

Supplementary Information

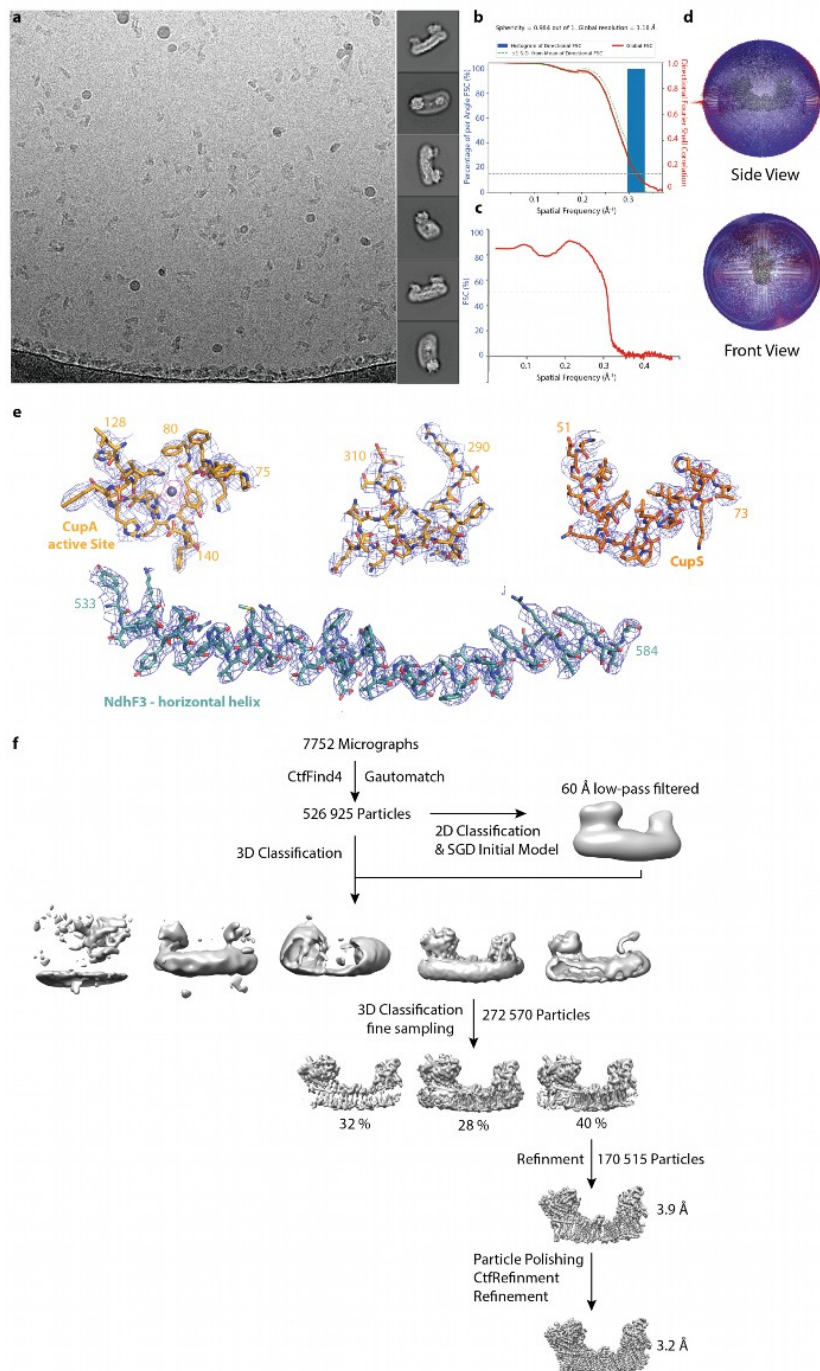
Supplementary Methods

Construction of the *T. elongatus* mutant. The generation of the CupS-TwinStrep-tag (TS) mutant was based on a CupS-StrepII-tag plasmid that was created by PCR amplification of the *cupS* (*tll0220*) gene and the corresponding upstream region using genomic DNA of *Thermosynechococcus elongatus* as template. Thereby at the 3'-end the StrepII-tag coding sequence as well as a SacII restriction site and at the 5'-end a SacI restriction site were fused by primer extensions (primers: P1-*for*-5'-AAAGAGCTCGGCCATAGCCAGATGTC-3'; P2-*rev*-5'-GGGCCGCGTATTATTTTCGAACTGCGGGTGG CTCCAAGCGCTGACTTTGTAGGGAATGTTG-3'). The product was ligated into a HincII linearized pUC18 plasmid. The *cupS* downstream region was amplified by use of PCR primers fusing a 5' PstI and 3' KpnI site (primers: P3-*for*-5'-GGGCTGCAGGGTATTAGCAGATTTCCC-3'; P4-*rev*-5'-CCCGGTACCCAATCAGGTCGTAGTTGC-3') and afterwards cloned into the plasmid pBluescriptSK(+) Km^R . The kanamycin resistance cassette and the *cupS* downstream region were then ligated via the restriction sites SacII and KpnI into the pUC18 plasmid containing the *cupS* upstream region (see above) resulting in pCupS-StrepII-tag construct. To generate the CupS-TS-tag mutant, *cupS* (*tll0220*) and the corresponding upstream region were amplified by PCR from pCupS-StrepII-tag, and thereby extended by half the sequence of the TS-tag at the 3'-end (primers: P1; P5-*rev*-5'-GCAGAACACCAGAACCACCGCCGCTGCCGCGCCTTTTTTCGAACTGCGGGTGGC-3'). The product was used as a template for a second PCR and thus extended with the other half of the TS-tag with the addition of the restriction site NotI to the 3'-end (primers: P1; P6-*rev*-5'-CCCGCGCCGCTTACTTCTCAAAT TGCGGATGAGACCACGCAGAACCACCAGAACCACC-3'). Finally, the StrepII-tag was replaced with the TS-tag via the restriction sites NheI and NotI. The resulting plasmid was transformed into *T. elongatus* according to Ref.¹ Full segregation of the mutant allele was confirmed by PCR (primers: P7-*for*-5'-GGATTAACGGACGCGATTAC-3'; P8-*rev*-5'-AGGCCGATAAGGCAGAACTA-3'). See Supplementary Table 4 for list of primers. The original strain of *Thermosynechococcus elongatus* BP1 originates from Ref. 2.

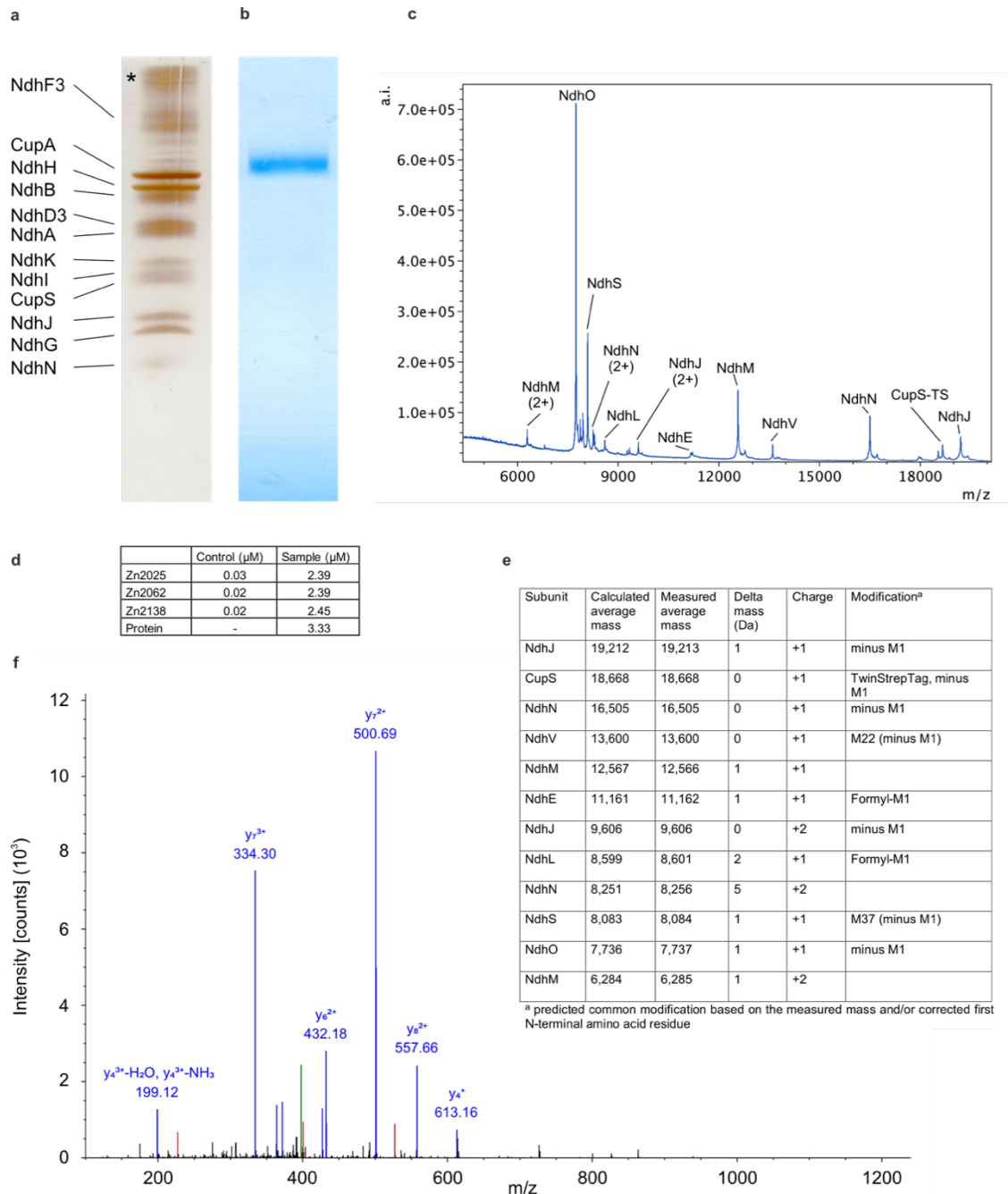
Culture conditions: *T. elongatus*. The CupS-TS-tag mutant was grown in BG-11 liquid medium³ with the addition of 80 $\mu\text{g ml}^{-1}$ of kanamycin, at a temperature of 45°C, and under illumination of increasing intensity of 50-200 $\mu\text{mol photons}$ (dependent on cell density). The cells were bubbled with 5% CO₂ until an optical density (OD₆₈₀) of 1.5 was reached, and then incubated only with compressed air to start the expression of NDH-1MS complex.

