}, *b*: “*up*” state from WT_{red}/–Na⁺, *c*: “*middle*” state from WT_{red}/–Na⁺, *d*: NqrB-T236Y_{ox}, *e*: NqrB-T236Y_{red}, *f*: NqrC-T225Y_{ox}, *g*: NqrC-T225Y_{red}, *h*: WT_{red} + KR, and *i*: WT_{red} + AD-42 are compared with WT_{red}, respectively. *j*: Maps for each helix with the structural model of NqrB-G141A_{red} + KR (in “*stable*” state) *k*: Maps for each helix with the structural model of NqrB-G141A_{red} + KR (in “*shifted*” state).



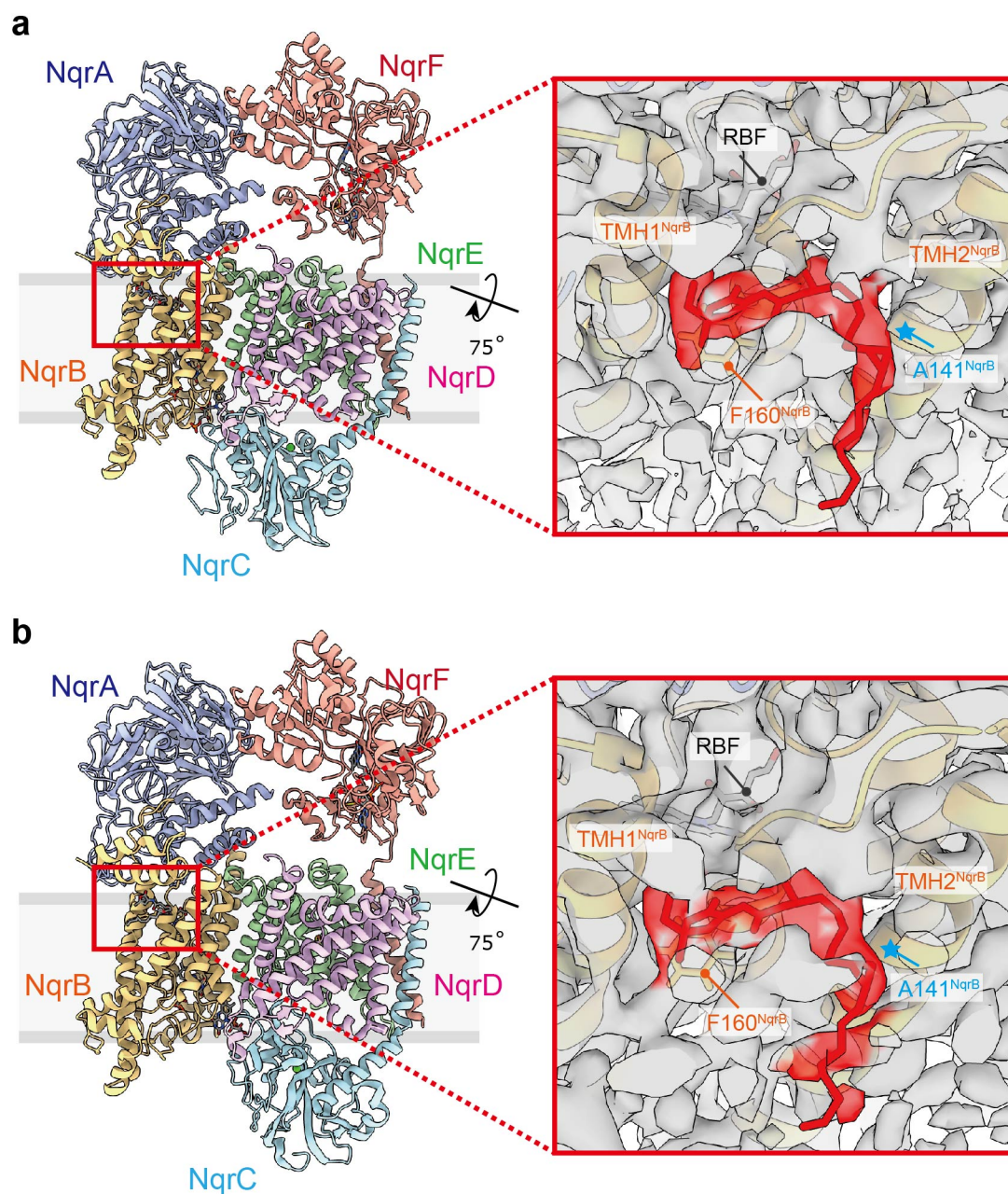
Supplementary Fig. 12: The binding site and density map of NADH. *a*, The NADH-binding site in the entire **WT_{red}**. *Red square* indicates the NADH-binding domain. *Dark red* residues indicate the Rossmann motif of the NADH-binding site. *b*, The Fo-Fc density map around NADH in NqrF; the density of NADH is colored *red*.



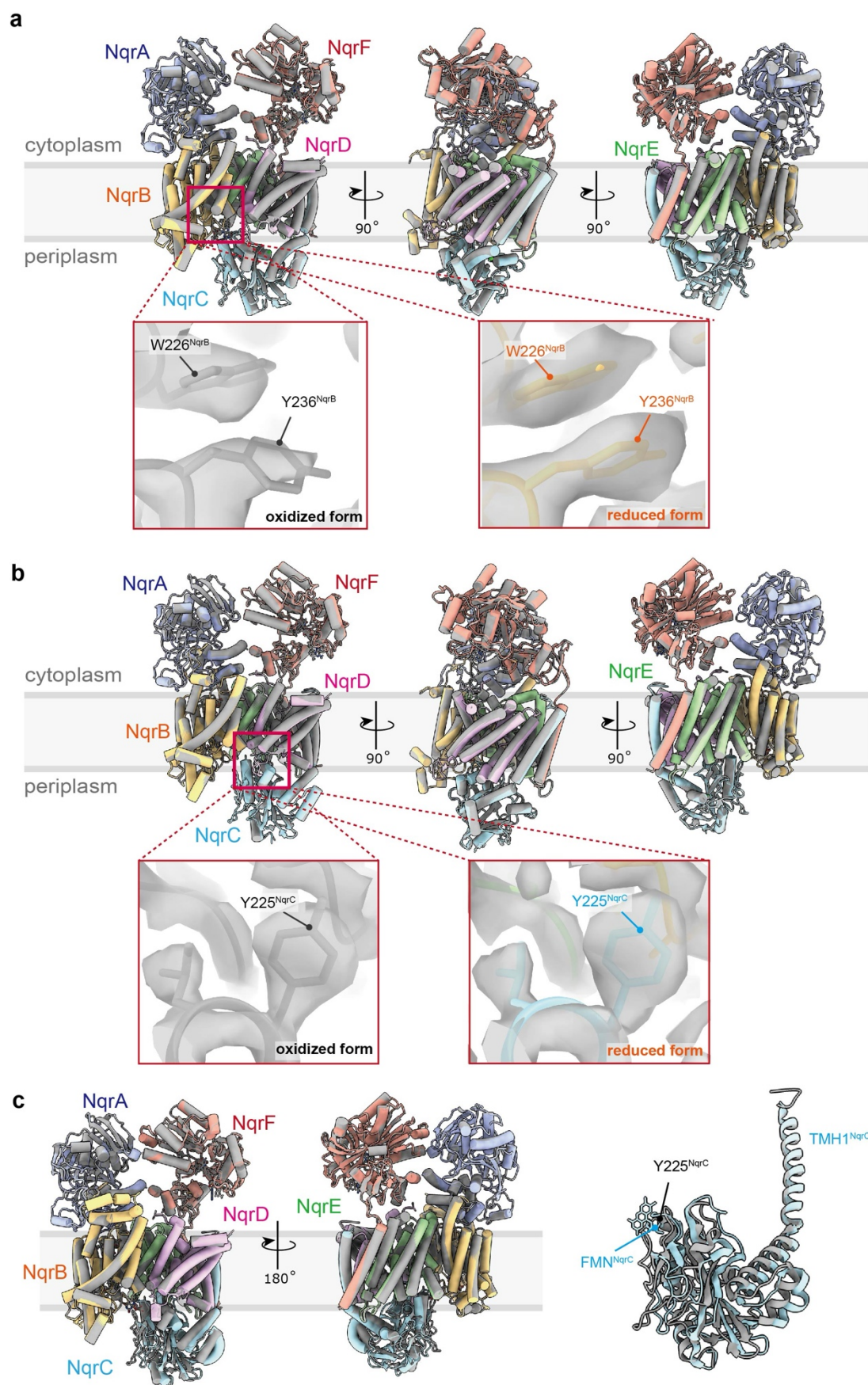
Supplementary Fig. 13: Structural superposition of the NqrF subunit. Structures of the NqrF subunit in the **WT_{ox}**, "**up**", "**middle**", and "**down**" states are superimposed. Each state is colored as follows: **WT_{ox}** (*light pink*), "**up**" state (*light brown*), "**middle**" state (*orange*), and "**down**" state (*red*), corresponding to the color key shown in the figure. This overlay highlights the conformational differences among the four states.



Supplementary Fig. 14: The structures of reduced Na⁺-NQR wild-type with bound inhibitors. *a*, Chemical structure of korormicin A (KR) and aurachin D-42 (AD-42). The *gray* and *colored* indicate the structures of WT_{ox} and WT_{red} with bound inhibitors, respectively. *b*, Comparison of WT_{ox} + KR and WT_{red} + KR. *c*, Comparison of WT_{ox} + AD-42 vs. WT_{red} + AD-42. The *colored* and *gray* structures correspond to WT_{red} and WT_{ox} with bound inhibitors, respectively.



