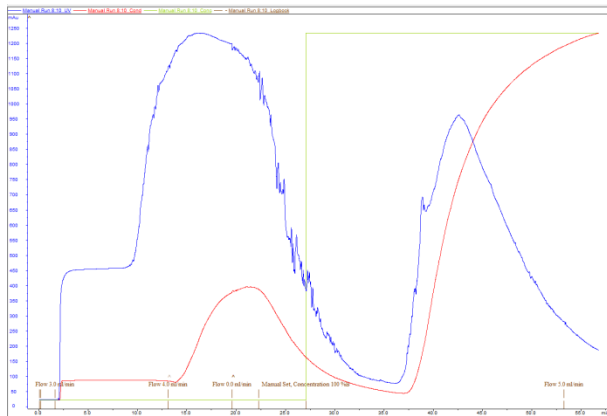
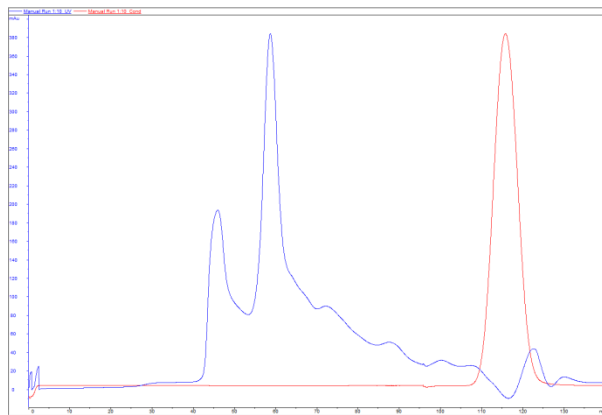
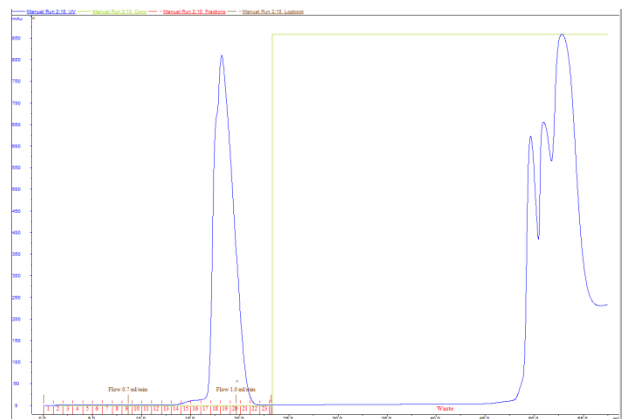
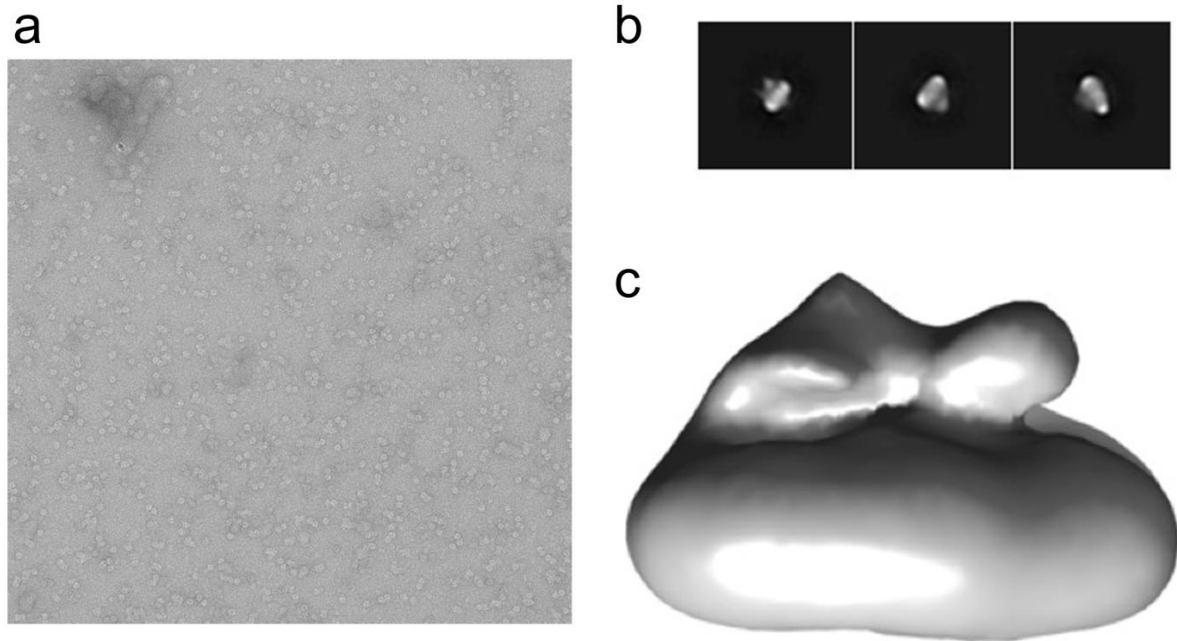


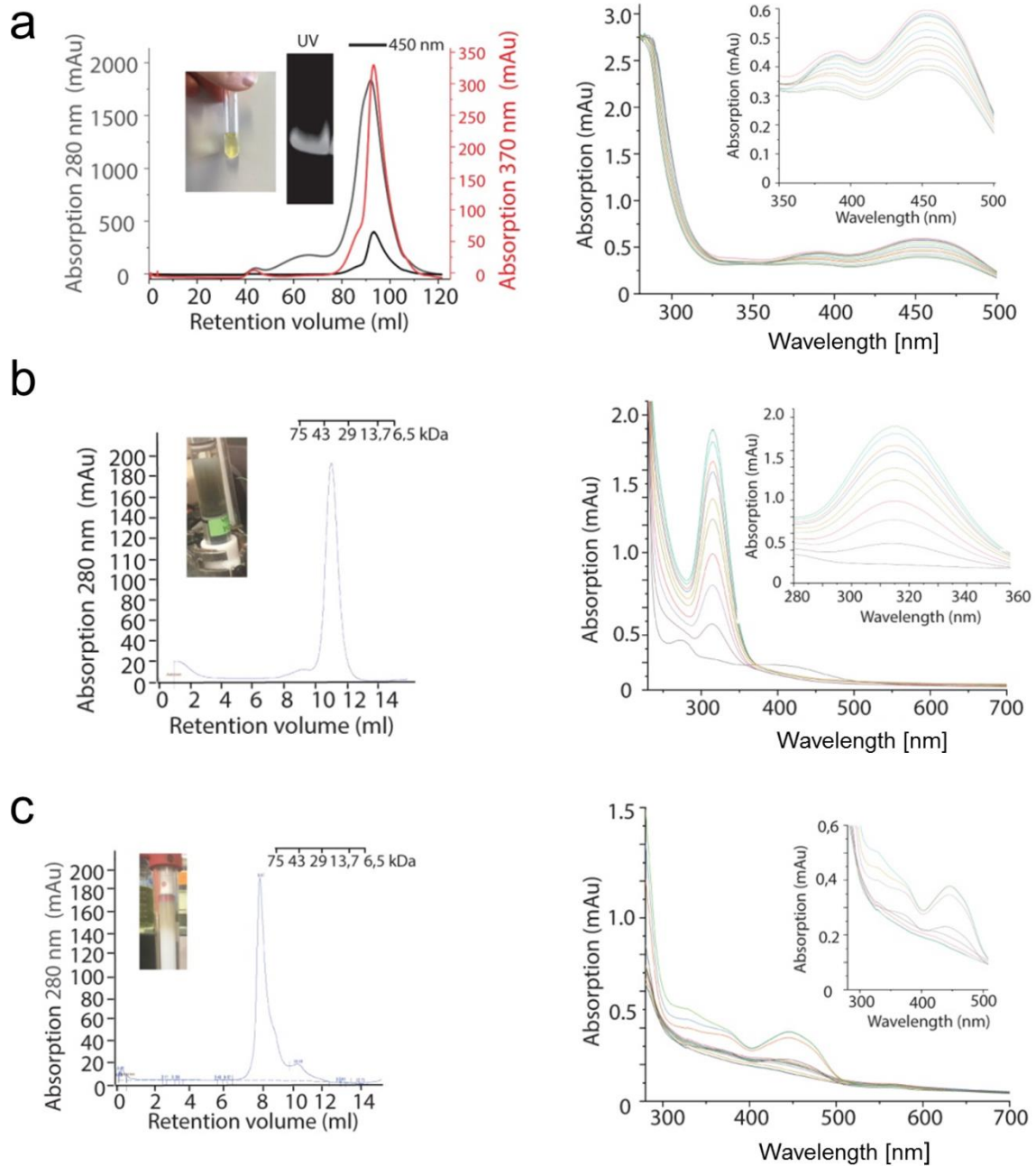
Supplementary data

a**b****c****d**

Supplementary Fig. 2: Elution profile of the chromatographic steps. **a** Hydroxyapatite. The solubilized membrane fraction was eluted with the flow-through (red-colored peak). **b** Reactive Red active agarose affinity: Rnf solution was applied to the column with a pipette and washed manually with a NaCl gradient. Rnf was eluted at 2M NaCl. The protein was collected according to the dark brown color of the Fe/S cofactors. **c** Superdex-200 gel filtration. Rnf activity was eluted with 57-62 ml (symmetric red-colored peak). **d** POROS HQ anion exchange resin. The pure Rnf complex is eluted after washing the anion exchange column with buffer A after 18 ml.



Supplementary Fig. 3: Negative-stain EM analysis of Rnf after purification. **a** EM micrograph. Data collections were performed eight times. **b** 2D classification. **c** 3D model. For negative-stain electron microscopy grid preparation was done under exclusion of oxygen in an anaerobic tent. The Rnf complex was negatively stained with 2% (w/v) uranyl formate. A 3 μ l protein sample (0.02 mg/ml) was pipetted onto freshly glow-discharged carbon-coated copper grids. Electron micrographs were collected using a TecnaiTM Spirit 12G TEM (ThermoFisher – formerly FEI) operating at 120 kV at a nominal 42,000-fold magnification and a calibrated pixel size of 2.68 Å (nominal defocus – 1.50 mm). A set of 200 micrographs were collected on a GATAN 4096 _ 4096 CCD detector by automated data collection with the Leginon software package⁵. Image processing, automatic particle picking, 2D classification and ab initio 3D map generation were performed with cisTEM⁶. The membrane part could be clearly distinguished from the soluble part, potentially consisting of RnfB and RnfC.