Purification of NDH-1MS complex. NDH-1MS was isolated from *T. elongatus* via TwinStrep(TS)/Streptactin affinity chromatography (IBA LifeSciences) with the TwinStrep-tag fused to the C-terminus of CupS (see above). The solubilisation of the thylakoid membranes and the purification of the complex were described earlier in detail.^{4,5} Within this study, minor modifications were introduced. In brief, thylakoid membranes were prepared according to Ref.⁶ and solubilised with 1% (w/v) glycol-diosgenin (GDN, Anatrace) and buffers for equilibration and elution were prepared with 0.02% (w/v) GDN. Strep-tagged protein complexes were concentrated with a spin concentrator (100 kDa cut off) and stored at -80°C until further analysis. For cryo-EM grid preparation, the sample was polished using size-exclusion chromatography.

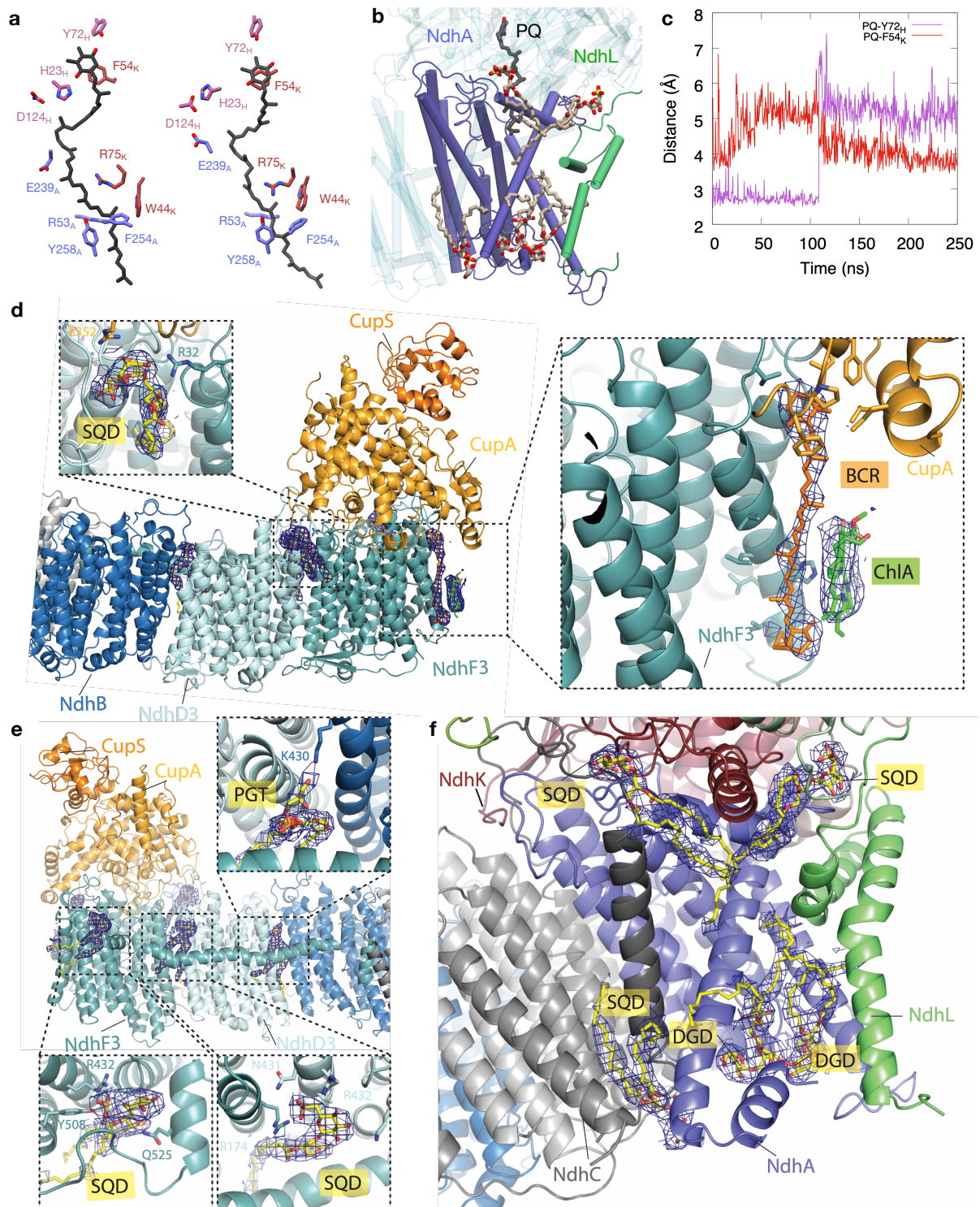
Zinc quantification. Zinc quantification was performed by inductively-coupled plasma optical emission spectrometry (ICP-OES) according to Ref. 6. In brief, the dried samples were dissolved in 1 ml 65% (w/w) HNO₃ at RT overnight, incubated at 60°C, 80°C and 100°C for 1 hr each and cooled down to 50°C before addition of 0.2 ml of 30% (v/v) H₂O₂. Then, the sample was heated again to 60°C and 100°C for 30 min each, cooled down to RT and ultrapure water was added to a final volume of 6 ml. The samples were analysed by ICP-OES with an iCAPDuo 6500 instrument (Thermo Fisher Scientific, Dreieich, Germany) after calibration with a multi-element standard. Emission specific for zinc was measured at 2025 nm, 2062 nm and 2138 nm.