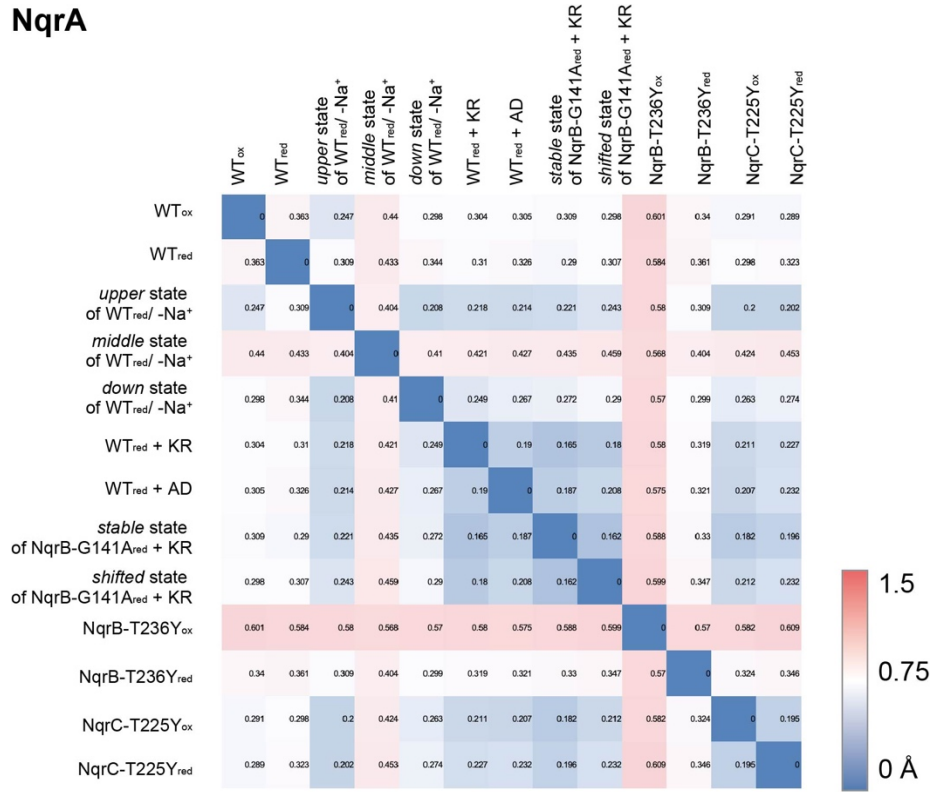
Supplementary Fig. 15: Structure of Na⁺-NQR with bound korormicin. The structure and density map of NqrB-G141A_{red} with bound KR. The density and model of KR are shown in *red*. *a*, The “*stable*” state. *b*, The “*shifted*” state.



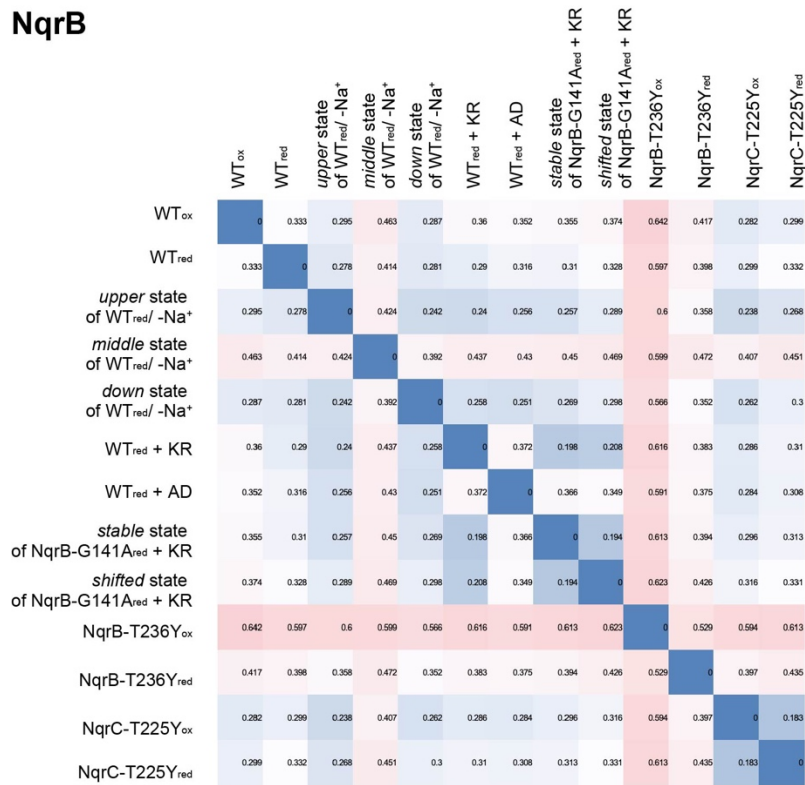
Supplementary Fig. 16 (continued)

Supplementary Fig. 16: The architecture of FMN deletions. *a*, Comparison of **NqrB-T236Y_{ox}** (*gray*) and **NqrB-T236Y_{red}** (*color*). *Red square* shows the position of the point mutation. NqrB-Y236 in each form is zoomed up with a density map in the bottom pictures. *b*, Comparison of **NqrC-T225Y_{ox}** (*gray*) and **NqrC-T225Y_{red}** (*color*). *Red square* shows the position of the point mutation. NqrC-Y225 in each form is zoomed up with a density map in the bottom pictures. *c*, Comparison of **NqrC-T225Y_{red}** (*gray*) and the “*shifted*” state of **NqrB-G141A_{red} + KR** (*color*). NqrC is highlighted in the right panel.

NqrA



NqrB



Supplementary Fig. 17 (continued)

NqrC

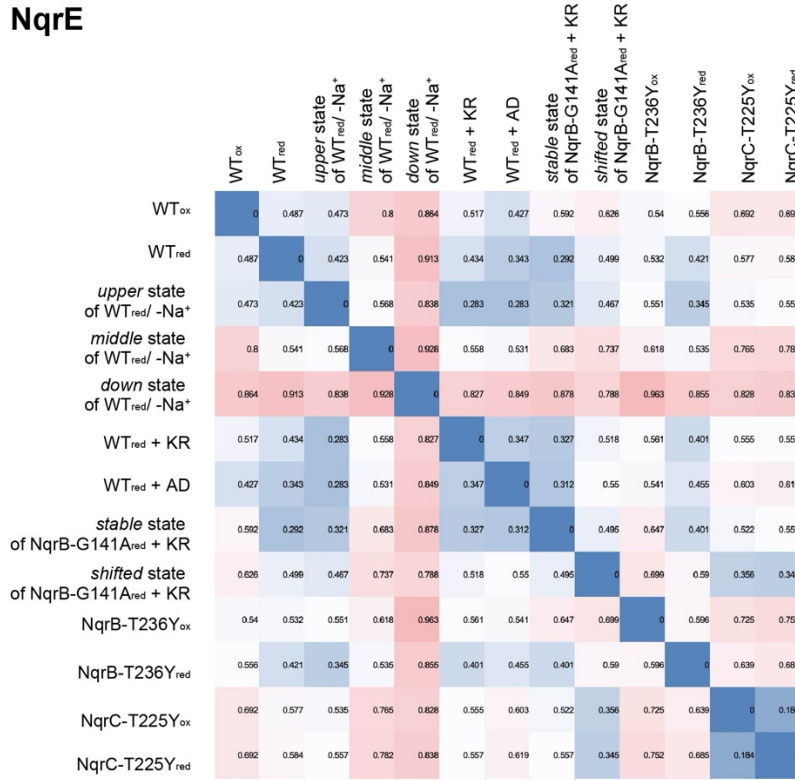
	WT _{ox}	WT _{red}	upper state of WT _{red} / -Na ⁺	middle state of WT _{red} / -Na ⁺	down state of WT _{red} / -Na ⁺	WT _{red} + KR	WT _{red} + AD	stable state of NqrB-G141A _{red} + KR	shifted state of NqrB-G141A _{red} + KR	NqrB-T236Y _{ox}	NqrB-T236Y _{red}	NqrC-T225Y _{ox}	NqrC-T225Y _{red}
WT _{ox}	0	0.467	0.422	0.58	0.572	0.45	0.462	0.447	1.353	0.656	0.534	0.552	0.632
WT _{red}	0.467	0	0.423	0.57	0.595	0.423	0.422	0.376	1.424	0.673	0.564	0.584	0.699
upper state of WT _{red} / -Na ⁺	0.422	0.423	0	0.524	0.496	0.336	0.336	0.336	1.353	0.65	0.451	0.559	0.633
middle state of WT _{red} / -Na ⁺	0.58	0.57	0.524	0	0.452	0.539	0.537	0.594	1.373	0.718	0.581	0.701	0.758
down state of WT _{red} / -Na ⁺	0.572	0.595	0.496	0.452	0	0.523	0.493	0.568	1.377	0.712	0.596	0.596	0.671
WT _{red} + KR	0.45	0.423	0.336	0.539	0.523	0	0.33	0.33	1.367	0.679	0.513	0.58	0.643
WT _{red} + AD	0.462	0.422	0.338	0.537	0.493	0.33	0	0.351	1.437	0.667	0.499	0.58	0.662
stable state of NqrB-G141A _{red} + KR	0.447	0.376	0.338	0.594	0.568	0.33	0.351	0	1.358	0.651	0.523	0.561	0.626
shifted state of NqrB-G141A _{red} + KR	1.353	1.424	1.353	1.373	1.377	1.367	1.437	1.358	0	1.323	1.377	1.202	1.221
NqrB-T236Y _{ox}	0.656	0.673	0.65	0.718	0.712	0.679	0.667	0.651	1.323	0	0.673	0.656	0.698
NqrB-T236Y _{red}	0.534	0.564	0.451	0.581	0.596	0.513	0.499	0.523	1.377	0.673	0	0.641	0.721
NqrC-T225Y _{ox}	0.552	0.654	0.559	0.701	0.596	0.58	0.68	0.561	1.202	0.656	0.641	0	0.474
NqrC-T225Y _{red}	0.632	0.699	0.633	0.758	0.671	0.643	0.662	0.626	1.221	0.688	0.721	0.474	0

