SUPPLEMENTARY INFORMATION

Table S1. Primers used for introduction of 6-Histidine affinity purification tag at the 3'-end of *petA* in *F. diplosiphon* SF33 (restriction sites are underlined, 6-Histidine tag is marked in bold, italicized letters).

Primer	Sequence (5' to 3')
petA-F-Ncol	GCT <u>CCATGG</u> GCTTGCTCAACATACTTCCACATG
petA-His-R3	GTGATGGTGATGGTGATGGAAATTCATTTCTGCGGCTTGGACTTTCTC
	AACCTGCTTCTT
petA-R-EcoRI	GCG <u>GAATTC</u> CCTACAAGGAAGAGACAAGCGTAT
petA-His-F3	CATCACCATCACCATCACTAAATTCTTTGCTAACTTACTT
	AGGCAATTTG
petAseq2	ACTTCCACTTCTGTCGGCTTTGCA
pJCF276seq2Ncol	ACAGACGGCATGATGAACCTGAAT
pJCF276seqEcoRI	TGTACCTATAACCAGACCGTTCAGC

Table S2. Inter-monomer Interactions in the Cyt $b_6 f$ Complex (PDB ID 4OGQ). Abbreviations, res1, res2, residues 1 and 2; Ch.1, Ch.2, Chains 1 and 2; aaProperty, amino acid property (phil, hydrophilic; phob, hydrophobic); #, amino acid number.

Mono	mer1			Mono	mer2				aaPro	perty
Res1	#	Atom	1 Ch.1	Res2	#	Atom	2 Ch.2	Dist(A	Å)Prop1	Prop2
TRP	7	CZ3	А	LEU	116	CG	А	3.86	phil	phob
TRP	7	CZ2	А	GLU	115	CD	А	3.92	phil	phil
PHE	8	CE1	А	LEU	116	CD1	А	3.96	phob	phob
GLU	10	OE2	А	LYS	112	NZ	А	3.58	phil	phil
ARG	11	NE	А	GLU	115	OE2	А	2.72	phil	phil
ARG	11	CG	А	PRO	113	0	А	3.70	phil	phob
ARG	11	NH2	А	LYS	112	CG	А	3.56	phil	phil
ARG	11	0	А	LYS	208	CE	А	3.54	phil	phil
LEU	12	CD2	А	MET	205	CE	А	3.74	phob	phob
LEU	12	CD2	А	LEU	116	CD1	А	3.72	phob	phob
LEU	12	CD2	А	PHE	201	CZ	А	3.99	phob	phob
LEU	12	0	А	LYS	208	CE	А	3.82	phob	phil
GLU	13	OE2	А	LYS	208	NZ	А	2.95	phil	phil
PHE	48	CZ	А	PHE	189	CZ	А	3.66	phob	phob
PHE	48	CE2	А	TRP	193	CE2	А	3.86	phob	phil
PHE	52	CB	А	PHE	189	CE2	А	3.58	phob	phob
PHE	52	CE1	А	VAL	190	CG2	А	3.36	phob	phob
THR	55	OG1	А	SER	185	OG	А	2.61	phil	phil
THR	55	0	А	THR	181	CG2	А	3.42	phil	phil
PHE	56	0	А	THR	181	CG2	А	3.41	phob	phil
PHE	56	CE1	A	SER	185	OG	A	3.34	phob	phil
PHE	56	CD2	A	ARG	182	NE	A	3.55	phob	phil
TYR	57	N	A	THR	181	CG2	A	3.88	phil	phil
TYR	57	OH	A	ARG	182	NH2	A	2.93	phil	phil
LYS	59	CE	A	GLN	1//	CG	A	3.50	phil	phil
LYS	59	CE	A	IHR	181	OG1	A	3.92	phil	phil
PRO	60	CG	A		60	CG	A	3.77	pnop	pnop
IHR	61	OGI	A	IHR	61	OGI	A	3.16	pnii	pnii
LYS	112	CG	A	ARG	11	NH2	A	3.50	pnii	pnii
	112		A	GLU	10	OE2	A	3.58	phil	phil
	115		A		11		A	3.70	priod	phil
	115		A		7		A	2.12	phil	prili phil
	115		A A		12		A A	3.92	phili phob	phili phob
	116		Δ		7	C73	Δ	3.86	phob	phil
	116		Δ	DHE	7 8		Δ	3.00	phob	nhoh
	177		Δ		50		Δ	3.50	phil	nhil
	181	061	Δ		59		Δ	3 01	phil	nhil
THR	181	CG2	Δ	THR	55	0	Δ	3.42	nhil	nhil
THR	181	CG2	Δ	PHF	56	õ	Δ	3.41	nhil	nhoh
THR	181	CG2	A	TYR	57	Ň	A	3.88	phil	phil
ARG	182	NH2	A	TYR	57	ОН	A	2.93	phil	phil
ARG	182	NE	A	PHE	56	CD2	A	3.55	phil	phob
		-							F	1