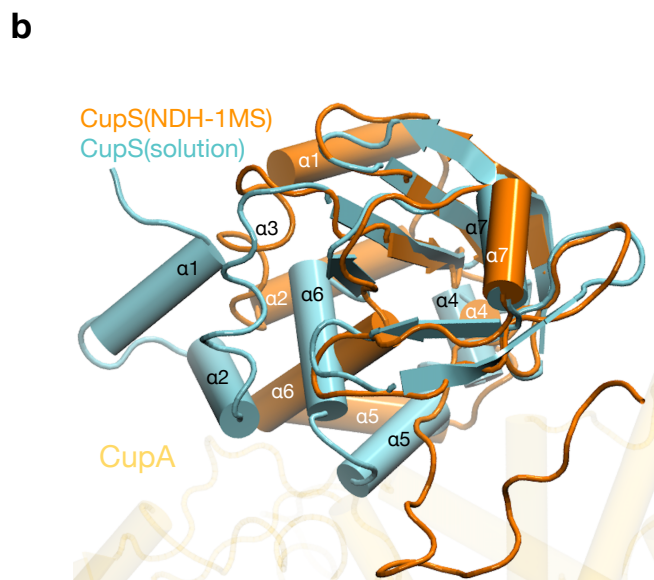
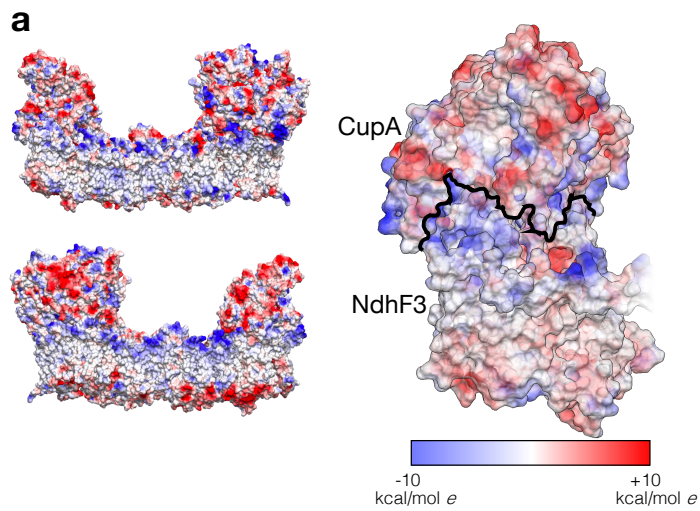
Supplementary Fig. 1 | Cryo-EM data collection, analysis, and classification scheme. **a)** Representative cryo-EM micrograph collected with an FEI Titan Krios microscope, operated at 300 kV and equipped with a K2 Summit camera. Representative reference-free 2D class averages are shown. **b)** 3DFSC (<https://3dfsc.salk.edu>) and preferred orientation analysis of the dataset with the red line, representing the estimated global FSC of $3.18 \text{ \AA} \pm 1 \text{ SD}$ (green dashed lines). A sphericity of 0.984 indicates an isotropic map. **c)** Model vs. map FSC for the final PHENIX real-space refined model. **d)** Angular distribution of the particles used for the final round of refinement. **e)** Representative regions of the photosynthetic complex I (in stick representation, same colours as in Fig. 1) and surrounding electron density maps are shown. Maps are displayed as a mesh using a contour level of up to 2 \AA around the atoms. Subunits and residue numbers are specified. **f)** The 526,925 particles selected from the dataset were 3D classified into five classes using as a reference an *ab initio* model generated by the SGD algorithm in RELION. A single class was selected and further sub-classified into three classes. The class consisting of the best aligning particles was subsequently auto-refined in RELION. After Bayesian particle polishing and CTF refinement, the particles were subjected to a final round of 3D refinement in RELION.



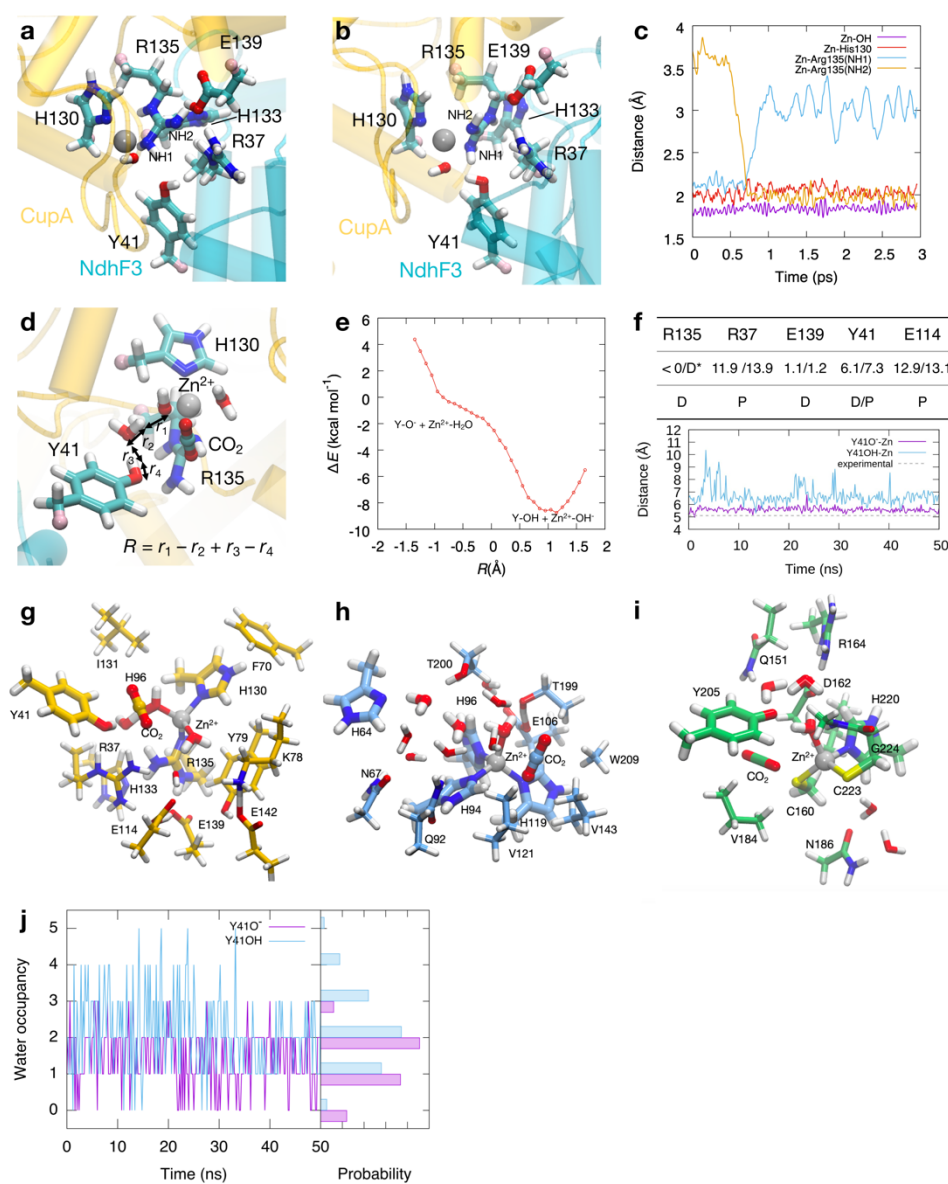
Supplementary Fig. 2 | Subunit composition and mass spectrometry analysis of NDH-1MS from *T. elongatus*. **a)** The purified NDH-1MS complex was analysed by SDS-PAGE (*probable protein aggregates), **b)** Blue-native PAGE, and **c/e)** MALDI-ToF mass spectrometry as described in Ref.⁵ (NdhJ, NdhM and NdhN are present as single and double charged (2+) ions) **d)** Zinc quantification by Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES) according to Ref.⁶ Zinc was detected at three specific wavelengths (2025 nm, 2062 nm and 2138 nm) and quantified in control (buffer only) and NDH-1MS sample after calibration with a zinc standard. Comparison with the protein concentration indicates a protein-to-zinc ratio of ~0.7. **f)** The CupA protein band (see panel **a**) was digested by trypsin and analysed by tandem mass spectrometry as described in Ref.⁵ Citrullination of arginine as well as oxidation of methionine and cysteine were allowed as dynamic modifications and only peptides that were identified with high confidence (false discovery rate >0.01) were considered as positive results. The peptide LLHHLWHDR₁₃₅ was the most abundant peptide (40 peptide spectrum matches vs. 1-23 PSMs) in the analysis and we have no indication for citrullination of Arg135.