NqrD

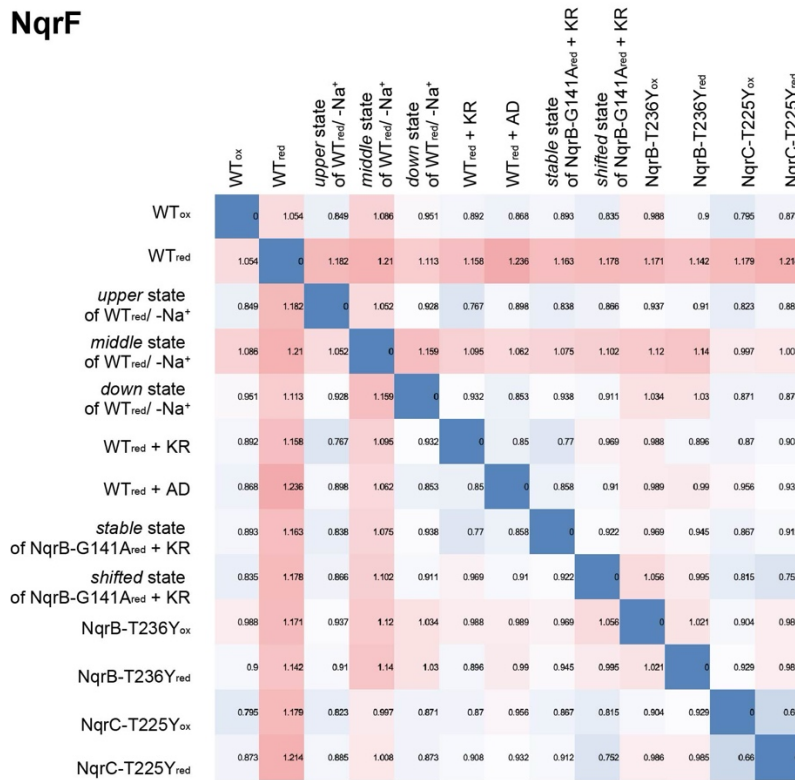
	WT _{ox}	WT _{red}	upper state of WT _{red} / -Na ⁺	middle state of WT _{red} / -Na ⁺	down state of WT _{red} / -Na ⁺	WT _{red} + KR	WT _{red} + AD	stable state of NqrB-G141A _{red} + KR	shifted state of NqrB-G141A _{red} + KR	NqrB-T236Y _{ox}	NqrB-T236Y _{red}	NqrC-T225Y _{ox}	NqrC-T225Y _{red}
WT _{ox}	0	0.403	0.389	0.588	0.506	0.408	0.377	0.431	0.703	0.556	0.458	0.777	0.77
WT _{red}	0.403	0	0.317	0.56	0.505	0.315	0.324	0.287	0.61	0.504	0.357	0.654	0.645
upper state of WT _{red} / -Na ⁺	0.389	0.317	0	0.558	0.493	0.282	0.257	0.294	0.562	0.505	0.305	0.652	0.638
middle state of WT _{red} / -Na ⁺	0.588	0.56	0.558	0	0.52	0.582	0.557	0.561	0.753	0.649	0.614	0.844	0.86
down state of WT _{red} / -Na ⁺	0.506	0.505	0.493	0.52	0	0.511	0.436	0.526	0.727	0.633	0.548	0.911	0.772
WT _{red} + KR	0.408	0.315	0.282	0.592	0.511	0	0.262	0.23	0.527	0.498	0.329	0.604	0.556
WT _{red} + AD	0.377	0.324	0.257	0.557	0.436	0.262	0	0.276	0.58	0.531	0.309	0.66	0.653
stable state of NqrB-G141A _{red} + KR	0.431	0.287	0.264	0.561	0.526	0.23	0.276	0	0.51	0.497	0.355	0.594	0.567
shifted state of NqrB-G141A _{red} + KR	0.703	0.61	0.562	0.753	0.727	0.527	0.58	0.51	0	0.728	0.544	0.37	0.376
NqrB-T236Y _{ox}	0.556	0.504	0.505	0.649	0.633	0.498	0.531	0.497	0.728	0	0.536	0.836	0.84
NqrB-T236Y _{red}	0.458	0.357	0.305	0.614	0.548	0.329	0.309	0.355	0.544	0.536	0	0.607	0.622
NqrC-T225Y _{ox}	0.777	0.654	0.652	0.844	0.811	0.604	0.66	0.594	0.37	0.636	0.607	0	0.239
NqrC-T225Y _{red}	0.77	0.645	0.638	0.86	0.772	0.566	0.653	0.567	0.376	0.84	0.622	0.239	0

Supplementary Fig. 17 (continued)

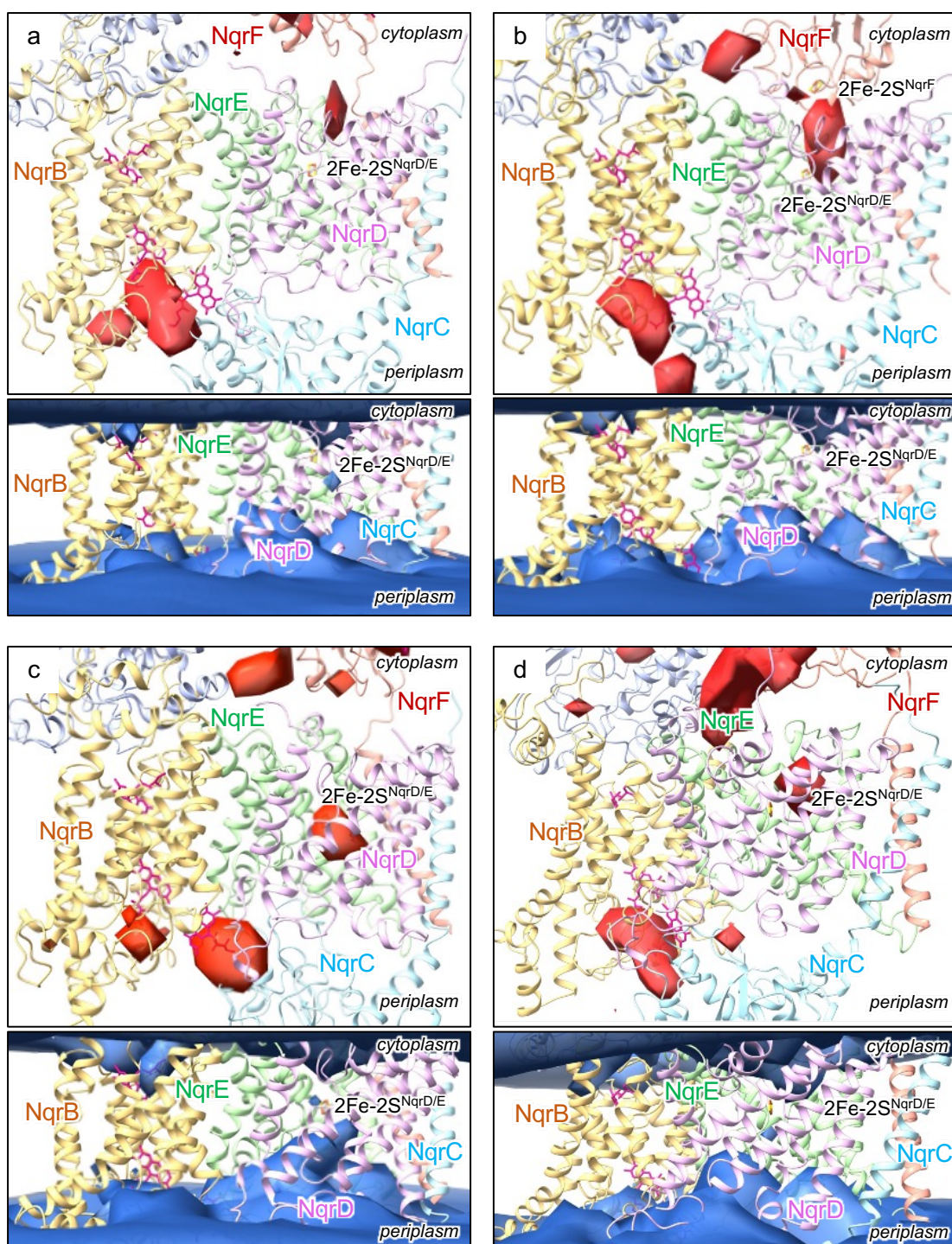
NqrE



NqrF



Supplementary Fig. 17: The structural similarities of each subunit of Na⁺-NQR.. The heat maps of NqrA-F subunits based on r.m.s.d value. The value is calculated with UCSF chimera.



Supplementary Fig. 18: Na^+ and water spatial densities from conventional MD simulations. Spatial densities from *a*: cMD3, *b*: cMD4, *c*: cMD5, and *d*: cMD6 simulations are shown. See **Supplementary Table 2** for details of each simulation. The density of Na^+ is shown in red. The relative iso-density surface of 3.5 to the bulk value is shown. The density of water is shown in blue. The relative iso-density surface of 0.3 to the bulk value is shown.

a NqrD

		TMH1		TMH2	
V. cholerae	---	MSSAKELKKS	VLAPVLDNNPIALQVL	GVCSALAVTTKLETA	FVMTLAVMFVTALSNF 57
C. trachomatis	---	MTNKSYLTYFTDALW	INNQPLIAI	LGISALAVTTT	VTTALTMGFAVSFVTGCSSF 57
B. fragilis	-	MSQLFSKKNKEVFAT	PLGLNNPVTQVL	LGISALAVTAKLEPA	IVMGLSVTVITAFSNV 59
P. aeruginosa	---	MAAQPTIREVLFPV	FQNNPIGLQI	LGISALAVTSNLKTAT	VMIAIALTLVTGFSNL 57
H. influenzae	---	MSGKTSYKDLLLAPI	AKNNPIALQI	LGISALAVTTKLETA	FVMAIAVTLVTGLSNL 57
K. pneumoniae	MAEQSDMKEVKRVL	VGPLIANNPIALQVL	GVCSALAVTTKLETA	FVMTIAVTLVTAFSSM 60	
Y. pestis	---	MADSKEIKRVLLSPL	FDNNPIALQI	GVCSALAVTTKLETA	LVMTLAVTLVTAFSSF 57
		TMH3		TMH4	
V. cholerae	FVSLIRNHIPNSVRI	IVQMAIIASLVIVDQIL	KAYLYDISKQLSVFVGL	LIITNCI	VMGR 117
C. trachomatis	VVSLLRKITPESVR	MIAQLIISLFFVILIDQFL	KAFFFTISKTLSVFVGL	LIITNCI	VMGR 117
B. fragilis	VISLLRKTI	PNRIRIIVQLVVAA	LVTIVSEVLKAFAYDV	SVQLSVYVGL	LIITNCI 119
P. aeruginosa	FISMIRRQIPSSIR	MIVQMVIASLVIVDQVL	KAYAYSLSKQLSVFVGL	LIITNCI	VMGR 117
H. influenzae	FVSLIRNYIPNSIR	IIVQLAIIASLVIVDQIL	KAYAYGLSKQLSVFVGL	LIITNCI	VMGR 117
K. pneumoniae	FISMIRHHIPNSVRI	IVQMAIIASLVIVDQVL	RAFAYETSKQLSVFVGL	LIITNCI	VMGR 120
Y. pestis	FISLIRNHIPNSVRI	IVQMVIASLVIVDQVL	RAYAYEISKQLSVFVGL	LIITNCI	VMGR 117
		TMH5			
V. cholerae	AEAFAMKSEPIPSF	IDGIGNGLGYGFVLM	TVGFFRELLGSGKLF	GLEVLPLISNG	----- 172
C. trachomatis	AESMARHVSP	IPAFLDGLGSGGLGYGW	LVCIISIRELFGFGTIL	GFRVIPEILYTSAAHP	177
B. fragilis	LEAFAMANGPWES	FLDGVGNGLGYAKIL	IIVAFFRELLGSGTLL	NFRIIPESFYK	----- 174
P. aeruginosa	AEAFAMANPPLVS	FFDGIGNGLGYGSAM	LLVLGVFVRELFAGKLYG	ISVLPVTNDG	----- 172
H. influenzae	AEAFAMKSPPVES	FVDGIGNGLGYGSML	IIVAFFRELLGSGKLF	GMTIFETIQNG	----- 172
K. pneumoniae	AEAYAMKSPPLAS	FMDGIGNGLGYGAIL	IIVGFLRELIGSGKLF	GITVLETQNG	----- 175
Y. pestis	AEAYAMKSPPIES	FMDGIGNGLGYGVIL	VLGVFVRELVGSGKLF	GVTVLETQNG	----- 172
		TMH6			
V. cholerae	GWYQPNGLMLLAP	SAFFLIGFMIWAIR	TFKPEQVEAKE	-----	210
C. trachomatis	DGYENLGLMVLAP	SAFFLLGIMIIVNI	IRAPKTKR	-----	213
B. fragilis	MGYINNGLMMLPP	MALIIICACIIWYQ	RSRCKELQEK	-----	210
P. aeruginosa	GWYQPNGLLLLPP	SAFFLIGLIIWAL	RTWKDQVEAPTYK	MAPQVSSKEAY	223
H. influenzae	GWYQANGLFLLAP	SAFFIIGFVIWGL	RTWKPEQKE	-----	208
K. pneumoniae	GWYQPNGLFLLAP	SAFFIIGLLIWA	LSWKPEQKE	-----	212
Y. pestis	GWYLPNGLFLLAP	SAFFIIGLLIWL	RTLKPAQIEKE	-----	209