SER	185	OG	А	PHE	56	CE1	А	3.34	phil	phob
SER	185	OG	Α	THR	55	OG1	А	2.61	phil	phil
THR	188	0	Α	PHE	189	CD1	А	3.36	phil	phob
PHE	189	CE2	А	PHE	52	CB	А	3.58	phob	phob
PHE	189	CZ	Α	PHE	48	CZ	А	3.66	phob	phob
PHE	189	CD1	Α	THR	188	0	А	3.36	phob	phil
VAL	190	CG2	А	PHE	52	CE1	А	3.36	phob	phob
PRO	192	0	А	TRP	193	CH2	А	3.91	phob	phil
TRP	193	CZ3	А	TRP	193	CZ3	А	3.48	phil	phil
TRP	193	CH2	А	PRO	192	0	А	3.91	phil	phob
TRP	193	CE2	А	PHE	48	CE2	А	3.86	phil	phob
TRP	193	CH2	А	ALA	196	CB	А	3.62	phil	phob
ALA	196	CB	А	TRP	193	CH2	А	3.62	phob	phil
PHE	201	CZ	А	LEU	12	CD2	А	3.99	phob	phob
MET	205	CE	А	LEU	12	CD2	А	3.74	phob	phob
LYS	208	NZ	А	GLU	13	OE2	А	2.95	phil	phil
LYS	208	CE	А	LEU	12	0	А	3.82	phil	phob
LYS	208	CE	А	ARG	11	0	А	3.54	phil	phil
TRP	7	CZ2	А	PHE	113	CE1	В	3.52	phil	phob

Table S3. Inter-monomer Interactions in the Cyt bc_1 Complex (PDB ID 3CX5). Abbreviations, res1, res2, residues 1 and 2; Ch.1, Ch.2, Chains 1 and 2; aaProperty, amino acid property (phil, hydrophilic; phob, hydrophobic); #, amino acid number.

