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Supplementary information

Structural basis of mitochondrial membrane bending by the I–II–III₂–IV₂ supercomplex

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Structural basis of mitochondrial membrane bending by I-II-III₂-IV₂ supercomplex

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SUPPLEMENTARY INFORMATION

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Supplementary Video 1. Coarse-grained molecular dynamics simulation of the *T. thermophila* supercomplex. First 800 ns of the MD simulation starting from an initially planar membrane reveals a deformation of the bilayer into a curved topology to accommodate the membrane protein complex.

Supplementary Video 2. Coarse-grained molecular dynamics simulation of pure lipid bilayer. Membrane shows fluctuations, however, a stable curved architecture (as in the case of supercomplex) is not observed.

Supplementary Video 3. Annular lipid shell of the *T. thermophila* supercomplex. Final frame of the coarse-grained MD-simulation with supercomplex and surrounding annular lipids shown, highlighting the curved shape of the lipid belt.

Source data for Extended Data Figure 2.

Supplementary Information Table 1. Data collection and model statistics

Data collection	CI	CII	CIII ₂	CIV ₂	CI-II-III ₂ -IV ₂
Microscope	Titan Krios				
Voltage (kV)	300				
Camera	K2 Summit				
Magnification	165,000				
Exposure (e-/Å²)	30.9				
Pixel size (Å)	0.83				
Defocus range (µm)	0.6-2.6				
Movies collected	26,063				
Frames / movie	20				
Data processing					
Initial particles	1,664,103				
Final particles	138,746				
Symmetry	C1	C1	C1	C1	C1
Map resolution (Å)	2.9	2.8	2.8	2.6	2.9
- FSC threshold	0.143	0.143	0.143	0.143	0.143
B-factor sharpening	46.8	49.1	52.2	45.4	49.9
EMDB ID	EMD-	EMD-	EMD-	EMD-	EMD-
Model refinement statistics					
CC (map/model)	0.82	0.82	0.81	0.84	0.79
Resolution (map/model)	2.99	3.3	3.18	2.89	3.36
- FSC threshold	0.5	0.5	0.5	0.5	0.5
Atoms (not H)	234277	41399	94264	436066	806006
Residues	13712	2409	5438	23792	45351
Lipids/CoQ	59/0	11/1	32/3	214/8	316/12
Model B-factor (Å²)					
- Protein	53.2	100.46	65.66	52.8	91.23
- Ligands	63.83	107.88	78.66	72.16	111.10
Rotamer outliers (%)	0.51%	0.62%	0.53%	1.18%	0.76
Ramachandran (%)					
- Outliers	0.01%	0.21%	0.00%	0.01%	0.02%
- Allowed	2.07%	3.28%	2.43%	1.75%	1.77
- Favoured	97.92%	96.51%	97.57%	98.25%	98.22
Clash score	4.12	3.91	5.68	4.22	4.63
MolProbity score	1.21	1.41	1.40	1.26	1.24
RMSD					
- Bonds (Å)	0.002	0.004	0.002	0.002	0.002
- Angles (°)	0.407	0.459	0.447	0.503	0.562
PDB ID					

Supplementary Information Table 2. List of proteins and comments

Subunit name	Gene name	UniProt ID	Encoded	Residues in total	Comments
Complex-I:					
ND1a	nad1_a	Q950Y3	mt	284	Corresponds to NuoH, Nqo8
ND1b	nad1_b	NP_149380	mt	59	Mt-ND1 C-terminal extension, separate gene
ND2a	ymf65	Q951A3	mt	360	Corresponds to NuoN, Nqo14
ND2b	nad2	Q951B2	mt	178	Mt-ND2 C-terminal extension, separate gene
Mt-ND3	nad3	Q950Z7	mt	121	Corresponds to NuoA, Nqo7
ND4	nad4	Q950X9	mt	505	Corresponds to NuoM, Nqo13
MT-ND4L	ymf58	Q950Z5	mt	116	Corresponds to NuoK, Nqo11
ND5a	nad5	Q950Z0	mt	750	Corresponds to NuoL, Nqo12
ND5b	ymf57	Q951C2	mt	100	Mt-ND5 C-terminal extension, separate gene
ND6	ymf62	Q950Y2	mt	255	Corresponds to NuoJ, Nqo10
NDUV1	TTHERM_00193910	Q23KE4	nuclear	474	Corresponds to NuoF, Nqo1
NDUV2	TTHERM_00335630	I7MEP0	nuclear	274	Corresponds to NuoE, Nqo2

