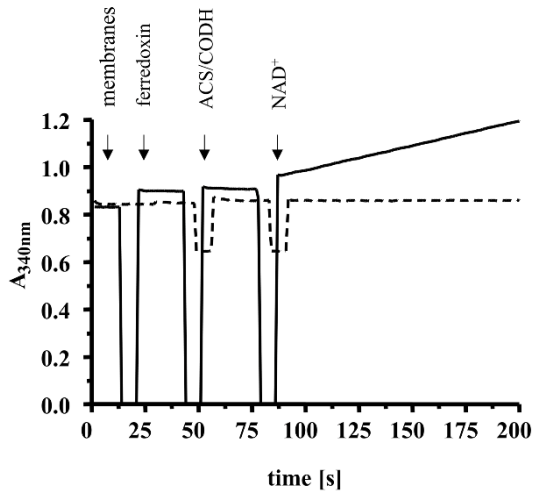


24 **Supplementary Figures**

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27 **Supplementary Figure 1. Membranes of *T. maritima* catalyze  $\text{Fd}^{2-}:\text{NAD}^+$ -oxidoreductase**

28 **activity.** 100  $\mu\text{g}$  membranes of *T. maritima* were added to 1 ml buffer (20 mM Tris-HCl, 20

29 mM NaCl 2 mM DTE, 2.2  $\mu\text{M}$  resazurin, pH 7.7) and ferredoxin: $\text{NAD}^+$  oxidoreductase was

30 measured as described in Material and Methods with (—) and without (---) addition of 30  $\mu\text{l}$

31  $\text{NAD}^+$  (100 mM). The absorption was followed at 340 nm.

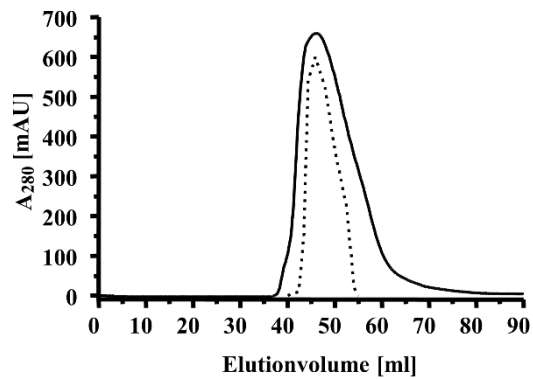
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38 **Supplementary Figure 2. Size exclusion chromatography of the Rnf/F<sub>1</sub>F<sub>o</sub>-ATP synthase**  
 39 **complex from *T. maritima*.** The Q-sepharose pool was applied on a Sephacryl S300 column  
 40 equilibrated with buffer C (50 mM Tris-HCl, 20 mM MgSO<sub>4</sub>, 150 mM NaCl, 2 mM DTE, 4.4  
 41 μM resazurin, 0.02 % DDM [w/v], 5 μM FMN, pH 8.0). The protein was separated with a  
 42 flowrate of 0.5 ml/min and the separation was followed by measuring the extinction at 280 nm  
 43 (—). After 46 ml the main protein eluted from the column in one peak. The dotted line (----)  
 44 indicates the ferredoxin:NAD<sup>+</sup> oxidoreductase activity. mAu, milli absorbance units.

