

526 **Supplemental Figure titles and legends**

527
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 *P. calidifontis* 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.

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

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

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

565
566 **Supplementary Figure 4.** Potential factors contributing to the thermal stability of ECNs, related to Figure 4.

567 (A) Disulfide bonds in *P. calidifontis* ECN. The full atomic model of one *P. calidifontis* ECN subunit is shown on
568 the left with the cryo-EM density corresponding to a single cytochrome subunit. Two disulfide bonds were
569 observed and highlighted in yellow circles. Those two sites are magnified on the right.

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

573
574 **Supplementary Figure 5.** *A. veneficus* ECN may have evolutionary link to cytochrome c552, related to Figure 5.

575 (A-B) Results of the HHsearch analysis queried with the sequences of AvECN (A) and AvECN-like protein from a
576 Planctomycetota bacterium (B). The GenBank accession number of (B) is MCH2104266. H(h), α -helix; E(e), β -
577 strand; C(c), coil.

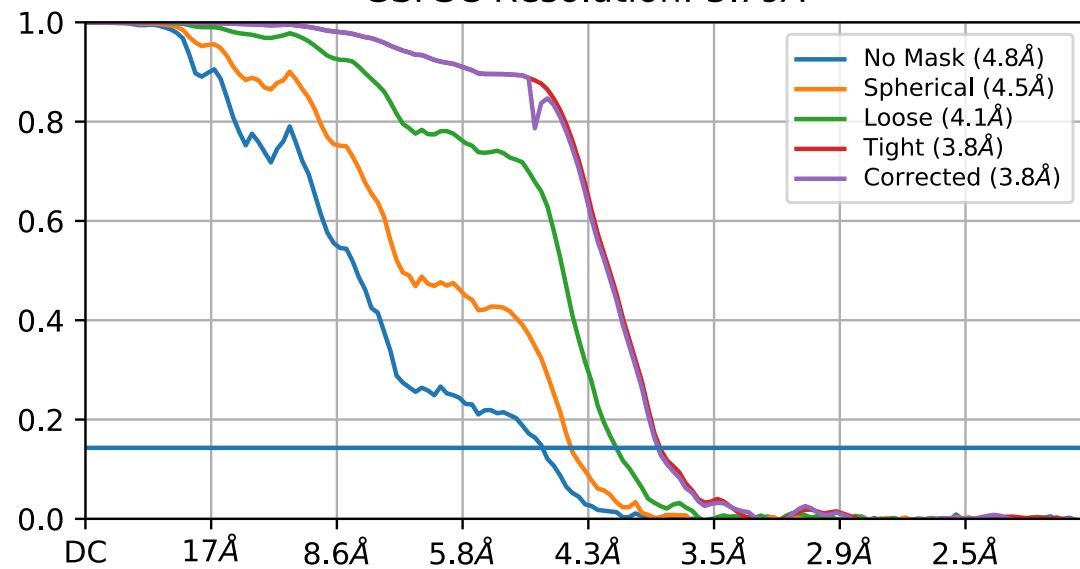
578 (C) Heme-based sequence alignment of *A. veneficus* ECN and *T. tepidum* cytochrome c552. Secondary structures
579 are labeled: α -helices in rounded rectangles, β -sheets in arrows, and loops in dashed lines.

