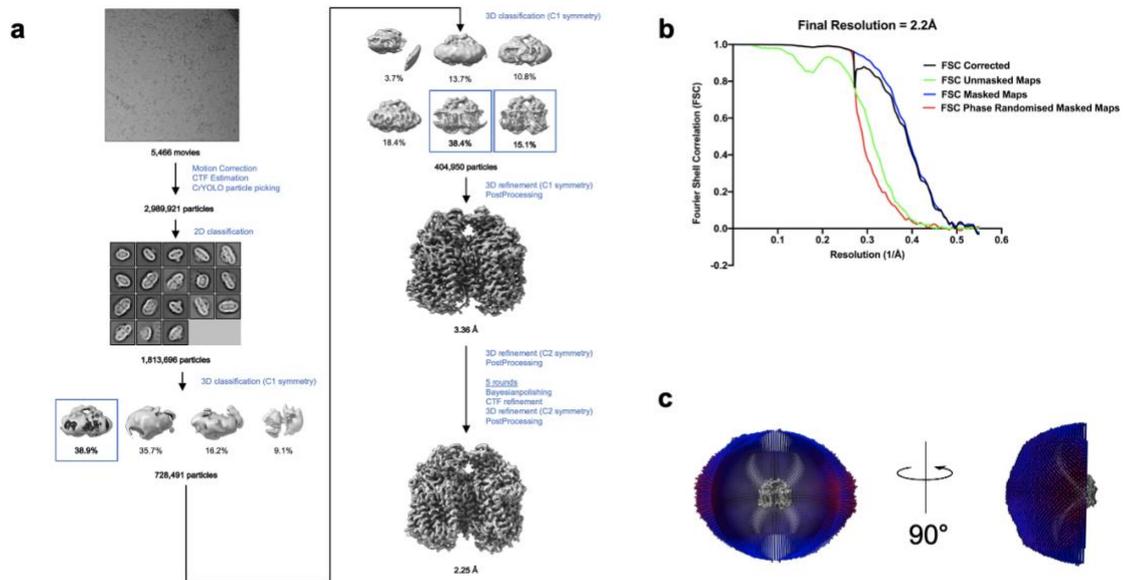
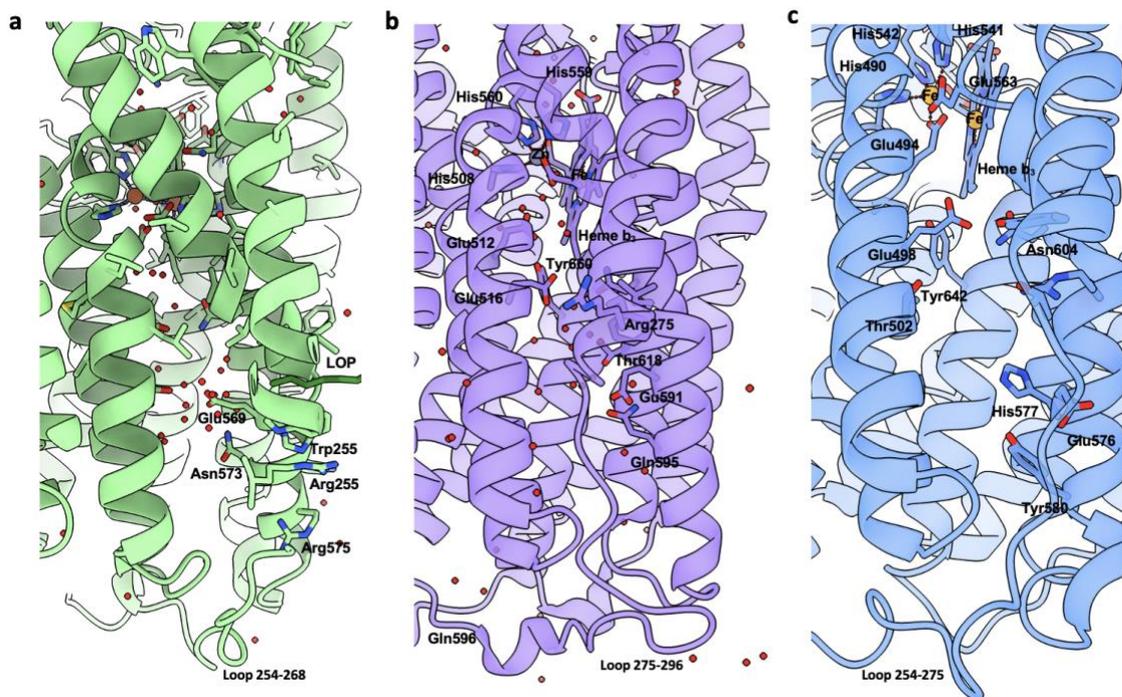