Supplementary Fig. 4: Separated production of the soluble subunits. **a** RnfG. RnfG of *C. tetanomorphum* was overproduced by co-expression with the *apbE* gene responsible for forming the covalent linkage of FMN with the protein^{7,8}. Without co-expression of *apbE* and supplementing the cultivation media with Mg^{2+} and FMN, respectively, RnfG is only produced in an aggregated form devoid of FMN. The *E. coli* cells were opened with a French pressure cell. After cell debris and membrane removal, the protein solution was applied to Ni-NTA agarose in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl and eluted with 250 mM imidazole. Then, the heterologous produced protein was applied to a Superdex-75 column (Hiload® 16/600 Superdex 75® pg, Sigma-Aldrich, Missouri/USA) for further purification and desalting. The yield was 30 mg RnfG from 5 g cells. The protein was pure and homogenous as derived from the gel filtration profile (left panel). Elution was monitored at 280 nm, 370 nm (red) and 450 nm

(small black peak) already indicating the presence of FMN. Its covalent binding became visible in the fluorescent SDS gel (left panel inset). A source data file is provided. The bound FMN could be stepwise reduced by dithionite monitored by UV/Vis spectral changes (right panel). The grey curve is related to RnfG as isolated. 10 x 3 μ L of dithionite (20 mM stock solution) was supplemented to the protein resulting in 10 curves (from green ascending to red). **b** RnfB. The soluble part of RnfB from *C. tetanomorphum* was successfully overproduced in *E. coli* BL21 (DE3) containing special plasmids for iron-sulfur cluster formation (pRKISC vectors)^{9,10}. Furthermore, *in vivo* iron-sulfur cluster reconstitution with various iron and sulfur sources were carried out as described¹¹. RnfB was kept anaerobically during the entire preparation performed by the procedure described for RnfG. RnfB detected at 280 nm is homogeneous (left panel) and pure. The yield was 80 mg RnfB from 5 g cells. The UV/Vis spectra indicate multiple iron-sulfur clusters that were stepwise reducible by dithionite (right panel). The brown curve describes RnfB as isolated. The curve after oxidizing RnfB with 12 μ M hexacyanoferrate (III) is drawn in grey and those after adding 8 x 3 μ L of dithionite (20 mM stock solution) ranges from violet to green. **c** RnfC. RnfC was produced and purified in the same way as RnfB. The yield was 10 mg RnfC from 5 g cells. All buffers for RnfC purification were supplemented with a flavin mixture (20 pM of riboflavin, FMN and FAD) to avoid loss during purification. RnfC was dominantly present as dimer in the gel filtration profile (left panel). The UV/Vis spectrum (right panel) indicates iron-sulfur clusters and a flavin shown in brown for isolated RnfC. The red and blue curve were obtained after oxidizing RnfC with 6 and 12 μ M hexacyanoferrate(III). Addition of 4 x 3 μ L of dithionite (20 mM stock solution) results in brown, violet, orange and blue curves. For measuring RnfC activity, NADH was oxidized with hexacyanoferrate (III).

a

AKB90213.1	<i>Y. pestis</i>	---MTESYLTI FIRA VFVENMALNFFLGMCTFLAISKKVETA FGLGLT VTALLAIATPLN	57
TWU82119.1	<i>H. influenza</i>	----MEHYISL FVKAVFIENMALSFFLGMCTFLAVSKKVSTAFGLGI AVTFVLGIAVPVN	56
ATD25238.1	<i>V. cholerae</i>	----MEHYISLLVKSIFIENMALSFFLGMCTFLAVSKKVKTSFGLGIAVIVLTISVPVN	56
GAD71947.1	<i>V. alginolyticus</i>	----MEHYISLLVKSIFIENMALSFFLGMCTFLAVSKKVKTSFGLGVAVVVVLTIAVPVN	56
sp Q8TSY0.1	<i>M. acetivorans</i>	MVKMAESLFTIFLEGVF IKNFLLIQFLGLCSFVGVT KDLSASGMSGAVVFMAMAATVS	60
ACR23746.1	<i>A. woodii</i>	-----MTLIFIMISAI FVNNFVLSRFLGICPFLGVSKQVETAVGMGVA VTFVMALASAIT	55
SQB91611.1	<i>C. tetanomorphum</i>	-----MSIFTIFISALLVNNFVLSRFLGICPFLGVSKKVETATGMGA AVTFVMALAAIMT	55
AKE29923.1	<i>T. maritima</i>	-----MKVFLLFFSAIFVNNFVLARFLGICPFLGVSKRLETATGMGIA VTFVMTVSAAIS	55
CAD44486.1	<i>P. stutzeri</i>	-----MEYALFLIGTVLVNNFVLVYFLGLCPFMGVSGKLDPSLGMGLATT LVMTLGGVSS	55
CAD6013402.1	<i>E. coli</i>	----MTDYLLLFVGT VLVNNFVLVKFLGLCPFMGVSKKLETAMGMGLATT FVMTLASICA	56
APQ65023.1	<i>S. enterica</i>	----MTDYLLLFVGT VLVNNFVLVKFLGLCPFMGVSKKLETAMGMGLATT FVMTLASICA	56
AZP58876.1	<i>P. aeruginosa</i>	----MTLALILVSAILVNNFVLVQFLGLCPFMGVSRKIETAIGLSLATT FVLTLAAMCS	56
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TWU82119.1		QLIYANVLKENALIEGVDLSFLNFITFIGVIAGLVQILEMVLDRFFPPLYNALGIFLP--	114
ATD25238.1		NLVYNLVLKPDALVEGVDLSFLNFITFIGVIAALVQILEMILDRFFPPLYNALGIFLP--	114
GAD71947.1		NLVYNLVLRENALVEGVDLSFLNFITFIGVIAALVQILEMVLDRFFPPLYNALGIFLP--	114
sp Q8TSY0.1		FALYNFILVP-----LKLEFLRTIAFIVVIAALVQVLEFIVRKHV PALYRSLGIYLP--	112
ACR23746.1		YVVQYAILDP-----LSLGYLQTI AFILIIAALVQLVEMI KSSPSLYQALGVYLPFL	109
SQB91611.1		FLVERFILIP-----LNIQYLS TLAFILVIASLVQFVEMVIKKVSPDLYKALGIYLP--	107
AKE29923.1		WFLDKLL-IS-----TGLEFLRTIVFILVIASFVQFVELFLKKTSPDLYEALGIFLP--	106
CAD44486.1		WLLERYVLQP-----LGLEFLRILSYILVIAGLVQLIEMI IRRVSPPLYRSLGIYLP--	107
CAD6013402.1		WLIDTWILIP-----LNLIYLR TLAFILVI AVVVQFTEMVVRKTS PVLYRLLGIFLP--	108
APQ65023.1		WLIDTWILIP-----LDLIYLR TLAFILVI AVVVQFTEMVVRKTS PALYRLLGIFLP--	108
AZP58876.1		HILQRYVLRP-----LDLEYLR TI GFILVI AVVVQFTEMLVVKKTS PLLYRVLGIFLP--	108
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TWU82119.1		-LIAVNCAIFGGVSMVQRD-YNFPESIVYGFSGGLGWMLAIVALAGLTEKMKYA-DIPA	171
ATD25238.1		-LITVNCAIFGGVSMVQRD-YSFAESVVYGFSGVWMLAIVALAGIREKMKYS-DVPP	171
GAD71947.1		-LITVNCAIFGGVSMVQRD-YNFAESIVYGFSGVWMLAIVALAGIREKMKYS-DVPP	171
sp Q8TSY0.1		-LITTNC AVLGA VLLNMND-YDLAQSVVFGVAAGLGYTVAMLMMAAIRERSDLVEVPKS	170
ACR23746.1		PLITTNC AVLGVALLINI QNE-YNFIE TIFNGVGAALGFTLAI VLFAGIRERLETS-AVPK	167
SQB91611.1		-LITTNC AVLGM AVINSNEK-YNLIQSIINSVGAALGFTLALVLLAGIREKMETNEYIPE	165
AKE29923.1		-LITTNC AILGMVLLNSLMK-LNFVEAVFHALGSGLG FALALVIFAGIREKMDLY-DLPE	163
CAD44486.1		-LITTNC AVLGVPLISVREG-HRLAEAGLFLGLGSALGFTLVMVIFAGLRERLALA-SVPA	164
CAD6013402.1		-LITTNC AVLGVALLNINLG-HHFLQSALYGFSAAVGFSLVMVLF AAIRERLAVA-DVPA	165
APQ65023.1		-LITTNC AVLGVALLNINLG-HHFLQSALYGFSAAVGFSLVMVLF AAIRERLAVA-DVPA	165
AZP58876.1		-LITTNC I VLG VALLNANKAEYGF LQATTQGF GAGLGFSLVLVLF AALRERIAA-DVPA	166
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AKB90213.1		GLQGLGIVFMTAGLMSLGFMFSFGIRL--	199
TWU82119.1		GLKGLGITTFISVGLMALGFMSFSGIQL--	198
ATD25238.1		GLRGLGITTFITAGLMA LGFMFSFGVQL--	198
GAD71947.1		GLRGLGITTFITVGLMALGFMSFSGVQL--	198
sp Q8TSY0.1		VGRGVTYAFFIATIMSMSFVNFFGVIPLE	199
ACR23746.1		ALEGFP IALLTAGLMAIAFLGFSGMKLG-	195
SQB91611.1		ALKGLPITLVTAGLMAIAFLGFQGLI---	191
AKE29923.1		PFKGTAIALITAGLLSLAFMGFGQMVKL-	191
CAD44486.1		AFAGAPIAFVTAGLLAMAFMGFAGLI---	190
CAD6013402.1		PFRGNAIALITAGLMSLAFMGFSGLVKL-	193
APQ65023.1		PFRGNAIALITAGLMSLAFMGFSGLVKL-	193
AZP58876.1		PFRGAAIGMITAGLMSLAFMGFSGLVRP-	194
* : . . : : : * : * * :			

b

EGT68456.1	<i>E. coli</i>	-MNAIWIAVA AVSLLGLA FGAILGYASRRFAVEDDPVVEKIDEILPQSQC GCGGYPGGRP	59
APQ65024.1	<i>S. enterica</i>	-MNTIWI AVGALALLGLVFGAILGYASRRFAVEDDPVVEKIDAILPQSQC GCGGYPGGRP	59
SUD83625.1	<i>P. stutzeri</i>	-MSLVLIA IALLALCLVCGAILGFAAVRFRVEGDP IAEQINALLPQTQC GCGGYPGGRP	59
AZP58875.1	<i>P. aeruginosa</i>	-MNGVFLAIGALLPICLAGGALLGYAAVRFVQGDPAEQVNALLPQTQC GCGGYPGGRP	59
AGL49173.1	<i>T. maritima</i>	--MEVIYSTLLAVLGF GFGAFLAYSAQRFKVEEDPRVKMIT EVLPGINCGAGGFAGGEEA	58
sp Q8TSX9.1	<i>M. acetivorans</i>	MSSVLINSIAVLAGLGF AVGVMLV IASKVKFKIDS NPLIDDVASLLPGANCGG GGFAGGAAA	60
ACR23747.1	<i>A. woodii</i>	MLNAILVPVGLILGVFLGIFIGLGLAIAAKVFVEYEDPRVPLVRAALPGANCGG GGLPGGDA	60
SQB91612.1	<i>C. tetanomorphum</i>	-MEGLLFPVLSLGG LGVVFGLLLGYASKKFAVEVDERVPMVRAALPGANCGG GGFAGGDA	59
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EGT68456.1	YAEAISCNGE	KINR	APGGEAV-MLKIAELLNVEPQPLDGE-----AQELTPARMV	109					
APQ65024.1	YAEAVGLQGE	KINR	APGGEAV-MLKIAELLNVEPQPCDGE-----EQQAAPVRML	109					
SUD83625.1	YAEAIA-GGDK	KINR	PPGGEAT-IQALADLLDVEPEPLDAA-----EGEVPP--RV	106					
AZP58875.1	YAEAIA-AGDK	KINR	PPGGEAT-IRALADLLDLEPEPLDAA-----E-ETPP--RV	105					
AGL49173.1	YAKAIVKGQAETNR	LPGRPQGV	EEKIKKILEEYKNVSS-----	97					
sp Q8TSX9.1	CAEAIVEQGAPVNS	CPVGGFEV-AKQIGALLGQEVTESEKEFFVVR	QGGNQHCTT--LY	117					
ACR23747.1	LAANIVGSSAAIDAC	FPVGGASC-AAVAEIMGMEAGSAVKKVATVI	QGTCTETAPN--RA	117					
SQB91612.1	YADAVVNAGAKPNG	FPVGGAAC-AAKIAEIMGVVDSSEPCKAYVK	QGTCDKAKE--KY	116					
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EGT68456.1	AVI-----	DENNCIGCTKCIQAC	CPVDAIVGATRAMHTVMSDLC	CTGCN	151				
APQ65024.1	AVI-----	DENNCIGCTKCIQAC	CPVDAIVGATRAMHTVMSDLC	CTGCN	151				
SUD83625.1	AYI-----	REAECIGCTKCIQAC	CPVDAIVGAQMHTVIVTECT	CTGCD	148				
AZP58875.1	AYI-----	REAECIGCTKCIQAC	CPVDAIVGAARLMHTVIADECT	CTGCD	147				
AGL49173.1					97				
sp Q8TSX9.1	DYHGVENCKVAL-MLCDSRKG	TYGCLGLGT	CVQACQFGALSMGEDGFPVVNKALCTSCG	176					
ACR23747.1	EYYGEMDCREAM-IASGSGSKGRYGCLGYGTCKAV	CPFDAIVIGEDGLPKVDPEKCTSCG		176					
SQB91612.1	EYYGAMTCVDAANIPGAGSKTG	FGCLGLGS	CVQVCAFDAIHV-ENGIADVDEEACTGCG	175					
EGT68456.1	LCVDP	CPPTHCSISLQPAETPDSWKWDLNTI-PVR-----IIPVEH	190						
APQ65024.1	LCVDP	CPPTHCSIELRPVNETPDSWKWDLNTI-PVR-----IIPVEQ	190						
SUD83625.1	LCVEPC	CPVDCIDMIELGSLNLSQSWKWDQPLA-PGQ-----LIASDR	187						
AZP58875.1	LCLEPC	CPVDCIEMRETPDDVRHWWKWPQSP---R-----LIASDR	184						
AGL49173.1				97					
sp Q8TSX9.1	NCIAACPNGVLT	FARDEKVVHL	CRSHDKGKDVKAV	EVG	CIGCKK	KEKE	C	PAGAIRVTE	236
ACR23747.1	KCVEACP	PKSIMTLVPEAQEVIVK	CHNFDKGKIARLS	CTTACIACGACVKA	CRFDAITVEN	236			
SQB91612.1	ACVSI	CPKSVIELTPMSKKVRIS	CNSHDKGIEVKNA	SVG	CLSCGL	CVRN	CP	SEAITMVN	235
EGT68456.1	HA-----								192
APQ65024.1	HA-----								192
SUD83625.1	EQAA-----								191
AZP58875.1	ERAA-----								188
AGL49173.1									97
sp Q8TSX9.1	FLAEIDQEKCTACGACVAIC	CPQKAIELR-----							264
ACR23747.1	NCAKIDYDKRQCYECVDKCPMNCISGDVEY	GKSTAYI	IEEN	IA	GL	AKN	IPVNAITG	296	
SQB91612.1	NLPVIDYDKCTQCGV	VGKCP	TKAIVNLNTNVSKEA---SNN-----					274	
EGT68456.1									192
APQ65024.1									192
SUD83625.1									191
AZP58875.1									188
AGL49173.1									97
sp Q8TSX9.1									264
ACR23747.1	EIKKPPYVIDHDM	IG	GI	FDK	RKSAIEMRP	NKTK			333
SQB91612.1									274