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

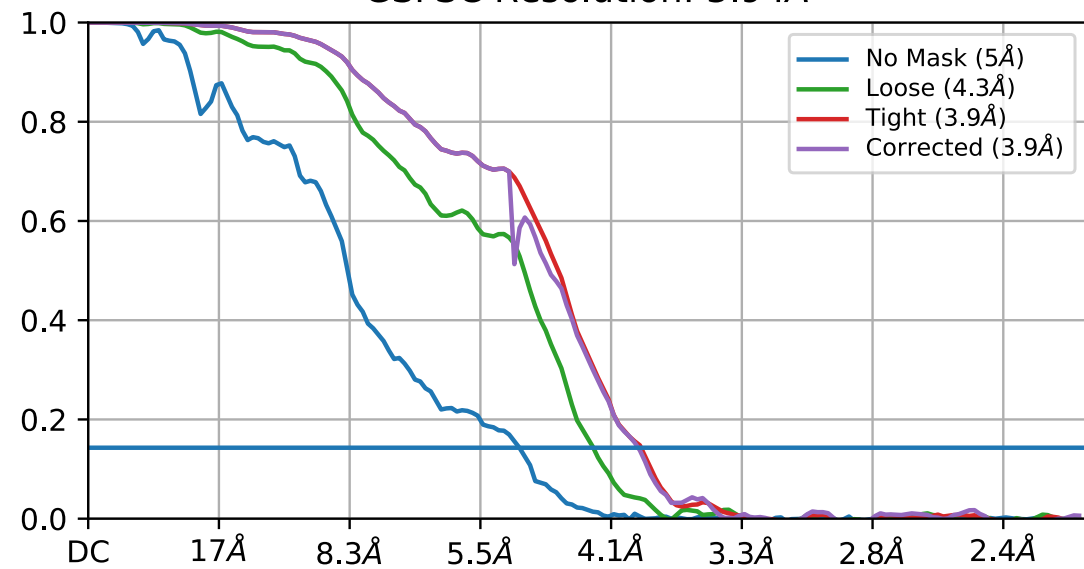
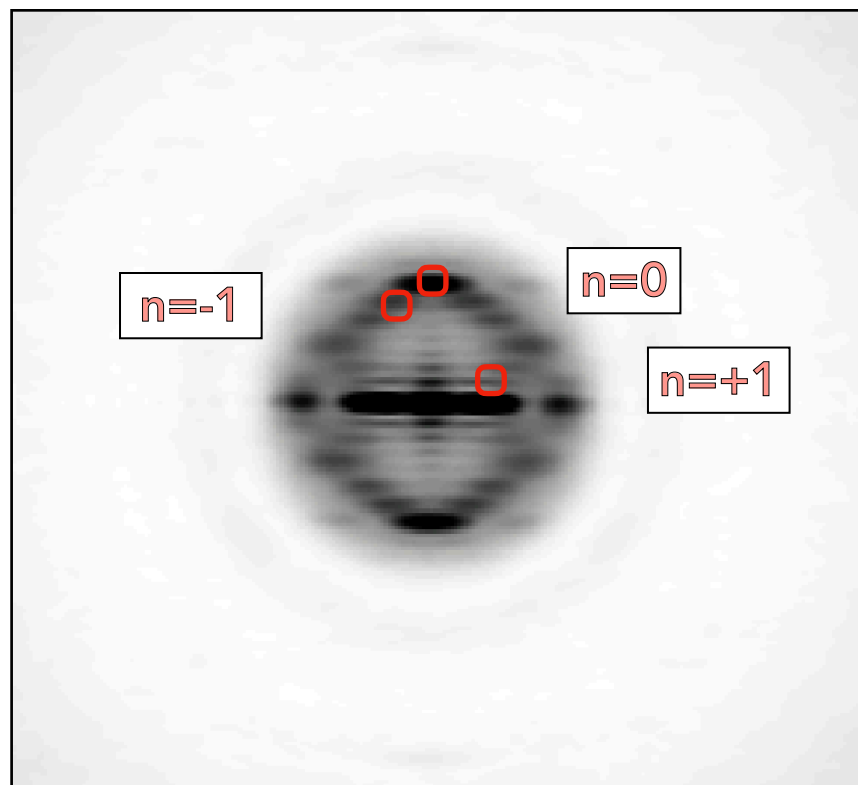
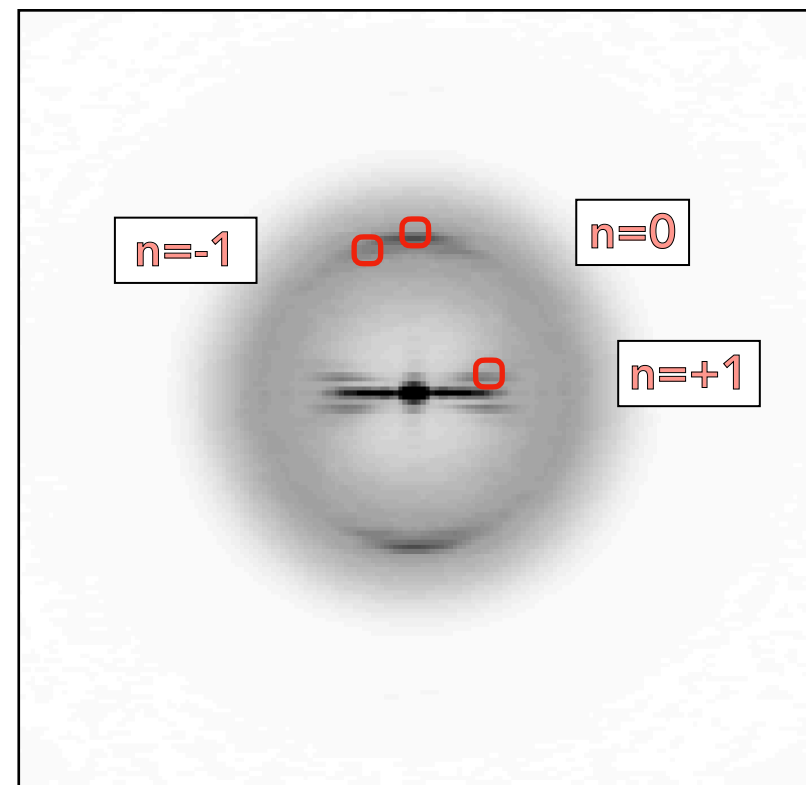
583 (E) The individual structures of *A. veneficus* ECN, *T. tepidum* cytochrome c552, and their alignment. Hemes are
584 hidden in this panel. The labeled secondary structures in (C) are colored accordingly. Other unaligned secondary
585 structures are kept grey. A magnified view of the aligned helices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

