Supporting Information

A quantitative assessment of (bacterio)chlorophyll assignments in the cryo-EM structure of the *Chloracidobacterium thermophilum* reaction center

Christopher J. Gisriel[1,†], David A. Flesher[2,†], Zhuoran Long[1], Jinchan Liu[2], Jimin Wang[2], Donald A. Bryant[3], Victor S. Batista[1], and Gary W. Brudvig[1,2,*]

[1]Department of Chemistry, Yale University, New Haven, CT 06520, USA.

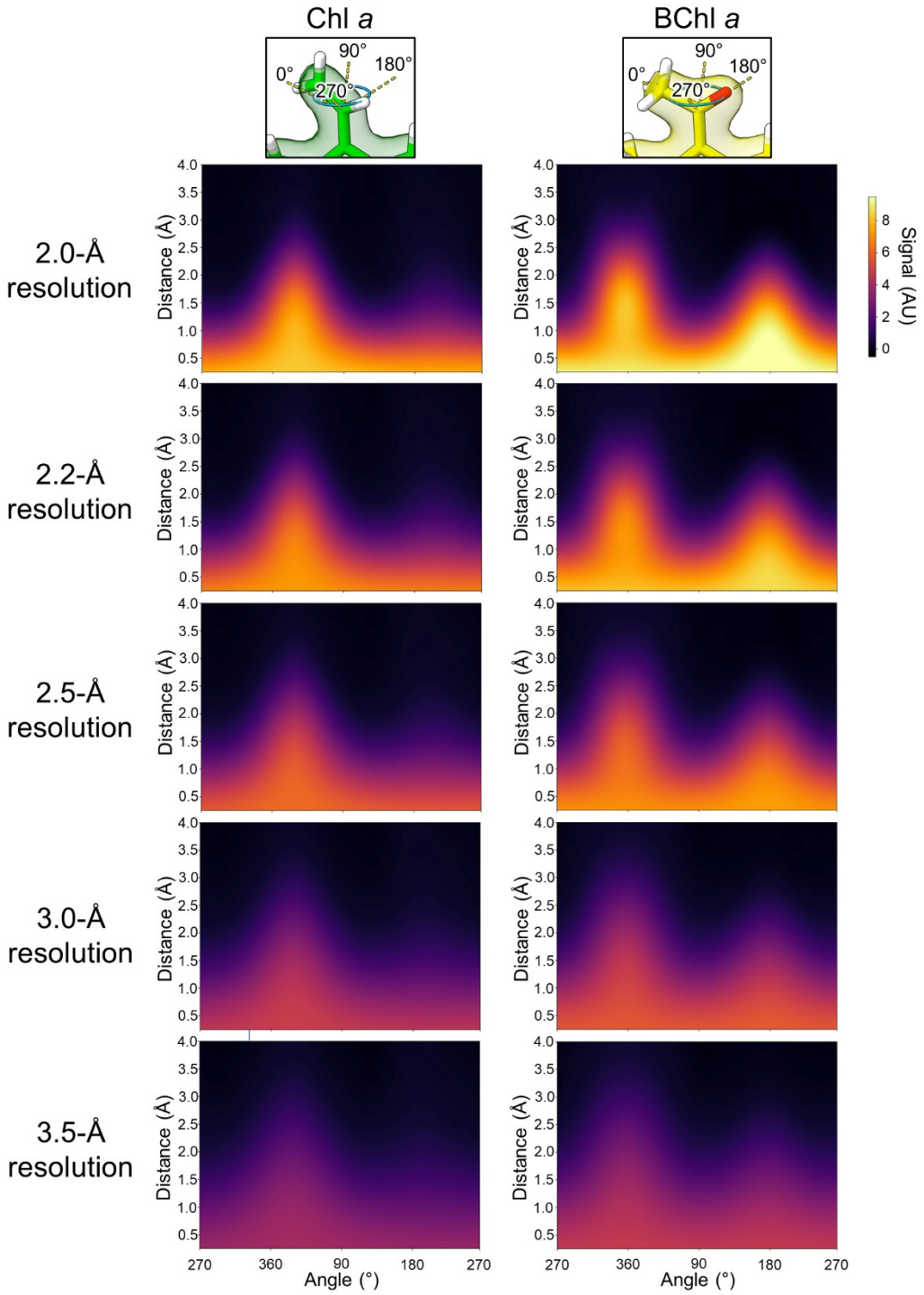
[2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.