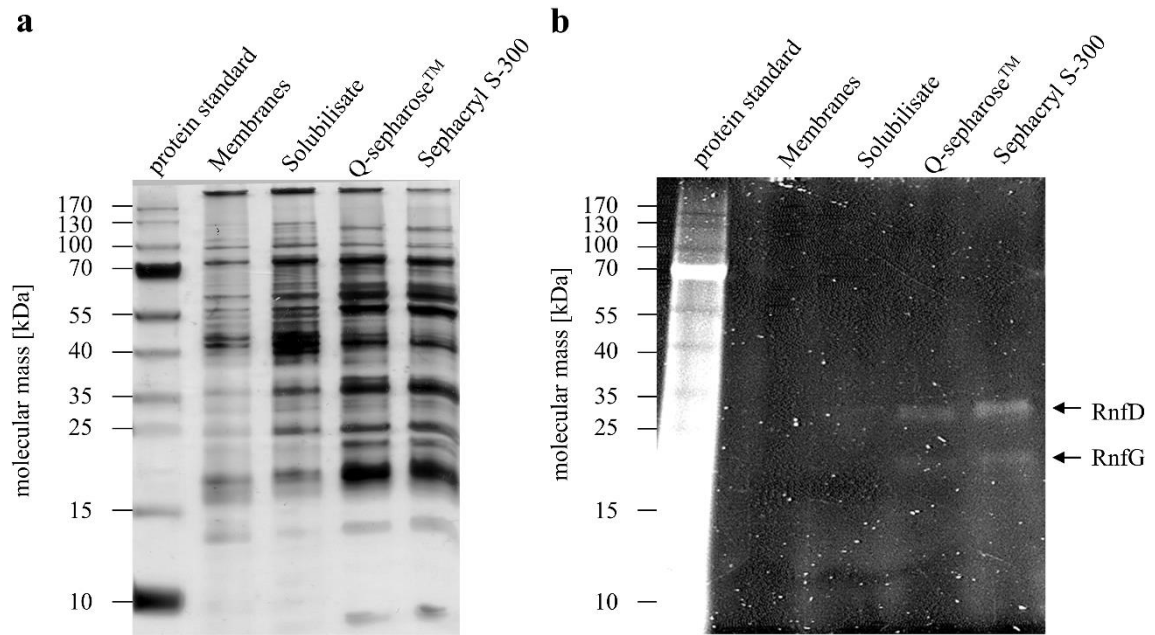
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51 **Supplementary Figure 3. Purification of Rnf/F<sub>1</sub>F<sub>0</sub>-ATP synthase complex from *T.***  
 52 ***maritima*.** 20 µg protein from each purification step were separated on a 12.5% SDS-gel  
 53 according to Laemmli *et al.*<sup>1</sup>. The proteins were visualized by staining with silver (a) or under  
 54 UV light (b). The “Page Ruler Prestained Protein Ladder” (Thermo Fisher Scientific, Waltham,  
 55 Massachusetts, USA) was used as protein standard.

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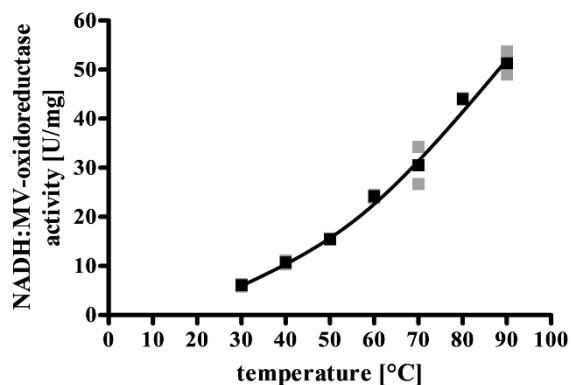
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66 **Supplementary Figure 4. Temperature dependence of the NADH:MV-oxidoreductase**  
67 **activity of the Rnf complex from *T. maritima*.** 2.5 µg of the purified protein was added to 1  
68 ml buffer (20 mM Tris-HCl, pH 7.7, 20 mM NaCl, 2 mM DTE, 4 µM Resazurin) and were  
69 incubated for 5 min at the corresponding temperature. The reaction was started by adding 10 µl  
70 MV (1 M) and 30 µl NADH (100 mM), and the absorption was followed by 604 nm. The data  
71 represent N = 2 independent experiments.