NDUS1	TTHERM_00194 260	Q23KA9	nuclear	718	Corresponds to NuoG, Nqo3
NDUS2	nad7	Q951B1	mt	442	Corresponds to NuoD, Nqo4
NDUS3	nad9_2	Q950Z3	mt	198	Corresponds to NuoC, Nqo5
NDUS4	TTHERM_00526 930	I7MK61	nuclear	185	Corresponds to AQDQ subunit
NDUS5	TTHERM_00012 9647	W7X4R4	nuclear	94	
NDUS6	TTHERM_00497 570	I7MK02	nuclear	132	Zinc-finger protein, contains a Zn ²⁺ ion
NDUS7	nad10	Q951B4	mt	162	Corresponds to NuoB, Nqo6
NDUS8	TTHERM_00294 640	I7MDW5	nuclear	236	Corresponds to NuoI, Nqo9
NDUA1	TTHERM_00455 560	I7MI60	nuclear	94	
NDUA2	TTHERM_00659 070	I7MA77	nuclear	103	
NDUA3	TTHERM_00148 710	I7M9B3	nuclear	135	
NDUA5	TTHERM_01161 000	Q23ND5	nuclear	206	
NDUA6	TTHERM_00933 070	I7M2Y3	nuclear	172	
NDUA7	TTHERM_00399 360	I7MIJ7	nuclear	282	
NDUA8	TTHERM_00464 930	I7MMF4	nuclear	238	

NDUA9	TTHERM_00557 760	I7MLH2	nuclear	362	NAD-dependent epimerase/dehydratase family protein
NDUA11	TTHERM_00945 210	Q24F24	nuclear	213	
NDUA12	TTHERM_00194 499	A4VDQ6	nuclear	194	
NDUA13	TTHERM_00149 260	I7M2U4	nuclear	175	
NDUAB1 -α	TTHERM_01005 100	Q22XT6	nuclear	138	Different isoform to NDUAB1-β
NDUAB1 -β	TTHERM_00470 710	I7MD12	nuclear	133	Acyl carrier protein
NDUB3	TTHERM_00446 569	A4VD20	nuclear	83	
NDUB4	TTHERM_00310 880	I7MG29	nuclear	126	
NDUB6	TTHERM_00430 000	Q231G0	nuclear	129	
NDUB7	TTHERM_00402 070	I7MIM0	nuclear	120	
NDUB8	TTHERM_00481 330	I7M855	nuclear	207	
NDUB9	TTHERM_00985 010	Q233X7	nuclear	189	
NDUB10	TTHERM_00193 750	Q23KG0	nuclear	188	
NDUB11	TTHERM_00114 380	Q22Z32	nuclear	214	
NDUB15	TTHERM_00061 4679	-	nuclear	147	Wrong gene annotation – change translation ORF to 5'-3' in frame 3

NDUC2	TTHERM_00160 690	Q22W63	nuclear	102	
NDUCA1	TTHERM_00136 440	I7M8Q7	nuclear	346	carbonic anhydrase-like protein (isoform 1)
NDUCA2	TTHERM_01005 010	Q22XU5	nuclear	257	carbonic anhydrase-like protein (isoform 2)
NDUCA3	TTHERM_00541 460	I7M6S0	nuclear	233	carbonic anhydrase-like protein (isoform 3)
NDUX1	TTHERM_00715 850	I7LT42	nuclear	150	
NDUFX	TTHERM_00161 210	Q22W11	nuclear	172	2Fe2S cluster-binding protein
NDUTX	TTHERM_01205 250	Q22AI5	nuclear	166	Thioredoxin, TRX family
NDUJ1	TTHERM_00938 750	Q22DR7	nuclear	317	DnaJ domain protein
NDUPH1	TTHERM_00697 370	Q24C39	nuclear	251	
NDUPH2	TTHERM_00193 950	Q23KE0	nuclear	189	
NDUTT1	TTHERM_00925 340	Q22E24	nuclear	516	lipid A-disaccharide synthase
NDUTT2	TTHERM_01000 190	Q24HK5	nuclear	333	Acyl-CoA synthetase (AMP-forming)/AMP-acid ligase II
NDUTT3	TTHERM_00268 000	I7LUQ4	nuclear	311	
NDUTT4	TTHERM_00295 430	I7MIE0	nuclear	212	
NDUTT5	TTHERM_00649 080	I7LT77	nuclear	205	

