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526 527	Supplemental Figure titles and legends
528	Supplementary Table 1. Cryo-EM and Refinement Statistics of P. calidifontis and A. veneficus ECNs, related to
529	Figure 1 and Figure 2.
530	
531	Supplementary Data 1. The tree analysis of AvECN homologs, related to Figure 5.
532	
533	Supplementary Figure 1. Fourier Shell Correlation (FSC) calculations and average power spectrum, related to
534	Figure 1 and Figure 2.
535	(A) The map:map FSC calculation of <i>P. calidifontis</i> ECN (0.143 cutoff)
536	(B) The average power spectrum from PcECN raw particles aligned to the same axis.
537	(C) The map:map FSC calculation of A. veneficus ECN (0.143 cutoff)
538	(D) The average power spectrum from AvECN raw particles aligned to the same axis.
539	
540	Supplementary Figure 2. ECN protein identification from cryo-EM maps, related to Figure 1 and Figure 2.
541	(A) AlphaFold2 predictions of multi-heme cytochromes containing three or more CxxCH motifs in P. calidifontis
542	were docked into the cryo-EM map corresponding to a single protein subunit. The color codes: α -helices are
543	yellow; β-sheets are blue; loops are green.
544	(B) AlphaFold2 predictions of multi-heme cytochromes containing three or more CxxCH motifs in A. veneficus
545	were docked into the cryo-EM map corresponding to a single protein subunit. The color code is the same as in
546	(A). The area of the AlphaFold2 predictions that cannot be possibly adjusted and fit into the map are highlighted
547	by red arrows.
548	
549	
550	Supplementary Figure 3. Protein sequences and cryo-EM densities of the heme c area in PcECN and AcECN,
551	related to Figure 3.
552	(A) Protein sequence of PcECN. CxxCH or CxCH motifs are highlighted in the cyan shade. The other histidines
553	outside those motifs coordinating heme c are highlighted in green. The predicted signal peptide is in the black
554	dashed line box. The predicted transmembrane region, not seen in the cryo-EM map, is highlighted in red

555 shade.

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(B) Zoomed-in views of the heme c area in PcECN. From left to right, heme 1 to heme 4. The protein-heme
color scheme remains the same compared to Fig. 1. Two cysteines and histidines bound to heme c are also
labeled.

(C) Protein sequence of AvECN. CxxCH motifs are highlighted in the cyan shade. The other histidines outside
 CxxCH motifs coordinating heme c are highlighted in green. The predicted signal peptide is in the black dashed
 line box.

(D) Zoomed-in views of the heme c area in AvECN. From left to right, heme 1 to heme 4. The protein-heme
color scheme remains the same compared to Fig. 2. Two cysteines and histidines bound to heme c are also
labeled.

565

Supplementary Figure 4. Potential factors contributing to the thermal stability of ECNs, related to Figure 4.
(A) Disulfide bonds in *P. calidifontis* ECN. The full atomic model of one *P. calidifontis* ECN subunit is shown on
the left with the cryo-EM density corresponding to a single cytochrome subunit. Two disulfide bonds were
observed and highlighted in yellow circles. Those two sites are magnified on the right.

(B-C) Surface modifications of PcECN (B) and AvECN (C) filaments. The density accounted for by atomic models
is colored in gray, and the extra density is colored in magenta. The protein backbones of ECN filaments are
shown in ribbons with protein cryo-EM densities.

573

Supplementary Figure 5. *A. veneficus* ECN may have evolutionary link to cytochrome c552, related to Figure 5. (A-B) Results of the HHsearch analysis queried with the sequences of AvECN (A) and AvECN-like protein from a Planctomycetota bacterium (B). The GenBank accession number of (B) is MCH2104266. H(h), α -helix; E(e), β strand; C(c), coil.

(C) Heme-based sequence alignment of *A. veneficus* ECN and *T. tepidum* cytochrome c552. Secondary structures
 are labeled: α-helices in rounded rectangles, β-sheets in arrows, and loops in dashed lines.

(D) The individual structures of *A. veneficus* ECN, *T. tepidum* cytochrome c552, and their alignment. Four hemes
 used for the alignments in both structures are highlighted in purple. The unused heme molecular in *T. tepidum* cytochrome c552 is colored orange. Proteins are colored grey.