Supplementary Fig. 19 (continued)

b NqrE

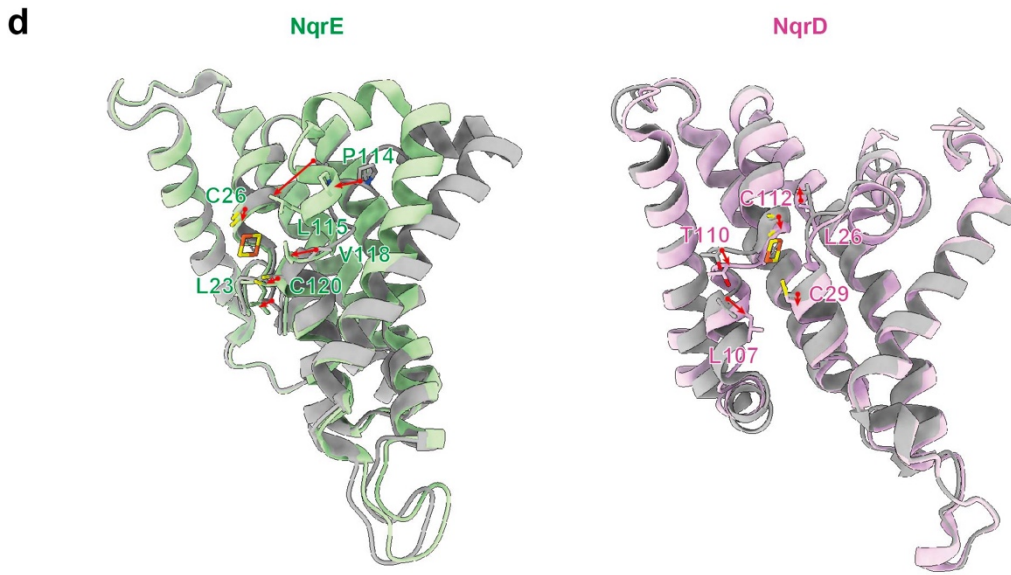
	TMH1	TMH2
V. cholerae	-----MEHYISLLVKSIF IENMALSF [*] FLGMC [*] TF LAVSKKVKTSFGLGIAVIVVLTISV 53	
C. trachomatis	MWSGDYSLLNLLGIFLQATFIQNIILLSTFLGMC [*] SYLACSSRLSTANGLGMSVALVLTITG 60	
B. fragilis	-----MEQLLSLFVRSIFVDNMIFAFFLGMCSYLAVSKNVKTAVGLGIAVTFVLVLT 53	
P. aeruginosa	-----MEHYISLFVKAVFVENMALAFFLGMCTFIAISKKVETAIGLGIIVVQTITV 53	
H. influenzae	-----MEHYISLFVKAVFIENMALSF [*] FLGMC [*] TF LAVSKKVSTAFGLGIAVTFVLGIAV 53	
K. pneumoniae	-----MAHYISLFVRAVFVENMALAFFLGMCTFLAVSKKVSTAFGLGVAVTVVGLAV 53	
Y. pestis	-----MEHYISLLVRAVFVENMALAFFLGMCTFLAVSKKVSTAFGLGIAVTVVLGISV 53	
	TMH3	
V. cholerae	PVNNLVYNLVLPD [*] ALV-----EGVDLSFLNFITFIGVIAALVQILEMILDRFFPPLYN 107	
C. trachomatis	SINWL [*] VHYFITKPGALAWLSPALANIDLSFLELIMFIVVIAAFTQILELLERFSRNLYL 120	
B. fragilis	PVNYLLQTKVLAANAI-----EGVDLSFLSFI [*] LFI [*] AVIAGIVQLVMVVERFSPSLYA 107	
P. aeruginosa	PANNLIYTYLLKDGALAWAG--LPEVDLSFLGLLSYIGVIAAIVQILEMLDKYVPSLYN 111	
H. influenzae	PVNQLIYANVLKENALI-----EGVDLSFLNFITFIGVIAGLVQILEMVLDKFMPSPLYN 107	
K. pneumoniae	PINNLVYNLVLRD [*] GALV-----EGVDLSFLNFITFIGVIAALVQILEMILDKYFPALYN 107	
Y. pestis	PANNLVYNLVLRD [*] GALV-----EGVDLSFLNFITFIGVIAAIVQVLEMILDRYFPALYN 107	
	TMH4	TMH5
V. cholerae	ALGIFLPLIT [*] VNCAIFGGVSFMVQRDYS-----FAESVYVGFGSGVGWMLAIVAL 157	
C. trachomatis	ALGIFLPLIAVNCAILGGVLFGITRNYP-----FLPMVVFSLGSGCGWMLAIVLF 170	
B. fragilis	SLGIFLPLIAVNCAIMGASLFMQQRITMDPSNPQAITGVGS [*] AVVYALGSGIGWLLAIVGL 167	
P. aeruginosa	ALGVFLPLITVNCAIMAGSLFMVERDYN-----LAESTVYGVGSGFSWALAIAAL 161	
H. influenzae	ALGIFLPLIAVNCAIFGGVSFMVQRDYN-----FPESIVYVGFGSGLGWMLAIVAL 157	
K. pneumoniae	ALGIFLPLIAVNCAIFGGVSFMVQRDYN-----FPESIVYVGFGSGIGWMLAIVAM 157	
Y. pestis	ALGIFLPLITVNCAIFGGVSFMAORDYN-----FPESIVYVGFGSGMGWMLAIVAL 157	
	TMH6	
V. cholerae	AGIREKMKYSDVPPGLRGLGITFITAGLMALGFM [*] SFSGVQL----- 198	
C. trachomatis	ATIREKLAYS [*] DPQHLRGTGISFIT [*] TGLMAMAFMGLTGIDISKPTTSKPAFVTNIATDSP 230	
B. fragilis	AAIREKMAYS [*] DPAPLKGLGITFITVGLMAMAFMCFSGLKL----- 208	
P. aeruginosa	AGIREKLKYSDVPEGLQGLGITFITIGLSLGFM [*] SFSGVQL----- 202	
H. influenzae	AGLTEKMKYADIPAGLKGLGITFISVGLMALGFM [*] SFSGIQL----- 198	
K. pneumoniae	AGIREKMKYANVPAGLRGLGITFIT [*] TGLMALGFM [*] SFSGVQL----- 198	
Y. pestis	AGIREKMKYANVPAGLQGLGITFISTGLMALGFM [*] SFAGVNL----- 198	
V. cholerae	----- 198	
C. trachomatis	QPNTHSSSEEPKAS 244	
B. fragilis	----- 208	
P. aeruginosa	----- 202	
H. influenzae	----- 198	
K. pneumoniae	----- 198	
Y. pestis	----- 198	

Supplementary Fig. 19 (continued)