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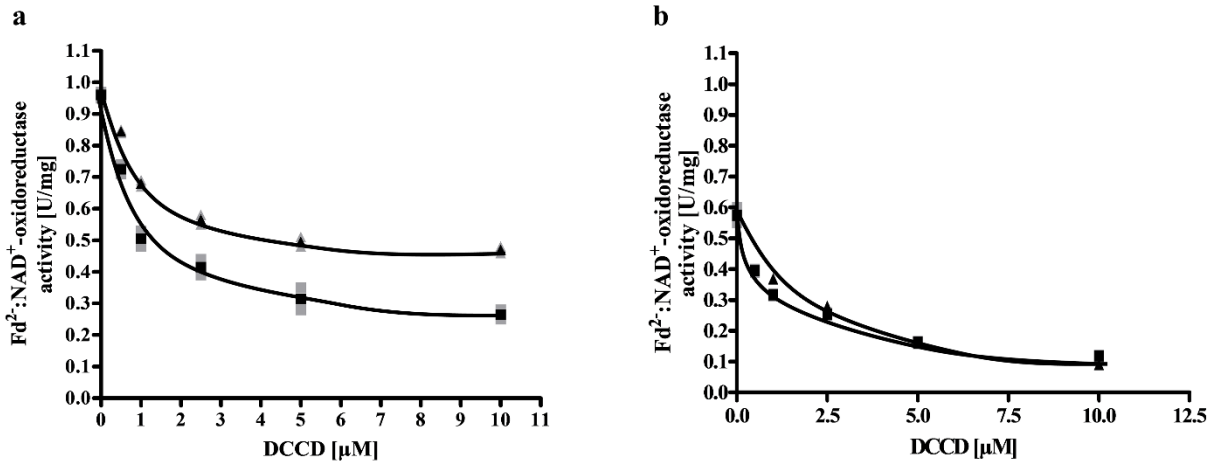
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84 **Supplementary Figure 5. Effect of  $\text{Li}^+$  (A) and  $\text{K}^+$  (B) on DCCD inhibition of**

85 **ferredoxin: $\text{NAD}^+$  oxidoreductase activity.** 14  $\mu\text{g}$  of purified Rnf complex was added to 1 ml

86 buffer (20 mM Tris-HCl, 2 mM DTE, 2.2  $\mu\text{M}$  resazurin, pH 7.7) containing 0 - 10  $\mu\text{M}$  DCCD

87 in absence (■) or presence of 20 mM LiCl (A) or KCl (B) (▲). The sample was incubated for

88 30 min at room temperature followed by 5 min at 60 °C and ferredoxin: $\text{NAD}^+$  oxidoreductase

89 was measured as described in Material and Methods. If LiCl or KCl was omitted during the

90 preincubation, 20 mM LiCl (A) or KCl (B) was added before the reaction start. The data

91 represent N = 2 independent experiments.

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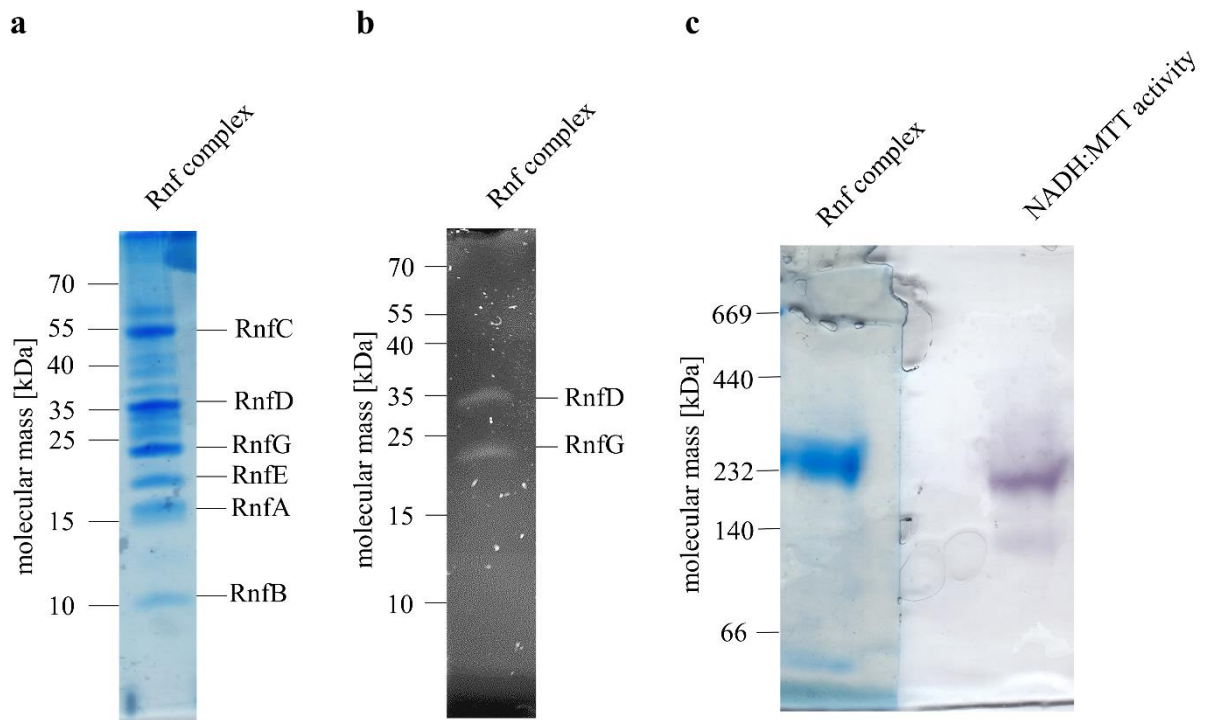
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101 **Supplementary Figure 6. Molecular mass of the purified Rnf complex and its subunit**  
 102 **composition.** 15  $\mu\text{g}$  of the purified enzyme was either separated in a native PAGE (c) or under  
 103 denaturing conditions in a SDS-PAGE (a). The gels were stained with Instant Blue<sup>TM</sup>  
 104 (Expedeon, Cambridgeshire, UK) or with a NADH:MTT in gel activity assay under native  
 105 conditions. The covalently bound FMN was visualized under UV-light (b).