(E) The individual structures of *A. veneficus* ECN, *T. tepidum* cytochrome c552, and their alignment. Hemes are
 hidden in this panel. The labeled secondary structures in (C) are colored accordingly. Other unaligned secondary
 structures are kept grey. A magnified view of the aligned helices is shown on the right

586











AcECN 241 NTTGNGSVNDASNWPGWYPYSW









262





В

С

P. calidifontis ECN



A. veneficus ECN

Disulfide bond 2

52E8_A cytochrome c552; cytochrome c, multiheme, purple sulfur bacteria, PHOTOSYNTHESIS; HET: HEC; 1.6A {Thermochromatium tepidum} Probability: 99.07%, E-value: 2.6e-10, Score: 104.42, Aligned cols: 73, Identities: 18%, Similarity: 0.388, Template Neff: 10.2

Α

В

Q s s sp pee d	COCCARHHEINERRINHHHHHURGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	
QQ F EXMM8<u>8</u>ABA AG16	61 24 24 INDIREBEEKEBAILAHRADIKAABET IGFHEINDTVEHHRABBERAARENOHRDHABBIGESSEDGISAHRAGEIAKAAAHLAGMACHEFG(262) 22 (262))
Q Coorservas	4242~~~C#C#CMCMMMMMMMMMMMMMMMMMMMMMMMMMMMMM)
	·····]:+[[++++++++++++++++++++++++++++++)
I _T Correries	41 ₄ 1~2262cC&cHcH222Was&azsH22H22H222222222222222222222222222222)
TT 55288AA	41 ₄ fs eleddaheredd ykkwrgsmakrakereledefiggevoortaekwrkregkffelddychafrodtardkerakterpryfelenkvach44.k ⁽⁴³⁵ /34 (435))
T ss dssp T ss dssp	^{CH} UHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH	
T_ss_pred T_ss_pred	^{СнинининининосhhuhhhcccChhнининининосссссссссссссссссссссссссссс}	

5ZE8_A cytochrome c552; cytochrome c,	multiheme, purple sulfur bacteria,	PHOTOSYNTHESIS; HET: HEC; 1.6A	{Thermochromatium tepidum
Probability: 100%, E-value: 2.1e-32,	Score: 260.17, Aligned cols: 250,	Identities: 22%, Similarity: 0	.343, Template Neff: 10.