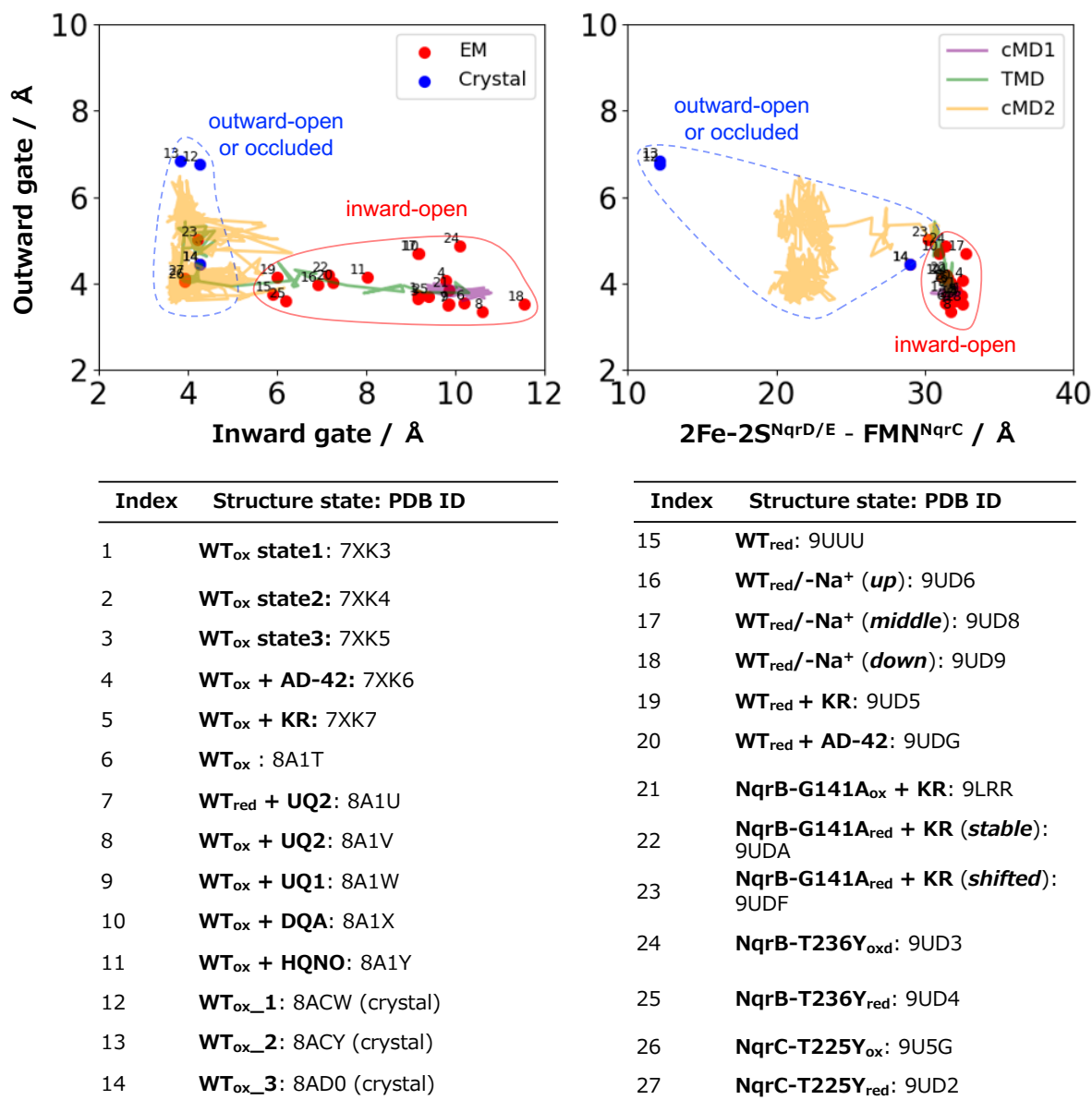
c Na⁺-NQR vs RNF

NqrD	MSSAKELKKSVLAPVLDNNPIALQVLGVCSALAVTTKLETAFVMTLAVMFVTALSNFFVS	60
RnfE	---MGVVSERLYNGIVKENATFVQVLGMCP TLAVTTSAINGIGMGLSATVVVLIGSNVVIS	57
NqrD	LIRNHIPNSVRIIVQMAITASLVIIVDQILKAYLYDISKQLSVFVG LIITNC IVMGRAEA	120
RnfE	LLKKVIPDEIRIPAYITVIATLVTVLQFLLQAYLPDLNKS LGIFIP LIVVNC IILGRAEA	117
NqrD	FAMKSEPIPSFIDGIGNGLGYGFVIMTVGFFRELLGSGKLFGLLEVLP LISNGGWYQPNGL	180
RnfE	YANKNSVGASFFDGLGMGLGFTVSLAALGIIREFLGTGKVFGAQITP-----DAFQPALI	172
NqrD	MLLAPSAFFLIGFMIWAIRTFKPEQVEAKE	210
RnfE	MILAPGGFFTLGILMAILNQRKLKKAKAK-	201
NqrE	MEHYISLLVKSIFIENMALSFFLGMCTFLAVSKKVKTSFGLGIAVIVVLTISVPVNNLVY	60
RnfA	-MSIFTIFISALLVNNFVLSRFLGICPFLGVSKKVETATGMGAAVTFVMALAAIMTFLVE	59
NqrE	NLVLPDALVEGVDSLNFITFIGVIAALVQILEMILDRFFPPLYNALGIFLPLITVNC	120
RnfA	RFIL-----IPLNIQYLSTLAFILVIASLVQFVEMVIKKVSPDLYKALGIYLP LITVNC	113
NqrE	AIFGGVSFMVQRDYSFAESVVYGFSGVGWMLAIVALAGIREKMKYSD-VPPGLRGLGIT	179
RnfA	AVLGMVINSNEKYNLIQSIINSVGAALGFTLALVLLAGIREKMETNEYIPEALKGLPIT	173
NqrE	FITAGLMALGFMSFSGVQL	198
RnfA	LVTAGLMAIAFLGFQGLI-	191

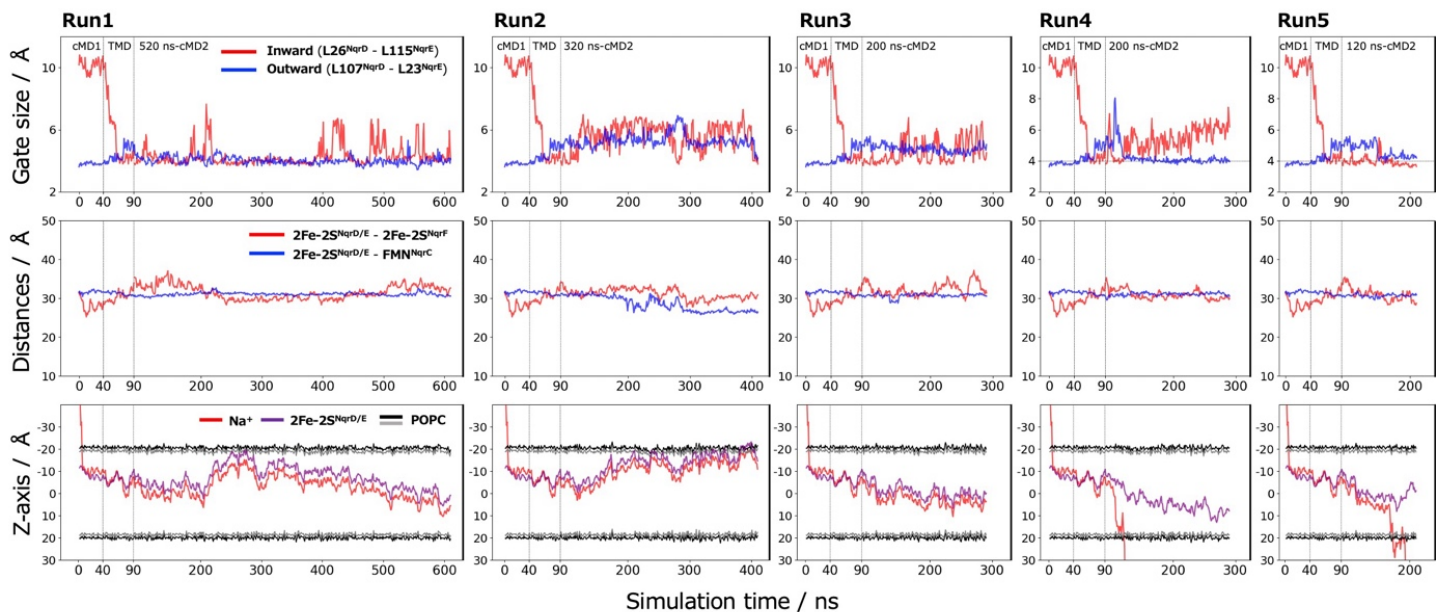
Supplementary Fig. 19 (continued)



Supplementary Fig. 19: Conserved Amino Acid Residues in NqrD and NqrE. Protein sequence alignments were generated using Clustal Omega (<https://www.ebi.ac.uk/jdispatcher/msa/clustalo>). Panels a and b display the alignments of NqrD and NqrE, respectively. Panel c compares NqrD/E of Na⁺-NQR from *V. cholerae* with RnfA/E of RNF from *A. woodii*. The *orange marker* indicates fully conserved residues, while the *yellow marker* signifies strong similarity (scoring > 0.5 in the Gonnet PAM 250 matrix), and the *light green marker* indicates weak similarity (scoring ≤ 0.5 in the Gonnet PAM 250 matrix). Four cysteines that coordinate an iron-sulfur cluster (Cys20 in NqrD and Cys20 in NqrE) are denoted with an *asterisk* (*). Essential residues for Na⁺ ion binding are indicated in *pink*: Leu26, Cys29, Leu107, Thr110, and Cys112 in NqrD; Leu23, Cys26, Leu115, Val118, and Cys120 in NqrE. Gray arrows show the alpha helices. Panel d highlights the conserved amino acid residues essential for Na⁺ translocation. The gray and colored structures correspond to the “shifted” state of NqrB-G141Ared + KR and WTox, respectively. Conserved residues implicated in Na⁺ translocation are highlighted in NqrE (left) and NqrD (right). Red arrows indicate the direction of residue displacement associated with the *inward/outward* transition.

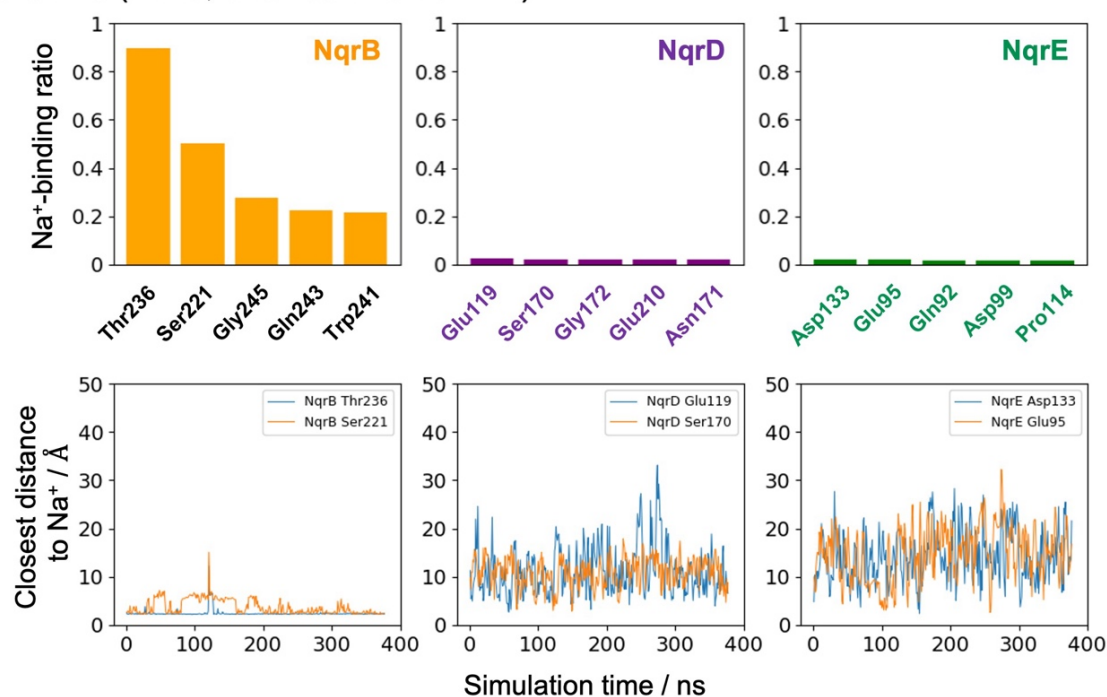


Supplementary Fig. 20: MD simulation trajectory compared to experimental structures. The size of the inward and outward gates is defined by the closest heavy atom distances: between Leu26 of NqrD and Leu115 of NqrE for the inward gate, and between Leu107 of NqrD and Leu23 of NqrE for the outward gate (see Fig. 4a). The *left* panel shows a 2D plot of the distances for the inward and outward gates. The *right* panel displays a 2D plot illustrating the distance between the cofactors (2Fe-2S in NqrD/E) and FMN in NqrC, along with the outward gate distance. The values can be found in the table at the bottom.