[3]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.

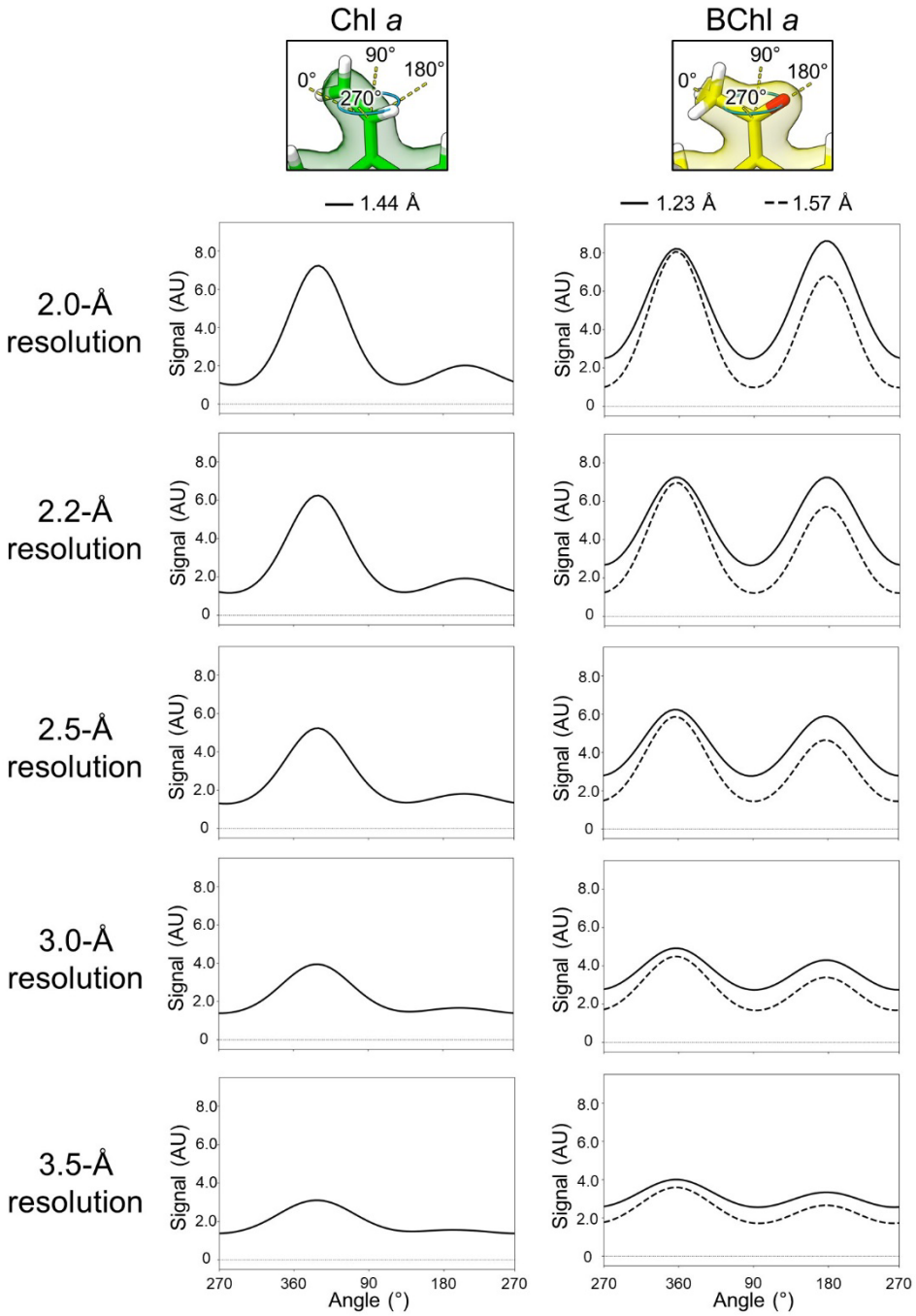
[†]These authors contributed equally.