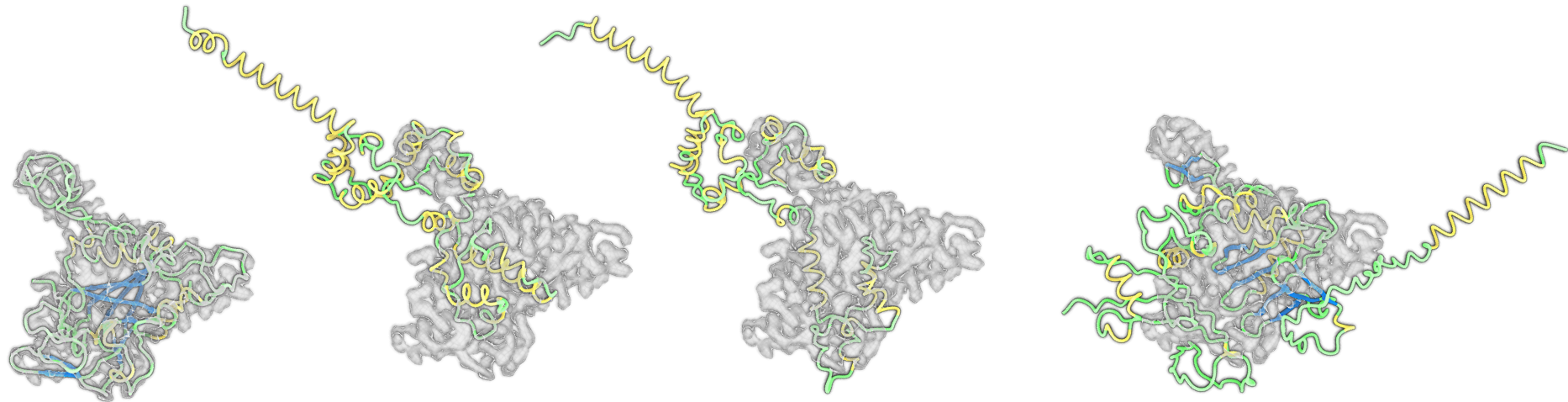
A

GSFSC Resolution: 3.79Å

*P. calidifontis* ECN (map:map)**C**

GSFSC Resolution: 3.94Å

*A. veneficus* ECN (map:map)**B****D**

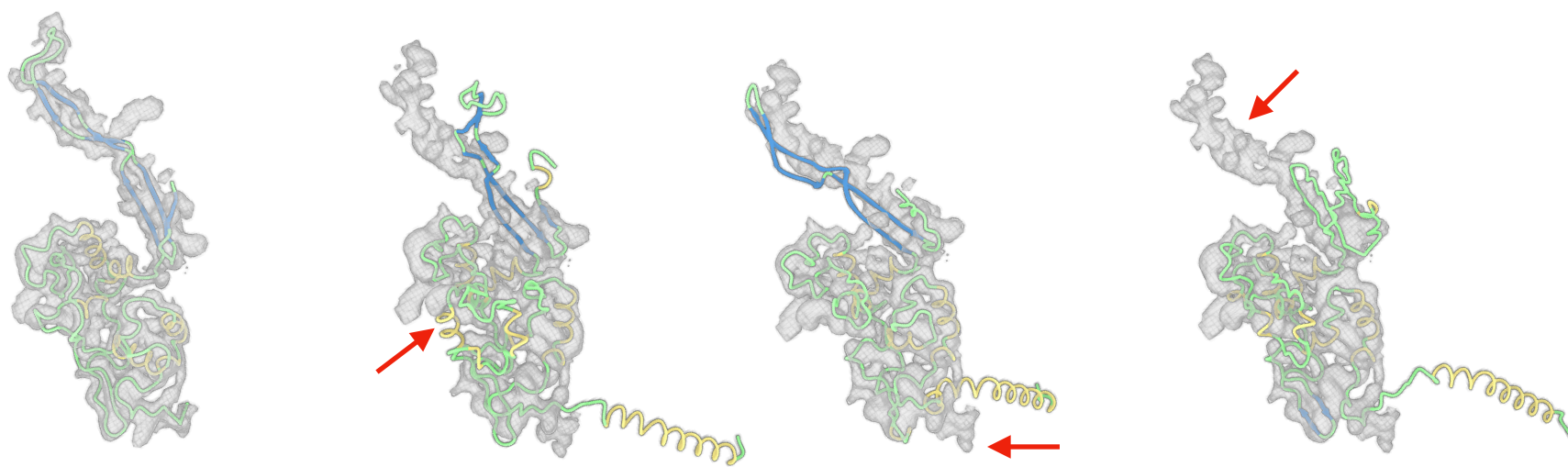
A

A3MW92