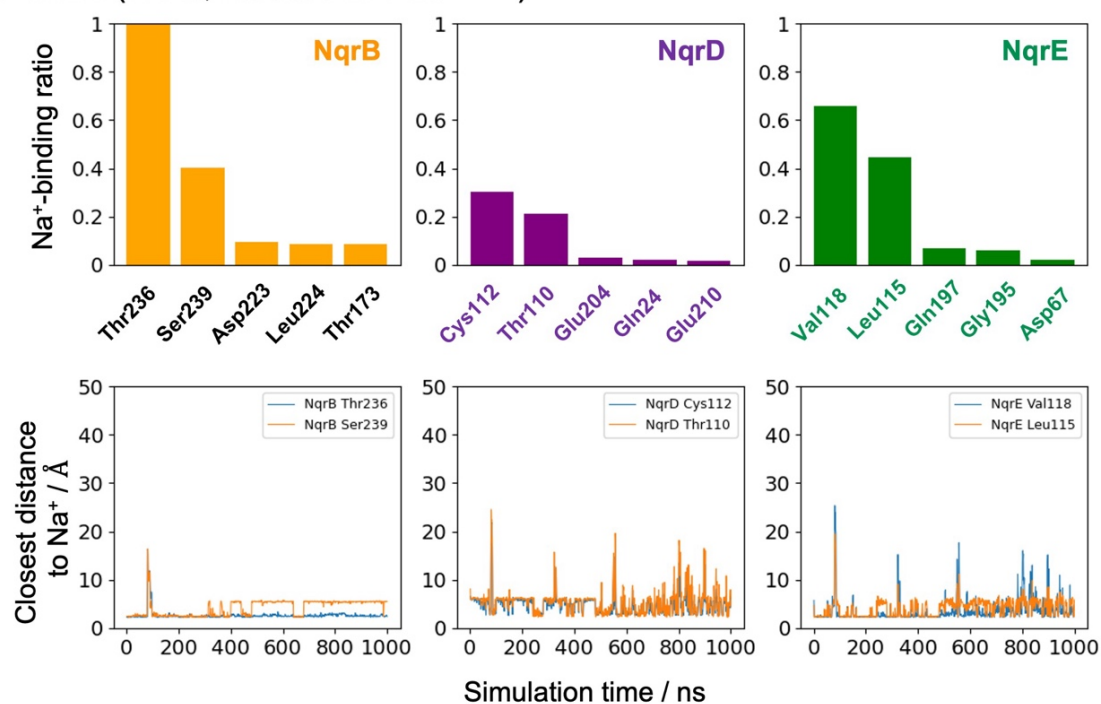


Supplementary Fig. 21: Additional five independent trajectories of cMD2 following TMD. *Upper column:* The inward gate and outward gate distances using a closest heavy-atom pair are plotted. *Middle column:* The distances between $2\text{Fe}-2\text{S}^{\text{NqrD/E}}-2\text{Fe}-2\text{S}^{\text{NqrF}}$ and $2\text{Fe}-2\text{S}^{\text{NqrD/E}}-\text{FMN}^{\text{NqrC}}$ using a closest heavy-atom pair are plotted. *Bottom column:* The z coordinates of the Na^+ and $2\text{Fe}-2\text{S}^{\text{NqrD/E}}$ in the NqrD/E transmembrane region are plotted with the membrane positions.

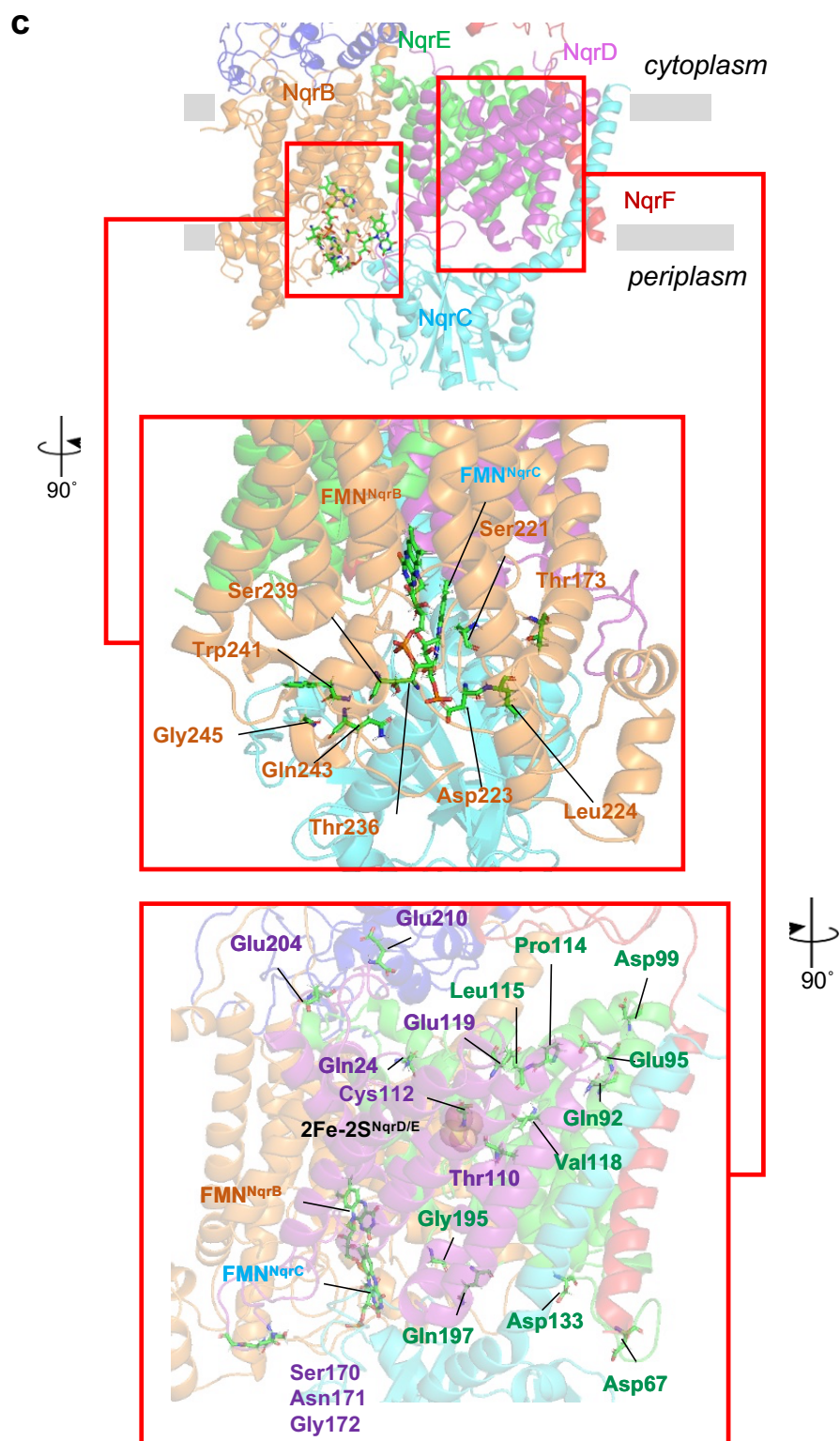
a cMD3 (7XK3, oxidized 2Fe-2S^{NqrD/E})



b cMD1 (7XK3, reduced 2Fe-2S^{NqrD/E})

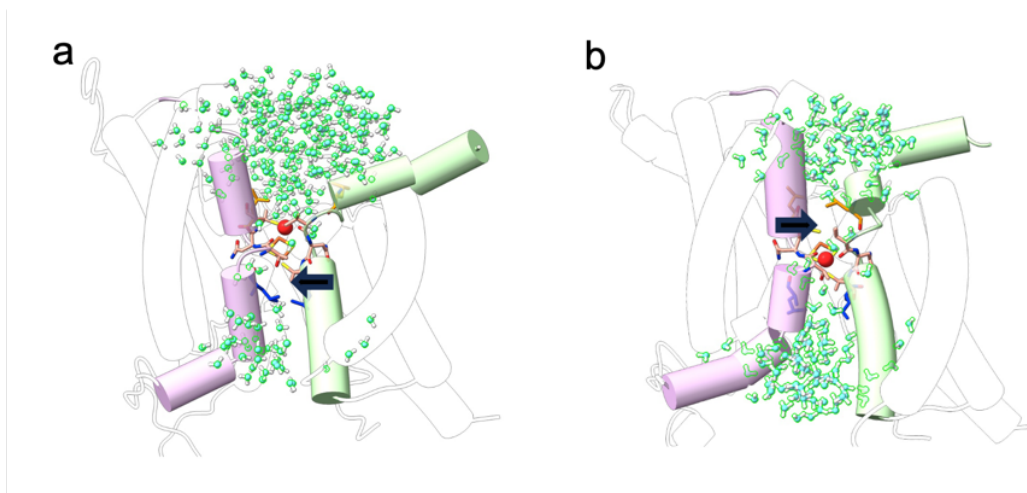


Supplementary Fig. 22 (continued)

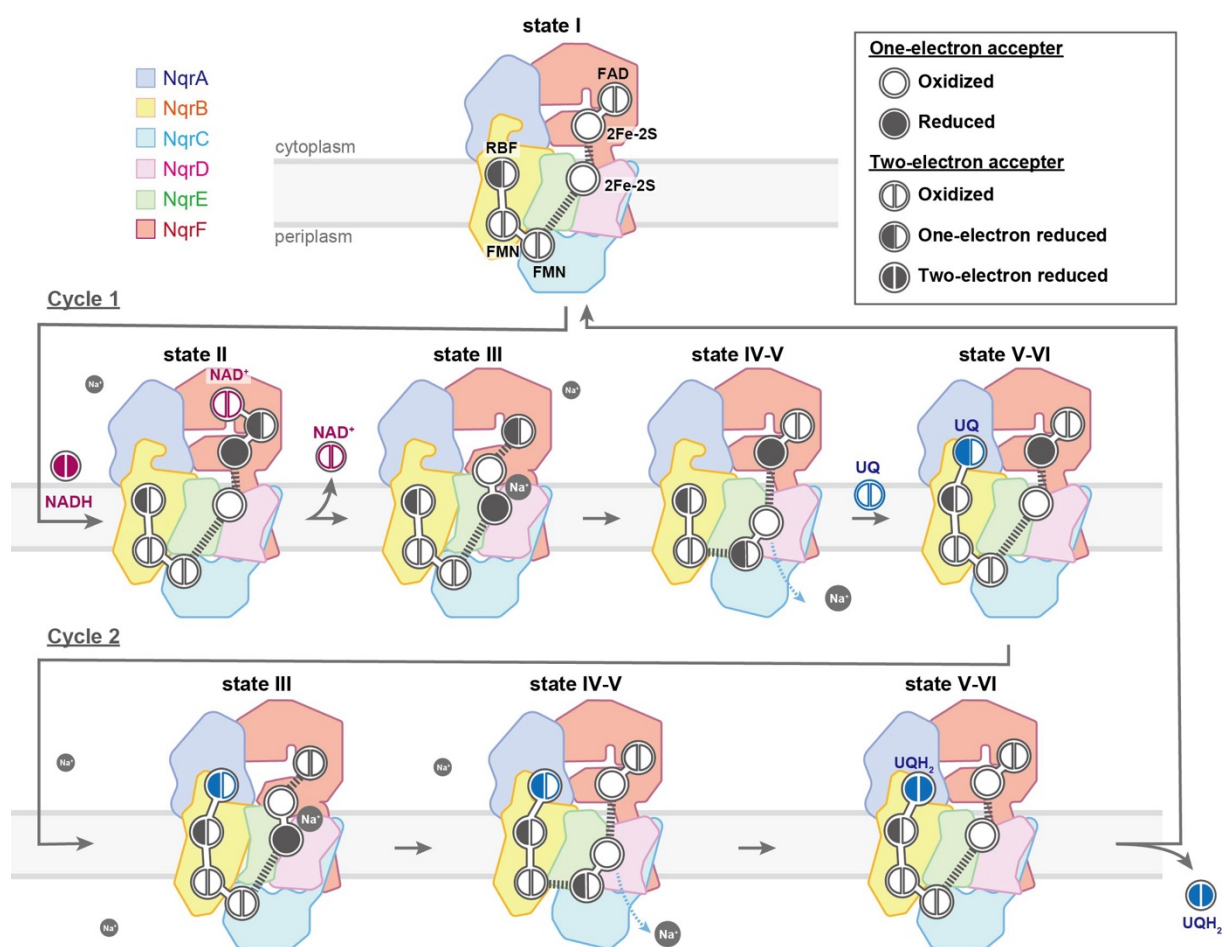


Supplementary Fig. 22 (continued)