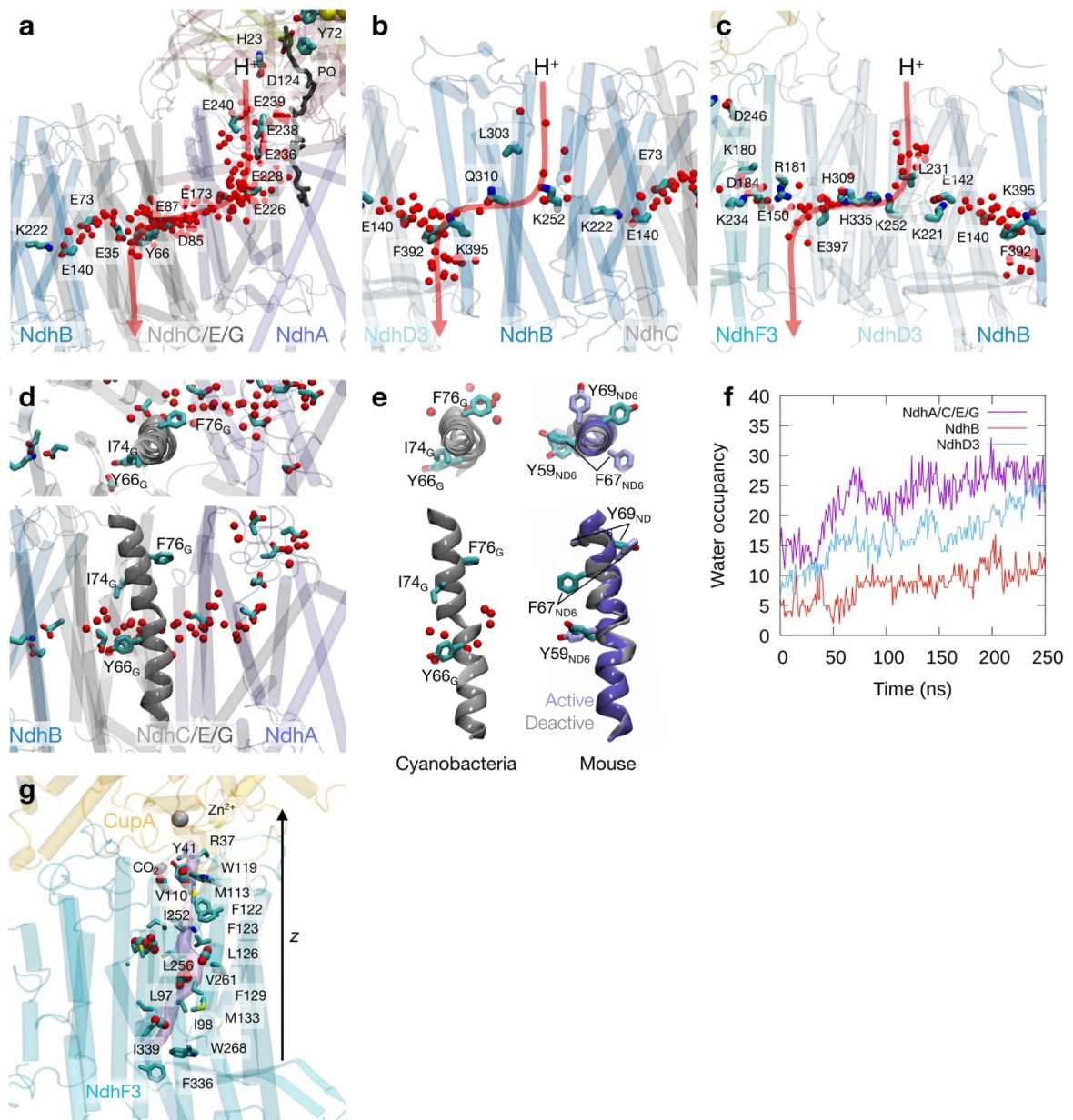
Supplementary Fig. 3 | Non-protein cofactors and lipids in the photosynthetic complex I. **a**) PQ binding site obtained from MD simulations. PQ in hydrogen-bond interaction with Tyr72 of NdhH subunit (*left*) and in stacking contact with Phe54 of NdhK (*right*). The figures show conserved residues of NdhH, NdhK and NdhA along the PQ-binding cavity. **b**) The PQ-cavity entrance is located in the NdhA subunit at the interface of three α -helices, in the proximity of NdhL (in green), and surrounded by experimentally observed lipid molecules. **c**) Distance of the PQ-headgroup to Tyr72 of NdhH and Phe54 of NdhK during 250 ns MD simulations. **d, e**) Experimentally resolved non-protein cofactors at the NdhF3/CupA interface from **e**) the front and **d**) backview. The binding area for the Chl *a*/ β -carotene (ChlA/BCR) motif is magnified. All densities are shown at 3 sigma value and using a contour level of up to 2 Å around the atoms. **f**) Lipid binding next to the PQ entry site. SQD – sulfoquinovosyl diglyceride; DGD - digalactosyl diacylglycerol; PGT – phosphatidylglycerol.



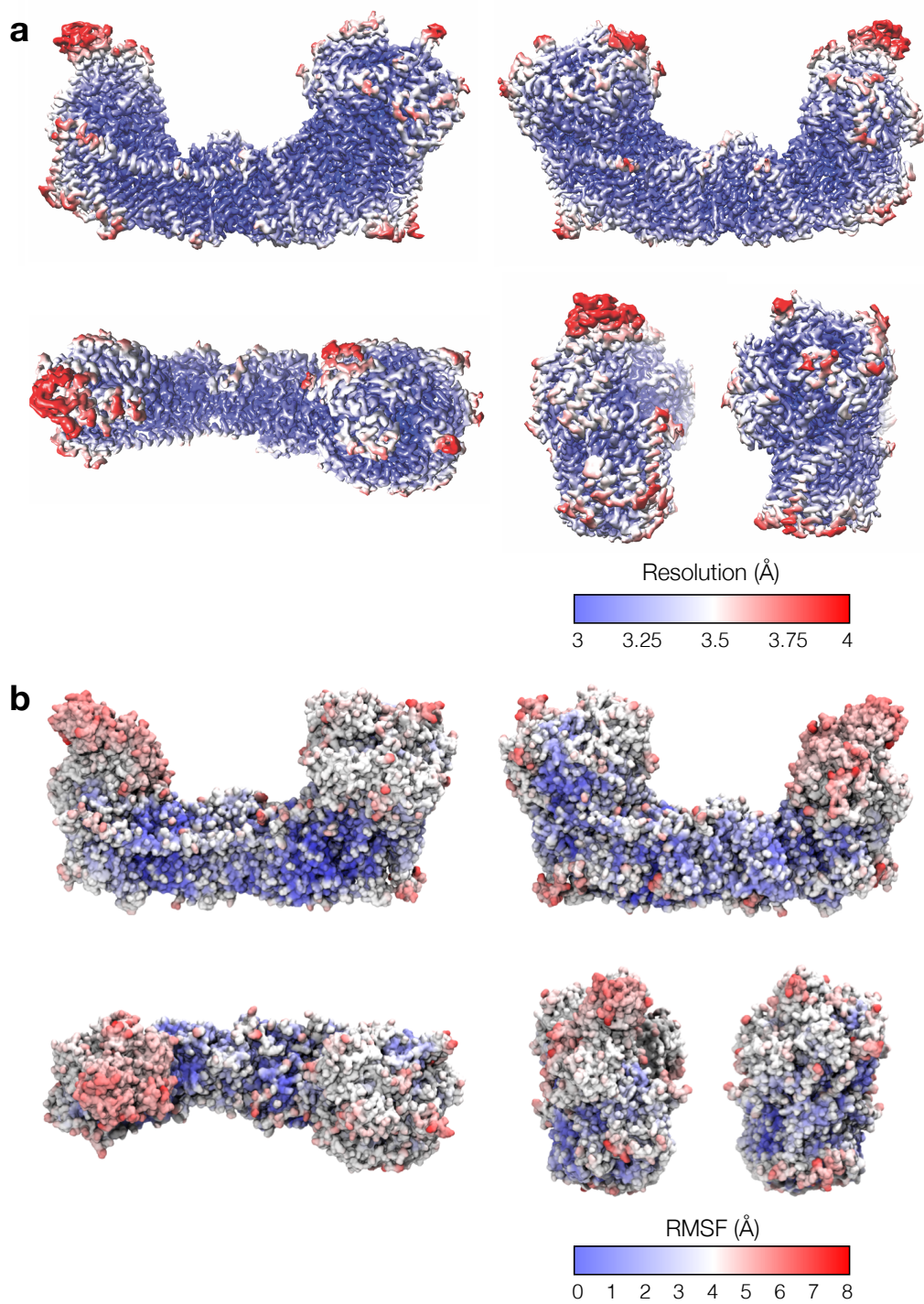
Supplementary Fig. 4 | CupA structure and interactions. **a**) Electrostatic potential (in kcal/mol *e*) at the surface of the complete NDH-1MS (left) and closeup of the CupA/NdhF3 interface (right). The negative area at the bottom of CupA (red) electrostatically interacts with the positive area (blue) at the top of NdhF3 (CupA-NdhF3 contact shown by a thick black line). **b**) CupS undergoes conformational changes from its solution structure (CupS in cyan, PDB ID: 2MXA⁹) upon binding to CupA (CupS in orange). The figure shows how helices $\alpha 1$, $\alpha 2$, and $\alpha 3$ in the N-terminal region, and $\alpha 5$ and $\alpha 6$ in the middle region, close upon the β -sheet.



Supplementary Fig. 5 | QM, QM/MM, and PBE calculations on CupA and CA. a-c) QM/MM calculations on a CupA/NdhF3 model. **a)** Arg135 (NH2) deprotonates by proton transfer to Glu139. The NH1 group of Arg135 remains coordinated to Zn²⁺. **b)** After *ca.* 1 ps QM/MM MD, the NH2 of Arg135 moves closer to Zn²⁺, further stabilizing the metal centre. **c)** Distance of first coordination sphere ligands to Zn²⁺ during the QM/MM MD simulations. **d, e)** QM/MM calculations of the proton transfer reaction from Zn²⁺-H₂O to Tyr41, showing **d)** the reaction coordinate, *R*, for the pT process and **e)** the pT energetics. **f)** *Top*: Predicted p*K*_a values of key residues in CupA and the NdhF3 interface during MDFP relaxation obtained from PBE continuum electrostatic models (see Methods). D - deprotonated residue; P - protonated residue; The second set of values correspond to calculations where R135 has been fixed in its deprotonated state (indicated with asterisk). *Bottom*: Distance of protonated Tyr41 (blue) and deprotonated Tyr41 (purple) to Zn²⁺ during MD simulations, and in the Cryo-EM structure (dashed grey line). **g-i)** DFT models of CO₂ hydration in CupA and CA. Optimised DFT models of **g)** CupA with *N*=185 atoms, **h)** αCA with *N*=155 atoms, and **i)** βCA with *N*=132 atoms. **j)** Water count between Zn²⁺ and Tyr41 during two MD trajectories.