Mono	mer1			Mono	mer2				aaPro	perty
Res1	#	Atom	1 Ch.1	Res2	#	Atom	2 Ch.2	Dist(Å)Prop1	Prop2
VAL	8	0	С	MET	199	CE	Ν	3.80	phob	phob
VAL	8	CB	С	ILE	203	CD1	Ν	3.99	phob	phob
TYR	9	ОН	С	ALA	200	CB	Ν	3.47	phil	phob
TYR	9	ОН	С	MET	196	0	Ν	2.62	phil	phob
TYR	9	CD2	С	MET	199	CE	Ν	3.91	phil	phob
TYR	9	ОН	С	VAL	116	CG2	Ν	3.50	phil	phob
TYR	9	CE1	С	THR	112	CG2	Ν	3.77	phil	phil
LEU	12	CD2	С	MET	199	CE	Ν	3.70	phob	phob
ILE	48	CD1	С	LEU	185	CG	Ν	3.67	phob	phob
MET	52	0	С	GLN	177	CB	Ν	3.36	phob	phil
MET	52	CE	С	ARG	178	CB	Ν	3.40	phob	phil
TYR	54	0	С	GLN	177	NE2	Ν	3.27	phil	phil
SER	55	OG	С	ASN	57	ND2	Ν	2.83	phil	phil
SER	55	OG	С	GLN	177	NE2	N	3.04	phil	phil
ASN	57	ND2	С	LEU	60	CD1	N	3.88	phil	phob
ASN	57	ND2	С	SER	55	OG	N	2.82	phil	phil
ASN	57	OD1	С	ASN	57	OD1	N	3.70	phil	phil
LEU	60	CD2	С	ASN	57	OD1	N	3.88	phob	phil
LEU	60	CD2	С	LEU	60	CD1	N	3.69	phob	phob
THR	112	CG2	C	TYR	9	CE1	N	3.68	phil	phil
VAL	116	CG2	C	IYR	9	OH	N	3.61	phob	phil
GLN	1//	NE2	C	IYR	54	0	N	3.33	phil	phil
GLN	1//	CB	C	MEI	52	0	N	3.26	phil	phob
GLN	1//	NE2	C	SER	55	OG	N	2.91	pnii	pnii
ARG	1/8	N	C		52	0	N	3.70	pnii	phob
	181		C		184	OH	N	2.76	pnop	phii
	182	CD1	C		52	CE	N	3.91	pnop	pnob
	184				181			2.70	pnii	pnop
	104				104		IN NI	3.70	phil	pnii phab
	104							3.30	phili nhah	phop
	100		C		104		IN NI	3.34 2.00	phob	phili
	100		C		100			3.09 2.02	phob	phob
	100	02	C		0		IN NI	3.03 2.67	phob	phob
	190		C		9			2.07	phob	phili
	199		C		0 12		IN NI	3.01 3.50	phob	phob
	100	CE	Ċ		۱ <u>۲</u>	CD2	N	3 00	phob	philop
	200		Ċ		9		N	3.33	nhoh	phil
	200		Ċ		8	CP	N	3.40 3.00	phob	phill
	203		U	VAL	0		IN	5.90	prion	hinn

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Bent trans-membrane helix of the ISP subunit (pink) in the cyt $b_6 f$ complex (PDB ID 40GQ). The PetL subunit (light brown) interacts with the ISP TMH on the n-side, through an ordered UDM detergent molecule (black/red sticks).

Figure S2. Low resolution mass spectroscopy (ESI-MS) analysis of the truncated ISP subunit from *F. diplosiphon*.

Figure S3. High resolution mass spectroscopy (ESI-MS) analysis of the truncated ISP subunit from *F. diplosiphon.* Ion-isolation of 14-charge precursor (inset) and electron-transfer dissociation tandem mass spectrum are shown.

Figure S4. Low resolution mass spectroscopy (ESI-MS) analysis of the full-length ISP subunit from *F. diplosiphon*.

Figure S5. High resolution mass spectroscopy (ESI-MS) analysis of the PetG subunit from *F. diplosiphon.* Ion-isolation of 3-charge precursor ion (inset) and collisionally activated dissociation tandem mass spectrum of the ion are shown.

Figure S6. High resolution mass spectroscopy (ESI-MS) analysis of the PetM subunit from *F. diplosiphon.* Ion-isolation of 3-charge precursor ion (inset) and collisionally activated dissociation tandem mass spectrum of the ion are shown.

Figure S7. High resolution mass spectroscopy (ESI-MS) analysis of the PetL subunit from *F. diplosiphon.* Ion-isolation of 3-charge precursor ion (inset) and collisionally activated dissociation tandem mass spectrum of the ion are shown.

Figure S8. High resolution mass spectroscopy (ESI-MS) analysis of the PetN subunit from *F. diplosiphon.* Ion-isolation of 3-charge precursor ion (inset) and collisionally activated dissociation tandem mass spectrum of the ion are shown.