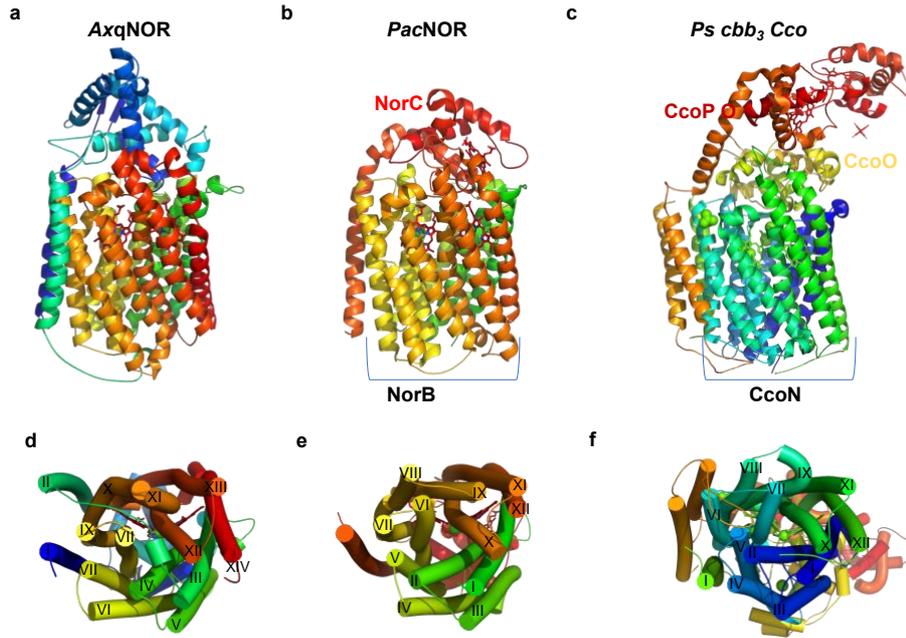
Supplementary information



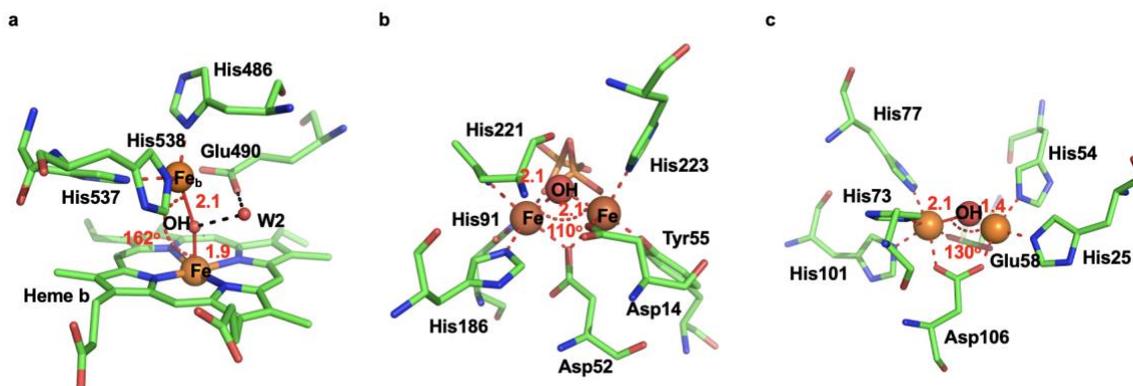
Supplementary figure 1: CryoEM analysis of quinol-dependent Nitric Oxide Reductase (qNOR) from *Alcaligenes xylosoxidans*. (A) Cryo-EM image processing workflow performed in RELION. Example micrograph, 2D classes, 3D classes and refined reconstructions are highlighted. **(B)** FSC curve for the final map. Final resolution was calculated using the gold-standard FSC cut-off of 0.143. **(C)** Euler angle distribution of refined particles.



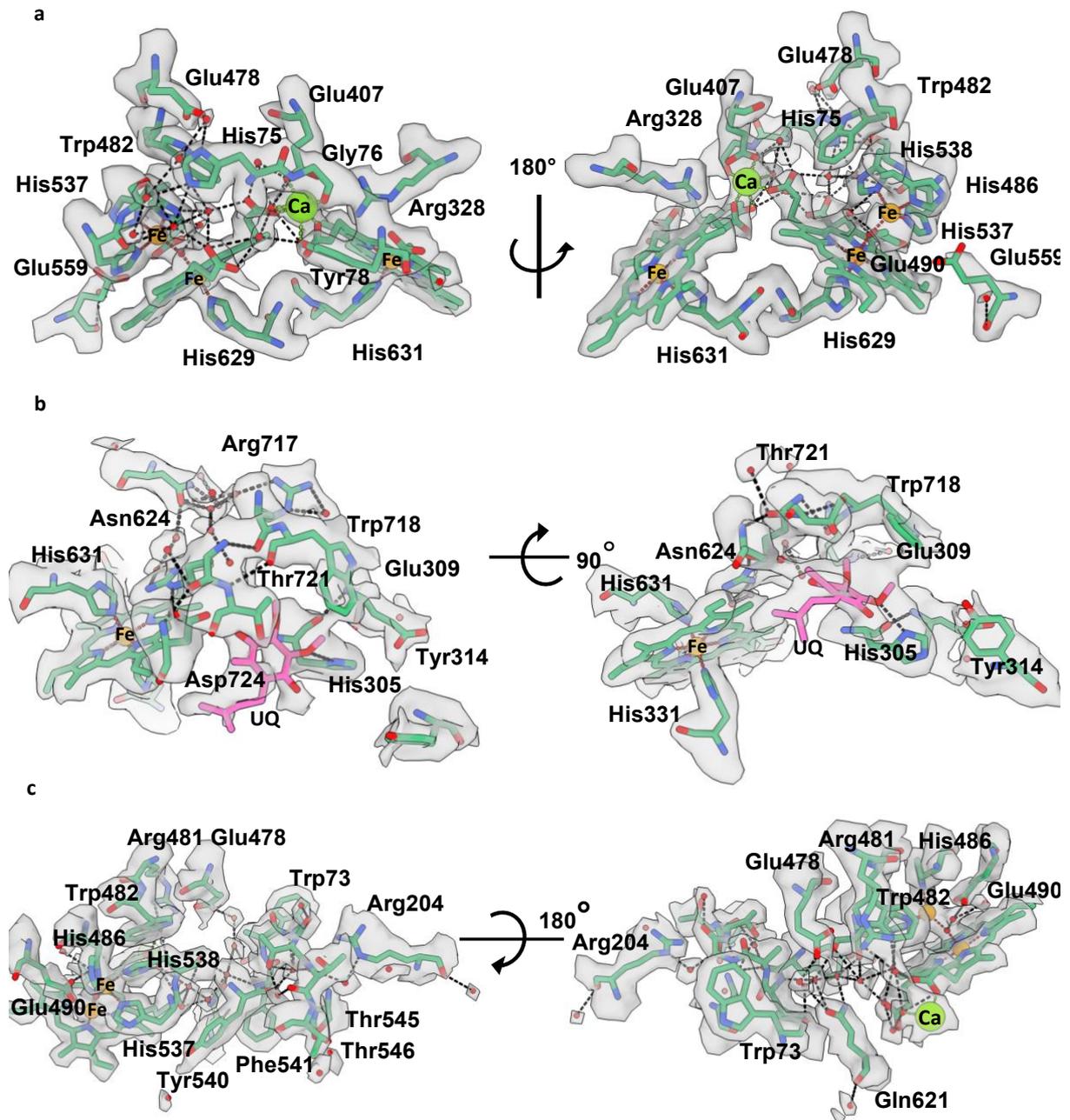
Supplementary figure 2. Comparison of the cytoplasmic part of product release channels at the dimeric interface in structurally characterized qNORs. (a) Product release path of qNOR from *Alcaligenes xylosoxidans* (AxqNOR) from the cytoplasmic side to the binuclear centre. AxqNOR coloured in green with residues lining the proposed path in AxqNOR shown as sticks. Fe atoms and water molecules are shown as orange and red spheres, respectively. Hydrogen bonds and metal co-ordination bonds shown as black and red dashed lines, respectively. This scheme is used throughout the figure. (b) Product release channel in qNOR from *Geobacillus stearothermophilus* (GsqNOR) (PDB ID: 3AYG) coloured in purple, with residues lining its proposed proton channel highlighted as sticks. Zn shown as grey sphere. (c) Product release channel in qNOR from *Neisseria meningitidis* (NmqNOR) coloured in blue, with residues lining its proposed proton channel highlighted as sticks (PDB ID: 6L3H).