Supplementary Fig. 6 | Proton and CO₂ channels formed during MD simulation. The figure shows water molecules (red sphere) averaged over 250 ns MD simulations in **a**) NdhC/E/G, **b**) NdhB, and **c**) NdhD3. **d**) Proton channel in NdhA/E/G and comparison to TM3 rotation in mouse complex I.¹⁰ **e**) TM3 of NdhG in NDH-1MS (grey, left). TM3 helix of ND6 of complex I from *Mus musculus* (right) in the deactive (grey) and active (purple) states. **f**) Hydration dynamics in the putative proton channels in subunits NdhB, NdhD3, and NdhA/C/E/G during MD simulations. **g**) The putative CO₂ gas channel (purple) is surrounded by hydrophobic and bulky residues, and connects to the Zn²⁺-binding site by conserved Tyr41, Arg37 and Glu114 of NdhF3 subunit.



Supplementary Fig. 7 | Dynamics of NDH-1MS inferred from the local resolution of the cryo-EM map and from MD simulations. a) The resolution was estimated using the local resolution function in RELION with default parameters and plotted using UCSF Chimera. Units are in (Å). **b)** Root-mean-square-fluctuations (RMSF, in Å) obtained from 250 ns MD simulations of NDH-1MS.

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

	NDH-1MS (EMDB-10513) (PDB ID: 6TJV)
Data collection and processing	
Magnification	105.000 x
Voltage (kV)	300
Electron exposure (e-/Å ²)	40.2
Defocus range (µm)	0.5-3.5
Pixel size (Å)	1.35
Symmetry imposed	C1
Initial particle images (no.)	526.925
Final particle images (no.)	170.151
Map resolution (Å)	3.20
FSC threshold	0.143
Map resolution range (Å)	2.98-7.26
Refinement	
Initial model used (PDB code)	6HUM, <i>de-novo</i>
Model resolution (Å)	3.23
FSC threshold	0.5
Model resolution range (Å)	3.23
Map sharpening <i>B</i> factor (Å ²)	-89.89
Model composition	
Non-hydrogen atoms	68541
Protein residues	4275
Ligands	15
<i>B</i> factors (Å ²)	
Protein	60.9
Ligand	64.8
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.109
Validation	
MolProbity score	2.2
Clashscore	11
Poor rotamers (%)	1.29
Ramachandran plot	
Favored (%)	88.00
Allowed (%)	11.48
Disallowed (%)	0.52

Supplementary Table 2 | Tandem-LC-MS analysis of NDH-1MS. For BN-PAGE, see Supplementary Fig. 2b.

Subunit^a	ORF	Mass (kDa)^b	TMH^c	XC^d	Coverage^e
NdhA	tlr0667	41.3	8	49.66	18.47
NdhB	tll0045	55.1	14	59.88	7.57
NdhC	tlr1429	15.0	3	4.67	15.91
NdhD	tlr0905	53.8	14	20.74	5.59
NdhE	tlr0670	11.1	3	20.05	14.85
NdhF	tlr0904	66.2	16	69.12	7.86
NdhG	tlr0669	21.6	5	5.35	16.50
NdhH	tlr1288	45.2	-	302.11	49.24
NdhI	tlr0668	22.4	-	151.24	57.14
NdhJ	tlr1430	19.3	-	142.17	59.52
NdhK	tlr0705	25.7	-	129.30	29.11
NdhL	tsr0706	8.6	2	5.42	11.84
NdhM	tll0447	12.6	-	133.40	49.55
NdhN	tlr1130	16.6	-	59.28	55.33
NdhO	tsl0017	7.9	-	27.22	57.14
NdhS	tlr0636	8.2	-	18.51	62.16
NdhV	tlr0472	13.7	-	10.34	19.20
CupA	tlr0906	50.9	-	268.37	49.24
CupS-TS	tll0220	18.8	-	109.15	55.31

^a results were filtered for known NDH-1MS subunits

^b calculated molecular weight

^c number of transmembrane helices

^d sequest protein score

^e sequence coverage (percent)