NDUTT6	TTHERM_00334 340	I7M1N8	nuclear	144	
NDUTT7	TTHERM_00637 590	Q22HE4	nuclear	143	
NDUTT8	TTHERM_00006 120	Q22SC4	nuclear	135	
NDUTT9	TTHERM_00653 670	Q23B10	nuclear	136	
NDUTT1 0	TTHERM_00616 320	I7MAF0	nuclear	127	
NDUTT1 1	TTHERM_00185 570	Q22T55	nuclear	113	
NDUTT1 2	TTHERM_00835 330	Q22E95	nuclear	93	
NDUTT1 3	TTHERM_00992 800	Q22DC2	nuclear	73	
NDUTT1 4	TTHERM_00399 460	I7MIK1	nuclear	71	
NDUTT1 5	TTHERM_00063 7389	-	nuclear	237	Wrong gene annotation – change translation ORF to 3'-5' in frame 1 Binds ADP-Mg ²⁺
NDUTT1 6	TTHERM_00124 3407	W7XFJ5	nuclear	119	Is required for I-IV ₂ interface stability. Stabilises ND5a Nt extension. Wrong gene annotation – change translation ORF to 3'-5' in frame 3
NDUTT1 7	TTHERM_00053 2499	-	nuclear	125	Wrong gene annotation – change translation ORF to 3'-5' in frame 2

Complex-II:					
SDHA	TTHERM_00047080	Q23DI3	nuclear	636	
SDHB	TTHERM_00241700	I7M403	nuclear	312	
SDHC	TTHERM_00387120	Q23RH8	nuclear	60	
SDHD	AF396436.1 (mt-genome sequence)	-	mt	43	Gene lies between ymf66 and ymf76 on mtDNA. Translation 3'-5' in frame 1
SDHTT1	TTHERM_00571650	Q24I09	nuclear	322	contains heme c
SDHTT2	TTHERM_00532090	Q248F8	nuclear	296	
SDHTT3	TTHERM_00283850	I7MEX7	nuclear	198	
SDHTT4	TTHERM_00658950	I7LX66	nuclear	195	Diphthamide synthesis protein
SDHTT5	TTHERM_00601860	Q22YL0	nuclear	114	
SDHTT6	TTHERM_00626980	Q23S01	nuclear	103	
SDHTT7	TTHERM_00713350	Q24CW6	nuclear	93	
SDHTT8	TTHERM_000287919	W7XBF5	nuclear	89	
SDHTT9	TTHERM_00637670	Q22HD6	nuclear	76	
SDHTT10	TTHERM_001034353	W7XF00	nuclear	62	