P. calidifontis ECN

A3MW94
AlphaFold prediction

A3MW99
AlphaFold prediction

A3MW89
AlphaFold prediction

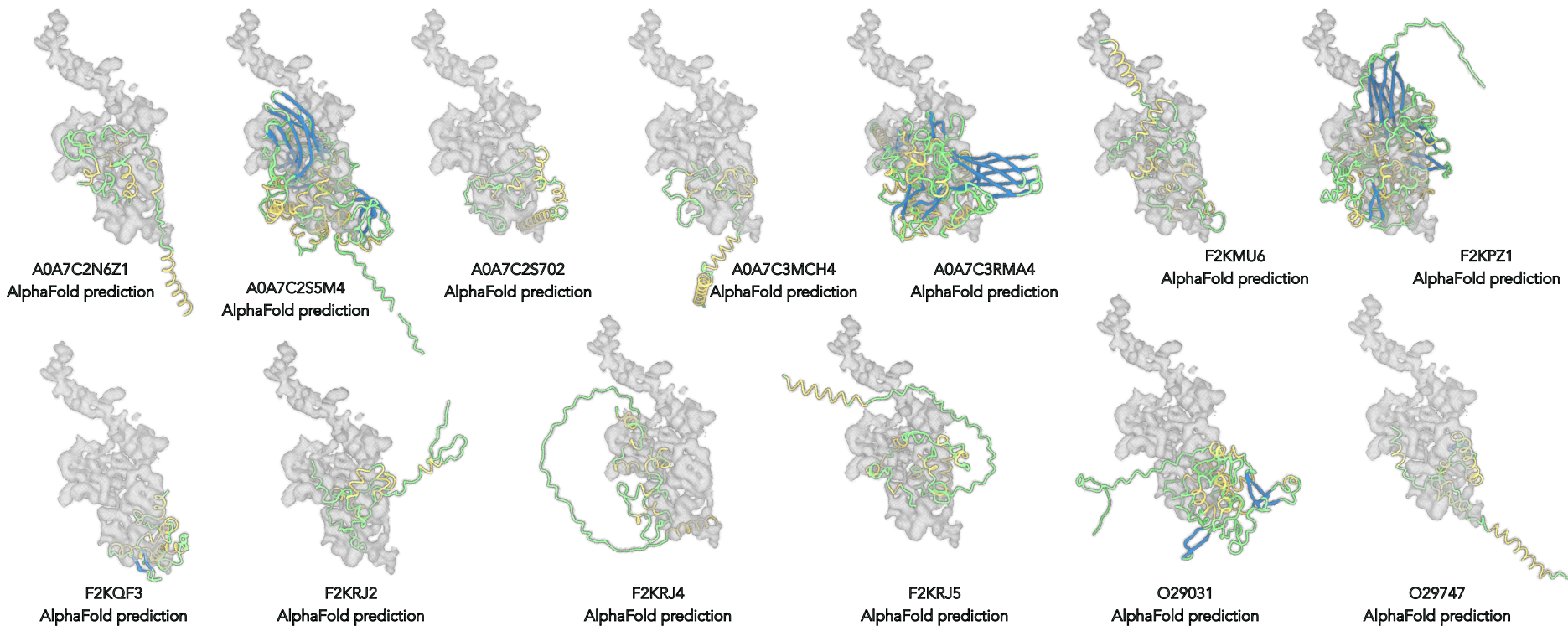
B

F2KMU8
A. veneficus ECN

F2KMU7
AlphaFold prediction

F2KPF5
AlphaFold prediction