Supplementary figure 3. Comparison of overall structures of AxqNOR, PacNOR and *Ps cbb₃ Cco*. (a) Overall structure of AxqNOR viewed parallel to the membrane shown as ribbon in various colors; heme *b* and heme *b₃* are shown as red sticks. Fe_B and calcium ions are represented as orange and green spheres, respectively. (b) Overall structure of cNOR from *P. aeruginosa* viewed parallel to the membrane (PDB code 3WFB). The NorB and NorC subunits are shown as ribbon in various colors and red, respectively. Heme groups are shown as red sticks. Fe_B and calcium ions are represented as orange and greens spheres, respectively. Average rmsd between 390 C α atoms of AxqNOR and NorB subunit is 4.3 Å; (c) Overall structure of *Ps cbb₃ cox* (PDB code 5DJQ) viewed parallel to the membrane shown as ribbon in various colors for subunit N, in yellow for subunit O; and in red for subunit P. Hemes *b* and *b₃* are shown as green sticks. (d) Arrangement of membrane-spanning helices of AxqNOR viewed from the periplasmic side. Transmembrane helices are indicated with Roman numerals in all panels. (e) Arrangement of membrane-spanning helices of *PacNOR* viewed from the periplasmic side. (f) Cytochrome *cbb₃* oxidase from (PDB code 5DJQ). Subunit N with helices I ~ XII is shown in multi color. Average rmsd between 278 C α atoms of AxqNOR and CoxN subunit is 8 Å Helices of subunit O and helices of subunit 3 are shown in yellow and orange-red, respectively.



Supplementary figure 4. Di-iron sites with bridging hydroxide ion. (a) Fe bi-nuclear site of AxqNOR. Non heme Fe is ligated by His486, His 537 and His 538 and OH ion with slightly distorted tetrahedral geometry. The hydroxide (OH) is bound in μ -oxo configuration between two metals at 2.1 Å distance to Fe_B and 1.9 Å to heme Fe, the distance between two Fe is 3.9 Å. Angle between two metal bonds is 162°. (b) Fe bi-nuclear site of Pig tartrate-resistant acid phosphatase type 5 (PDB: 5UQ6 [<https://doi.org/10.2210/pdb5UQ6/pdb>]); the distance between two Fe is 3.4 Å. (c) Fe bi-nuclear site of human Hemerythrin (PDB: 2HMQ [<https://doi.org/10.2210/pdb2HMQ/pdb>]), the distance between two Fe is 3.2 Å. Residues are shown as sticks, Fe ions as orange spheres, water molecules and OH ion as red spheres.



Supplementary figure 5: The cryo-EM map for key residues surrounding key elements and residues (a) AxqNOR calcium site. Cryo-EM map shown with threshold set to 0.023. (b) Key residues around the AxqNOR electron donor binding site. Ubiquinone electron donor shown as pink sticks. Cryo-EM map shown with threshold set to 0.1. (c) AxqNOR NO entry site and surrounding residues. Cryo-EM map shown with threshold set to 0.020. AxqNOR residues shown as green sticks. Cryo-EM map shown as a translucent grey surface. Waters, iron ions and calcium ions shown as red, orange and green spheres, respectively.