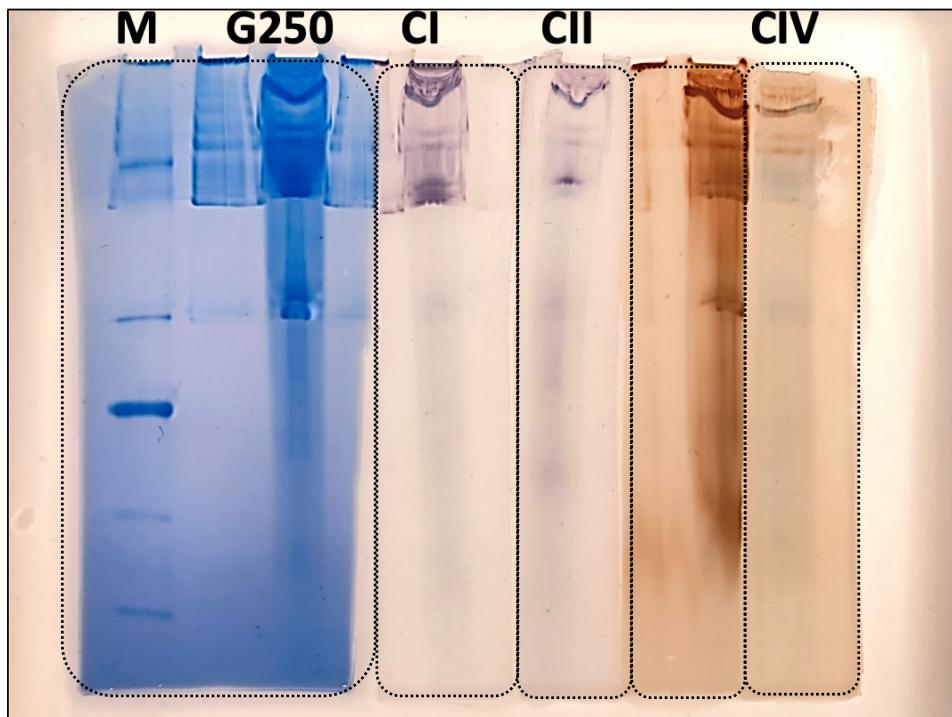
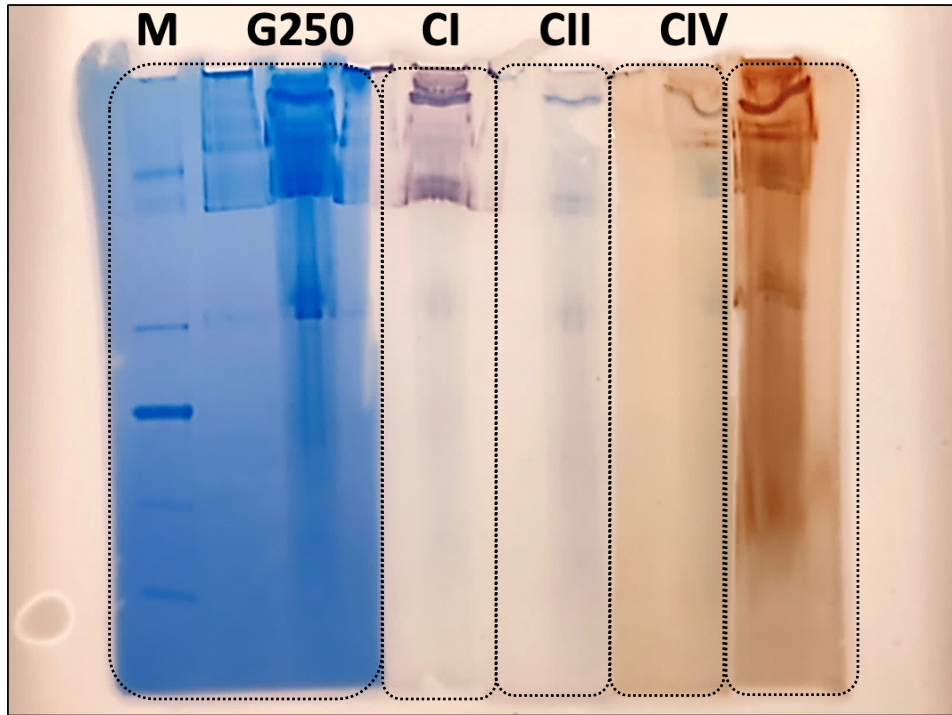
SDHTT11	DY684362.1 (EST, mRNA sequence)	-	nuclear	46	Gene translation should be 3'-5' in frame 1
Complex- III:					
MPP- β	TTHERM_00502 380	I7MGU2	nuclear	513	
MPP- α	TTHERM_00836 690	I7MJ25	nuclear	482	
COB	cob	Q950Z1	mt	426	contains heme bL and bH
CYC1	TTHERM_00918 500	Q24IM5	nuclear	319	Contains heme c
UQCRFS 1	TTHERM_00295 080	I7MIC7	nuclear	269	Contains 2Fe2S cluster
UQCRH	TTHERM_00194 690	Q23K66	nuclear	86	cytochrome bc1 hinge proteins
UQCRQ	TTHERM_00765 330	I7M484	nuclear	130	
UQCR9	TTHERM_00456 790	I7MM45	nuclear	119	
UQCR10	TTHERM_00218 930	I7MFL6	nuclear	62	
UQCRTT 1	TTHERM_00382 330	Q23F81	nuclear	328	
UQCRTT 2	EV837551.1 (EST, mRNA sequence)	-	nuclear	41	Gene translation should be 5'-3' in frame 3
UQCRTT 3	-	-	-	66	Unidentified subunit

Complex-IV:					
COX1	cox1	Q950Y4	mt	688	
COX2	cox2	Q950Y9	mt	604	
COX3a	yfm67	Q950Y7	mt	453	structurally replaces H1 of COX3
COX3b	yfm68	Q950Y6	mt	594	
COX5B	THERM_00378620	Q23FF5	nuclear	637	
COX6A	THERM_000723218	W7XCY5	nuclear	130	
COX6B	THERM_00568030	Q24I72	nuclear	230	
COX6C	THERM_00046170	Q23DS4	nuclear	103	
COX7A	THERM_00151250	I7MGF9	nuclear	133	
COX7C	THERM_000470561	W7X287	nuclear	236	
COX6BL	THERM_00218340	I7LVX0	nuclear	88	
COX17L	THERM_01043280	Q22CI1	nuclear	990	CTF/NF-I domain-containing protein
NDUA4	THERM_001001528	W7WZP1	nuclear	220	(Named COXTT13 in Zhou et al.)
COXMC1	THERM_00127269	A4VDV3	nuclear	346	Oxoglutarate/malate translocator protein