F2KRJ1
AlphaFold prediction



A0A7C2N6Z1
AlphaFold prediction

A0A7C2S5M4
AlphaFold prediction

A0A7C2S702
AlphaFold prediction

A0A7C3MCH4
AlphaFold prediction

A0A7C3RMA4
AlphaFold prediction

F2KMU6
AlphaFold prediction

F2KPZ1
AlphaFold prediction

F2KQF3
AlphaFold prediction

F2KRJ2
AlphaFold prediction

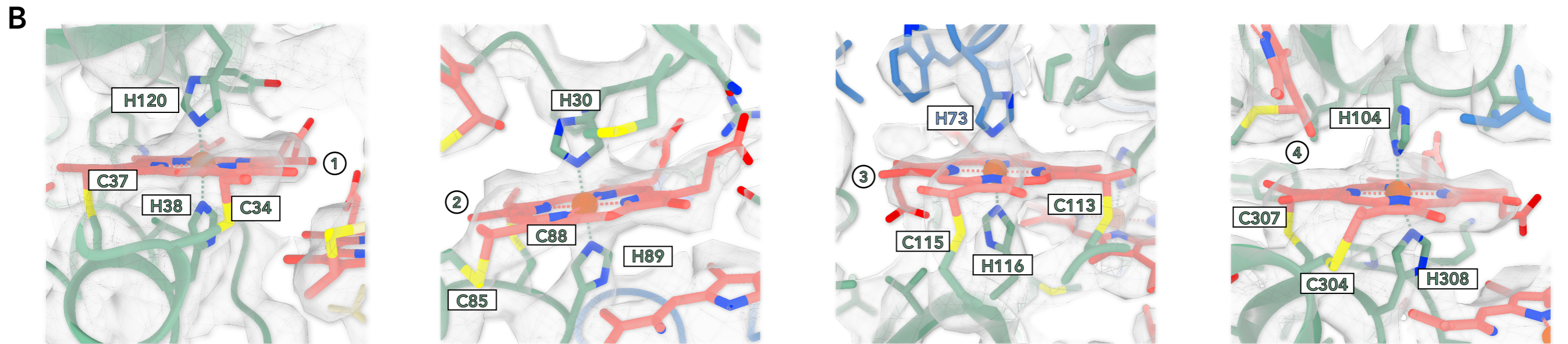
F2KRJ4
AlphaFold prediction

F2KRJ5
AlphaFold prediction

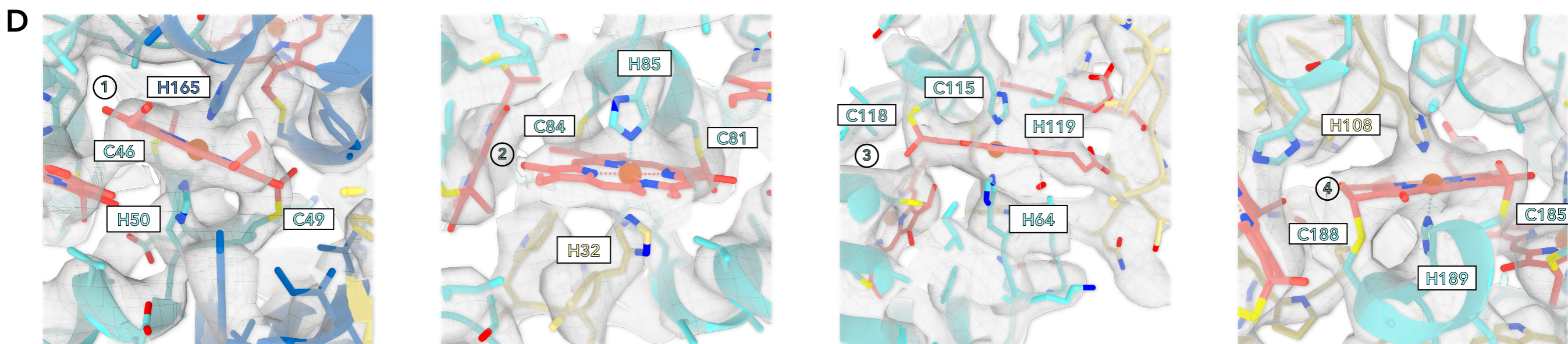