AxqNOR	-----MGPYRRLWFTLI AVLAVT FAL LGFYGGEVYRQAPPI PEEVASA	43
GsqNOR	MEVNR TVSPNIQTGRKTTNSFLKS--ILIFTILISSTVLLVGGYWIFKEMAPRPKVEVRSE	58
NmqNOR	-----MGQYKLLWYLLFAVLAVCFITLILGYMGSEVYKAPPYPEQVVSA	43
	. : : * : : : : * * : : : * * : * * :	
AxqNOR	DGTRLFGRDDILDGQTAWQSIGGMQLGSIWGHCAQAPDWTADWLHRELMAWLDAARDA	103
GsqNOR	SGEVLMTKETIIGGQAVFQKYGLMDYGTVLGHCSYMGPDYTAELKVTYEGMDYKAKER	118
NmqNOR	SGKVLMAKDDILAGQSAWQTTGGMEVGSVLGHCAQAPDWTADWLHRELSAWLDTAQQT	103
	. * * : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
AxqNOR	HGRDYQQLDAPAAQLREQLKAEYRANRADAAGCKLTLSPRRAQVAQTEAYYDQLFSDA	163
GsqNOR	YNKPFADLTDEKSIIREQVIKEMRKNRYNPVTDVVLVLTDAQVYGLEKVRDYRDVFTNG	178
NmqNOR	YGKPFDEVSPPEQAVLKRRLADEYRNQSRIKEDGSSVVISDTRVKAIESILPYYHGVYGD	163
	: : : : : : : : : * * : : : : : : : : : : : : : : * * : : :	
AxqNOR	PALHRSRENYAMKENT-----LPDANRRRQMTHTFFWTAWAAGTEREGTSVITYNNW	215
GsqNOR	DWGLKKG--LIKESDMKANRAVADSDQIQIADFFWTAWLSTLRIGDEITYNNW	236
NmqNOR	PALQTTREHFAMKNNT-----LPSQAREKLFDFFWTWSWASTNRPEDETFITYNNW	215
	. : : : : : : : : * * : : : : : : : * * * * : * * * : * * * * :	
AxqNOR	PHEPLIGNHPSENVMWSIISVVVLLAGILLIWAFAFLR--GKEEPPAPARDPLTT	272
GsqNOR	PYYEDAGNTMSFSAYVWWSGASVTILLIFIGIILYVYRYQLSMQAYAEKFPV-IDLRR	295
NmqNOR	VHEPLINNVPPTTENYMSFTSVVLLLMGIGLLMWYSFLT--KHEEVE--VPTEDPIK	270
	* : . * : . * * * : * : * : * : : : . * * . :	
AxqNOR	FALTPSQALGKYFLVVALFGFQVLLGGFTAHTYVGGQKFGID-----LSQWFPYSL	326
GsqNOR	QPLTPSQVAKGYFVVVALFVQTFMGALLAHTYVGGQKFGID-----IYDILFPNI	350
NmqNOR	VQPLTPSQALGKYFLVVALFVQVLLGGFTAHTYVGGQKFGIDEALGFEMSDFWFPYAL	330
	***** * : : . * * * * : * : * : * : * : * : * : * : * : * : * : :	
AxqNOR	VRTWIIQSALFWIATGFLAAGLFLAPLNGRDPKYQKAGVDILFWALVVLVVGSGFAGNY	386
GsqNOR	AKGYHLQLAIFWIATAWLGMGIFIAPLV-GGQEPKQGLLVDLFWALVVLVVGSGMIGQW	409
NmqNOR	TRTWIIQSALFWIATGFLTAGLFLAPIVNGGKDPKQAGVNFILYIALFIVVGGSYAGNF	390
	: : : * * * * * : * * * : * : * : * : * : * : * : * : * : * * * * :	
AxqNOR	LAIQIMPDPDLNFWLGHQGYIYVDLGRWLQIGKFGICFWLVLMLRGI VPAALRTPGGDKN	446
GsqNOR	LGVNGYLGNE-WFLLGHQGWYIELGRWQIILVVGMLLWLFVFRGVKRGKRESDKGG	468
NmqNOR	FALTHILPPEFNFVFGHQGYIYLDLGRFWQLLLMVGLLLWLFMLRCTVSAFKEKGV DKN	450
	: : : : : * : * * * * : * : * : * : * : * : * : * : * : * : * : :	
AxqNOR	LLALLTASVGAIGLFGAGFFYGERHTLTVMEYRWVIVHLWVCGFFEVFATTALAFIFS	506
GsqNOR	LIHLLFYSAIAVPPFYIFAFFIQPDNFTMADFWRWVILHLWVCGIFEVFAVVVIGFLLV	528
NmqNOR	LLAIFVASMVGVFYAPGLFYGEKSPVAVMEYRWVIVHLWVCGFFEVFATAAFVY	510
	* : : * * : . * * : * : : : : * : * : * : * : * : * : * : * : * : * :	
AxqNOR	TLGLVSRMATTASLASASFLMGGIPGTFHHLVYAGTTPVMAVGSFSALEVVP L I V L	566
GsqNOR	QLRLVTKKSTVRALYFQFTILLGSGVIGIHHYYNGSPVWIALGAVFSALEVIPLTLL	588
NmqNOR	NMGFVRRSTASTALAAAIFMLGGVPGTLHHLVYSGSTASMAI GACFSALEVVP L L L	570
	: : * : : : : : : : : * * * : * : * : * : * : * : * * * * * * * * :	
AxqNOR	GHEAWENWRLKTRAPWENLKWPLMCFVAVAFWNMLGAGVFGFMINPPVSLYIIGLNTT	626
GsqNOR	ILEAVEQYKMMRDGANFPYKATFWFLISTAIWNLVGAGVFGFLINLPAVSFEHQFLT	648
NmqNOR	GREAYEHWYSQHLSEWAKRLRWPLMCFVAVAFWNMIGAGVFGFLINPPIISLFYIIGLNTS	630
	* * : : : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : :	
AxqNOR	PVHAHAALFGVYGFALGFTLLVLRIRPQYALS PGLMKLAFWGLNLGLALMI FTSLLPI	686
GsqNOR	PAHGHAAAMGVYGMFAIAVLLYSLRNI V KPEAWNDKWLKFS CWMLNIGLAGMVVITLLPV	708
NmqNOR	AVHAHAALFGVYGFALGFTLLVLRVARYLKNVQFDDKLMTWGFWLLNGLVGMIAISLLPV	690
	. * * : * * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
AxqNOR	GLIQFHASVSEGMWYARSEAFMQDDILKTLRGRVTFGDDVFLGALAMVVQVILGLLSGK	746
GsqNOR	GILQMKFAFIHGYSRSPSFLQDDVVQNLVAVPDTIFLIGVVALLVFAIKALFHLR	768
NmqNOR	GVIQYASITHGLWYARSEEFQMEILDTLRVTAAADLIFIGGACVAIQATKIVFGRD	750
	* : * : . . * * * * : * * : * : * : * : * : * : * : * : * : * : * :	
AxqNOR	----- 746	
GsqNOR	KPTHGEGEELPVANHWMKDRKNSLE 794	
NmqNOR	K----- 751	