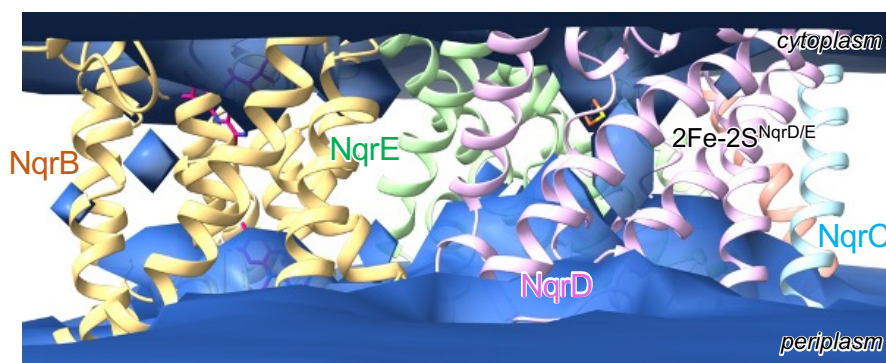
Supplementary Fig. 22: Analysis of amino acid residues that are involved in Na⁺ binding. a-b, Simulation is calculated at the scale of 400 ns. Contact ratio corresponds to the ratio of Na⁺-binding frame to total frame. Residues showing > 0.01 of contact ratio and the < 3 Å of distance to were listed up. The *top* panels show the top five residues that have high Na⁺ binding ratios for NqrB, NqrD, and NqrE, respectively. The Na⁺ binding was defined through the distance being less than 3Å. The *bottom* panels show the time course of the closest distances between the residues and Na⁺. **a,** Analysis of MD simulation with oxidized cofactors (cMD3). **b,** Analysis of MD simulation with reduced 2Fe-2S^{NqrD/E} cofactor (cMD1). **c,** The locations of amino acid residues in NqrB and NqrD/E that show a high Na⁺ binding ratio in MD simulations. The *top* panel indicates the relative positions of the two regions containing amino acid residues with a high Na⁺ binding ratio in TMHs. The *middle* panel shows the region around the FMN binding site in NqrB. The *bottom* panel shows the region of TMHs^{NqrCDEF} bundle.



Supplementary Fig. 23: Water orientation in the cytoplasmic-open *a*. and in the periplasmic-open state *b*. The oxygen and hydrogen atoms of water are shown in cyan and white, respectively. The arrow points to a spatial gap in water.*gap in water*.



Supplementary Fig. 24: The proposed catalytic mechanism of Na⁺-NQR. One-electron cofactors (e.g. 2Fe-2Ss) are shown as *full circles*; two-electron cofactors (e. g. NADH, FAD, FMNs, RBF, and UQ) are shown as two *semicircles*. *Black filled circles* or *semicircles* indicate reduction by one electron. When cofactors are close enough for electron transfer, the connection is shown as a *solid white bridge*; distances too long for electron transfer are shown with *dashes*. Since the oxidized state of RBF is reported to be a stable semiquinone, it is shown as two *semicircles*, one of which is always *black*. All snapshots were assigned to step I–IV described in the discussion.



Supplementary Fig. 25: Water spatial density from conventional MD (cMD1). The accessibility of water is shown in the model based on **WT_{ox}** (PDB ID; 7XK3) containing reduced 2Fe-2S^{NqrD/E}. The relative water density with iso-density surface of 0.1 to the bulk value is shown in blue.

Supplementary Table 1: Statistics of cryo-EM data, refinement, and validation of Na⁺-NQR.

	WT _{red}	NqrC-T225Y _{ox}	NqrC-T225Y _{red}
Data collection and processing			
Magnification	81,000	81,000	81,000
Voltage / kV	300	300	300
Total dose / e ⁻ Å ⁻²	60	60	60
Pixel size / Å	0.88	0.88	0.88
Defocus range / μm	-0.8 to -2.0	-0.8 to -2.0	-0.8 to -2.0
Symmetry imposed	C1	C1	C1
Recorded movies	6,086	13,302	13,316
Initial particles	1,391,844	2,497,690	2,378,419
States			
EMDB ID	EMDB-64518	EMDB-63872	EMDB-64059
PDB ID	9UUU	9U5G	9UD2
Final particle	245,271	579,365	1,436,839
Resolution (FSC _{0.143}) (Å)	3.1	2.7	2.6
Map sharpening B-factor (Å ²)	68.1	89.6	100.8
Model refinement			
Model resolution (FSC _{0.5}) (Å)	3.6	2.7	2.6
Composition			
Non-hydrogen atoms	14694	14765	14763
Residues	1892	1900	1900
Ligands	FMN: 2, FES: 2, RBF: 1, FAD: 1, NAI:1, CA: 1	FMN: 1, FES: 2, RBF: 1, FAD: 1, CA: 1, LMT: 2	FMN: 1, FES: 2, RBF: 1, FAD: 1, CA: 1, LMT: 2
Waters	0	7	5
Bond length (Å)	0.010	0.005	0.005
Bond angles (°)	0.999	0.608	0.698
Clash score	5.09	3.07	3.54
MolProbity score	1.65	1.33	1.61
B-factor overall			
Protein	119.27	120.69	50.57
Ligand	162.52	134.18	60.48
Ramachandran plot (%)			
Favored	94.36	96.50	96.56
Allowed	5.59	3.5	3.34
Outlier	0.05	0.00	0.11
Rotamer outlier (%)	0	0.90	1.61

Supplementary Table 1 (continued)

	NqrB-T236Y _{ox}	NqrB-T236Y _{red}
Data collection and processing		
Magnification	81,000	81,000
Voltage / kV	300	300
Total dose / e ⁻ Å ⁻²	60	60
Pixel size / Å	0.88	0.88
Defocus range / µm	-0.8 to -2.0	-0.8 to -2.0
Symmetry imposed	C1	C1
Recorded movies	4,440	8212
Initial particles	1,026,158	2,496,421
States		
EMDB ID	EMDB-64060	EMDB-64061
PDB ID	9UD3	9UD4
Final particle	79,750	319,845
Resolution (FSC _{0.143}) (Å)	3.8	3.3
Map sharpening B-factor (Å ²)	106.9	125.5
Model refinement		
Model resolution (FSC _{0.5}) (Å)	4.1	3.4
Composition		
Non-hydrogen atoms	14680	14720
Residues	1894	1894
Ligands	FMN: 1, FES: 2, RBF: 1, FAD: 1, PEE:2, CA: 1, LMT: 1	FMN: 1, FES: 2, RBF: 1, FAD: 1, CA: 1, LMT: 2
Waters	0	5
Bond length (Å)	0.004	0.003
Bond angles (°)	0.693	0.587
Clash score	9.88	6.23
MolProbity score	2.28	1.65
B-factor overall		
Protein	91.91	41
Ligand	101.43	70.18
Ramachandran plot (%)		
Favored	94.74	96.71
Allowed	5.26	3.24
Outlier	0.00	0.05
Rotamer outlier (%)	3.37	1.36

Supplementary Table 1 (continued)

	WT _{red} /- Na ⁺		
Data collection and processing			
Magnification	81,000		
Voltage / kV	300		
Total dose / e ⁻ Å ⁻²	60		
Pixel size / Å	0.88		
Defocus range / μm	-0.8 to -2.0		
Symmetry imposed	C1		
Recorded movies	13,302		
Initial particles	3,653,368		
States	“up” state	“middle” state	“down” state
EMDB ID	EMDB-64063	EMDB-64064	EMDB-64065
PDB ID	9UD6	9UD8	9UD9
Final particle	1,244,065	100,990	77,726
Resolution (FSC _{0.143}) (Å)	2.6	3.8	3.1
Map sharpening B-factor (Å ²)	101.8	108.2	70
Model refinement			
Model resolution (FSC _{0.5}) (Å)	2.7	3.9	3.2
Composition			
Non-hydrogen atoms	14743	14706	14665
Residues	1894	1894	1894
Ligands	FMN: 2, FES: 2, RBF: 1, FAD: 1, CA: 1, LMT: 2	FMN: 2, FES: 2, RBF: 1, FAD: 1, PEE:3, CA: 1, LMT: 1	FMN: 2, FES: 2, RBF: 1, FAD: 1, CA: 1
Waters	2	0	0
Bond length (Å)	0.004	0.005	0.003
Bond angles (°)	0.566	1.035	0.639
Clash score	2.50	8.71	3.40
MolProbity score	1.20	2.19	1.29
B-factor overall			
Protein	11.7.64	39.13	116.59
Ligand	129.35	55.90	141.01
Ramachandran plot (%)			
Favored	97.02	96.28	97.13
Allowed	2.98	3.71	2.82
Outlier	0.00	0.00	0.05
Rotamer outlier (%)	0.58	3.5	0.84

Supplementary Table 1 (continued)

NqrB-G141A _{red} + KR		
Data collection and processing		
Magnification	81,000	
Voltage / kV	300	
Total dose / e ⁻ Å ⁻²	60	
Pixel size / Å	0.88	
Defocus range / μm	-0.8 to -2.0	
Symmetry imposed	C1	
Recorded movies	5,086	
Initial particles	1,384,337	
States	"stable" state	"shifted" state
EMDB ID	EMDB-64066	EMDB-64068
PDB ID	9UDA	9UDF
Final particle	520,594	74,083
Resolution (FSC _{0.143}) (Å)	2.6	2.9
Map sharpening B-factor (Å ²)	86.0	68.1
Model refinement		
Model resolution (FSC _{0.5}) (Å)	2.8	3.1
Composition		
Non-hydrogen atoms	14963	15124
Residues	1921	1922
Ligands	IQT (KR):1, FMN: 2, FES: 2, RBF: 1, FAD: 1, CA: 1	IQT (KR):1, FMN: 2, FES: 2, RBF: 1, FAD: 1, PEE:2, CA: 1, LMT: 2
Waters	37	17
Bond length (Å)	0.197	0.196
Bond angles (°)	2.777	2.764
Clash score	2.84	6.13
MolProbity score	1.13	1.90
B-factor overall		
Protein	121.19	106.42
Ligand	119.94	92.40
Ramachandran plot (%)		
Favored	97.75	95.13
Allowed	2.25	4.71
Outlier	0	0.16
Rotamer outlier (%)	0.38	1.98

Supplementary Table 1 (continued)

	WT _{red} + KR	WT _{red} + AD
Data collection and processing		
Magnification	81,000	81,000
Voltage / kV	300	300
Total dose / e ⁻ Å ⁻²	60	60
Pixel size / Å	0.88	0.88
Defocus range / μm	-0.8 to -2.0	-0.8 to -2.0
Symmetry imposed	C1	C1
Recorded movies	4,881	5,096
Initial particles	2,157,492	1,072,343
States		
EMDB ID	EMDB-64062	EMDB-64069
PDB ID	9UD5	9UDG
Final particle	285,018	390,507
Resolution (FSC _{0.143}) (Å)	2.9	2.7 (J43)
Map sharpening B-factor (Å ²)	93.5	106.4
Model refinement		
Model resolution (FSC _{0.5}) (Å)	3.0	2.7
Composition		
Non-hydrogen atoms	14496	15132
Residues	1921	1919
Ligands	IQT (KR):1, FMN: 2, FES: 2, RBF: 1, FAD: 1, CA: 1, LMT: 2	ONI (AD):1, FMN: 2, FES: 2, RBF: 1, FAD: 1, PEE:2, CA: 1, LMT: 2
Waters	37	61
Bond length (Å)	0.003	0.004
Bond angles (°)	0.630	0.624
Clash score	3.14	4.59
MolProbity score	1.26	1.40
B-factor overall		
Protein	136.23	123.78
Ligand	142.62	124.47
Ramachandran plot (%)		
Favored	97.17	97.06
Allowed	2.62	2.78
Outlier	0.21	0.16
Rotamer outlier (%)	0	0.38

Supplementary Table 2: List of atomistic MD simulations.