<i>T. elongatus</i> (BP-1)/1-437	1 MVQAMERPSSAKL PFLDPLADIIYHLEAGGALIPDPVNLMKIIGMYKAVSI PMDFYWRDLYLGERVFINLFFPKYFPTKELFEL	88
<i>Synechocystis</i> sp. (PCC 6830)/1-431	1 ---MTTLTPKATLPESTHFPADVYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAERVFLNLRFFKYFLPQYVLDL	85
<i>Synechococcus</i> sp. (PCC 6301)/1-430	1 ---MTVTTRTTPLPFSQHRFAEVYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAEQVFLNIPAFKYFISQYVLDL	85
<i>M. aeruginosa</i> (TAIHU98)/1-431	1 ---MTGTLIKAKI PPSHTEFADIHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAERVFLDLFFKYFLPQYVLDL	85
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	1 MVQAMERPSSAKL PFLDPLADIIYHLEAGGALIPDPVNLMKIIGMYKAVSI PMDFYWRDLYLGERVFINLFFPKYFPTKELFEL	88
<i>S. elongatus</i> (PCC 7942)/1-430	1 ---MTVTTRTTPLPFSQHRFAEVYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAEQVFLNIPAFKYFISQYVLDL	85
<i>S. cyanosphaera</i> (PCC 7437)/1-434	1 MVQTPIKQPTAKL PPSHQFADVYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAERVFLDLFFKYFLPQYVLDL	88
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	1 MVQTPIK-PTGII PPSNHQFAEVYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAERVFLDLFFKYFLPQYVLDL	87
<i>Xenococcus</i> sp. (PCC 7305)/1-433	1 MVQT-SPKSTSKI PPSHTEFADIHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAERVFLDLFFKYFLPQYVLDL	87
<i>Nostoc</i> sp. (PCC 7524)/1-440	1 MVQ-TPDKPVTKI PPSQHEFADIYHLEAGGSLPDPENLMQIIGIYKAVAVPMDFYWRDLYIAEQVFLNIFGFFKYFISKEVLER	87
<i>T. elongatus</i> (BP-1)/1-437	89 PHHYAGDTADLRIWRGPAHAHPELMEFIEIKGET-GKMPRLHHLWDRINMEFSEDLARAMMWHRM---GGQLDIYLDSEYKAAAD	171
<i>Synechocystis</i> sp. (PCC 6830)/1-431	86 ANHYAGETADLRIWRGEASAHPELLEFMEKGNIT-TKMPKLFHHLWDRINMEFAEACQAMLWHGRDM-GMGKFDYLDSEYKANAD	171
<i>Synechococcus</i> sp. (PCC 6301)/1-430	86 PHSYAGDNADLRIWRGPATAHPELLEFMQKRGELKRLKPKLHHLWDRINMEFAEACMDAMLWHQGM---GGRFNDYLDSEYRQAD	170
<i>M. aeruginosa</i> (TAIHU98)/1-431	86 QHHYAGDNADLRIWRGTGSAHPELLEFMDKGGT-RKMPRLHHLWDRINMEFAEACQAMLWHGRDM-GWGLFDAYLDSEYRQAD	171
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	89 PHHYAGDTADLRIWRGPAHAHPELMEFIEIKGET-GKMPRLHHLWDRINMEFSEDLARAMMWHRM---GGQLDIYLDSEYKAAAD	171
<i>S. elongatus</i> (PCC 7942)/1-430	86 PHSYAGDNADLRIWRGPAHAHPELLEFMQKRGELKRLKPKLHHLWDRINMEFAEACMDAMLWHQGM---GGRFNDYLDSEYRQAD	170
<i>S. cyanosphaera</i> (PCC 7437)/1-434	89 HHHYAGDDADLRIWRGEATAHPELLEFMEKGGT-TKMPKLFHHLWDRINMEFAEACMDAMLWHGRDM-GWGKFDALDTEYKANAD	174
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	88 HHHYAGDDADLRIWRGEATAHPELLEFMEKGGT-GKPKLFHHLWDRINMEFAEACMDAMLWHGRDM-GWGKFDALDSEYKANAD	173
<i>Xenococcus</i> sp. (PCC 7305)/1-433	88 PHHYAGENADLQVWRGRASAHPELLEFMEKGGT-SKMPKLFHHLWDRINMEFAEACMDAMLWHGRDM-GWGKFDALDTEYKANAD	173
<i>Nostoc</i> sp. (PCC 7524)/1-440	88 HHHYAGDDADLRIWRGEATAHPELLEFMEKGGT-FKPKLFHHLWDRINMEFAEACMDAMLWHGRDM-HRMYAVPNQFDALDSEYKANAD	174
<i>T. elongatus</i> (BP-1)/1-437	172 KAIRAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	259
<i>Synechocystis</i> sp. (PCC 6830)/1-431	172 KAIRAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	259
<i>Synechococcus</i> sp. (PCC 6301)/1-430	171 RAIRAYFQGNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	258
<i>M. aeruginosa</i> (TAIHU98)/1-431	172 RAIKAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	259
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	172 KAIRAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	259
<i>S. elongatus</i> (PCC 7942)/1-430	171 RAIRAYFQGNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	258
<i>S. cyanosphaera</i> (PCC 7437)/1-434	175 RAIKAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	262
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	174 KAIKAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	261
<i>Xenococcus</i> sp. (PCC 7305)/1-433	174 RAIKAYFKRNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	261
<i>Nostoc</i> sp. (PCC 7524)/1-440	175 KAIKAYFQGNPMLGLYKLFDFLFCARQATMMNVLGLFEWVMAVVFPEISDRYDEGSITSVKDAMNVLVNGIFAIAGSPIYHHVYI	262
<i>T. elongatus</i> (BP-1)/1-437	260 DDEVHVLVPEKKGEMWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	347
<i>Synechocystis</i> sp. (PCC 6830)/1-431	260 DGECELIIPKSKGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	347
<i>Synechococcus</i> sp. (PCC 6301)/1-430	259 NGECFELIIPKSAGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	346
<i>M. aeruginosa</i> (TAIHU98)/1-431	260 DGCYELIIPKSKAFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	347
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	260 DDEVHVLVPEKKGEMWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	347
<i>S. elongatus</i> (PCC 7942)/1-430	259 NGECFELIIPKSAGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	346
<i>S. cyanosphaera</i> (PCC 7437)/1-434	263 RGCEYELIIPKSKGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	350
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	262 RGCEYELIIPKSKGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	349
<i>Xenococcus</i> sp. (PCC 7305)/1-433	262 DDECYELIIPKSKGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	349
<i>Nostoc</i> sp. (PCC 7524)/1-440	263 RGCEYELIIPKSKGFTWLYEAAFPYVEAVFYRSPFRGKTSYNAQAKVPEDEKDFHYGILYADVFPVGTAGIPPLLLMQDMYHFLPQY	350
<i>T. elongatus</i> (BP-1)/1-437	348 LKDYFHQCRGEDDILVQLGIAFHCHAMTYTISAVLQATRAAFYVPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	435
<i>Synechocystis</i> sp. (PCC 6830)/1-431	348 LIEYYQKHCRGEDDMLVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	429
<i>Synechococcus</i> sp. (PCC 6301)/1-430	347 LLEEYRQHCRGEDDILVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	428
<i>M. aeruginosa</i> (TAIHU98)/1-431	348 LIDYQQQCRGEDDILVQLGISTFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	429
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	348 LRDYFHQCRGEDDILVQLGIAFHCHAMTYTISAVLQATRAAFYVPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	435
<i>S. elongatus</i> (PCC 7942)/1-430	347 LLEEYRQHCRGEDDMLVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	428
<i>S. cyanosphaera</i> (PCC 7437)/1-434	351 LQYQYQHCRGEDDILVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	432
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	350 LIEYYQKHCRGEDDILVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	431
<i>Xenococcus</i> sp. (PCC 7305)/1-433	350 LQYQYQHCRGEDDILVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	431
<i>Nostoc</i> sp. (PCC 7524)/1-440	351 LVDDYQYCRGEDDILVQLGITFORSMYNVTISAVICALETALLYPLDDPNPKHLKMAHFFEAQMDRFLRFPQYGAIEACKIRNVDPN	438
<i>T. elongatus</i> (BP-1)/1-437	436 YL	437
<i>Synechocystis</i> sp. (PCC 6830)/1-431	430 YR	431
<i>Synechococcus</i> sp. (PCC 6301)/1-430	429 YR	430
<i>M. aeruginosa</i> (TAIHU98)/1-431	430 YR	431
<i>Thermosynechococcus</i> sp. (NK55a)/1-437	436 YL	437
<i>S. elongatus</i> (PCC 7942)/1-430	429 YR	430
<i>S. cyanosphaera</i> (PCC 7437)/1-434	433 YR	434
<i>Cyanothece</i> sp. (ATCC 51142)/1-433	432 YR	433
<i>Xenococcus</i> sp. (PCC 7305)/1-433	432 YR	433
<i>Nostoc</i> sp. (PCC 7524)/1-440	439 YR	440

Supplementary Table 3 | Multiple sequence alignment (MSA). of the CupA (top) and NdhF (bottom). Colouring conservation threshold: 50%. Colouring method: clustal. Colouring conservation threshold: 90%. The MSAs were performed with ClustalW.⁸