NO entry channel; heme, Fe_b ligation; Ubiquinol binding; Ca binding ligands

Supplementary figure S6A. Sequence alignment of AxqNOR, NmqNOR and GsqNOR. Homology between AxqNOR (PDB:8BGW [<https://doi.org/10.2210/pdb8BGW/pdb>]) and NmqNOR(PDB:6L3H [<https://doi.org/10.2210/pdb6L3H/pdb>]) is 58.7%; Homology of AxqNOR and GsqNOR is 33.3%. Residues or NO entry channel are highlighted in grey; heme Fe_b and F_B ligands in cyan; Ubiquinol binding site in magenta and Ca binding ligands in Green; fully conserved residues are marked with *. Alignment is performed by CLUSTRALW EBI server.

Redox sites

AxqNOR	478	E	Y	W	R	W	I	V	H	L	W	-	-	V	E	G	F	535	T	F	H	H	L	Y	626	T	P	V	H	A	H
NmqNOR	482	E	Y	W	R	W	V	V	H	L	W	-	-	V	E	G	F	539	T	L	H	H	L	Y	630	S	A	V	H	A	H
GsqNOR	500	D	F	W	R	W	I	I	H	L	W	-	-	V	E	G	I	557	I	G	H	H	Y	Y	648	T	P	A	H	G	H
PacNOR subunit B	199	K	F	Y	W	W	V	V	H	L	W	-	-	V	E	G	V	256	T	G	H	H	Y	F	344	T	A	A	H	G	H
Cytochrome bo3 subunit 1	277	L	I	W	A	W	G	H	P	E	V	Y	I	L	I	L	331	W	L	H	H	F	F	416	L	I	A	H	F	H	

Ubiquinol sites

AxqNOR	302	F	T	A	H	Y	T	718	W	G	R	T	F	G	D	V	V
NmqNOR	300	L	T	A	H	Y	T	722	W	V	R	T	A	A	D	L	I
GsqNOR	325	L	L	A	H	Y	Y	740	L	V	R	A	V	P	D	T	I
PacNOR subunit B	36	I	M	G	L	Q	Y	438	W	L	R	E	G	A	G	V	V
Cytochrome bo3 subunit 1	60	M	Y	I	I	V	A	496	M	I	A	A	S	G	A	V	L

KEY	
W	IDENTICAL MATCH
W	POSITIVE MATCH
W	NEGATIVE MATCH

Ca binding

AxqNOR	73	W	G	H	G	A	Y	Q	326	L	V	R	T	W	405	G	Y	E	Y	V	557	A	L	E	V	V
NmqNOR	73	L	G	H	G	A	Y	Q	330	L	T	R	T	W	409	G	Y	E	Y	L	561	A	L	E	V	V
GsqNOR	88	L	G	H	G	S	Y	M	350	I	A	K	G	Y	427	G	W	E	Y	I	579	A	L	E	V	I
PacNOR subunit B	-	-	-	-	-	-	-	-	55	V	A	R	M	V	136	G	I	V	I	V	278	A	L	E	P	L
PacNOR subunit C	68	L	G	E	G	A	Y	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Cytochrome bo3 subunit 1	38	F	G	K	W	-	-	-	101	Q	I	F	T	A	220	-	M	T	M	F	349	G	I	T	T	M
Ps cbb3 cox	-	-	-	-	-	-	-	-	55	R	L	R	P	L	120	S	K	E	Y	A	277	L	I	L	L	A

NO channel

AxqNOR	71	S	I	W	G	H	204	R	E	G	T	S	V	T	Y	T	N	540	Y	F	A	G	T	T	619	Y	I	Q	G	L
NmqNOR	71	S	V	L	G	H	204	R	P	D	E	T	F	T	Y	T	N	544	Y	F	S	G	S	T	623	Y	I	Q	G	L
GsqNOR	86	T	V	L	G	H	225	R	I	G	D	E	I	T	Y	T	N	542	Y	Y	N	G	S	P	641	F	E	H	G	Q
PacNOR subunit B	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	261	F	W	I	G	V	P	337	Y	T	H	G	T	
PacNOR subunit C	66	T	L	L	G	E	136	-	-	-	-	-	-	D	T	N	-	-	-	-	-	-	-	-	-	-	-			
Cytochrome bo3 subunit 2	0	-	-	-	-	-	72	-	S	N	K	D	A	K	Y	S	P	336	F	T	M	G	A	G	409	V	L	H	N	S

Supplementary figure 6B. Sequence fragments conservation for AxqNOR, NmqNOR, GsqNOR, PacNOR subunits B and C and subunit 2 of COX. Identical residues are colored in dark yellow, positive matches by light yellow. The upper sequences were aligned by pairwise alignment against AxqNOR sequence using BLAST and scored according to the BLOSUM62 score matrix. The bottom sequences were not similar enough to AxqNOR for pairwise alignment, so were aligned by multiple sequence alignment with the upper sequences using Clustal Omega. These aligned sequences were not scored by a matrix and so were only marked for identical residues.