C

WP_000103965.1	V. cholerae	MSTIIFGVVMFTLIIILALVLVILFAKSKLVPTG-----DITISINGDPEKAIV-----	48
AAM04101.1	M. acetivorans	-----MKRSLHSKEVANLSDV--IKIDKLPEKAIIPMRQHD	34
AKE29919.1	T. maritima	-----MLTFKGGVHPPELKEWSKDKPIERAPLPQKVVFVLSNHA	39
ACR23742.1	A. woodii	-----MNVKHGTFKGGIHPYRKESTAEVPLGFGKKPEMVIIPMSLHI	43
KAJ52772.1	C. tetanomorphum	-----MELLTFKNGVHPHGHKHYTENKPIEEYLPKGDIVIPMSQHI	41
CAD6007421.1	E. coli	-----MLKLFSAFRKNKIWDFNGGIHPPEMKTQSNGTPLRQVPLAQRFVILPKQHI	51
ALE62000.1	S. enterica	-----MLKLFSAFRDKKIWVFDGGIHPPEMKTQSNGTPLRQVPLAPRFVILPKQHI	51
AEJ04398.1	P. stutzeri	-----MTSLKVWDIHGGIHPPEHKELSNRTPIQPAPLPKRLILPLAQHL	44
KYO98541.1	P. aeruginosa	-----MSALHAFPGGLCLPANKERSTALPIQQAPLAQRYIVPLGQHI	42
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WP_000103965.1			48
AAM04101.1		GIACAPLVKKGAEVIVGQKLGECEGSDLAYVHSPFCGTVNSIELMP--NPS-GKRILSVV	91
AKE29919.1		GNPAKPVVSPGDEVKTQGVIGEPGFISAYLHSPVTGRVLEIKKIL--HPILGKPIEAIV	97
ACR23742.1		GAPCTPIVKKGDTVFLGQRVGEPNGFVSVPVHASVSGKVIAVEERP--HAS-GDRVMSVV	100
KAJ52772.1		GAPAEPIVKKGDRVLVGQKIGEAQGFVSANIHASVSGTVKNVAPVT--LFN-GVKSTAVI	98
CAD6007421.1		GAEGELCVSVGDKVLRGQPLTRGRGKM-LPVHAPTSGTVTIAIAPHSTAHPS-ALAELSVI	109
ALE62000.1		GAEGELCVSVGDRVLRGQALTRGRGRM-LPVHAPTSGTVTIAIAPHSTAHPS-ALAELSVI	109
AEJ04398.1		GAPAEPCVALGEHVLKGQIIVASGFVSAALHAPTSGVVSFIGPQYPHVS-GMLANAIV	103
KYO98541.1		GAPARPCVEVGQAVLKGQTIAPDGTVSAALHAPTSGTVVGAHPYPHAS-GLPAPAIIV	101

WP_000103965.1	-----TQPGGKLLTALAGAVFVSSACGGGGSCGQCRVKIKSGG	87
AAM04101.1	LTPSECAQTVD----FVPEKDAPPSRLIEI IKEAGI----VEY----YEKPTYLALKP	137
AKE29919.1	IERTSDDEWVHI---ETGDFERMSKEEILEI IKKAGI----VGLGGAMFPTHVKLSPPP	149
ACR23742.1	IESDGL-DTIDPSIKPYGTLEDMDADAIKKMVLNAGI----VGLGGATFPPTHVKLAIPP	154
KAJ52772.1	IENDGQYEEIETEKRDYTK---LSNEEI INI IKEAGI----VGMGGATFPPTHVKLAPPP	150
CAD6007421.1	IDADGEDCWIPRD--GWADYRTRSREELIERIHQFGV----AGLGGAGFPTGVKLGQGG-	161
ALE62000.1	IDADGEDRWIERE--GWSDDYRAHSREALIERIHQYGV----AGLGGAGFPTGVKLGQGG-	161
AEJ04398.1	IDSDGEDRWIELE--PHLDYRALERPALLELRQAGI----SGLGGAGFPTAVKLTTPPP	156
KYO98541.1	IASDGLERWTTELH--PCPDFRAESPLALLERIRAAGI----GGLGGAGFPTAAKLAARP	154
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WP_000103965.1	GDILPTELDHISKGEA--REGERLACQVAVKADMDLELPEEIFGVKKWECTVISNDNKAT	145
AAM04101.1	GKRIDTLLMNATF--PLIT---HAYLSSLDKVLLEGFKLMLEASGISR--GVIVLRADDKE	190
AKE29919.1	EKKVDTLIVNGAECEPVLTI DHRLMLERAEDILQGILIMMKVLGVQK--AVVGVESNKMD	207
ACR23742.1	DKKVDCCVVLNGAECEPYLTADHHLMTSQAQKVVVMGLKAMKSVGVEK--GFTGVEDNKTD	212
KAJ52772.1	DKNIDSIVVNAACEPEPYLTCDHRMMLEKTNEIVEGLKIVLKLFPKAT--GYIGIEDNKMN	208
CAD6007421.1	GDKIETLIINAAACEPYITADDRMQDCAAQVVEGIRILAHILQPRE--ILIGIEDNKPKQ	219
ALE62000.1	GDKITTLIINAAACEPYITADDRMQDCAAQIVEGIRILAHILQPRE--VLIGIEDNKPKQ	219
AEJ04398.1	TQIRITLIINGTECEPYITADDLMLREKAELVAGIEILAHLIQPDQ--VLIGIEDNKPE	214
KYO98541.1	AEKIHTLVVNGAECEPYISADDLMLRERATQVLGGIDILVQILRPEE--VLVGIEDDKPE	212
	. : : : : . . : : . : : .	
WP_000103965.1	FIKELKLAIPDGGSPVFRAGGYIQIEAPAHVVKYADFVPEKYRGWDKFNLFYRESKVD	205
AAM04101.1	SIKAFKNAKVDGKPLTVAPI--VGM----RHADYYLEDVEDQII--VVAAGKITYPPTMM	242
AKE29919.1	AYHNLKKVFKG-YPVDVALL-----RTKYPQGAE	235
ACR23742.1	AIEALVKAIGNDSRLEVYSL-----HTKYPQGAE	241
KAJ52772.1	AIKAMQEAVNKIANIEVKAV-----KTKYPQGAE	237
CAD6007421.1	AISMLRAVLADSNDISLRVI-----PTKYPSGGA	248
ALE62000.1	AISMLRAVLADAHDISLRVI-----PTKYPSGGA	248
AEJ04398.1	AIAAVRAAIGER-PFVLKVF-----PTKYPSGGE	242
KYO98541.1	AIAALGAALGER-PYRIVAL-----PTRYPSGGE	240
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AKE29919.1	KQLIYAITGRMVPRGGPLMDVGVVVQNVGTCAVKEAVVDGKPLVERGM-TV-SGDAVKN	293
ACR23742.1	KQLIAAITGREVPSPGALPADAGVVVMNVGTAAQIAESMITGLPLYKRYL-TC-TGDAIKN	299
KAJ52772.1	KQLIYAITKREVPSGGPLPADAGCIVQNVDTIYEIYNVAVNGKPLTSRVV-TV-TGDAIKE	295
CAD6007421.1	KQLTYILTGKQVPHGGRSSDIGVLMQNVGTAYAVKRAVIDGEPITERVV-TL-TGEAIA	306
ALE62000.1	KQLTRILTGKQVPHGGRSSDIGVLMQNVGTAYAVKRAVIDGEPITERVV-TL-TGEAVSR	306
AEJ04398.1	KQLIQILTGEVPSGGPLPADIGMLCQNVGTCAIHDVLLGKPLISRIT-TL-TGEALAR	300
KYO98541.1	RQLIQLLTGEVPPADGLPADIGILCQNVGTAAVHDAVVLGRPLISRIT-TL-AGGALER	298
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WP_000103965.1	----KCTISGPFGEFFAK----DTDAEMVFIGGGAGMAPMRSHIFDQLRLKLSKRKMSYW	308
AAM04101.1	PKTVIVKFGTPIKDVI EACGGYKGE PGKVI VNGSMGGVAV-----YT	341
AKE29919.1	QKNLIVRIGTPVKQDVIDYCGGIDENTERVILGGPMMGISI-----TN	335
ACR23742.1	PQTIEIRIGVPFQSVIDQCGGFSSEPGKVISGGPMMGVQTQ-----FV	341
KAJ52772.1	PKNLRFKIGTSVRELVEAAGGFAEEPLKVISGGPMMGMAM-----YS	337
CAD6007421.1	PGNVWARLGTTPVRHLLNDAGFCPSADQMVMIMGGPLMGFTL-----PW	348
ALE62000.1	PGNVWARLGTTPVRHLLNDAGFCPSADQMVMIMGGPLMGFTL-----PW	348
AEJ04398.1	PMNVEALIGTPAGELLAFAGLEQNRLNRLIMGGPMMGFTL-----PS	342
KYO98541.1	PMNVEVLIGTPVHELLAFAGLAEGRLERVLIMGGPMMGFAL-----PD	340
	: . . : : *	
WP_000103965.1	YGARSKREMFYVEDFDGLAA-----ENDNFVWH CALS -----DPQPEDNWTGYTG	353
AAM04101.1	DEAPV-----VKNTVGIVVQTEAEVL-RDEATV C IHCAR C VDV C PMNLLPGRIAAMADM	394
AKE29919.1	LDIPV-----MKGTSGITAFLP-KKS--RPQKPCIRCSEC VQV CPMNLQPYLLYLLSTK	386
ACR23742.1	TDIPV-----MKGTSGILCLTK-ESAKIATPSN C IHC GK CVG V CP I HLQPLNIAEYSQR	394
KAJ52772.1	LDVPS-----TKGTSGLVCLTK-KVAEIEEESN C INC GK CVG V CPMNLMP T KLATASAV	390
CAD6007421.1	LDVPV-----VKITNCLLAPSANELGEPQEEQ S CIRC SAC ADAC AD PADLLPQQLYWFSGK	402
ALE62000.1	LDVPV-----VKITNCLLAPSVTEMGAPQEEK S CIRC SAC ADAC AD PADLLPQQLYWFSGK	402
AEJ04398.1	LDVPV-----IKTINCLLASTLEELPPPPALP C IRC GE CAE AC PVSLLPQQLHFFALG	396
KYO98541.1	LSVPL-----IKTCNCLLAGDATELPEPVAMP C IRC GDC AQV C PVSLLPQQLHFFALG	394
	: : . * . *	
WP_000103965.1	FIHNVL YENYLKDHEAPED C EY Y M C GPPMMNAAVINMLKNLGV EEN ILLDDFGG-----	408
AAM04101.1	GMFDRCREYFALN C IE G GA A -VV C PAKR-----HLVQLIRYSKLQIMNQKNETVEATE	447
AKE29919.1	RKYDEAVENGLMD C IE G GS T -YT C PSKI-----EHVRYIKLAKTVYRATRGRRR----	435
ACR23742.1	NMWDKCESNNAMD C IE G GS S -YI C PAKR-----TLVSSIRVAKREIIAQRRKGN----	443
KAJ52772.1	SNLDMFNEFSGRDC C IE G GS S -FV C PARR-----HLLQIRIRSGKKAVSKKK-----	435
CAD6007421.1	QQHDKATTHNIAD C IE G GA A -WV C PSNI-----PLVQYFRQEKAEIAAIRQEEKRAAE	455
ALE62000.1	QQHDKATAHNIAD C IE G GA A -WV C PSNI-----PLVQYFRQEKAEIAAIRLEEKRAAE	455
AEJ04398.1	QEHEQLKAHLFD C IE G GA A -YV C PSSI-----PLVQYYRAAKAEIRELQKQQAHEH	449
KYO98541.1	DEHEQLLAHLNFD C IE G GA A -YV C PSSI-----PLVQYYRASKAEIREQRQKLLKAEQ	447
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WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	AKARFEARQARLEREKAARLERHKSAAV---QPAAKDKDAIAAALARVKEKQAQATQPI	511
ALE62000.1	AKARFEARQARLEREKAARLARHKSAAV---QPAAKDQDAIAAALARVKEKQAQATQPV	511
AEJ04398.1	SKQRFELRQERLRRAEQEKEAERKARAERAARAKAAQGESAQVVS-----DTTAA--	499
KYO98541.1	SRERFEQRQARLRRDEERRAAERAQRAEKAALARAQAERE EAAP-----ATAVDPV	499
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	VIKAGERPDNSAI IAAREARKAQARAKQAE LQQTNDAA TVADPRKTAVEAAIARAKARKL	571
ALE62000.1	VIQAGSQPDNSAVIAAREARKAQARAKQA AHPMADSAI PGDDPSKA AVEAAIARAKARKQ	571
AEJ04398.1	-----TPSAAPKS-----GLSETQKKLIEASMAQVALKKA	530
KYO98541.1	QA-----AIERARARKQAG-----SGSERLKR LKIEASMARVALKKA	536
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	EQQQANA-----EPEQQVDPRKAAV	591
ALE62000.1	EQ-QAGS-----EPAEPVDPRKAAV	590
AEJ04398.1	EKQLAAHDTPELQAQVADLRKAAEAAQ QALDAVM--QENASAAPTVSPADDEALKKAKI	587
KYO98541.1	EKQLLGHDTP EQHGLVAELRAAAEAADKALADAEASLPRDLPSAPPAALDDEALKKAKA	596
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	EAAIARAKARKLEQQQANAEPEE---QVDPRKAA---VEAAIARAKARKLEQQQANAEPE	645
ALE62000.1	EAAIARAKARKQEQ-QAGSEPVE---AVDPRKAA---VEAAIARAKARKQEQQ-TGSEPA	642
AEJ04398.1	EAAMLKAQIRKLEKL--EAPDDDQQAELARLRQQLHEAEQALAAAQ-----SAAPAPA	638
KYO98541.1	QAAMARAQLKRSEKAFGEAPGAEQRATLDELRAEVERCEATLARLE-----RHAPKPA	649
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	QQ-----VDPRKAAVEAAIARAKARKREQQPAN-----	673
ALE62000.1	EA-----VDPRKAAVEAAIARAKARKQEQQT-G-----	669
AEJ04398.1	AKPA-DDEALKKAKIELAMKRAELKKA EKAGAQEAELSRLRDALQAAEQALHAAEDASHK	697
KYO98541.1	APGDDGQAALKRAKIALV GKRAALKKAEQAGVMDSELERLRGELQAAERDLHAAEDACGK	709
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	-----AEPEEQVDPRKAA-VEAAIARAKARKLEQQQANAVPEEQVDPRKAAVAAA IAR	725
ALE62000.1	-----SEPAEPIDPRKAA--VEAAIARAKARKQEQQ-AGSEPAEPADPRKAAVAAA IAR	720
AEJ04398.1	PAPELVRTSKPGIDERQRELKTEVAFARADLRKLERDEQT-EPT-----TLEAARLR	748
KYO98541.1	PAPELVRIDKRFPVDPRTRELKTELAYARAALKKLERLANA-DAA-----ALAAARAR	760
WP_000103965.1	-----	408
AAM04101.1	-----	447
AKE29919.1	-----	435
ACR23742.1	-----	443
KAJ52772.1	-----	435
CAD6007421.1	AQAKKAAQQKVVNED---	740
ALE62000.1	VQAKKAAQQKVVNED---	735
AEJ04398.1	LSEA----ERQLADYQQS	762
KYO98541.1	LSAA----ERALTEHGTE	774