Simulation	Initial structure	Reduced cofactor	NqrD/E conformation		Length / ns
			initial	final	
cMD1	7XK3	2Fe-2S ^{NqrF} , 2Fe-2S ^{NqrD/E}	inward	inward	40 (1,000)
TMD	<i>cMD1</i>	2Fe-2S ^{NqrF} , 2Fe-2S ^{NqrD/E}	inward	outward	50
cMD2	<i>TMD</i>	FMN ^{NqrC}	outward	(inward)	800 520 320 200 200 120
cMD3	7XK3	none	inward	inward	400
cMD4	WT _{red} /-Na ⁺ (down)	2Fe-2S ^{NqrF} , 2Fe-2S ^{NqrD/E}	inward	inward	400
cMD5	NqrB-G141A _{red} + KR (<i>shifted</i>)	2Fe-2S ^{NqrF} , 2Fe-2S ^{NqrD/E}	outward	outward	400
cMD6	NqrB-G141A _{red} + KR (<i>stable</i>)	FAD, 2Fe-2S ^{NqrF} , 2Fe-2S ^{NqrD/E} FMN ^{NqrC}	inward	inward	400

Supplementary Table 3: Na⁺ binding site by MD simulation.^a

Subunit	Binding site	Contact ratio
WT_{ox}: full oxidized		
NqrB	Thr236	0.90
	Ser221	0.50
	Gly245	0.27
	Gln243	0.22
	Trp241	0.22
	Asp223	0.21
	Ser239	0.18
	Ala248	0.12
NqrD	Glu119	0.025
	Ser170	0.023
	Gly172	0.022
	Glu210	0.022
	Asn171	0.020
NqrE	Asp133	0.023
	Glu95	0.020
	Gln92	0.017
	Asp99	0.016
	Pro114	0.016
WT_{red}: reduced 2Fe-2S^{NqrF} and 2Fe-2S^{NqrD/E}		
NqrB	Thr236	1.0
	Ser239	0.46
	Ser221	0.14
	Asp223	0.12
	Gln240	0.074
NqrD	Cys112	0.37
	Thr110	0.24
	Glu164	0.058
	Lys202	0.031
	Arg199	0.030
NqrE	Val118	0.11
	Thr117	0.029
	Leu113	0.018
	Val73	0.017
	Glu92	0.017

“down” state of WT_{red}/ -Na⁺: reduced 2Fe-2S^{NqrF} and 2Fe-2S^{NqrD/E}		
NqrB	Thr236	1
	Ser239	0.88
	Asp223	0.18
	His13	0.051
	Gly234	0.046
NqrD	Cys112	0.084
	Thr110	0.079
	Val24	0.063
	Glu210	0.041
	Glu204	0.030
	Gly172	0.025
NqrE	Val118	0.31
	Leu115	0.11
	Cys26	0.063
	His3	0.018
	Leu198	0.012
“shifted” state of NqrB-G141A_{red}: reduced FAD, 2Fe-2S^{NqrF}, 2Fe-2S^{NqrD/E} and FMN^{NqrB}		
NqrB	Thr236	0.94
	Ser239	0.34
	Ser221	0.078
	Glu106	0.046
	His12	0.031
NqrD	Thr110	0.49
	Glu210	0.028
	Cys112	0.027
	Glu204	0.020
	Gln100	0.016
NqrE	Val118	0.48
	Cys120	0.47
	Asp67	0.015
	Glu71	0.013
	Glu2	0.013
WT_{ox}: reduced 2Fe-2S^{NqrF} and 2Fe-2S^{NqrD/E}		
NqrB	Thr236	1.0
	Ser239	0.40
	Asp223	0.095

	Leu224	0.088
	Thr173	0.086
	Thr227	0.086
	Ser44	0.048
NqrD	Cys112	0.30
	Thr110	0.21
	Glu204	0.029
	Gln24	0.021
	Glu210	0.019
NqrE	Val118	0.66
	Leu115	0.45
	Gln197	0.067
	Gly195	0.060
	Asp67	0.021
“shifted” state of NqrB-G141A_{red}: reduced 2Fe-2S^{NqrF} and 2Fe-2S^{NqrD/E}		
NqrB	Thr236	0.95
	Ser239	0.60
	Trp226	0.11
	His101	0.052
	His12	0.042
NqrD	Thr110	0.58
	Cys112	0.19
	Glu210	0.027
	Gly172	0.025
	Asn171	0.018
NqrE	Val118	0.57
	Cys120	0.55
	Gln197	0.082
	Ala68	0.019
	Leu198	0.018
“stable” state of NqrB-G141A_{red}: reduced FAD, 2Fe-2S^{NqrF}, 2Fe-2S^{NqrD/E} and FMN^{NqrC}		
NqrB	Thr236	1
	Ser239	0.33
	Gly234	0.15
	Pro269	0.15
	Asp223	0.056
NqrD	Chs112	0.13

	Thr110	0.086
	Gly172	0.026
	Glu204	0.026
	Glu210	0.025
NqrE	Val118	0.85
	Leu115	0.72
	Lys33	0.055
	Glu172	0.039
	Gln197	0.028
“shifted” state of NqrB-G141A_{red}: reduced 2Fe-2S^{NqrF} and 2Fe-2S^{NqrD/E}		
NqrB	Thr236	0.95
	Ser239	0.60
	Trp226	0.11
	His101	0.052
	His12	0.042
NqrD	Thr110	0.58
	Cys112	0.19
	Glu210	0.027
	Gly172	0.025
	Asn171	0.018
NqrE	Val118	0.57
	Cys120	0.55
	Gln197	0.082
	Ala68	0.019
	Leu198	0.018
“stable” state of NqrB-G141A_{red}: reduced FAD, 2Fe-2S^{NqrF}, 2Fe-2S^{NqrD/E} and FMN^{NqrC}		
NqrB	Thr236	1
	Ser239	0.33
	Gly234	0.15
	Pro269	0.15
	Asp223	0.056
NqrD	Chs112	0.13
	Thr110	0.086
	Gly172	0.026
	Glu204	0.026
	Glu210	0.025
NqrE	Val118	0.85

	Leu115	0.72
	Lys33	0.055
	Glu172	0.039
	Gln197	0.028

^a Simulation is calculated at the scale of 400 ns. Contact ratio corresponds to the ratio of Na⁺-binding frame to total frame. Residues showing > 0.01 of contact ratio and the < 3 Å of distance to were listed up.

Supplementary Table 4: MD simulation checklist.

Reliability and reproducibility checklist for molecular dynamics simulations	Yes	No	Response (Please state where this information can be found in the text)
*All boxes must be marked YES by acceptance unless “Response not needed if No”.			
1. Convergence of simulations and analysis			
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations (<i>e.g.</i> time-course analysis)?	☒	☐	It is described in “A proposed mechanism for Na ⁺ translocation” and shown in Fig. 4c and Supplementary Fig. 20 that the gate sizes are equilibrated.
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?	☒	☐	It is described in “Molecular dynamics simulation” section how we split equilibration and production runs.
1c. Are there at least 3 simulations per simulation condition with statistical analysis?	☒	☐	It is described in “A proposed mechanism for Na ⁺ translocation” and shown in Supplementary Fig. 21.
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?	☒	☐	It is described in “A proposed mechanism for Na ⁺ translocation” and shown in Supplementary Fig. 21 that the simulation results are independent of initial configuration.
2. Connection to experiments			
2a. Are calculations provided that can connect to experiments (<i>e.g.</i> loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure factors, diffusion coefficients, bulk modulus and other mechanical properties, <i>etc.</i>)?	☒	☐	The experimentally determined conformational transition leads to Na ⁺ translocation in our simulations as described in “A proposed mechanism for Na ⁺ translocation”.
3. Method choice			
3a. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?	☒	☐	Our simulations contain membranes and membrane proteins.
3b. Is it described in the text whether the accuracy of the chosen model(s) is sufficient to address the question(s) under investigation (<i>e.g.</i> all-atom vs. coarse-grained models, fixed charge vs. polarizable force fields, implicit vs. explicit solvent or membrane, force field and water model, <i>etc.</i>)?	☒	☐	It is described in “Molecular dynamics simulation” section that the all-atom force fields used in this study have been sufficiently tested for membrane proteins.

3c. Is the timescale of the event(s) under investigation beyond the brute-force MD simulation timescale in this study that enhanced sampling methods are needed?	☒	☐	
If YES, are the parameters and convergence criteria for the enhanced sampling method clearly stated?	☒	☐	They are described in “Molecular dynamics simulation” section.
If NO, is the evidence provided in the text?	☐	☐	
4. Code and reproducibility			
4a. Is a table provided describing the system setup that includes simulation box dimensions, total number of atoms, total number of water molecules, salt concentration, lipid composition (number of molecules and type)?	☒	☐	The information is described in “Molecular dynamics simulation” section.
4b. Is it described in the text what simulation and analysis software and which versions are used?	☒	☐	It is described in “Molecular dynamics simulation” section.
4c. Are other parameters for the system setup described in the text, such as protonation state, type of structural restraints if applied, nonbonded cutoff, thermostat and barostat, etc.?	☒	☐	They are described in “Molecular dynamics simulation” section.
4d. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	☒	☐	They are uploaded to https://github.com/OkazakiLab/nqr_mdfiles
4e. Is there custom code or custom force field parameters?	☒	☐	Response not needed if No
If YES, are they provided as supplementary files or in a public repository?	☒	☐	They are uploaded to https://github.com/OkazakiLab/nqr_mdfiles