riobability. 100%	, i varue.	Zife 52, Scole, 200.17, Alighed Cols, 250, Identifies, 220, Similarity, 0.545,	тешрт	acei
Q ss pred		ccCCChhhHHHcCHHHHHHcCHhHHHhcCHHHHHHHhhhhcccccCCCChHHccCCCCCC		
O MCH2104266.1	45	LEGWEOHECAACHREVTREWASSLHALAWVDEHYOEDMKKVRRKKSCYGCHIPEPLHGFAGFIP	108	(341
0 Consensus	45		108	(341
-		+ .+++.+ . +.+++.++		
T Consensus	37	~~~~~C~~CH~~~~~~~~~~~~~~~~~~~~~~~~~~~~	113	(435
T 5ZE8 A	37	IHRVSSELCANCHEDIYROWKGSMHAKSTALSDFIHGLFYOOEVGDPTAEGMVHKKSGKFPLCLYCHAPNAARDOTT	113	(435
T ss dssp		GGGCCHHHHHTTCHHHHHHTSTTTTCSTTTCHHHHHHHHHH		
T ss pred		CCCCCChHHHHHhchHHHHhcChhHhhhcccChhHHHHHHHhCCcccccccc		
0 ss prod		CCCCccbuubccccccuuubcccccc		
Q 35_preu 0 MOU2104266 1	100		1 / 2	(2/1
Q MCH2104200.1	109	IFDERVERVERVERVERVERVERVERVERVERVERVERVERVE	142	(341
Q Consensus	109	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	142	(34)
	114		1 0 0	(425
T Consensus	114		192	(433
T SZE8_A	114	-KLDAHPAYTEGVNCVACHTLKTYNGIQDAEGKLRYGIXAYDLSDRLQAPIGFPKELEKLKARSDDLFGGAVDAVPDQKP	192	(435
T ss_assp		-CTTSCHHHHTCSCHHHHTEEEECCSBCTTSCBCCGGGGEEECSSEEESSCCCCCHHHHHCCC		
T ss_pred		-cccucchincucchinccceecuccucuceeccccccccccucucchilli Hillinccccccucucucu		
Q ss_pred	1.40	CCCcccccCCccCcccccccccnHHHHccccCCCCCccnnHHhcCcccCCCCcnnCCCccccc	0.00	10.44
Q MCH2104266.1	143	DAHPSEKNKSFTSEGANRLCIACHATNIGPVIGVAKGFNETQVAKDGGSCVGCHMAIVERS	203	(341
Q Consensus	143	~~ <u>h</u> ~~~~ <u>C</u> H~~~~ <u>C</u> H~~~~~ <u>C</u> H~~~~~~ <u>C</u> H~~~~~~ <u>C</u> H~~~~~~ <u>C</u> H~~~~~ <u>C</u> H~~~~ <u>C</u> H~~~~~ <u>C</u> H~~~~ <u>C</u> H~~~~ <u>C</u> H~~~~ <u>C</u> H~~~~ <u>C</u> H~~~ <u>C</u> H~~ <u>C</u> H~	203	(341
		.+		
T Consensus	193	~~h~~~~~s~~~s~~~~c~~CHmp~~~~	266	(435
T 5ZE8_A	193	NPHLGEPVELDGKTIPALTMEANPRQLRTSDACMGCHDQRDNPQGVPLCQTGKEFIAGGSQVACQTCHMPVAGG-	266	(435
T ss_dssp		CTTSSSCEEETTEEECCCCEEECHHHHHSGGGTHHHHSCEECTTSCEEECHHHHHHHHTCCCCHHHHHSCEETT-		
T ss_pred		-2000000cccc0ccccccchhcccccccHHHc00000000ccccchHHhc00CccccccccCch		
Q ss_pred		cCCCCCCCCCCCCcccccCCCCCHHHHhceeEEEE=-eCCE-EEEEEEcCCCCCC-CCCceEEEEEEEEECCC		
Q MCH2104266.1	204	HAIDPETGEASPVRVGRSHLLQTPRDPAFLRQAFGIRAHLRDGKVILSIENRTGHRVP-GLAQRTLELRFKLLSGD	278	(34)
Q Consensus	204	~~~~v~v~v~n~~d+~~f~~r~~r~~r~~r~~r~~r~~r~v~v~n~~d+~~r~r~r~r~r~r~r~r~r~r~r~r~r~r~r~r~r~	278	(34)
		.++ . . .++.++ +++++++		
T Consensus	267	v-v-v-v-N-~gH-~Ptg-~-r-~l~v-d~~	331	(435
T 5ZE8_A	267	FADHSMGGGHHEAMLKRSIVFDLTTKADKEKIAAQVWIRNLQPHAMPTGAPFRNLYLKLTAYDAS	331	(435
T ss_dssp		EECCCCCCTTCHHHHHTEEEEEEEEEEEEEEEEEE		
T ss_pred		CCCCCCCCCCCCCCHHHHhhcEEEEEecCCCCCeEEEEEEecCCCCCCCCCCCCCcceeEEEEEECCC		
Q ss_pred		CCEEEEEEEceEEEEEec		
Q MCH2104266.1	279	GETLEEIRHEAVLAEGDHD 319	(341)	
Q Consensus	279	g 319	(341)	
		++ ++++ ++++++ .		
T Consensus	332	g~~~~~d~~i~~~~~v~~~l~y~ 405	(435)	
T 5ZE8 A	332	GEVLWONAADHPSKDDPRAYFAYGLADDOGNPAPPPTATKPGDDTRLOPHETRELNYEIPAKGVALVRGELYYN 405	(435)	

DDPRAYFAYGLADDOGNPAPPPTATKPGDDTRLOPHETRELNYEIPAKGVALVRGELYYN T ss dssp T ss_pred





С AcECN_A.veneficus NISGTGNNLPCEKCHADVFAEFKNNPGAHKTIGGGTDT-----VEHIRAACGECHRTSVVGTFASGDGTSATPGQEAHAAAT-----IACMACHEFGPNGNAPYSG-(39aa)-CytC552_T.tepidum EPIHRVSSELCANCHEDIYRQWK--GSMHAKSTALSDP-(49aa)-AYTEGVNCVACHTLKTYNGIQDAEGKLRYGIKAYDLSDR-(63aa)-DACMGCHDQRDNPQGVPLC------226 228 231 234 200 203 205 207 210 240 AcECN_A.veneficus AHQTEIERAVEDKTLIDSNEACIACHTYVP------VKINWTHKVSLEF CytC552_T.tepidum ----QTGKEFIAGGSQVACQTCHMPVA-(20aa)-IVFDLTTKADKE--VKINW<mark>T</mark>HKVSL<mark>E</mark>FNCTY-(11aa)-HYNVTNWTVNGTRYTTVF<mark>G</mark>NTTG<mark>N</mark>GSVNDASNWPGWYPYSW--------KIAAQV<mark>W</mark>IR<mark>N</mark>LQPHAMPT<mark>G</mark>APFRNLYLKLTAYDASGEVLWQ-(98aa)-