NdhF3 <i>T. elongatus</i> /1-611	1 MLQSFADTIVLIPFYSLAGMVLSE----LIWSPGTRKTRGPRPAGY--LNILLTFVSVFHALLATVAIANQ--PPQYLHWTLVDVAGLHLD	82
NdhF4 <i>T. elongatus</i> /1-609	1 MPNSLLETSSWVPCYGLVGAALT--LWATGYVRRTPRPAAY--FNLLMTVVAFGHGFLLQOTRSG--ATATVHWLQAPGLDLS	82
NdhF1 <i>T. elongatus</i> /1-656	1 -MEPLYQYAWLIPVLPFLGALVGFGLIAFSETFKLRRRPAIFIM---ALMALIAGHSLTLFWSVQGHLPFT--QMIEMAAAGNLHIA	84
NdhF3 <i>Synechocystis</i> sp./1-615	1 MLESLSRIIWLVPYVALLGALLA----VWSPGLTRQTRGPRPAGY--ISTLMTFVAFHLSLLVLIHWQ--PAIDLSFPLHAAADLEIN	82
NdhF4 <i>Synechocystis</i> sp./1-634	1 MSDFLLQSSWVIFPFYGLIGSILS----LPWSFRILIKQTRGPRPAGY--FNVFMTLVSATHGVALSALWQ--PSEQVIFHMLQVADLDT	82
NdhF1 <i>Synechocystis</i> sp./1-681	1 -MELLYQLAWLIPVLPFLFAGATVVGILISFNQATNKLRQINAVFII---SCLGAALVMSGALLWDQIQGHASYA-QMIEMASAGSFHIE	84
Nqo12 <i>T. thermophilus</i> /1-606	1 ----MALLGTILLPLGLFALLGLFGKRMREPLPGVLAASGLV----ASFLLGAGLL-----LGGARFQA---EWL----PCIP	64
NuoL <i>E. coli</i> /1-613	1 ----MNMLALTIILPLIGVFLFASRGRWSENVAIVGVGSLAALVTAFIGVDF--ANGETYSQPLWNTMMSVGFDFNIG	76
N5_M. musculus/1-607	1 -MNIPTTSIILLI-FILLSPLI-----ISMNSLNKHINFP-----LYTTTSIKFSFIISLPLMLFFHNMEYMITWHVMTMNSMEIK	77
NdhF3 <i>T. elongatus</i> /1-611	83 IPVEISILTTTALMILITANLMAQVFAVGMEMDVGWAF--FALLALFEGGMGALVLLDSLFFNVVVLIELITLTYLLI--GLVFNQPLVVTG	172
NdhF4 <i>T. elongatus</i> /1-609	83 FSLLINSVTTGAMELVTGLSILAQIFALGYLEKDWGMA--FALMGFFEAALSGLAISDS--LLSGLLEMLTSLTYLLI--GFVYAOPLVVKKA	172
NdhF1 <i>T. elongatus</i> /1-656	85 MGYVIDPLAALMLVITVAVFLVMLVSDGMAHPDGYV--FFAYLSLFGSSMLGLVSPNVLQVYI--FWELVGMCSYLLI--GFWYDRKSAEAA	174
NdhF3 <i>Synechocystis</i> sp./1-615	83 FDLKISTVNIALLVILITGLNLGAQIYAIYGLERDWGWA--FFSMLMFEAGLCTVLCNSLFFSFFVVLIELITLGTYYLIGVFNQSLVVTG	172
NdhF4 <i>Synechocystis</i> sp./1-634	83 LAVEISPVSLGALSVVGTISFLVQIFGLCYMEKDWSLAF--FYGLLGFEEAALGCLALSDFLSLYGELLEMLTSLTYLLVGFVWYAKQALVA	172
NdhF1 <i>Synechocystis</i> sp./1-681	85 MGYVIDHLSALMLVITVAVFLVMLVITDGYMAHPDGYV--FFAYLSLFASSMLGLVSPNVLQVYI--FWELVGMCSYLLI--GFWYDRKSAEAA	174
Nqo12 <i>T. thermophilus</i> /1-606	65 FSLLLDNLDSGFMILLVTVGVGFLIHVYAIYMGDPDGYSS--FFAYFNLFIAMMLTLVLADSYPMVLTGVEGVLGASFLYIGFHWKPKQYADS	154
NuoL <i>E. coli</i> /1-613	77 FNLVLDGLSLTMSLVTGVLFIHMVYASWVRGEGEYS--FFAYTNLFIASMVLVLDLNLMLLWLGVEGVLGCSYLLI--GFVYTDPRNGAA	166
N5_M. musculus/1-607	78 MSFKTDFPSLFTSVALFVWISIQFSSV--MHSBPIN--FKYLTLELITMLLITSAANNMFLQITGVEGVLGMSFLIIGWYGRTDANTA	167
NdhF3 <i>T. elongatus</i> /1-611	173 ARDAFLTKRFGDILLMGVLAITYPLAGSWNYYDAAWAATAQV---NSTLITLICALIAGPCKCA--FPHLHWLDEAMEGPIEASIL--	257
NdhF4 <i>T. elongatus</i> /1-609	173 ARDAFLTKRFGDILLMGVVALGSLAGSYDFPHLYEWAQANL---PDGWFLLGLALIAAGPTKCA--FPHLHWLDEAMEGPNPASIM--	257
NdhF1 <i>T. elongatus</i> /1-656	175 AQKAFVTVNFGDIFGLLGMVGLFWATGDFDFPAGMGLTELVTNGLLSPSLAAILAIVLFLGPVAKSAA--FPHLHWLDEAMEGPIEASIL	257
NdhF3 <i>Synechocystis</i> sp./1-615	173 ARDAFLTKRFGDILLMGVVALPLAGSWNYYDAAWAASADL---NPTAATLLCLALIAAGPTKCA--FPHLHWLDEAMEGPIEASIL	264
NdhF4 <i>Synechocystis</i> sp./1-634	173 ARDAFLTKRFGDILLMGVVALSSYQGLTFPSQLDNWASTVPP---TGITATLLGLSLIAGPTKCA--FPHLHWLDEAMEGPNPAGIM--	257
NdhF1 <i>Synechocystis</i> sp./1-681	175 CQKAFVTVNFGDIFGLLGLLGLYATGSPDFGTIGRLEGLVSSGVLGSAIAAILAIVLFLGPVAKSAA--FPHLHWLDEAMEGPIEASIL	264
Nqo12 <i>T. thermophilus</i> /1-606	155 ARKAFIVNRIICDGLGFMGLMALLWALYGTLSISELKEAMEGPLK-N---PDLLALAGLLFLGAVGKSAIIPMHWLDEAMEGPIEASIL	240
NuoL <i>E. coli</i> /1-613	167 AMKAFVTVNFGDIFLAPALFILYNEGLTFNPREMVLVLAFAHFDG---NNMLMWTMLLGLGAVGKSAIIPMHWLDEAMEGPIEASIL	253
N5_M. musculus/1-607	168 ALQAILNYRIGDIFGLIAMWESLNMSWELQ---IMFSNNN---DNL--IPLMGLLIAATKCA--FPHLHWLDEAMEGPIEASIL	247
NdhF3 <i>T. elongatus</i> /1-611	258 RNAVVVATGAWLVKLPVLSLSPVALTALVLSV--ALGTLIATAV--DTRKALSIVLSAYMGVPIAVGLKPEGLIAVFLVILTYSLAMA	347
NdhF4 <i>T. elongatus</i> /1-609	258 RNSVVVAAAGAYILIKLOPLVACPGANIALIAITV--AISELSVSIAG--IDIKRALSHTSVYLVLFVIGVGNWDFALFVLLTHAIAKA	347
NdhF1 <i>T. elongatus</i> /1-656	265 HAATMVAAGVFLIARMPFVFEQLQVMTTIAWTGAF--AFMGATIAIT--NDIKRSLAYSTISQLGYMVMGCVGAYSAGLPHLMTTHAYFKA	354
NdhF3 <i>Synechocystis</i> sp./1-615	258 RNTLVVATGAWLVKLPVLSLSPVALTALVLSV--AIGASLIAIAI--DTRKFLSYVVSAYMGLVPIAVTGGQGETALQLIFTYTFAMA	347
NdhF4 <i>Synechocystis</i> sp./1-634	258 RNSVVVSAAGAYVLIKLOPLVETLSIASKTLVILVTL--VVMVMSLIAIAI--DTRKSLSHSTSVYLVLFVAVGLGQVDFLFLPAHAIAKA	347
NdhF1 <i>Synechocystis</i> sp./1-681	265 HAATMVAAGVFLVARMYPVEFPIPVVMTTIAFTGCT--AFGLATIAIT--NDIKRSLAYSTISQLGYMVMAMGIGAYSAGLPHLMTTHAYFKA	354
Nqo12 <i>T. thermophilus</i> /1-606	241 HAATMVTAGVYLIARSSFLVSLPDSVYLAIVAVGLL--IAYGALSFAFG--DTRKIVAYSTISQLGYMFLAAGVAYVWVFLPHMTTHAFFKA	330
NuoL <i>E. coli</i> /1-613	254 HAATMVTAGVYLIARTHGLFVPEVHLVILVAVGLL--IAGPAALV--DTRKSLAYSTISQLGYMFLAAGVAYVWVFLPHMTTHAFFKA	340
N5_M. musculus/1-607	248 HSSTMVAGIFLLVRFHPLTNNNFILTTMLCLGAI--TLFTAICALT--DTRKIVAYSTISQLGIMMVTLENNQPHLAFHICHTAFFKA	337
NdhF3 <i>T. elongatus</i> /1-611	348 VLMMSIGSITWNSVT-----DRLRLGLWSRR--ISGISFLVGSAGLLAVPPL--ASFFPQAEELDTAFAG---LPWVGGVLLMNT	424
NdhF4 <i>T. elongatus</i> /1-609	348 LLMFMSIGSIVMTNS-----DLTEGLGGERMATSFAVIGGLSLI--GLPL--GAFWSFYRGIYYWQT---MPWLVGLIIVNL	424
NdhF1 <i>T. elongatus</i> /1-656	355 MLFLGSGSVIHSMEGVVGHNDPLA--DMRYMGLRKYM--ITGATFLVGLCLAISGVPPF--AGFWSKDEILGAVFHA---NPAMWLLTWLTA	420
NdhF3 <i>Synechocystis</i> sp./1-615	348 IIVMCGVGIILNNTV-----DLITQYGLWSRR--ISGLSYLVGVASLIALPPF--GTFWAWLKAENLATS---SPLLVGLVLLVNL	424
NdhF4 <i>Synechocystis</i> sp./1-634	348 LLMFMSIGSITFTSG-----NITMETLNNRMEVTTTSFVVSAGLLAVPPL--GMFTVWVWVMSGLVAV---SWPILLALLVNL	424
NdhF1 <i>Synechocystis</i> sp./1-681	355 MLFLCSGSVIHSMEGVVGHNDPILA--DMRIMGRLKYM--ITATCFLIGTLATC--IIPP--AGFWSKDEILGALFQA---NPLLWFVWATAG	440
Nqo12 <i>T. thermophilus</i> /1-606	331 LLFLASGSVIALHGG-----DVRMKGGLWKHL--PTRWHALIGALAGLPL--SGFWSKDAI LAATLTYPGVGGVYVAGLLVAV	411
NuoL <i>E. coli</i> /1-613	344 LLFLASGSVILACHH-----ENIFKMGGLRKSILVLYVCLVGGAAISALPLVTAAGFWSKDEILGAGMA---NGHINMLVAVAGLVAF	423
N5_M. musculus/1-607	338 MLFMCSSITHSLAD-----DDIRKMENTKIM--FTSSCLVIGSLAIT--GMPFL--TGFSKDLI--EAINTC--NTNAWALLITLIATS	417
NdhF3 <i>T. elongatus</i> /1-611	425 FAAFSLG--TEFLVWVGGEVKPMTA-----RSPEVFWPMILPMTVDLGLVHLPLPMA	475
NdhF4 <i>T. elongatus</i> /1-609	425 LTAVNLT--VFRVFLGPAQPKTR-----RAPEVTFPLAVPMVTLISLMLVFPFLLQ	475
NdhF1 <i>T. elongatus</i> /1-656	441 LTAFYMF--MYFMTFEGKFRNVPPEQE-----HHHSHHAAVPHESPWTMLPLVLAIPSTLIGFVGT	505
NdhF3 <i>Synechocystis</i> sp./1-615	425 LTAFNVT--GFCLIFGGEAKPMTV-----RSPGELWALVPMVTVGFALHLSLILK	475
NdhF4 <i>Synechocystis</i> sp./1-634	425 FSALNLT--VFRVFLVFLGPKPQKTR-----RAPEVWPWMAVPMVSLIIVTLVPIAPL	475
NdhF1 <i>Synechocystis</i> sp./1-681	441 MTAFYMF--MYFMTFEGGFRNDQAEKDVGLQFYGLLPNFGGAMNPKELDHEAGHDHSHSSEPHESPMTMTFPLMALAVPSVLIGLGR	530
Nqo12 <i>T. thermophilus</i> /1-606	412 LTMAYM--WFVFLVFLGEEGRH-----H-----HPHEAPPVLMVLLHLLGSLVAGYLAL	461
NuoL <i>E. coli</i> /1-613	424 MTSLYTF--MIFIVFHGKEQIH-----A-----HAVKGVTHSLPIVLLIL--STFVGALLV	472
N5_M. musculus/1-607	418 MTAMYSM--IIEVFTMTKPRFPPLIS-----INENDPDLNMPKRLAFSGIFAGFVIS	469
NdhF3 <i>T. elongatus</i> /1-611	476 R-----FDWVIWTPQLSATAAALITALL--LWVAAWVYLGKAIKPKV---QFPLPSVQN-----LLAYDFY	533
NdhF4 <i>T. elongatus</i> /1-609	476 R--V-----Q--LLEPTIDWTI---VALVVSGLACILLGGFVTLKRSWTRPI---KVLRFVQD-----LLAYDFY	532
NdhF1 <i>T. elongatus</i> /1-656	506 P--FNNLFEVFIHAPGEEKVAEHAVDLTFEL--ILGSSVGI--LGMCTIVAYLMLKGTFPSPQ--AI-----AKAIQP-----LYQFSLHKWY	581
NdhF3 <i>Synechocystis</i> sp./1-615	476 Q--G-NLLPD-----FADINWGL-----SSVLIASSLLVGGSAFYLYLNPKITKPI---DLPLPVQVN-----FFAYDLV	534
NdhF4 <i>Synechocystis</i> sp./1-634	476 Q--W-SFNLSATYPLG---LTSPTVQWA-----MPLMLVAGITCILLGSLMPLRRNLSRSS---RLRPLVQD-----LFAYDQV	541
NdhF1 <i>Synechocystis</i> sp./1-681	531 P--WANQFEAFIHPGEVVEHAEFEWGEFY--VMAGNSIGIALIGITVASLMYVQHKFDPK--VL-----AEKFPS---LYQSLINKWY	606
Nqo12 <i>T. thermophilus</i> /1-606	462 PHPLPNVLEPFLKPALAEVE--AHH-----L-SLGAEWGLIASAAVA--LLGLWAGVFFPQ-----RKVFPA--WYLAFAEASREAFY	533
NuoL <i>E. coli</i> /1-613	473 P--PLQGVLPQTEP---AH--GSM-----L-TLEITSGVAVVGLL--AAWLWLGKRTLVTSIANSAPRGLLTKWVYNAW-----G	540
N5_M. musculus/1-607	470 YNIPPTSIPVLTMP-----W-FLLKTTALIIISVGLFALE---LNNLTMKLSMKNANPYSFST-----LLGF	528
NdhF3 <i>T. elongatus</i> /1-611	534 TPPLYRATV-----VGVVDMISRTAWDFRTFVDGTGNAGVVTLLGGDRLKYSTT--QSQAM--ILTIIMGIAITLVI---AICWPLLA--	611
NdhF4 <i>T. elongatus</i> /1-609	533 IEELYRYTV-----VWAVRSLQSLSAWVDRHIVDRVNTGAASLVGGLLKYSGASQSQAM--LLLVFVIGLALGG---AIAWALL--	609
NdhF1 <i>T. elongatus</i> /1-656	582 FDELYEAVF-----IKCCRRLLARQVLEVDVNVVDGVNVLGFTVMTVGTGELKYLQNGRAQFALIVL--IAGVGFVI---PSVQ--T--	656
NdhF3 <i>Synechocystis</i> sp./1-615	535 TDKFYKLT-----VAVIDSIRLNIWDFKTFVDGVNLIIGIVTIFSGQSLKYNVSQ--QTFVLSIVLGLTLIGA---FLSYSLGQ	613
NdhF4 <i>Synechocystis</i> sp./1-634	542 LDKIYGATV-----VAAVAATAKISTWFDVYVLDGIVNLVSLVTFSGSALKYVNT--QSQPMLLTLVGVALLIWFSLGQWMAIRQ	623
NdhF1 <i>Synechocystis</i> sp./1-681	607 FDDLVDKLF-----VQGSRRVARQIMEVDYKVDGAVNLTGLVTLVSGEGLKYLENGRAQFALIVF--GAVLGFVI---VFSL--T--	681
Nqo12 <i>T. thermophilus</i> /1-606	534 VDRAYNALI-----VNPFLKAAEAALFYGDRLSSYFGL--GGAARSLGQGLARLQTFYLRVVALLTVLGLALLLGV---MRW-----	606
NuoL <i>E. coli</i> /1-613	541 FDLWLYDKVF-----VKPFLGLAWLL---KRDPLNSMNIIPAVLSRFAGKCLLSENGYLRVWVMSMIGAVVVAL---LMLV--R--	613
N5_M. musculus/1-607	529 PPSIIHRTIPMKSLNLSLKTSLTLLDLVLEKTIIPKSTSTL-----HTNMTTITNOKGLIKL--FMSFLINIILIIIL---YSIN--LE	607
NdhF3 <i>T. elongatus</i> /1-611	-----	
NdhF4 <i>T. elongatus</i> /1-609	-----	
NdhF1 <i>T. elongatus</i> /1-656	-----	
NdhF3 <i>Synechocystis</i> sp./1-615	614 AF-----	615
NdhF4 <i>Synechocystis</i> sp./1-634	624 FWSSWLSLILP-----	634
NdhF1 <i>Synechocystis</i> sp./1-681	-----	
Nqo12 <i>T. thermophilus</i> /1-606	-----	
NuoL <i>E. coli</i> /1-613	-----	
N5_M. musculus/1-607	-----	