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GAD71712.1	V. alginolyticus	MSLKHYFEKATPKFGPGGKYHKFYPLFEAVETIFYTPGKVNQGMTHVRDSIDLKRMIMV	60
AAX87224.1	H. influenza	MGLKNLFEEKMEPAFLPGGKYSKLYPIFESIYTLTYPGTVTHKNTHVRDALDSKRMIMTV	60
AKB05379.1	V. cholerae	MGLKKFLEDIEHHFEPGGKHEKWFALYEAAATLFYTPGLVTKRSSHVRDSVDLKRIMIMV	60
AKB88576.1	Y. pestis	MGLKNFLEKIEHHFEAGGKLEKYYPLYEAAATIFYTQGVTPGASHVRDAIDLKRMIMLV	60
WP_011020707.1	M. acetivorans	-----MTSFTVSPPPHIKKKIFIKNLIWSR	25
AKE29920.1	T. maritima	-----MKLISAYAPHLREEDDVRKIMLDV	24
AFA48979.1	A. woodii	-----MNELNLTVSSSPHIRAKHSTASIMQNV	27
SQB91608.1	C. tetanomorphum	-----MSETTMYTVSSSPHIRAKDQTQSIMRDV	28
CAC03727.1	P. stutzeri	-----MIRSSVDRIMLHV	13
AZP58873.1	P. aeruginosa	-----MALPRPTSPHARGSNRTPAIMRLV	24
EFF12894.1	E. coli	-----MVFRIASSPYTHNQRTSRIMLLV	24
KPF39873.1	S. enterica	-----MVFRIASSPYTHNQRTSRIMLLV	24
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GAD71712.1		WLAVFPAMFWGMYNIGQQASDALLYGMEQASAQALVSSWQLSWVW--GSLDAVSQAGWES	119
AAX87224.1		FLALFPAIFYGMYNVGNQAI PALNQLG---NLDQLIANDWHYALASSLGLDLTANATWGS	117
AKB05379.1		WLAVFPAMFWGMYNAGGQAI AALNHLYSGDQLAAIVAGNWHYWLTEMLGGTMSSDAGWGS	120
AKB88576.1		WFAMFPAMFWGMYNVGYQAI PALNQLYSGAELQQVIAGDWHYRLAQLMGASITPDAGWAS	120
WP_011020707.1		IVAILPTISAAAVYFFGF AALGNIIAS---ILG-----	54
AKE29920.1		LIALSPNVIGAAVYFFGWYALFLCIAGA---VIG-----	54
AFA48979.1		IIAILPALAVAGYVFGWLWALALVAIC---VIS-----	56
SQB91608.1		VIALLPATIIAGVYFFKLQGLLVILAS---VLS-----	57
CAC03727.1		CLAILPTITAWGLYLFGWPAIYLWLLT---CAS-----	42
AZP58873.1		LGACVPGLLTLTWLYGPGTLLNLAWA---SLV-----	53
EFF12894.1		LLAAVPGIAAQLWFFGWGTLVQILLA---SVS-----	53
KPF39873.1		LIALLPISIAAQTWFFGWGTLFQIVLA---AIT-----	53
		* * :	
GAD71712.1		KMWLGATYFLPVYATVFIVGGFWEVLF AIVRK----HEVNEGFFVSSILFALILPPTIP	174
AAX87224.1		KMALGAIFFLPIYLVVFTVCTIWELFSVVRG----HEVNEGFMVSTILFALIVPPTLP	172
AKB05379.1		KMLLGATYFLPIYATVFIVGGFWEVLF CMVRK----HEVNEGFFVTSILFALIVPPTLP	175
AKB88576.1		KMLLGAAVFLPIYAVVFLVGGFWEVVSIVRK----HEINEGFFVTSILFSLIVPPTLP	175
WP_011020707.1		-----AVGIEFVIQKAFNKKL--TIMDGNAIYLGILLALICPPTLP	93
AKE29920.1		-----ELFDIFVMRYLRGVKD--FVPDGS GAVTGLLLANVSTRLP	93
AFA48979.1		-----SVATEAVIQLLKKPI--TVNDWSAVVTGVLLEALNLPINAP	95
SQB91608.1		-----CVVAEYIWQKASKKKV--TVGDYSAVVTGLLALNVPASIP	96
CAC03727.1		-----AVACEAACLYLLGRPLR--RLLDGSALLSGWLLALTLPWPAP	82
AZP58873.1		-----ALACEAAMLALRRPPGVFLKDGSA LV TALLLAVALPPYAP	94
EFF12894.1		-----ALLAEALVLKLRKQSV AATLKDNSALLTGLLLAVSIPPLAP	94
KPF39873.1		-----ALVAEAIVLRRLRKQSVASHLQDYSALLTGLLLAVSIPPLAP	94
		: . *::: *	
GAD71712.1		LWQAALGITFGIIVAKEIFGGTGRNFLN PALAGRAFLYFAYPANMSGGAV-----	224
AAX87224.1		LWQAALGITFGIIVAKEIFGGVGRNFMN PALAGRAFLFFAYPAQISGDTV-----	222
AKB05379.1		LWQAALGITFGVIVAKEVFGGTGRNFLN PALAGRAFLFFAYPAQISGDLV-----	225
AKB88576.1		LWQAALGISFGVIGKEIFGGTGRNFLN PALAGRAFLFFAYPAQISGDLV-----	225
WP_011020707.1		AWMIFIGGAFAGVGKHAFFGGIGSYTFHPSLAAWVFLSLAWAQDMLPGTIPILSS----	148
AKE29920.1		FWAFLGLVLFALGIGKHVFGGLGQNIFN PALVGRAFL LISFPTYMTTWVVPAGAF----	148
AFA48979.1		WWIGVGSVF AIAIVKQCFGGGLGQNFIN PALAARAFLLASWPGHMTSTAYI-----	146
SQB91608.1		LWIPVVGGFFAIIVVKQFFGGGLGQNFVN PALAARAFLLASWPVQMTSWT-----	145
CAC03727.1		WWIAVGGSMF AIGIKQLYGGVGQNVFN PAMLARVALLIAFPLQMTTWPALPLGTGEGAP	142
AZP58873.1		WWLTIVATFFALVFGKHYGGGLGQNPFN PAMLG YVVALVSFPLEMTRWPSDLSAL--GLP	152
EFF12894.1		WWWVVLGTVFAVIAKQLYGGGLGQNPFN PAMIGYV VLLISFPVQMTSWLPPHEIA-VNIP	153
KPF39873.1		WWWVVLGTGF AIIAKQLYGGGLGQNPFN PAMIGYV VLLISFPVQMTSWLPPYEIA-ATTP	153
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GAD71712.1		-----WVAADGYSGATPLSQWYEGGQSALI--NNMTGQTISWG	260
AAX87224.1		-----WTAADGFSGATALSQWSQGGQGALQ--HTVTGAPITWM	258
AKB05379.1		-----WTAADGYSGATALSQWAQGGAGALI--NNATGQTITWM	261
AKB88576.1		-----WTSADGFSGATPLSQWSVNGSHSLV--NTVSGQPITWM	261
WP_011020707.1		-----F-----S-----	150
AKE29920.1		-----WKSPADVTAATPLALFKEHGVF----TPYWDL----	177
AFA48979.1		-----PLTDTVTATPLALLKAGETGSM--PSTLDL----	175
SQB91608.1		-----LDGVTATPLAILKGNEATGAAAPDLMSV----	174
CAC03727.1		GWLEGLRITFAGGAL-----ADGLSGATALGHLQTELT LGHSAAQILDGHFALL	191
AZP58873.1		DSLREFLG-----LATRPDAWAHATALDVLKTRD--SLTVDELFAG----	191
EFF12894.1		GFIDAIQVIFSGHTASGGDMNTLR LGIDGISQATPLDTFKTSVRAGHSVEQIMQY-----	208
KPF39873.1		DMLDTLRMIFTGHTASGGDMTLLRIGIDGISQATPLDTFKTSVRAGHSVEQIMQY-----	208
GAD71712.1		DAFVGNLPGSMGEVSTLLIALTGLVLIFMKIASWRIVAGVLVGLIGASLLNNAIG---SD	317
AAX87224.1		DAFVGNLPGSMGEVSTLAIITGGAVIVFTRIAAWRIIAGVMIGMIATSTLFLNIG---SE	315
AKB05379.1		DAFIGNIPGSIGEVSTLALMIGAAFIYVMGIASWRIIGGVMIGMILLSTLFLNVIG---SD	318
AKB88576.1		DAFLGYIPGSIGEVSTLMIITGGAIIFGRVASWRIVAGVMIGMIATAYLFWNIG---ST	318
WP_011020707.1		DLILENGAGFLTDVSPILVLAGVILILVKYIEWRIPLSYLLTTVILALV-----L	201
AKE29920.1		F--IGKVGGSLGETSALLITGFIYLLLRKRVKIFIPVSYIGTVLVFSSIAIYLMN--PRY	233