	cov	pid	1	1 100
1 Alcaligenes	100.0%	100.0%	MGPYRRLWF	TLIAVLAVTFALLGYYGGEVYQAPPPIEVSASDTRFLGRDDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
2 Polaromonas	99.9%	81.2%	MGSYKLLWF	TLIGLVIVTFSSLLGYGTEVYQAPPPIAQVTPDGLVDFRGGILGQTAWQSIGMQLGSIWGHGAYQAPMNTA
3 Ralstonia	99.6%	80.3%	MGPYKLLWF	TLIAVLAVTFALLGYYGGEVYQAPPPIAQVTTDGLKFLTAEDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
4 Hydrogenophaga	99.5%	76.8%	MLGKLVIVTFSSLLGYGADVYQAPPPIAQVSTAEGALFTRDDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA	
5 Bordetella	99.7%	81.1%	MGPYRRLWF	TLIGLVIVTFSSLLGYGGEVYQAPPPIAQVVAHGGVLFDRGGILGQTAWQSIGMQLGSIWGHGAYQAPMNTA
6 Comamonas	99.9%	76.2%	MGSYRKLWF	TLIAVLAVTFSSLLGYGTEVYQAPPPIQVATAGGVLYTQESILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
7 Pseudomonas	99.5%	76.9%	MGEYRKLWF	TLIAVLAVTFSSLLGYGTEVYQAPPPIQVQSTGSLFTNDDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
8 Thauera	100.0%	79.5%	MGNRYRKLWF	MLIGLVIVTFSSLLGYGTEVYQAPPPIQVIVSDGGVLYTHGILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
9 Chitiniphilus	99.9%	78.3%	MGPYRKLWF	TLIAVLAVTFSSLLGYGTEVYQAPPPIQVQVQVNGPVLFTADDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
10 Cupriavidus	99.9%	81.4%	MGLYRKLWF	TLIAVLAVTFSSLLGYGTEVYQAPPPIQVVAEGTILFSGDDILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
11 Oceanimonas	99.6%	75.4%	MANYRKLWF	LLLVVLTFTAILGYGFEVYQAPPPIAQVVTENGTLTTHGILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
12 Haehella	99.3%	76.2%	MAEYRKLWF	LLIAVLAVTFSSLLGYGTEVYQAPPPIQVVAATGCTIFFTGHILDQTAWQSIGMQLGSIWGHGAYQAPMNTA
13 Neisseria	99.9%	58.7%	MGOYKLLWF	TLIAVLAVTFSSLLGYGTEVYQAPPPIQVVSASGVLMMKDDILAGQSAWQTGGMEVGSVLGHGAYQAPMNTA
14 Geobacillus	99.3%	34.3%	MEVNRITVSPNIQTGRKTTNSFLKSLITFTLISSTVLLVGGYVIFKMAPRPKVRSSEGLVMTKTIIGQAVFKYGLMDYGTVLGHGAYQAPMNTA	
consensus/100%				
consensus/90%				
consensus/80%				
consensus/70%				

	cov	pid	101	2 200
1 Alcaligenes	100.0%	100.0%	DWLHRELMAWDLAAADAHGRDYGQLDAPAQAALREQLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
2 Polaromonas	99.9%	81.2%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
3 Ralstonia	99.6%	80.3%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
4 Hydrogenophaga	99.5%	76.8%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
5 Bordetella	99.7%	81.1%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
6 Comamonas	99.9%	76.2%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
7 Pseudomonas	99.5%	76.9%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
8 Thauera	100.0%	79.5%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
9 Chitiniphilus	99.9%	78.3%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
10 Cupriavidus	99.9%	81.4%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
11 Oceanimonas	99.6%	75.4%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
12 Haehella	99.3%	76.2%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
13 Neisseria	99.9%	58.7%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
14 Geobacillus	99.3%	34.3%	DWLHRELTAWDLAAAEFGKDFAVLDAGDQAMLRAHLKAEYRANRADAAGGKLTLSRRRQAQAQTEAYYQDLSFAPALHRSRENYAMKENTLPDA	
consensus/100%				
consensus/90%				
consensus/80%				
consensus/70%				

	cov	pid	201	3 300
1 Alcaligenes	100.0%	100.0%	ERRRQHTFFFWTAWAATERPGE	TSVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
2 Polaromonas	99.9%	81.2%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
3 Ralstonia	99.6%	80.3%	ERRRQHTFFFWTAWAATERPGE	HTVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
4 Hydrogenophaga	99.5%	76.8%	ERRRQHTFFFWTAWAATERPGE	SVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
5 Bordetella	99.7%	81.1%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
6 Comamonas	99.9%	76.2%	ERRRQHTFFFWTAWAATERPGE	ADVGGSGATYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
7 Pseudomonas	99.5%	76.9%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
8 Thauera	100.0%	79.5%	ERRRQHTFFFWTAWAATERPGE	TSVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
9 Chitiniphilus	99.9%	78.3%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
10 Cupriavidus	99.9%	81.4%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
11 Oceanimonas	99.6%	75.4%	ERRRQHTFFFWTAWAATERPGE	SNVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
12 Haehella	99.3%	76.2%	ERRRQHTFFFWTAWAATERPGE	SVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
13 Neisseria	99.9%	58.7%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
14 Geobacillus	99.3%	34.3%	ERRRQHTFFFWTAWAATERPGE	QVYTYNNWPHPLIGNHPSSNIVWSIASVVLLAGVIGLLIWAWAFLRKKEE
consensus/100%				
consensus/90%				
consensus/80%				
consensus/70%				

	cov	pid	301	4 400
1 Alcaligenes	100.0%	100.0%	TFALTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
2 Polaromonas	99.9%	81.2%	TFALTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	VSNWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
3 Ralstonia	99.6%	80.3%	TFALTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	MSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
4 Hydrogenophaga	99.5%	76.8%	IAAGLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
5 Bordetella	99.7%	81.1%	TFALTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	VSNWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
6 Comamonas	99.9%	76.2%	SVLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
7 Pseudomonas	99.5%	76.9%	SVLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
8 Thauera	100.0%	79.5%	SVLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
9 Chitiniphilus	99.9%	78.3%	AAVPLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
10 Cupriavidus	99.9%	81.4%	LSVPLTPSQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
11 Oceanimonas	99.6%	75.4%	TFKLTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
12 Haehella	99.3%	76.2%	SFALTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
13 Neisseria	99.9%	58.7%	SKVLTSPQALGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
14 Geobacillus	99.3%	34.3%	RKQPLTPSQKAGQYFLVLVALFQVLLGGFTAHTYVGGQFYGD	LSQWFPYSLVTRWHIQSALFWIATGFLAAGFLAPLINGGRDPYFQ
consensus/100%				
consensus/90%				
consensus/80%				
consensus/70%				