Figure S9. High-resolution top-down mass spectrometry of small sub-units of *F. diplosiphon* SF33 b_6f complex. Selected fractions from LC-MS+ analysis were analyzed by static nanospray according to *Materials and Methods*. Product ion assignments for known proteins were made using ProSightPC operated in single protein mode with a 15-ppm mass accuracy threshold and with the delta mass feature deactivated. Product ion assignments from collisional activated dissociation (CAD) experiments are labeled with 135° slashes to the top left (*b*-ions) or

bottom right (v-ions) and from electron transfer dissociation (ETD) experiments with 90° slashes to the top left (*c*-ions) or bottom right (*z*-ions), as described in the main text 64 . (A) The 14579 Da component (Table 1) matched to 46 product-ions of electron transfer dissociation of a precursor-ion of monoisotopic mass 14567.0896 Da for a P-Score of 2.2x10⁻⁴³ identifying this species as an N-terminal truncation product of the Rieske subunit. (B) Cleavage site of ISP subunit in cyt $b_6 f$ complex (PDB ID 2E74). Tyr41 and Phe42 are located proximal to the membrane-water interface. (C) The 4026 Da component matched to 41 product-ions of collisionally activated dissociation (CAD) of a precursor-ion of monoisotopic mass of 4023.1578 Da for a P-Score of 3.32 x 10⁻⁶⁴ identifying this species as *F. diplosiphon* SF33 PetG. Shading of the initiating Met residue indicates formylation. (D) The 3578 Da component matched to 16 product-ions of collisionally activated dissociation (CAD) of a precursor-ion of monoisotopic mass of 3575.8351 Da for a P-Score of 7.8 x 10⁻²³ when matched to the Nostoc azollae PetM sequence, identifying this species as F. diplosiphon SF33 PetM. (E) The 3255 Da component matched to 27 product-ions of collisionally activated dissociation (CAD) of a precursor-ion of monoisotopic mass of 3256.0387 Da for a P-Score of 2.6 x 10⁻⁵³ when matched to a custom PetL sequence, identifying this species as F. diplosiphon SF33 PetL. (F) The 3261 Da component matched to 33 product-ions of collisionally activated dissociation (CAD) of a precursor-ion of monoisotopic mass of 3259.7588 Da for a P-Score of 5.6 x 10⁻⁴⁶ identifying this species as PetN of F. diplosiphon SF33.

Figure S10. Alignment of cyt b_6 polypeptide sequences from unicellular prokaryotes (*Synechocystis* PCC 6803, and *Synechococcus* PCC 7002), filamentous prokaryotes (*Nostoc* PCC 7120, *Fremyella diplosiphon* SF33, and *Mastigocladus laminosus*), a unicellular eukaryotic alga (*Chlamydomonas reinhardtii*), and higher eukaryotes (*Arabidopsis thaliana*, and *Spinacia oleracea*) sources. Secondary structure assignment and residue numbering are based on the cyt $b_6 f$ complex crystal structure from *Nostoc* PCC 7120 (PDB ID 4OGQ, chain A). Residues involved in inter-monomer contacts are highlighted with arrows. Color code: red- complete conservation, yellow- partial conservation, and white- no conservation.

Figure S11. Alignment of subIV polypeptide sequences from unicellular prokaryotes (*Synechocystis* PCC 6803, and *Synechococcus* PCC 7002), filamentous prokaryotes (*Nostoc* PCC 7120, *Fremyella diplosiphon* SF33, and *Mastigocladus laminosus*), a unicellular eukaryotic alga (*Chlamydomonas reinhardtii*), and higher eukaryotes (*Arabidopsis thaliana*, and *Spinacia oleracea*) sources. Secondary structure assignment and residue numbering are based on the cyt b_6f complex crystal structure from *Nostoc* PCC 7120 (PDB ID 40GQ, chain B). Residues

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involved in inter-monomer contacts are highlighted with arrows. Color code: red- complete conservation, yellow- partial conservation, and white- no conservation.