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NqrD V.c. MSSAKELKKSVLAPVLDNNPIALQVLGVCALAVTTKLETAQVMTLAVMFTALSNFFVS 60
Rnfe V.c. ---MSENRTLMLNGMWNPNPALVQLLGLCPLLAVSSVTNVALGLGIATLLVVGSNVTVS 87
Rnfe T.m. ---MSR-LRELTKGIKENPTYVQVLGMCPTLAVTTSAINGLGMGLATTAVLTMNSNVIS 86
Rnfe A.w. ---MNF-MKNLTRGIIIRENPTFVVLVGLMCPTLAVTTSAINGMGMGLATMLVVLIGSNVAIS 86
      .      :      : :** : :**:* ***:.. .: : :* . * ** . :*

NqrD V.c. LIRNHIPNSVRRIIVQMAIIASLVIVVDQILKAYLYDISKQLSVFVGLIITNCIVMGRAEA 120
Rnfe V.c. LVRDYPKKEVRIPVFVMIASLVITCVQLLMNAYAGLYLSLGIPIPLIVTNCIIIGRAEA 117
Rnfe T.m. LIRKIVPKIRIPIFIVVIASFVIMIDLLMHGFAYDLWKTGLGFIPLIVVNCIIMGRAES 116
Rnfe A.w. ALRKVIPDNIRIPAFVIVVIASFVIVVGMMLMKAYVPALDAALGIFIPLIIVVNCIILARAEA 116
      :* :*..** : :***:* : : : : : *..* : **:.***:..***:

NqrD V.c. FAKMSEPIPSFDIGIGNGLGYGFVLMTVGPFRELLGSGKLPGLVLEVLPLISN-----G 172
Rnfe V.c. FASKNDVLPAAALDFWVGLGNTSVLVVVLGSLREIIGNGLTFDGDALLGEWAKVLRIEVF 177
Rnfe T.m. FASKHGVLDSMLDGLGVGLGFTGSLVLLGSVRELFNGNTIFGYKV-----WEL----- 164
Rnfe A.w. FAFSNGIADSFADAVGMLGFTLALTLGSIRESILGAGSIFGFSL-----FGA----- 164
      ** . : *.. ** * : * : **:* *..*

NqrD V.c. GWYQFNGLMMLLAPSAFFLIGFMIWAIRTFKPEQVEAKE----- 210
Rnfe V.c. HFDSAFLLALLPPGAFIVGVFLIAAKSVID-KQIARQPKQKQAIERARVTNV 230
Rnfe T.m. ----KIFLEILPPGAYITLGLLSALFTYIG-IRKKRGEAK----- 200
Rnfe A.w. A-YEPVLLMILPPGAFITLGLLIGLINWKT-KKA----- 196
      * :* *..* :* : :

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115 **Supplementary Figure 7. Sequence alignment of NqrD from *V. cholerae* (*V. c.*) with Rnfe**  
116 **from *A. woodii* (*A. w.*), *T. maritima* (*T. m.*) and *V. cholerae*.** The conserved acidic amino  
117 acids are marked in red and the amino acid that is potentially involved in Na<sup>+</sup> binding is  
118 highlighted in gray. Positions with a star (\*) are fully conserved residues. Positions with a colon  
119 (: ) are residues with high similarities (reference value > 0.5 in Gonnet PAM 250 Matrix), dots  
120 (.) are residues with low similarities (reference value < 0.5 in Gonnet PAM 250 Matrix).