O29031
AlphaFold prediction

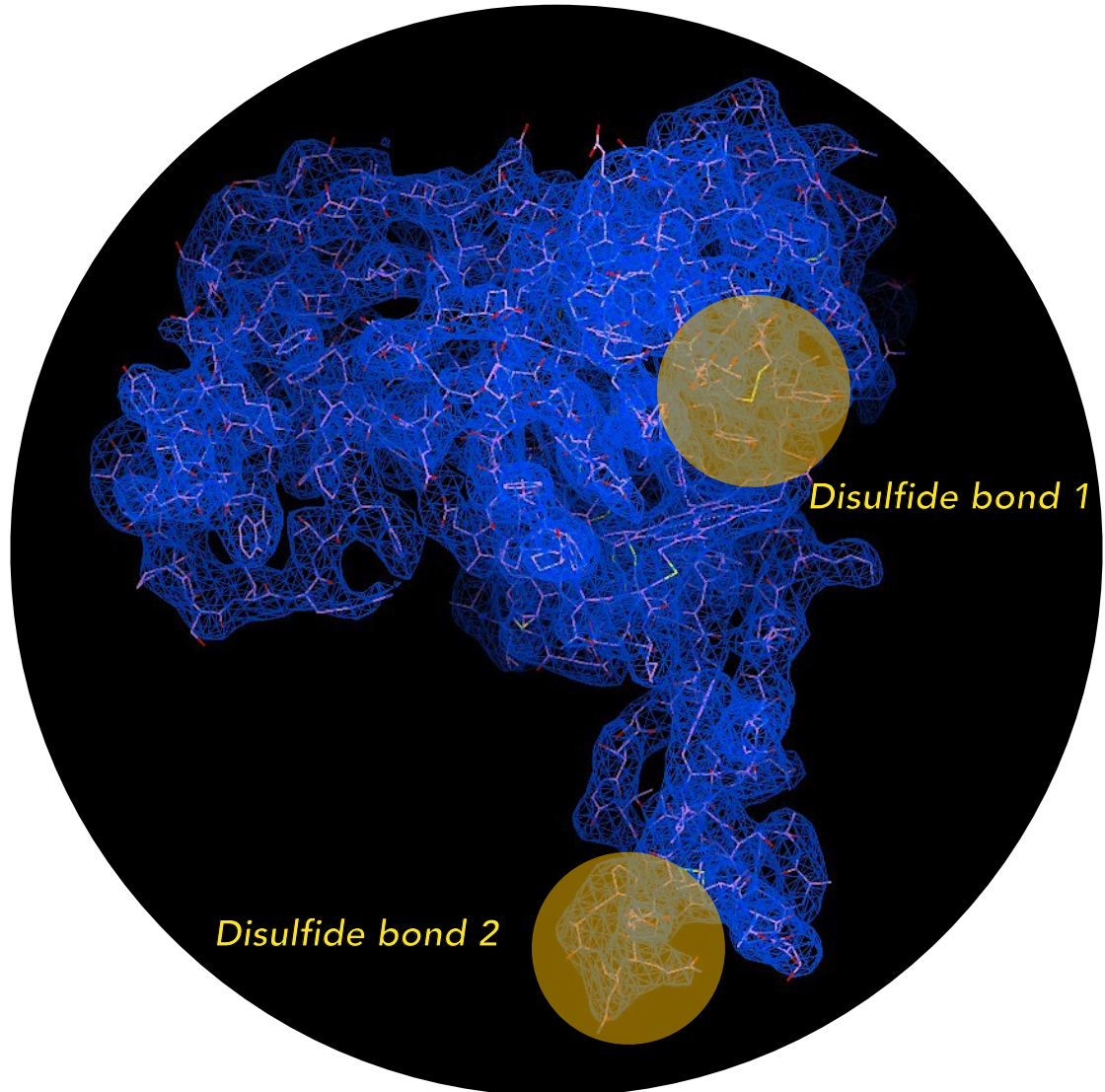
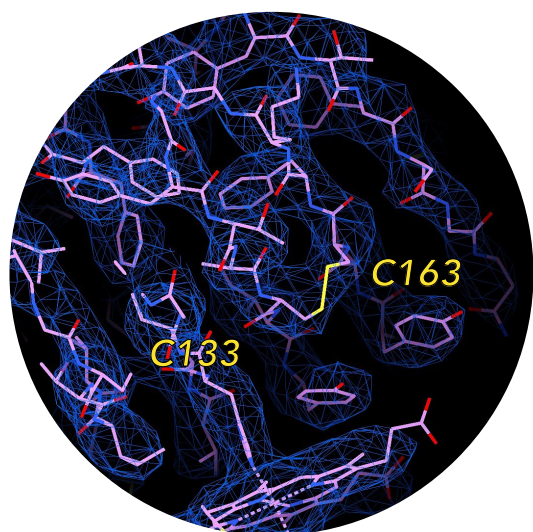
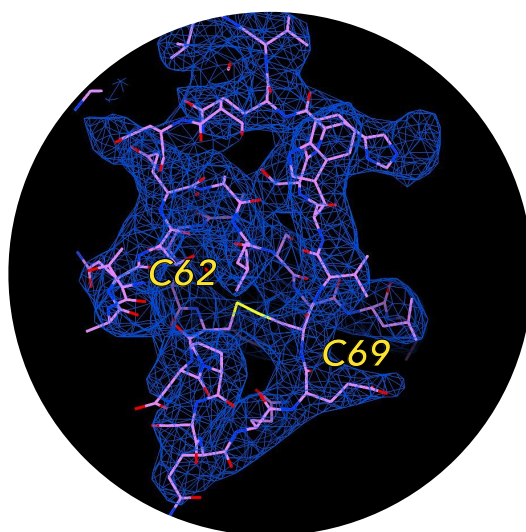
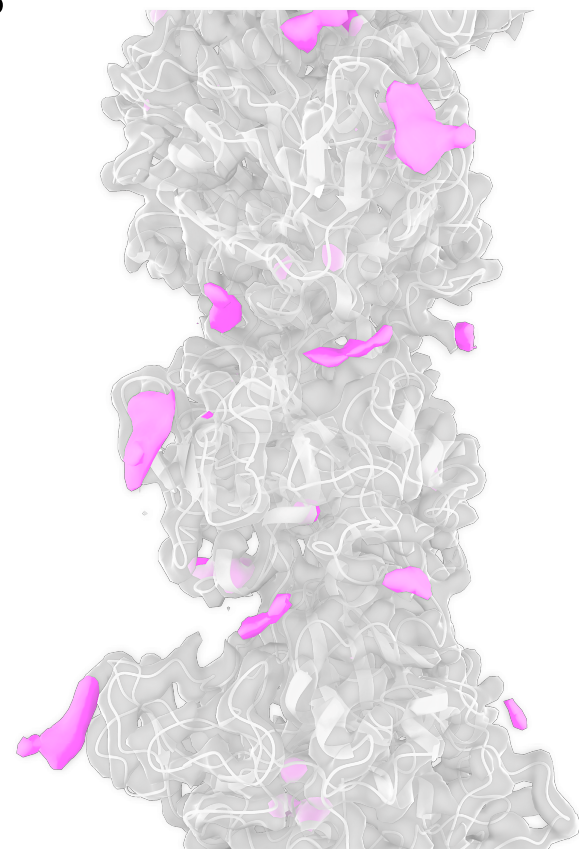
O29747
AlphaFold prediction