Figure S12. Alignment of cyt b_6 polypeptide sequence (from the cyt b_6f complex) with the homologous N-terminal domain of the cyt *b* subunit (of the cyt bc_1 complex). Sequences of the cyt *b* (bc_1) polypeptide represent prokaryotic anoxygenic photosynthetic bacteria (*Rhodobacter capsulatus*, and *Rhodobacter sphaeroides*), a unicellular eukaryote (*Saccharomyces cerevisiae*), and higher eukaryotes (*Gallus gallus*, and *Bos taurus*). Residue numbering is follows the sequence of the cyt b_6 polypeptide from *Nostoc* PCC 7120. Residues involved in inter-monomer interactions in the cyt b_6f complex of *Nostoc* PCC 7120 (PDB ID 4OGQ) are highlighted with arrows. Color code: red- complete conservation, yellow- partial conservation, and white- no conservation. For clarity, partial sequences of the cyt *b* polypeptide, which shares homology with the cyt b_6 subunit, are shown in the figure.

Figure S13. Alignment of subIV polypeptide sequence (from the cyt b_6f complex) with the homologous C-terminal domain of the cyt *b* subunit (of the cyt bc_1 complex). Sequences of the cyt *b* (*bc*₁) polypeptide represent prokaryotic anoxygenic photosynthetic bacteria (*Rhodobacter capsulatus*, and *Rhodobacter sphaeroides*), a unicellular eukaryote (*Saccharomyces cerevisiae*), and higher eukaryotes (*Gallus gallus*, and *Bos taurus*). Residue numbering is follows the sequence of the subIV polypeptide from *Nostoc* PCC 7120. The residue involved in inter-monomer interactions in the cyt b_6f complex of *Nostoc* PCC 7120 (PDB ID 4OGQ) is highlighted with an arrow. Color code: red- complete conservation, yellow- partial conservation, and white- no conservation. For clarity, partial sequences of the cyt *b* polypeptide, which shares homology with the subIV polypeptide, are shown in the figure.















m/z



m/z

A c1 - F t I - P - P - A - A - G - G - A - G - G - A - T + A - K + D + E - L - G t N - D - V - S - V - z114c26 - S - K - F + L + A - S - H - N - V - G - D + R + S - L - V + Q + G + L - K - G + D - P - T + Y + L - z89c51 - V + V - E - S - K - E - A - I - G + D - Y - G - I - N - A - I - I - T - H - L - G - C - V - V - P - z64c76 - W - N - V - A - E - N - K - F - K - C - P - I - H - G - S - Q - Y + D + A - T + G + K - V - V - R + z39c101 - G - P + A - P - L - S - L + A + L - A - H - T - K - V - E - D + D - K - V - V - V - T - P - W - T - z14c126 - E - T - D - F - R - T - G - E - E - P - W - A - Z 1

C b1 - MtvtEtPtLttstGtItvtLtGtL-ItvtvtTtLtstG-LtFtYtA-A-y13 b26 - Y-KtQtY-KtR-P-T-E-L-G-G- y1

D b1 - M-G-S-E-I-L-N-A-A-M L S-F t G L L I F V t G t W-G-I-G-A-L-y10 b26 - L-L-K-I-Q-G-A-E F E- y1

F b1 - M-A-I L L T L L G W V V S L L L V V F T W - S - I - A M - V V W G - y5 b26 - R N - G - L - y1









	120	130	140	150
Nostoc_subIV	e i e n <mark>v</mark> n k <mark>f o</mark> n	P FR RPVATT <mark>V</mark>	/FLF. GTLVT	L <mark>WLG</mark> IGAA.L <mark>PI</mark> DKSL
Rhodobacter_sph	WLDTSPVRSG	RYRPMFKIY	TULLADEVIL	T <mark>WVG</mark> AQQTTF <mark>PY</mark> D
Rhodobacter_cap	WLDTSKVRSG	AYRPKFRMWE	TWFLVLDFVVI	TWVGAMPTEY <mark>PY</mark> D
Saccharomyces	FTDRSVVRGN	TEKVLSKFF	FEIFVENFVLL	G <mark>QIG</mark> ACHVEV <mark>PY</mark> V
Gallus	FLHKSKQRTM	TERPLSQTLE	FWLLWANLLII	T WIG SQPVEH <mark>PF</mark> I
Bos_taurus	LLHTSKQRSM	MERPLSQCLE	TU ADL <mark>LTL</mark>	T WIG GQPVEH <mark>PY</mark> I
	*			