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NqrB V.c. MGLKKFLEDIEHHPFGGKHEKWFALYEAAATLFYTPGLVTKRSSHVRDSVDLKRIMIMV 60
RnfD V.c. -----MAFFIASPPHLRSKRSTADVMRWV 24
RnfD T.m. -----MKLISAYAPHLREDDVRKIMLDV 24
RnfD A.w. -----MNELNLTVSSSPHIRAKHSTASIMQNV 27
      :   :   : * * . . . : * *

NqrB V.c. WLAVFPAMFWGMYNAGGQAIALNHLVSGDQLAAIVAGNWHYWLTEMLGGTMSSDAGWGS 120
RnfD V.c. LVCALPGLIAQT----- 36
RnfD T.m. LIALSPAIVIGAA----- 36
RnfD A.w. IIALPALAVAG----- 39
      :. *.:

NqrB V.c. KMLLGATYFLPIYATVFI-----VGGFWEVLFMVRKH---E-VNEGFFVTSILFALIV 170
RnfD V.c. -----YFFGYGTLLIQLLLAISVAVALEAGINLLRKRSPISALRDYSAVVTAWLLAVAI 89
RnfD T.m. -----YFFGWYALFLCIAGAVIGELFDIF-VMRVLRGVKDFVDPGSGAVTGLLLAMNV 88
RnfD A.w. -----YVFGLWALALVAICVI-SSVATEA-VIQKLLKKPITVNDWSAVVTGVLLAFNL 90
      *.: :   :   :   :   :   :   :   :   :   :   : * * . *.:

NqrB V.c. PPTLPLWQAALGITFGVVVAKEVFGGTGRNFLNPALAGRAFLFFAYPAQISGDLV----- 225
RnfD V.c. PPLSPWVWVVIIGLIFAIIVIAKHLYGGLGQNPFPNAMIAYVVLISFPVQMTSWMAPIKLT 149
RnfD T.m. STRLPPWAFLLGLVFAALGIGKHFVGGGLGQNI FNPALVGRAFLLISFPTVMTTWVVPAGGF 148
RnfD A.w. PINAPFWIIVGVSVFAIAIVKQCFGGLGQNFINPALAARAFLLASWPGHMTSTA----- 144
      * * : * * : * : * * * * : * * : * * :

NqrB V.c. WTA-----ADGYSGATALSQWAQGGAGALINNAIGQTI 258
RnfD V.c. AEPSSLVDSFSLIFGGFDSGLSLQQIRTGIDGITMATPLDAIKT---SLKAGHTMSET 205
RnfD T.m. WKS-----PADVVTAATPLALFKE-----HGVFT 172
RnfD A.w. YIP-----LTDIVTATPLALLKA-----GETGSMP 170
      * : * * *

NqrB V.c. TWMDAFIGN--IPGSIGVSTLALMIGAAFIIVVMGIASWRIIGVMIGMILLSTLFNVIG 316
RnfD V.c. LTQPQPSGFGAGIC---WVWNIAYLLGGLILLKLRIRWHIPVMMLAGLVFTALLAQLF 262
RnfD T.m. FYWDLFIGK--VGGSLGKETSALLLIIGFIYLLLRKRVKIFIPVSYIGTVLVFSSIAAYLMN 230
RnfD A.w. STLDLFTGLNGVYGCIGKISALALLIGGLYLYIKGII SWRIPTIYLLTIAIFAL----- 224
      * * : * : : * : : * : : * : : :