A *PcECN* 1: MKKFPALITTL LLLAVFVAA^TYGPYSYN^HPTNC^ISCHSNSTGTANSQALSGLTSGPAAGACDPSQQECVWS^HHQVLKGTD 80
PcECN 81 VWKK^CINCHVAIWNSINSGPGNV^HSGLLNSYGCACHAVA^HVGYGNPTDGYTACIYFYVPRLLSTATPGYFGAKPTLDFRNV 160
PcECN 161 YICFKGTPEGTYTFSGNAPTS LMQLLESKGEVTVKALLVGYDKYANGTVKAKSSAADFLETDFFSALEQAGIFRYEWGTA 240
PcECN 241 SGAVLKNPSVRTHPLTEEAPNGETIVMGVFDIHTGDFILVAPYAPYSRAPYYLPVAVNPGVAA^CFNCHFVYQGQLGTAKV 320
PcECN 321 MEVGGVWKIGIPADVLNSLTDPHKIVMPAAQAAGGGVAPN^LSLVALLATATLLGGAF^LLRRRAQ 385



C *AcECN* 1: MNGGKLALLMVTLAAMGTLVLPSTTS^LFLGQ^HMWYNI SGTGNNLP^CEKCHADVFAEFKNNPGA^HKTIGGGTDTVEH IRAA 80
AcECN 81 ^CGECHRTSVVGTFASGDGTSATPGQEA^HAAATIA^CMACHEFGPNGNAPYSGAPVAGGF DNVT TDTASSPYN DNGD TTYG 160
AcECN 161 TKEA^HQTFIERAVEDKTLIDSNEA^CIACHTYVPVKINWTHKVSLEFNCTYEYNTGTSGVTTHYNVTNWTVNGTRYTTVFG 240
AcECN 241 NTTGNGSVNDASNWP GWYPYSW 262



A*P. calidifontis* ECN*Disulfide bond 1**Disulfide bond 2***B***P. calidifontis* ECN**C***A. veneficus* ECN