AFA48979.1	FTGLNGVYGCIGEISALALIGGLYLIYKGIISWRIPTIYLLTIAIFALL----	227
SQB91608.1	F--IGHVGGCIGETSALALIGGAYLFYKHIIDWRIPVSFIGTTFIFTAI----	226
CAC03727.1	PAFLGYSGSGIGETSELLILGGLWLLALRIIHWEIPLGMLLTVGALAALANQI-DPQVH	250
AZP58873.1	NPAFGHLGGAGSEWVNLAFLGGLFLLWRRFLTWHAPLGMLAGLFAMSLLFWNGSGSDSH	251
EFF12894.1	PIYSGILAGAGQWVNLAFLAGGVWLLWQKAIKRWHPFSFLVTALCATLGWLFS-PETL	267
KPF39873.1	PIYSGALAGVGWQWVNLAFLVGGVFLWQKAIKRWHPVSFLLTLALCAALGWLFS-PATL	267

GAD71712.1	SNPMFSMPFYWHFVIGGVAEGTFEMATDPVSAFTNNGKWAYGLLIGVMTIFIRVLNPAY	377
AAX87224.1	TNPMFSMPWHWHFVLGGFAEGMFMATDPVSASFNTNGKWWYGALIGVMAVLI RTVNPAY	375
AKB05379.1	TNAMFNMPWHWHFVLGGFAEGMFMATDPVSASFNTSGKWAYGILIGVMCIVLRVNPAY	378
AKB88576.1	TNPLFAMPWYWHFVLGGFAEGMFMATDPVSASFNTGKWWYGGLIGVMCIVLRVANPAY	378
WP_011020707.1	GDPL-----AYVVSSTFLLGVFFIATEVTVTSPVTQNGRIVYGILCGFLTVIYGYFSGNY	255
AKE29920.1	GDPL-----FHLSSGGLMGALFEMATDMVTSPITAKGQVIFGIGCGVLTMAIRLF-GAY	286
AFA48979.1	QDPI-----VHMVSGGVMGGAFFMATDYASSPVTAKGQIIYAIGCGLITMIIRLY-GGY	280
SQB91608.1	SSPV-----YELFAGGLMGALFEMATDYATSPITPLGRIIFGVGCVITSLIRIF-GGY	279
CAC03727.1	GGGL-----FHLTSSGGLMGALFIATDPVTSPISRSGRLLFAIGCGALVFVIRSW-GNF	303
AZP58873.1	GSPL-----FHLFSGATMGGAFFIVTDPVSGATSNRGRVLFGLGVGLTYVIRAW-GGY	304
EFF12894.1	ASPQ-----IHLSSGATMGGAFFILTDPVTASTTNRGRVIFGALAGLLVWLIRSF-GGY	320
KPF39873.1	ASPQ-----LHLSSGATMGGAFFILTDPVTASTTNRGRVIFGALAGVVLVWLIRSF-GGY	320

GAD71712.1	PEGIMLAILFANLFAFLFDYLVKESNIKRRMKRARPS-----	414
AAX87224.1	PEGMMLAILFANLFAPIFDYIVVQANIKRRRARTNG-----	411
AKB05379.1	PEGMMLAILFANLFAFLFDHVVERNIKRRRLARYGKQ-----	415
AKB88576.1	PEGMMLAILFANLFAFLFDYVVVQANIKRRKARGE-----	413
WP_011020707.1	VWGTLYALLLSNAVAPFIELKTLPKPMGGVANE-----	288
AKE29920.1	PEGVSFSILFMNALVPLIDRYTRPRIFGEVKK-----	318
AFA48979.1	PEGCSYSILLMNVA TPLIERTKERIYGVTKIKKEAKA-----	318
SQB91608.1	PEGVSYSILVMNLFVPLIERWTAPKIFGKVK-----	310
CAC03727.1	PEAVAFVLLMNALVPLIDRCRPRAYGRNARGKPLVAAKWTRQVKEVDKV	354
AZP58873.1	PDGVAFVLLMNLAAPTIDYYTRPRTYGHRKAERGFKAGD-----	344
EFF12894.1	PDGVAFVLLANITVPLIDYYTRPRVYGHRKG-----	352
KPF39873.1	PDGVAFVLLANITVPLIDYYTRPRVYGHRKG-----	352

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TWU82118.1	H. influenza	-----MSGKTSYKDLLAPIAKNNPIALQILGIC	SALAVTTKLETAFAVMAIAVT	49
AKB89713.1	Y. pestis	-----MADSKIEIKRVLLSPLFDNNPIALQILGVCS	SALAVTTKLETAALVMTLAVT	49
ATD25239.1	V. cholerae	-----MSSAKELKKSVLAPVLDNNPIALQVLGVCS	SALAVTTKLETAFAVMTLAVM	49
GAD71946.1	V. alginolyticus	-----MSSAQNVKKSILAPVLDNNPIALQVLGVCS	SALAVTTKLETAFAVMTLAVT	49
AAM04104.1	M. acetivorans	-----MYPHRRADMNPISSEFIRGITKDNPTFGLVLGLC	PTLAVTTSVENGIGMAMGTL	53
AGL49171.1	T. maritima	-----MSRLRELTGKI IKENPTYVQVLGMC	PTLAVTTSAINGLGMGLATT	45
ACR23745.1	A. woodii	-----MNFMKNLTRGI IRENPTFVLVLGMC	PTLAVTTSAINGMGMGLATM	45
SQB91610.1	C. tetanomorphum	-----MGVVSERLYNGIVKGNATFVQVLGMC	PTLAVTTSAINIGMGLSAT	46
CAC03729.1	P. stutzeri	MSSQCGSADVTAPKPKGLFNYFSSALWDYNVALVQMLALCP	ALAVTTTATNGLGMGLATT	60
AZP58871.1	P. aeruginosa	-----MSEQDFREIARNGLWRNPNGLVQLLGLC	PLLGTSTVNALGLGLATT	48
CAD6013372.1	E. coli	-----MSEIKDVIVQGLWKNNNSALVQLLGLC	PLLAVTSTATNALGLGLATT	46
CCW74136.1	S. enterica	-----MSEIKDIVVQGLWKNNNSALVQLLGLC	PLLAVTSTATNALGLGLATT	46

TWU82118.1	LVTGLSNLFSVSLIRNYIPNSIRIIVQLAIIASLVIVVDQILKAYAYGLSKQLSVFVGLII	109
AKB89713.1	LVTAFSSFFISLIRNHIPNSVRIIVQMVI IASLVIVVDQVLRAIYAYEISKQLSVFVGLII	109
ATD25239.1	FVTALSNFFVSLIRNHIPNSVRIIVQMAIIASLVIVVDQILKAYLYDISKQLSVFVGLII	109
GAD71946.1	FVTALSNFVSLSIRNHIPNSVRIIVQMAIIASLVIVVDQVLKAYLYDISKQLSVFVGLII	109
AAM04104.1	FVLVGSNNMVS AIRKGIPGTVRLPVEIIVVIATFVTIVDMVMEAFTPDLYTSLGVFIPLIV	113
AGL49171.1	AVLTMSNVVISLIRKIVDPKIRIPFIVVIASEFVTMIDLMLHGFAYDLMLKTLGLFIPLIV	105
ACR23745.1	LVLIGSNVAISALRKVIPDNIRIPAFVVVIA SFVTIVGMLMKAYVPALDAALGIFIPLIV	105
SQB91610.1	VVLIGSNVVISL LKKVIPDEIRIPAYITVIATLVTLVQLFLLQAYLPDLNKS LGIFIPLIV	106
CAC03729.1	LVLMITNAIISALRHSISPVRNPLMIGI IAGVVTLIDMAINAWMHELYKVILGLFIALIV	120
AZP58871.1	LVLACSNAAVSLVRGAVSEAIRLPFVMI IAVLTTCIELLMQAWTYELYQVLGIFIPLIT	108
CAD6013372.1	LVLTLTNLTISTLRHWTPAEIRIPIYVMI IASVVS AVQMLINAYAFGLYQSLGIFIPLIV	106
CCW74136.1	LVLTLTNLTVSALRRWTPAEIRIPIYVMI IASVVS AVQMLINAYAFGLYQSLGIFIPLIV	106

TWU82118.1	TNCIVMGRAEAFAMKSPVESFVDGIG-NGLGYGSMIIIVAFFRELIGSGKLF-----	162
AKB89713.1	TNCIVMGRAEAYAMKSPPIESFMDGIG-NGLGYGVILVLVGFVRELVGSGKLF-----	162
ATD25239.1	TNCIVMGRAEAFAMKSEPIPSFIDGIG-NGLGYGFVLMVTGVFFRELIGSGKLF-----	162
GAD71946.1	TNCIVMGRAEAFAMKSAVPVSLIDGIG-NGLGYGFVLTITGVFFRELFGSGKLF-----	162
AAM04104.1	VNCIVIGRAEAYALKNGVFYSIIDALG-EGTGFLLVILIGGIRELLGTGIIDP-----	166
AGL49171.1	VNCIIMGRAESFASKHGVLD SMLDGLG-VGLGFTGSLVLLG SVRELFGNGTIFG-----	158
ACR23745.1	VNCIILARAEAFASFNGIADSFADAVG-MGLGFTLALTILGSIREILGAGSIFG-----	158
SQB91610.1	VNCIILGRAEAYANKNSVGSFFDGLG-MGLGFTVSLAALGIREFLGTGKVF-----	159
CAC03729.1	TNCAVLGRAESFCSNRNVPVPSILDGRRAWASGFTWVLVIGGIREILGQRARCSPPRRL	180
AZP58871.1	TNCIVLGRAEFAAKNGVLRASFDGLL-MGLGFALVLLVGLGREL LQGOTLLA-DMHLL	166
CAD6013372.1	TNCIVVGRAEFAAKKGPALSDGFS-IGMGATCAMFVLGSLREI IGNGLTFD-GADAL	164
CCW74136.1	TNCIVVGRAEFAAKKGPWLSALDGFS-IGMGATGAMFVLGSLREILGNGLTFD-GADSL	164