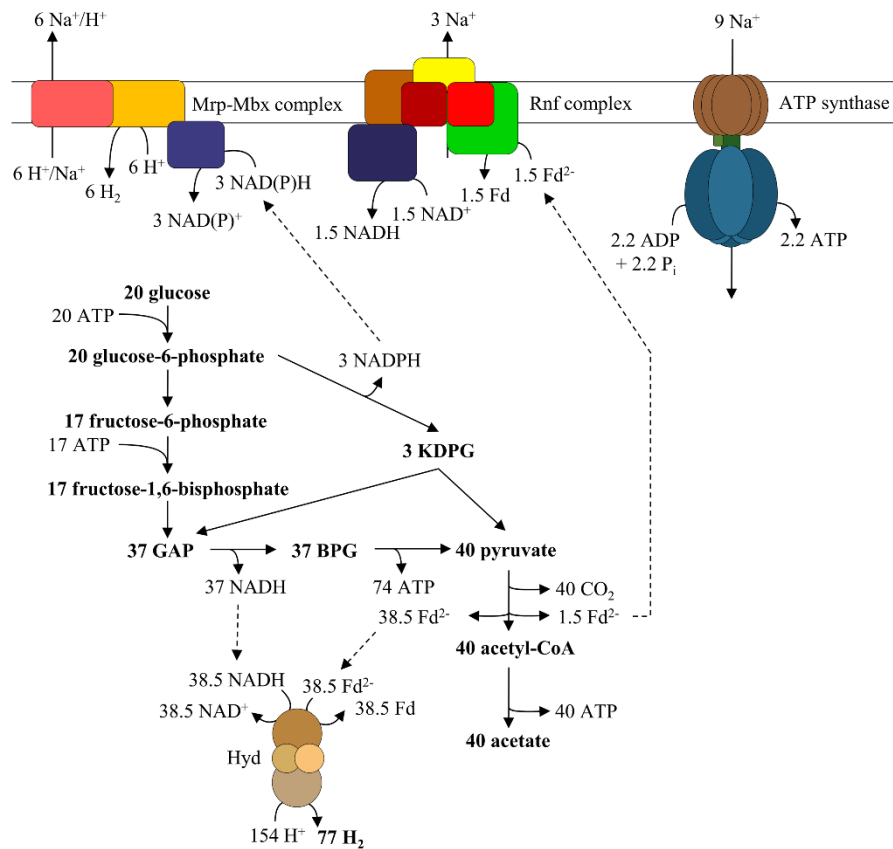
NqrB V.c. SDTNAMFNMPWHHLVGGFAFGMFFMATDPVSASFNSGKWAYGILIGVMCVLIRVVNP 376
RnfD V.c. PGT----TASPMIHLLSGATMLGAFFIATDPVSASITDKGRLLIYGFFIGAMVFLIRSW-G 317
RnfD T.m. P-R----YGDPLFHLLSGGLMLGALFMATDMVTSPITAKGQVIFGIGCGVLTMAIRLF-G 284
RnfD A.w. L-V----GQDPVIVHMVSGGVMLGAFMATDYASSPVTAKGQIIYAIGCGLITMIIRLY-G 278
      *.: * . : * : * * * : : * . * : : : * : . *

NqrB V.c. AYPGGMMLAILFANLFAPLFDHVVVERNIRRLARYGKQ- 415
RnfD V.c. GFPDGVAFVLLANMCVPLIDYTKPRTYGH----- 348
RnfD T.m. AYPGVSFSILFMALVPLIDRYTRPRIFGEVKK----- 318
RnfD A.w. GYPGCSYSILLNVAATPLIERFTKERIYGVTKIRKEKA 318
      :.: * : * * : * * : : . *