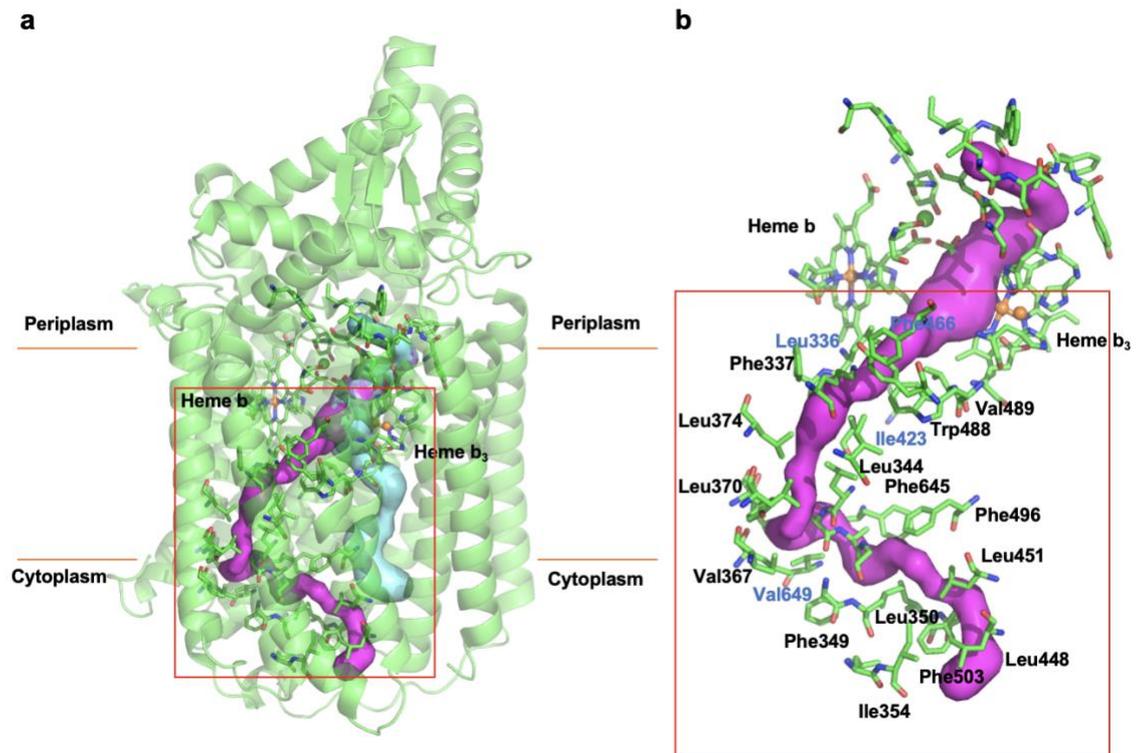
	cov	pid	401	5 500
1 Alcaligenes	100.0%	100.0%	AGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FAGICFWLWMLRGIVPALRT-PGGNLLALLTASVGAIGLFY
2 Polaromonas	99.9%	81.2%	AGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGILLWLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
3 Ralstonia	99.6%	80.3%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	LAGIVZLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
4 Hydrogenophaga	99.5%	76.8%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	MAGILLWLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
5 Bordetella	99.7%	81.1%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	YVIGLLWLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
6 Comamonas	99.9%	76.2%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FAGIVZLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
7 Pseudomonas	99.5%	76.9%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGAVFWLLMLRGVVPALL-RGGNLLALLTASVGAIGLFY
8 Thauera	100.0%	79.5%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FVGVWLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
9 Chitiniphilus	99.9%	78.3%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGILLWLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
10 Cupriavidus	99.9%	81.4%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGIVZLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
11 Oceanimonas	99.6%	75.4%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGIVZLVLMRGVVPALL-RGGNLLALLTASVGAIGLFY
12 Haehella	99.3%	76.2%	LGVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGAVFWLLMLRGVVPALL-RGGNLLALLTASVGAIGLFY
13 Neisseria	99.9%	58.7%	AGVFLYIALFTVVGSGYAGNFALHILPPEFNFWLGHQGYEYDGLRQWIG	FTGAVFWLLMLRGVVPALL-RGGNLLALLTASVGAIGLFY
14 Geobacillus	99.3%	34.3%	LLVDILFVALVIVVGSFAGNYLAIQIMPDLNFWLGHQGYEYDGLRQWIG	FTGAVFWLLMLRGVVPALL-RGGNLLALLTASVGAIGLFY
consensus/100%				
consensus/90%				
consensus/80%				
consensus/70%				

	cov	pid	501		600
1	Alcaligenes	100.0%	100.0%	AGFFYGERTHLTVYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
2	Polaromonas	99.9%	81.2%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
3	Ralstonia	99.6%	80.3%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
4	Hydrogenophaga	99.5%	76.8%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
5	Bordetella	99.7%	81.1%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
6	Comamonas	99.9%	76.2%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
7	Pseudomonas	99.5%	76.9%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
8	Thauera	100.0%	79.5%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
9	Chitiniphilus	99.9%	78.3%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
10	Cupriavidus	99.9%	81.4%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
11	Oceanimonas	99.6%	75.4%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
12	Hahella	99.3%	76.2%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
13	Neisseria	97.9%	58.7%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
14	Geobacillus	99.3%	34.3%	AGLFLYGERTHLSVMYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
	consensus/100%			AGFFYGERTHLTVYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
	consensus/90%			AGFFYGERTHLTVYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
	consensus/80%			AGFFYGERTHLTVYRWWVHLWV	GFVFATTALAFIFSTLGLVSR
	consensus/70%			AGFFYGERTHLTVYRWWVHLWV	GFVFATTALAFIFSTLGLVSR