TWU82118.1	----MTIFETIQNGGWYQANGLFLLAPSAFFIIGFVIWGLRTWKPEQQEK-----	208
AKB89713.1	----VTVLETQNGGWYLPNGLFLLAPSAFFIIGLLIWGLRTLKPAQIEKE-----	209
ATD25239.1	----LEVLPISNGGWYQPNGLMLLAPSAFFLIGFMIWAIRTFKPEQVEAKE-----	210
GAD71946.1	----LEVLPISNGGWYQPNGLMLLAPSAFFLIGFLIWVIRILKPEQVEAKE-----	210
AAM04104.1	----FGMT-LINLSGVINPAMFMTMSPGAFLTIAVLMITIVNRRQQKAAKGG-----	213
AGL49171.1	----YKVV-----EL---KIFLEILPPGAYITLGLLSALFTYIGIRKKRGEAK-----	200
ACR23745.1	----FSLF-----GAAYEPVLLMILPPGAFITLGLLIGLINWKTCKA-----	196
SQB91610.1	----AQIT-----PDAFQPALIMILAPGGFFTLGILMAILNQRKLKAK---AK-----	201
CAC03729.1	LGEHFRWL-EITVLPGFQGILLAILPPGAFIVLGFVLAFAKRVVDRRAERRIRTHGELVV	239
AZP58871.1	FGPAAADW-KIQPPFQYQGFLAILPPGAFIMLGLLIALKNRIDESLAERAKVQAGDVPA	225
CAD6013372.1	LGSWAKVL-RVEIFHTDSPFLLAMLPPGAFIGLGLMLAGKYLIDERMKKRRAEAAAERAL	223
CCW74136.1	LGGWAKVL-RVEIFHTDSPFLLAMLPPGAFIGLGLMLAVKYLIDEMKKRRAETA-PSAV	222
	: : *...: ...:	

TWU82118.1	-----	208
AKB89713.1	-----	209
ATD25239.1	-----	210
GAD71946.1	-----	210
AAM04104.1	-----	213
AGL49171.1	-----	200
ACR23745.1	-----	196
SQB91610.1	-----	201
CAC03729.1	LQ-----	241
AZP58871.1	TQRQVRVTVGIV	238
CAD6013372.1	PNGETGNV----	231
CCW74136.1	PAGETGKV-----	230

f

AAX87225.1	H. influenza	-----MAKFNKDSVGGTILVLLSLVCSIIIVAGSAVMLKPAQ	38
AKB87827.1	Y. pestis	-----MASDKPRNNDISGKTLVVVILCLVCSVVVAGAAVGLKAKQ	41
KKP21788.1	V. cholerae	-----MCSIIVSAAAVGLRDKQ	17
GAD71945.1	V. alginolyticus	-----MASNNDISIKKTLGVVIGLSLVCSIIIVSTAAGVGLRDKQ	37
CAC03728.1	P. stutzeri	MNELTQTTPVADGNEPFFTRPGLVETWRERSYQALSGLVLCALVAALLGNQLTHQRI	60
AZP58872.1	P. aeruginosa	-----MDAATRRSMLRNALLGLFALVGVGLVALVQQFTEARI	38
CAD6006652.1	E. coli	-----MLKTIRKHGITLALFAAGSTGLTAAINQMTKTTI	34
CCW74137.1	S. enterica	-----MLKTIRKHGITLALFAAGSTGLTAVINQMTKSTI	34
AKE29921.1	T. maritima	-----MKDILKTGLILMVFTAISGLFLGLVYVGVKGI	33
sp Q8TSY2.1	M. acetivorans	-----MSDSKEITKVIVTMVVISAVAALLALTYTPTQAQL	36
ACR23744.1	A. woodii	-----METKEKVQIDWKVVKLGLILFVISAVAACALALTNVYTAGTI	43
SQB91609.1	C.tetanomorphum	-----MKKVSSPKLGMVLLLIAAVCGILGGVQVTAEFI	35

AAX87225.1	EEQKLLDKQKNILNVAGLLQAN---TNVKETYAKFIEPRFVDLATGEYTQQADDS----	90
AKB87827.1	QEQRLLDKQRNIALAVAGLLQPRMLAEVQQAFATRIEPRLLDLQSGEFLKQDPATFDRSQ	101
KKP21788.1	KENALDKQSKILQVAGIEAKG--SKQIVELFNKSIEPRLVDFNTGDFVEGDAANYDQRK	75
GAD71945.1	KANAVLDKQSKIIVEVAGIDANG--K-KVPELFAEYIEPRLVDETGNFTEGNASTYDQRE	94
CAC03728.1	VDAERQDRLAVLRQVLP-----QALYDNDPLADAFNVED--	94
AZP58872.1	AEAQREARGRALLELP-----PGSYDNHPLDSQVPTFA--	72
CAD6006652.1	AEQASIQQKALFDQVLP-----AERYNNALAQSCYLVTA--	68
CCW74137.1	HEQALQQQHALFDQVLP-----PDRYNNNLQESCYLVDA--	68
AKE29921.1	QEADNAAKLSAIKFVLK-----DPLTGDYL-VDEKEIEE--	66
sp Q8TSY2.1	KLLQAEQQKEAMKEILP-----QA--ADFEPTVGSEVDD--	68
ACR23744.1	EEMNVQTNVVARQEVLP-----KA--ADFEAVPAKDVEK--	75
SQB91609.1	AIQNKKTLDANKAILP-----EA--SEFAEKT-----	61

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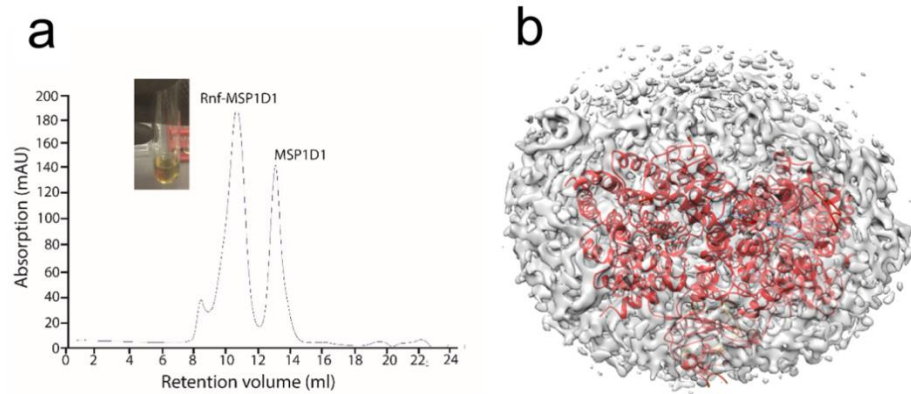
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KKP21788.1	AAKEASESIKLTAEQDKAKIQRRANVGVVYLVK-DGDKTSKVI-----LPVHGNG--	124
GAD71945.1	ASKDAERSIALTPPEEDVADIRRRANTAVVYLVK-DQDEVQKVI-----LPMHKGK--	143
CAC03728.1	-----AEL---G---LIEVYPARRAGQLTATAFQI-----STVGYG	124
AZP58872.1	-----PKLLGLDA---PRPAYVARLHGQASAVILQAS-----APDGYS	107
CAD6006652.1	-----PEL-GK-G---EHRVYIAKQDDKPVAAVLEAT-----APDGYS	101
CCW74137.1	-----PAL-GK-G---THRVFIARKDDKPVAIIIEAT-----APDGYS	101
AKE29921.1	-----I---VKKTGIET-VVLKEYKEGVVGLPLYEFVTKDGRNAYVLSGYAPGFG	112
sp Q8TSY2.1	-----DGNPVVLYYKGVDSGNVVGYYVERN-----Q-VGAQ	99
ACR23744.1	-----I---ASEIGMEKPEELLEVIYIGKSNGEVVGYTVKGT-----PTSGYA	114
SQB91609.1	-----DIKGEIVLGVTEGKSGSDLKGYTIKVA-----P-KGYA	94

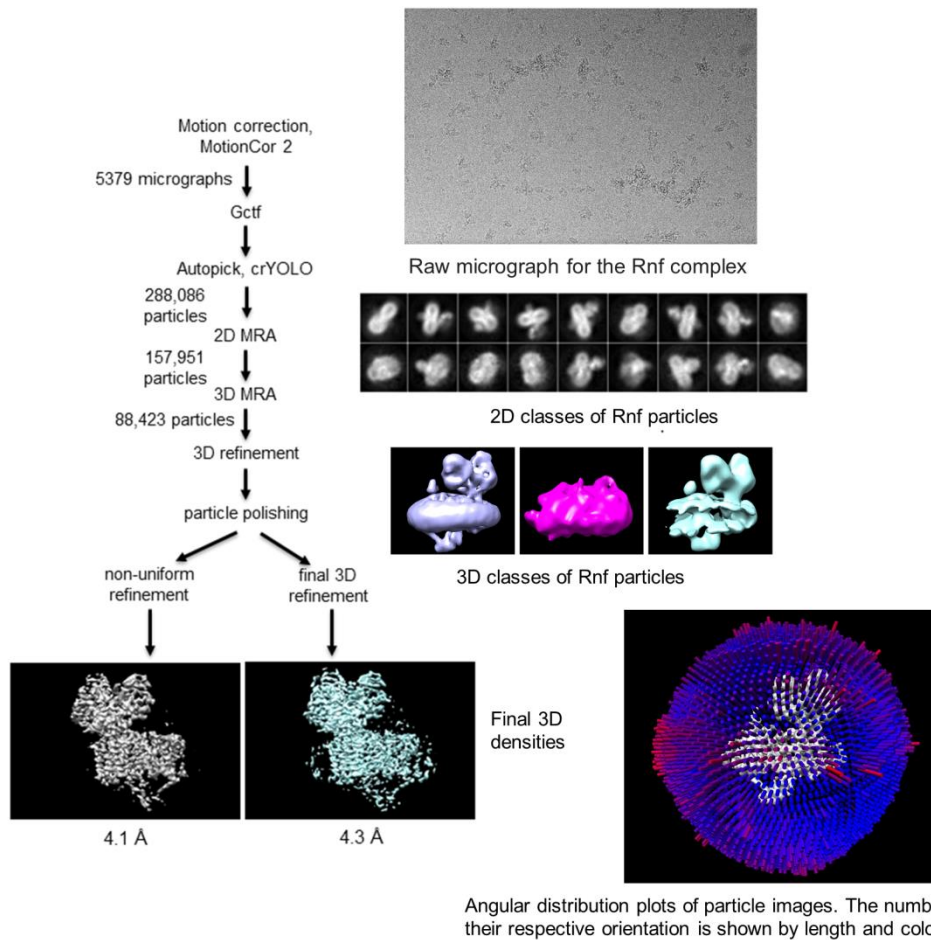
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AKB87827.1	-LWSMMYAFVAIDTDGKTVRGITYYDHGETPGLGGEIEN--PIWRNQWIGKRLFDDQGQP	208
KKP21788.1	-LWSMMYAFVAVETDGNVTSGLTITYYEQGETPGLGGEVEN--PAWRAQWVGKKLFDENHKKP	181
GAD71945.1	-LWSMMYAFVAVETDGNVTSAITYYEQGETPGLGGEVEN--PSWRDQFQIGKKLYNEDHQP	200
CAC03728.1	GPIVQFIALD---SE-GRILGVRVLSHKETPGLADKIEVTRSDWIKAFDGLSLASTPLDQ	180
AZP58872.1	GAIQLLVGVT---AQ-GRLLGVRVVAHKETPGLGDRIELAKSPWVHGFDGKSLGDPADAG	163
CAD6006652.1	GAIQLLVGAD---FN-GTVLGRVTEHHETPGLGDKIELRLSDWITHFAGKKISGADDAH	157
CCW74137.1	GAIQLLVGAD---FN-GTVLGRVTEHHETPGLGDKIERRLSDWITHFSGKTISGENDTH	157
AKE29921.1	GNVTVVACFIKTEDGFMLNSVRVIDYSQETPGLGAKIGE--ESIQRFFFPVPEGLKNGL	170
sp Q8TSY2.1	GMIQLLAGIS---SDFSTITGFQVMKHSETPGLGALITT--PEFQQGFVDLPVADTS---	151
ACR23744.1	GEVQVLTGIS---AD-GVITGITIIKSNETPGLGAKASG--V-WNDQFTGKSAKEEL---	164
SQB91609.1	GAIEMMVGVS---TE-GKVTGIKILNHAETPGLGANATD--PKFSGQYANKPAK-EL---	144
	*****:	
AAX87225.1	----AIRIVKG----QAPQ-DEHSIDGLSGATLTGNGVQGTFFNYWFSKDGFGPYLEKLHS	241
AKB87827.1	----AIRIVKG----RAPANDPHAVDGLSGATLTSGVQNSFNFWLGENGFGPFLKKVRE	260
KKP21788.1	----AIKIVKG----GAPQSGEHGVDGLSGATLTSGVQNTFDFWLGDMDGFGPFLTKVRD	233
GAD71945.1	----AIKVVKG----GAPQSGEHGVDGLSGATLTSGVQHTFDFWLGDMDGFGPFLAKVRD	252
CAC03728.1	-----WAVKKDGGQFDQFAGATITPRAIVKGVLRAL-----EFQARQST	219
AZP58872.1	-----WAVKKDGGTDFDQFAGATVTPRAVVRAVHKAL-----RYFDANRE	202
CAD6006652.1	-----WAVKKDGGDFDQFTGATITPRAVVNAVVRAG-----LYAQTLP	196
CCW74137.1	-----WAVKKDGGDFDQFTGATITPRAVVNAVVRAG-----LYAESLPA	196
AKE29921.1	RVDKDAGLPKGSPEELKKQGVKVSVMGTGATITPRAVVTALNLMY-----RYLEEVSK	224
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ACR23744.1	VV-----VKGTTKE-----GSNEIQAITGSTITSKAVTSGVNMSI-----QVYQNL	207
SQB91609.1	KV-----VKG-AAS-----GEDEIVAITGATITSKAVTLGVNEAI-----KFYDTKLK	186
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AAX87225.1	GAN-----	244
AKB87827.1	GALKNG-----	266
KKP21788.1	GGLN-----	237
GAD71945.1	GELN-----	256
CAC03728.1	AQSNQETRP---	228
AZP58872.1	RLLAPEEEAAGHE	214
CAD6006652.1	QLSQ-LPACGE-	206
CCW74137.1	QLPH-LTACGE-	206
AKE29921.1	-----	224
sp Q8TSY2.1	-----	188
ACR23744.1	-----	207
SQB91609.1	GGK-----	189