COXMC2	TTHERM_00621 600	Q23M99	nuclear	318	2-oxoglutarate/malate carrier protein
COXMC3	TTHERM_00112 650	Q22ZA6	nuclear	330	
COXBP	TTHERM_00016 360	Q22RF2	nuclear	685	BBC53 chromosome condensation regulator RCC1
COXTIM 1	TTHERM_00052 8460	W7X3D6	nuclear	72	Tim10/DDP family zinc finger proteins
COXTIM 2	TTHERM_00070 3379	W7XDM 6	nuclear	72	part of TIM9.10 hexamer
COXTIM 3	TTHERM_00433 490	Q231A8	nuclear	93	part of TIM9.10 hexamer
COXTIM 4	TTHERM_01289 060	Q22A35	nuclear	68	part of TIM9.10 hexamer
COXTIM 5	TTHERM_00028 510	Q22N23	nuclear	81	part of TIM9.10 hexamer
COXTIM 6	TTHERM_00805 850	Q233U0	nuclear	72	part of TIM9.10 hexamer
COXFS	TTHERM_00133 510	I7M8P0	nuclear	188	Fe-binding zinc finger CDGSH type protein
COXAC	TTHERM_00695 750	Q24C97	nuclear	127	
Ymf70	ymf70	Q950Y0	mt	89	
Ymf75	ymf75	Q951A7	mt	190	
COXTT1	TTHERM_00361 490	Q22PJ5	nuclear	490	
COXTT2	TTHERM_00721 790	Q22FX8	nuclear	473	Protein phosphatase 2C

COXTT4	TTHERM_00338 280	I7M1Q4	nuclear	402	SURF1 family protein
COXTT5	TTHERM_00046 440	Q23DP7	nuclear	385	TraB family protein
COXTT6	TTHERM_00047 230	Q23DG8	nuclear	348	
COXTT7	TTHERM_00675 650	Q23DZ5	nuclear	318	
COXTT8	TTHERM_00070 850	I7LTZ4	nuclear	318	SURF1 family protein
COXTT9	TTHERM_00525 160	I7LY65	nuclear	252	
COXTT10	TTHERM_00420 130	I7MD70	nuclear	234	
COXTT11	TTHERM_00093 9159	W7X4J9	nuclear	231	
COXTT12	TTHERM_00455 090	I7M3P9	nuclear	215	
COXTT14	TTHERM_00530 650	I7LZX8	nuclear	210	
COXTT15	TTHERM_00641 250	Q23F08	nuclear	193	
COXTT16	TTHERM_00218 840	I7M8Y9	nuclear	175	AAA protein fold
COXTT17	TTHERM_00049 040	Q23D87	nuclear	173	
COXTT18	TTHERM_00218 570	I7MKT6	nuclear	173	
COXTT19	TTHERM_00433 830	Q230X6	nuclear	170	
COXTT20	TTHERM_00794 470	Q23VY4	nuclear	158	

COXTT21	TTHERM_00938 940	Q22DP8	nuclear	154	
COXTT22	TTHERM_00691 100	I7MFV5	nuclear	149	
COXTT23	TTHERM_00666 370	Q23TE5	nuclear	124	
COXTT24	TTHERM_00161 000	Q22W32	nuclear	122	
COXTT25	TTHERM_00151 580	I7M9E7	nuclear	105	
COXTT26	TTHERM_00485 790	I7LTF1	nuclear	90	
COXTT27	TTHERM_00030 2101	W7XDH 2	nuclear	212	(Including subunit COXTT3 in Zhou et al.)
COXTT28	TTHERM_00093 3397	W7X912	nuclear	171	



Source data for Extended Data Figure 2. CN-PAGE was performed to separate protein assemblies within the final sucrose cushion sample. After electrophoresis, the gel lanes were cut and incubated with different reagents to reveal the presence of proteins in the marker (M) and sample (G250) lanes, active complex I (CI), complex II (CII) or complex IV (CIV) within remaining sample lanes. The lanes were then juxtaposed to reconstruct the full gel. The two CN-PAGE gels were obtained from two separate supercomplex preparations.