Supplementary Table 3 (contd.) | Multiple sequence alignment (MSA) of the CupA (top) and NdhF (bottom). Colouring conservation threshold: 50%. Colouring method: clustal. Colouring conservation threshold: 90%. The MSAs were performed with ClustalW.⁸

Supplementary Table 4 | List of primers for the construction of the *T. elongatus* CupS-TwinStrep-tag mutant.

Primer Name	DNA Sequence (5' to 3')	Description	PCR ^a
P1- <i>for</i>	AAAGAGCTCGGCCATAGCCAGAT GTC	<i>cupS</i> (<i>tll0220</i>), US region and StrepII-tag incl. 5'-end SacI and 3'-end SacII restriction site, amplified from genomic DNA	1,975
P2- <i>rev</i>	GGGCCGCGGTTATTTTCGAACTG CGGGTGGCTCCAAGCGCTGACTTT GTAGGGAATGTTG		
P3- <i>for</i>	GGGCTGCAGGGTATTAGCAGATTT CCC	<i>cupS</i> (<i>tll0220</i>) and DS region incl. 5'-end PstI and 3'-end KpnI restriction site, amplified from genomic DNA	1,512
P4- <i>rev</i>	CCCGGTACCCAATCAGGTCGTAGT TGC		
P5- <i>rev</i>	GCAGAACCACCAGAACCACCGCCG CTGCCGCGCCTTTTCGAACTGCG GGTGGC	<i>cupS</i> (<i>tll0220</i>), US region and 1/2 TS-tag incl. 5'-end SacI and 3'-end SacII restriction site, amplified from pCupS-StrepII-tag (with P1- <i>for</i>)	1,998
P6- <i>rev</i>	CCCGCGGCCGCTTACTTCTCAAATT GCGGATGAGACCACGCAGAACCAC CAGAACCACC	<i>cupS</i> (<i>tll0220</i>), US region and TS-tag incl. 3'-end NotI restriction site, amplified from product P1/P5 (with P1- <i>for</i>)	2,037
P7- <i>for</i>	GGATTAACGGACGCGATTAC	Segregation check	2,148 (WT 842)
P8- <i>rev</i>	AGGCCGATAAGGCAGAACTA		

^a PCR product length in basepair, US = Upstream, DS = Downstream

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