	cov	pid	601		700
1	Alcaligenes	100.0%	100.0%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
2	Polaromonas	99.9%	81.2%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
3	Ralstonia	99.6%	80.3%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
4	Hydrogenophaga	99.5%	76.8%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
5	Bordetella	99.7%	81.1%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
6	Comamonas	99.9%	76.2%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
7	Pseudomonas	99.5%	76.9%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
8	Thauera	100.0%	79.5%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
9	Chitiniphilus	99.9%	78.3%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
10	Cupriavidus	99.9%	81.4%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
11	Oceanimonas	99.6%	75.4%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
12	Hahella	99.3%	76.2%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
13	Neisseria	97.9%	58.7%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
14	Geobacillus	99.3%	34.3%	IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
	consensus/100%			IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
	consensus/90%			IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
	consensus/80%			IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL
	consensus/70%			IVLGHAEANMRLNLTAPMNL	NPLMCFVAVAFWNNL

	cov	pid	701		800
1	Alcaligenes	100.0%	100.0%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
2	Polaromonas	99.9%	81.2%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
3	Ralstonia	99.6%	80.3%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
4	Hydrogenophaga	99.5%	76.8%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
5	Bordetella	99.7%	81.1%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
6	Comamonas	99.9%	76.2%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
7	Pseudomonas	99.5%	76.9%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
8	Thauera	100.0%	79.5%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
9	Chitiniphilus	99.9%	78.3%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
10	Cupriavidus	99.9%	81.4%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
11	Oceanimonas	99.6%	75.4%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
12	Hahella	99.3%	76.2%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
13	Neisseria	97.9%	58.7%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
14	Geobacillus	99.3%	34.3%	MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
	consensus/100%			MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
	consensus/90%			MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
	consensus/80%			MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS
	consensus/70%			MATFAGLNLGLVLMIFTSLLPIG	IQFHASVSEGLWYARS

Supplementary figure 6C. Sequence alignment of representative AxqNOR homologs. *Alcaligenes xylosoxydans* (uniprot A0A0D6H8R3); *Polaromonas* sp. (A0A1H0FNK7); *Ralstonia* sp. (A0A1H0U9C7); *Hydrogenophaga intermedia* (A0A1L1PKZ3); *Bordetella genomosp* (A0A261SF53); *Comamonas terrigena* (A0A2A7UR79); *Thauera* sp (A0A2W6XYG8); *Pseudomonas putida* (A0A3M8T612); *Chitiniphilus eburneus* (A0A4U0PXB0); *Cupriavidus taiwanensis* (B3RCN9); *Geobacillus stearothermophilus* (B3Y963); *Oceanimonas* sp.(H2G1F4); *Hahella chejuensis* (Q2SP13). Consensus between residues is highlighted by % and color; % of identity between sequences is shown in "pid" column. Alignment is performed by CLUSTALW EBI server.



Supplementary figure 7. Putative product release pathway in AxqNOR. (a) Location of the putative product release path from binuclear active site to the cytoplasm is shown in purple and based on CAVER 3.0 analysis. Water channel is shown in cyan. (b) Hydrophobic residues surrounding the channel and heme molecules are shown as sticks. Iron and Ca ions are represented by orange and green spheres. Part of the channel directed to the cytoplasm is indicated by the red square. Majority of the residues lining the path are strictly conserved. Residues with black labels are strictly conserved; residues with blue labels are semiconserved.

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

Data Accession	
PDB	8BGW
EMDB	16041
Data Collection	
Microscope	FEI Titan Krios
Voltage (kV)	300
Detector	Falcon 4
Energy filter slit width (eV)	10
Nominal magnification	130k
Pixel size (Å/pixel)	0.91
Defocus range (µm)	-0.9 - -2.7
Exposure time	6.11
Frames	44
Exposure rate (e ⁻ pixel ⁻¹ s ⁻¹)	4.61
Electron exposure (e ⁻ /Å ²)	34.90
Dose per frame (e ⁻ /Å ²)	0.8
Micrographs collected	5466
Reconstruction	
Software	RELION 3.1
Particles used in refinement	404,950
Symmetry	C2
Overall resolution when FSC=0.143 (masked) (Å)	2.2
Map sharpening B-factor (Å ²)	-47.68
Model Refinement	
Software	REFMAC5
Non-hydrogen atoms	12845
Protein residues	11649
Ligands	746
Average B factors (Å ²)	61.128
Protein	63.29
Ligands and water	91.95
R.M.S. deviations	
Bond length (Å)	0.012
Bond angle (Å)	1.649
Ramachandran statistics (%)	
Outliers	0.27
Allowed	5.47
Favoured	94.26
MolProbity score	2.36
ClashScore	15.19
Poor rotamers (%)	2.41
Model vs. Map FSC	0.854

Supplementary Table 2: Comparison of Ca ligands and bond distances in AxqNOR, its mutant and *PacNOR*.

Ca ligands in AxqNOR (<i>PacNOR</i>)	2.2 Å AxqNOR 8BGW	3.9 Å AxqNOR 6QQ5	3.3 Å AxqNOR Variant 6QQ6	2.7 Å <i>PacNOR</i> 300R
Water	2.3	-	-	2.1
OH Tyr78(73*)	2.5	2.6	2.7	2.6
O Gly76(71*)	2.3	2.4	2.8	2.3
O2A Heme b ₃	2.3	2.6	2.1	2.6
O1D Heme b	2.2	2.3	2.5	2.4
O2D Heme b	(3.7)	2.7	2.5	(3.8)
OE2 Glu407(135)	2.1	2.5	2.4	2.8
OE1 Glu407(135)	2.2	2.9	3.0	2.6
* residues numbers for <i>PacNOR</i>				