Supplementary Fig. 5: Sequence alignment of Nqr from *Vibrio cholerae*, *Vibrio alginolyticus*, *Yersinia pestis* as well as *Haemophilus influenza*, and *Rnf* from *Acetobacterium woodii*, *Escherichia coli*, *Thermotoga maritima*, *Salmonella enterica*, *Methanosarcina acetivorans*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa* and *C. tetanomorphum*. Nqr subunits are highlighted in gray. **a** RnfA, Cys25 and Cys113 are highlighted in red. **b** RnfB. [4Fe-4S]_{RnfB1-6} clusters are highlighted in red, yellow, gray, green, blue and magenta based on the Rnf structure of *C. tetanomorphum*. The [4Fe-4S] cluster content varies in Rnfs from different microorganisms. *T. maritima* RnfB carries only the first [4Fe-4S] cluster of FdI, *C. tetanomorphum*, in addition, the five [4Fe-4S] clusters of FdII+III and *A. woodii* RnfB even two further [4Fe-4S] clusters in a new FdIV segment¹². *E. coli* Rnf host besides the FdI segment (red) only the FdII unit with one of the two [4Fe-4S] clusters (gray) differently ligated compared with *C. tetanomorphum* FdII. **c** RnfC. The two [4Fe-4S] clusters were marked in yellow and green. **d** RnfD. Residues of constriction were highlighted in green, Asp248 and Asn124 in magenta, those contacting FMN in yellow and Thr153 in blue. **e** RnfE. Cys26 and Cys109 are highlighted in red. **f** RnfG. Thr164 is shown in blue. According to the genome sequence¹³ the Rnf complex has a molecular mass of 169.253 kDa (RnfA 20.584 kDa; RnfB 27.923 kDa, RnfC 47.103 kDa, RnfD 32.968 kDa, RnfG 19.478 kDa, RnfE 21.197 kDa).



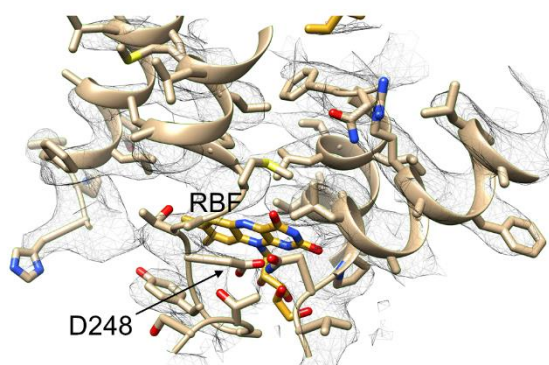
Supplementary Fig. 6: Rnf in nanodiscs. **a** Reconstitution of Rnf into nanodiscs MSP1D1^{14,15} replacing the lipid DMPC. Lipid-filled and Rnf-filled nanodiscs could be separated by gelfiltration (Superdex 200 HR 10/300 GL) **b** Cryo-EM map (top view) indicating the disordered but visible nanodisc belt around a Rnf model (red ribbon).



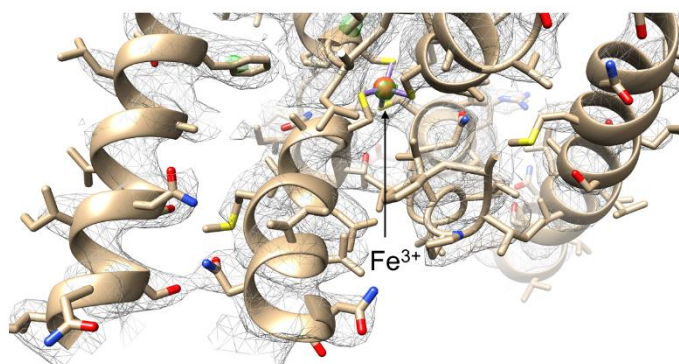
Supplementary Fig. 7: Workflow of Rnf structure determination using Relion¹⁶ for data processing.

Data collection was done on a Titan Krios EM at 300 kV equipped with a Gatan K3 detector with a pixel size of 0.837Å at 105000x magnification. Data processing is a multi-step process described in the Method section. The figure also shows the major 2D and 3D classes based on multiple reference alignment (MRA) and the angular distribution of the particles in the micrographs that represents an indicator for the quality of 3D refinement.

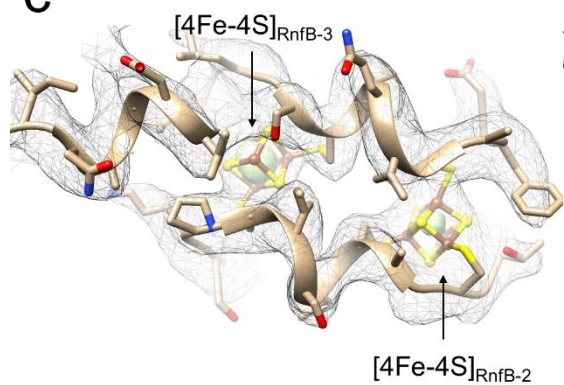
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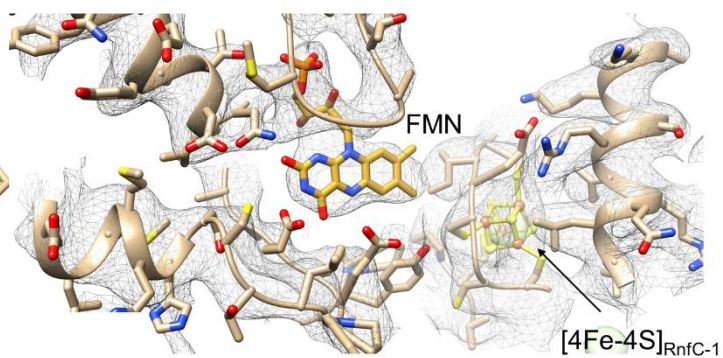
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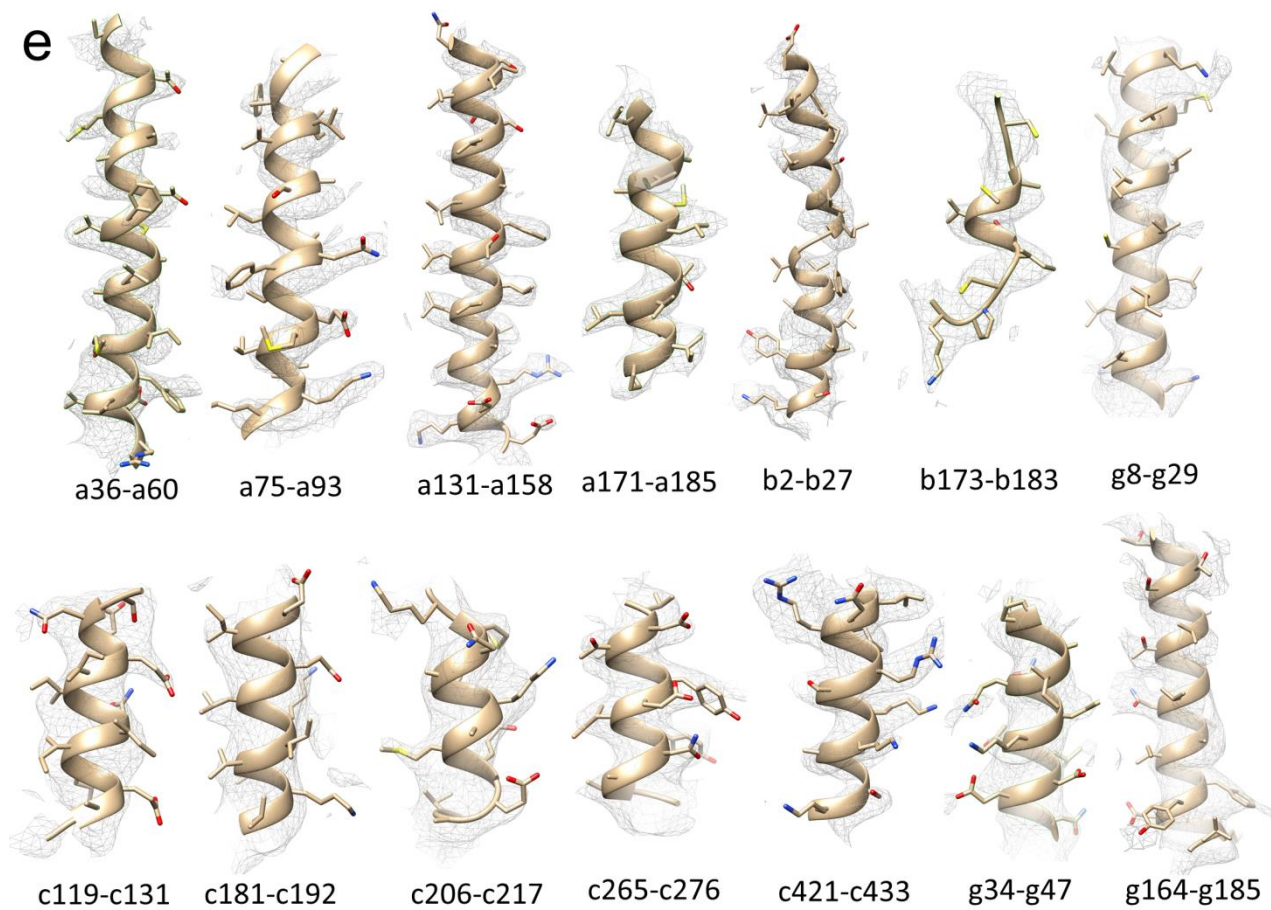
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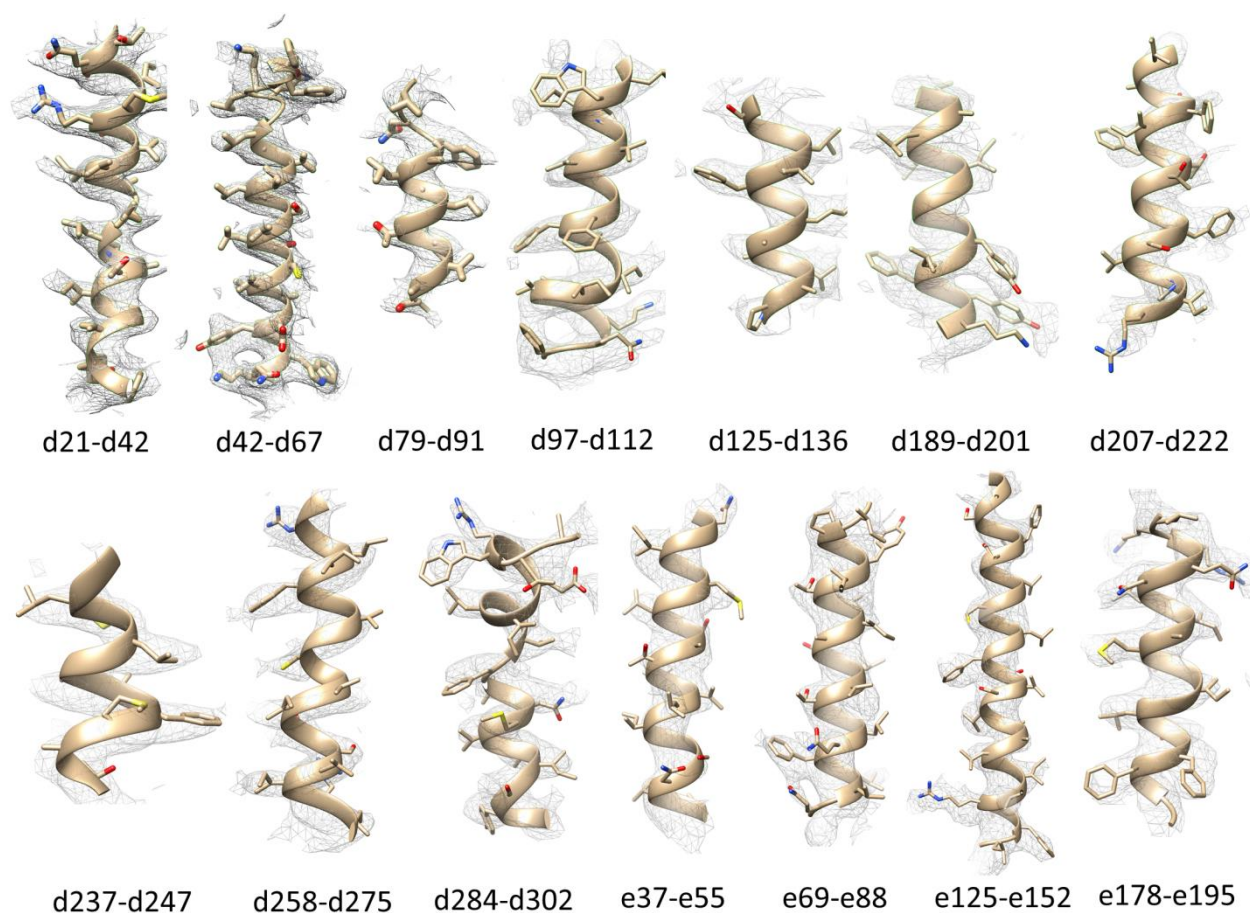