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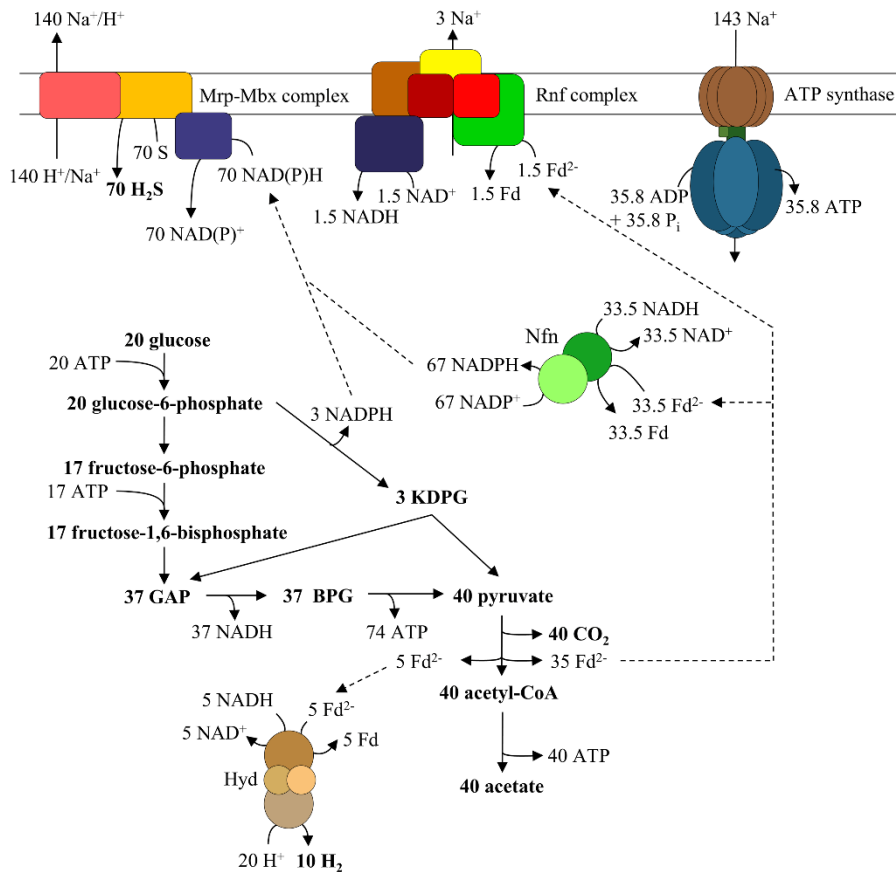
133

134 **Supplementary Figure 9. Sequence alignment of NqrB from *V. cholerae* (*V. c.*) with RnfD**  
135 **from *A. woodii* (*A. w.*), *T. maritima* (*T. m.*) and *V. cholerae*.** The conserved acidic amino  
136 acids are marked in red and the amino acid that is potentially involved in Na<sup>+</sup> binding is  
137 highlighted in gray. Positions with a star (\*) are fully conserved residues. Positions with a colon  
138 (: ) are residues with high similarities (reference value > 0.5 in Gonnet PAM 250 Matrix), dots  
139 (.) are residues with low similarities (reference value < 0.5 in Gonnet PAM 250 Matrix).



140

141 **Supplementary Figure 10. A model of glucose metabolism linked to a Mrp-Mbx and Rnf**  
 142 **complex in *T. maritima*.** Glucose is oxidized *via* glycolysis and Entner-Doudoroff pathway  
 143 and acetate is produced. NADPH generated in the glucose-6-phosphate dehydrogenase reaction  
 144 is oxidized by a Mrp-Mbx complex by producing H<sub>2</sub>, thereby establishing a Na<sup>+</sup> or H<sup>+</sup> gradient<sup>2</sup>.  
 145 Electrons from Fd<sup>2-</sup> are shuffled to NAD by the Rnf complex, thereby generating an additional  
 146 Na<sup>+</sup> gradient; the Na<sup>+</sup> gradient is used by the F<sub>1</sub>F<sub>0</sub> ATP synthase to generate ATP. A potential  
 147 H<sup>+</sup> gradient could be converted to a secondary Na<sup>+</sup> gradient *via* a Na<sup>+</sup>/H<sup>+</sup> antiporter and then  
 148 used for ATP synthesis or the ATP synthase may use Na<sup>+</sup> and H<sup>+</sup> simultaneously<sup>3</sup>. All other  
 149 reducing equivalents are used by the electron bifurcating/confurcating hydrogenase (Hyd) to  
 150 generate H<sub>2</sub>. KDPG, 2-keto-3-desoxy-6-phosphogluconate, GAP, glyceraldehyde 3-phosphate,  
 151 BPG, bisphosphoglycerate. Please note that the ion/electron and ion/ATP stoichiometries are  
 152 based on thermodynamics (see also<sup>4</sup>).