d

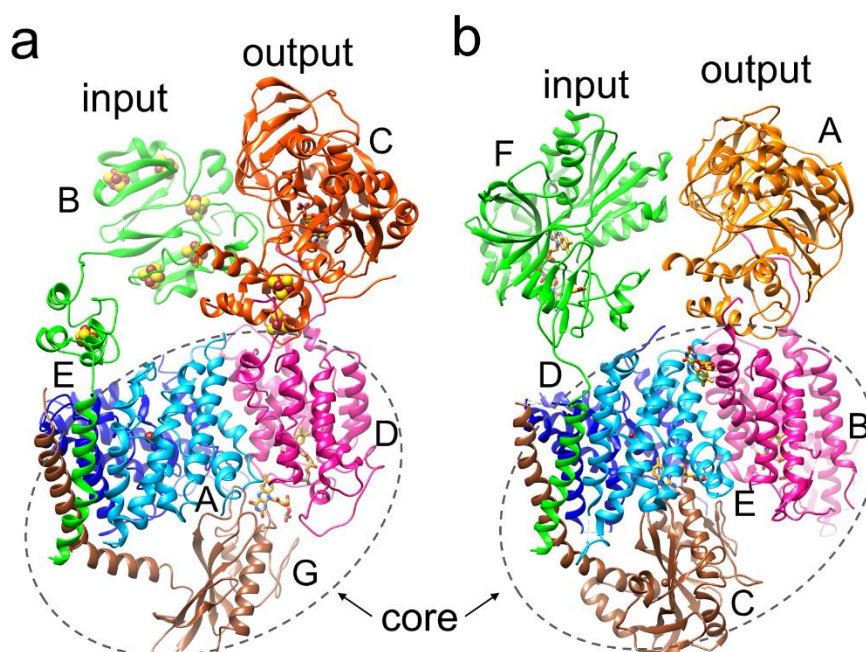


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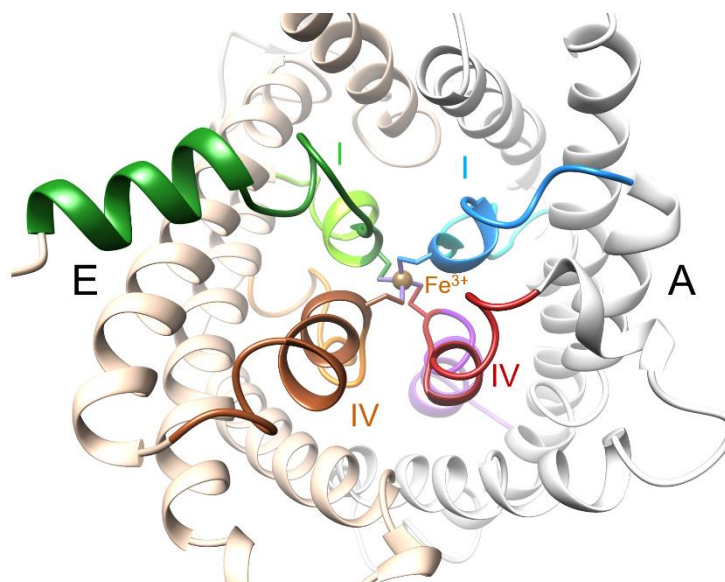




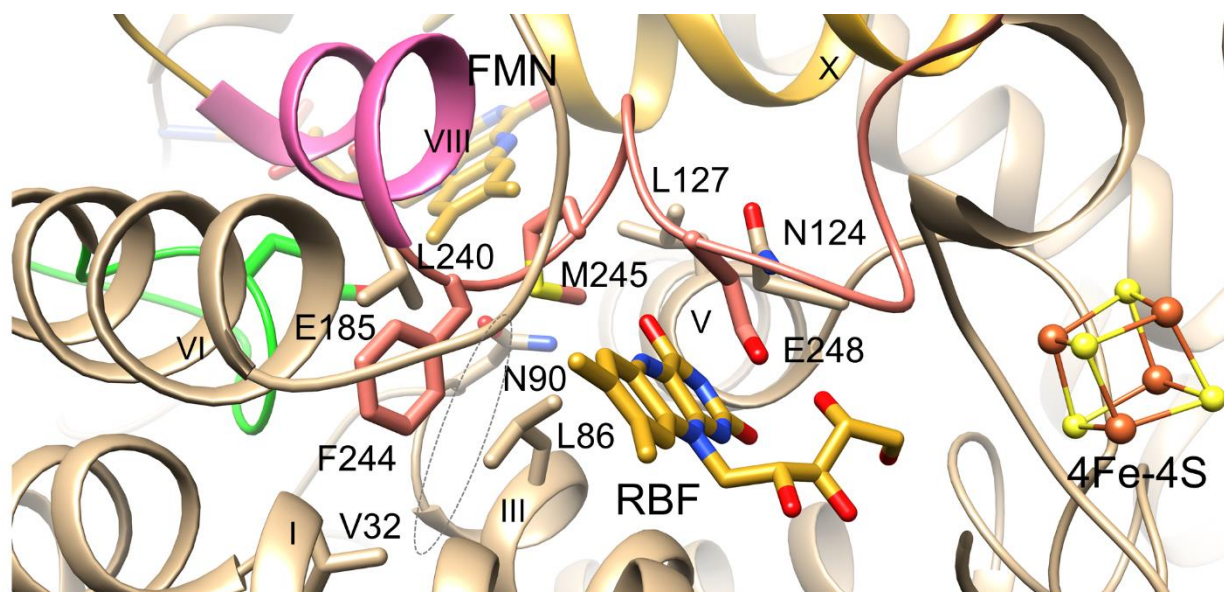
Supplementary Fig. 8: The density map at 4.27 Å resolution. Main chain modeling was feasible except for residues 31-95 of RnfB. The profile of some characteristic side chains was sufficient for reliable interpretation, which allowed the approximate assignment of those in between. Solely, the side chains of the N-terminal helix of RnfB were set to zero. Regions **a** around the riboflavin (RBF) and Asp248 of RnfD, **b** around the potential Fe ion between RnfA and RnfE, **c** around the $[4\text{Fe-4S}]_{\text{RnfB-II}}$ and $[4\text{Fe-4S}]_{\text{RnfB-III}}$ clusters and **d** around FMN of RnfC.. The main chain was drawn in the Ribbon style and the side chains and cofactors as sticks and spheres. Contour levels 1 and 2 are drawn as gray mesh and as green surface, respectively. **e** Density and model of all helices of the Rnf complex.



Supplementary Fig. 9: Comparison between the architecture of Rnf **a** and Nqr **b**. The core complexes RnfADEG and NqrEBDC are structurally and functionally highly related. RnfB/NqrF (green) and RnfC/NqrA (orange), the input and output modules in the Na^+ pumping direction, are spatially arranged in the same manner relative to the core complex implicating that the two NAD binding subunits RnfC and NqrF have to be attached to different sides. It is worth to note that the structurally related RnfC and NqrA perform different catalytic reactions.



Supplementary Fig. 10: Hourglass-like architecture of RnfAE. Helices I and IV of RnfA and RnfE are kinked at the central Fe^{3+} binding site are extended outwards towards the extra- and intracellular monolayer forming an hourglass-like arrangement. Half-helices 13:24 and 25:33 of RnfA are drawn in sky blue and cyan; half-helices 103:113 and 114:128 of RnfA in dark red and purple; Half-helices 5:14 and 27:34 of RnfE are highlighted in dark green and green, half-helices 96:108 and 109:122 of RnfE in brown and orange.



Supplementary Fig. 11: Detailed view of the region between FAD_{RnfD} and the [4Fe-4S]_{RnfC-2} cluster in the closed-channel enzyme state. The key player is helix 237:246 (VIII) (purple) non-covalently joined with helices X (gold), VII and IX (the latter not shown). Loops that follow helix VIII and occlude the extracellular half-channel are drawn in salmon and green. In the main text the electron flow from Fd_{red} to NAD⁺ coupled with an uphill Na⁺ transport was already described in detail. In the reverse direction FAD_{RnfD} and the riboflavin (RBF) interspace is again the scene of the central coupling process. Reduction of RBF (from neutral semiquinone to reduced hydroquinone in Nqr¹⁷) might result in a repulsion between the negatively charged C-terminal end of helix VIII (purple) including Asp248 and the electron-rich reduced riboflavin that softens helix VIII. As a consequence, the cytoplasmic Na⁺ binding site localized in this region is impaired while Na⁺ can reach the hydrophobic barrier (dashed ellipse) from the extracellular half-channel because the occlusion loop (green) is postulated to be refolded into two helix windings when RnfG is attached to RnfAE (Fig. 5). Driven by the high extracellular concentration, Na⁺ binding (assumed next to Asn90, Glu185 and the tip of the occlusion loop) induces a rearrangement of the already disengaged helix VIII together with several other helices including helix 284:293 (X) (gold) thereby adjusting the strained open-channel state. Concurrently, Na⁺ diffuses through the widened hydrophobic barrier and perhaps through an opened cytoplasmic half-channel. Finally, FMN_{RnfD} accepts an electron from reduced riboflavin, which triggers a subsequent strained-to-relaxed state transition. In parallel, RnfG is rotated to RnfD and the occlusion loop (green) blocks the access to the constriction.

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Source data file for supplementary Fig. 3a