153

154 **Supplementary Figure 11. A model of glucose metabolism linked to a Mrp-Mbx and Rnf**  
 155 **complex in *T. maritima* under high H<sub>2</sub> partial pressure and presence of sulfur.** Glucose is  
 156 oxidized *via* glycolysis and Entner-Doudoroff pathway and acetate is produced. Most of the  
 157 reducing equivalents are used from the NADH-dependent Fd<sup>2-</sup>:NADP<sup>+</sup>-transhydrogenase (Nfn) to  
 158 confurcate electrons to NADP<sup>+</sup>. NADPH generated in the glucose-6-phosphate dehydrogenase and  
 159 transhydrogenase reaction is used by a Mrp-Mbx complex to reduce elemental sulfur to H<sub>2</sub>S,  
 160 thereby establishing a Na<sup>+</sup> or H<sup>+</sup> gradient<sup>2</sup>. A potential H<sup>+</sup> gradient could be converted to a  
 161 secondary Na<sup>+</sup> gradient *via* a Na<sup>+</sup>/H<sup>+</sup> antiporter and then used for ATP synthesis or the ATP  
 162 synthase may use Na<sup>+</sup> and H<sup>+</sup> simultaneously<sup>3</sup>. Further electrons from Fd<sup>2-</sup> are shuffled to NAD  
 163 by the Rnf complex, thereby generating an additional Na<sup>+</sup> gradient; the Na<sup>+</sup> gradient is used by the  
 164 F<sub>1</sub>F<sub>0</sub> ATP synthase to generate ATP. All remaining reduction equivalents are used by the electron  
 165 bifurcating/ confurcating hydrogenase (Hyd) to generate H<sub>2</sub>. KDPG, 2-keto-3-desoxy-6-  
 166 phosphogluconate, GAP, glyceraldehyde 3-phosphate, BPG, bisphosphoglycerate. Please note that  
 167 the ion/electron and ion/ATP stoichiometries are based on thermodynamics (see also<sup>4</sup>).

168 **Supplementary Tables**169 **Supplementary Table 1. Purification of the Rnf F<sub>1</sub>F<sub>0</sub>-ATP synthase complex from *T.***  
170 ***maritima*.**

Sample	Protein (mg)	Fd <sup>2</sup> :NAD <sup>+</sup> -oxidoreductase activity (mU/mg)	total-activity (U)	enrichment	yield (%)
Membranes*	2200	97	213	1	100
Solubilisate	304	594	181	6.1	85
Q-Sepharose <sup>TM</sup>	47	1340	63	13.8	30
Sephacryl S-300	13	2100	27	21.6	13

171 \*Membranes were prepared from 30 g (wet weight) of cells

172

173 **Supplementary Table 2. Proteins identified by LC-MS/MS and MALDI-TOF in the**  
174 **ATPase + Rnf complex preparation.**

Protein	Accession number	Protein Identification Propability	#Peptides	% Coverage
electron transporter RnfC [Thermotoga]	gi 490184336	100%	14	43%
electron transporter RnfG [Thermotoga]	gi 740209784	100%	8	31%
electron transporter RnfD [Thermotoga]	gi 740191358	32%	1	3.2%
ATP synthase epsilon chain [Thermotoga]	gi 490183443	100%	9	58%
ATP synthase subunit beta [Thermotoga]	gi 490183445	100%	27	60%
ATP synthase F1 subunit gamma [Thermotoga]	gi 490183448	100%	21	59%
ATP synthase subunit alpha [Thermotoga]	gi 490183450	100%	39	59%
ATP synthase subunit delta [Thermotoga]	gi 490183452	100%	13	54%
ATP synthase subunit B [Thermotoga]	gi 490183458	100%	19	71%
ATP synthase subunit c [Thermotoga]	gi 490183460	100%	3	32%
F <sub>0</sub> F <sub>1</sub> ATP synthase subunit A [Thermotoga]	gi 490183462	100%	3	7.9%

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176

177 **Supplementary Table 3. Proteins identified by LC-MS/MS and MALDI-TOF in the Rnf**  
 178 **complex preparation.**

<b>Protein</b>	<b>Accession number</b>	<b>Protein Identification Propability</b>	<b>#Peptides</b>	<b>% Coverage</b>
electron transport complex subunit RnfC [Thermotoga maritima]	G4FHF8_THEMA	100%	18	49%
electron transport complex subunit RnfG [Thermotoga maritima]	Q9WY88_THEMA	100%	12	44%
electron transport complex subunit RnfD [Thermotoga maritima]	Q9WY87_THEMA	100%	1	3.1%
electron transport complex protein RnfB [Thermotoga maritima]	G4FHG3_THEMA	100%	3	23%
electron transport complex subunit RnfA [Thermotoga maritima]	Q9WY90_THEMA	99%	1	5.8%

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181 **Supplementary References**

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