*To whom correspondence should be addressed: gary.brudvig@yale.edu

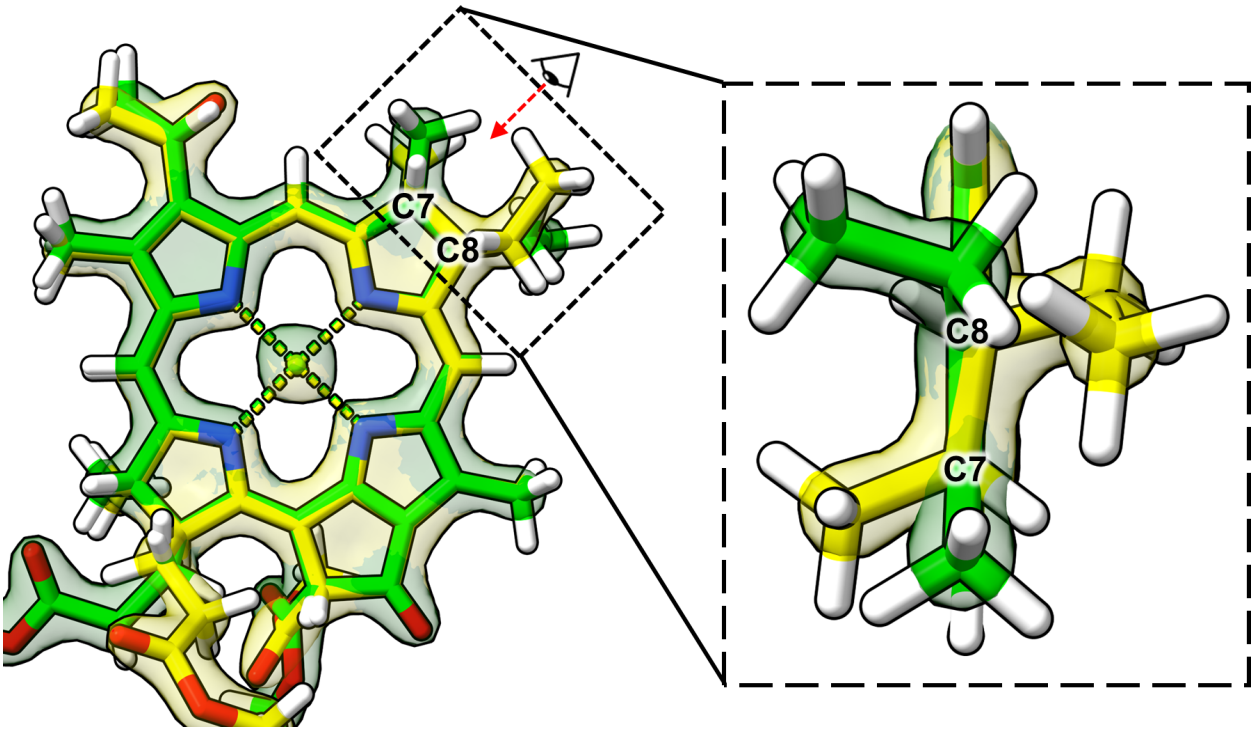
Supplementary Fig. 1 Heat maps of Chl *a* and BChl *a* C3 cone scans moving away from the C3[1] carbon atom derived from calculated cryo-EM maps at 2.0-, 2.2-, 2.5-, 3.0, and 3.5-Å resolutions.
Supplementary Fig. 2 Cone scans of Chl *a* and BChl *a* C3 at expected bond distances derived from calculated cryo-EM maps at 2.0-, 2.2-, 2.5-, 3.0, and 3.5-Å resolutions.
Supplementary Fig. 3 Perspective of C7 and C8 carbons in Chl *a* and BChl *a* molecules of theoretical ESP maps.
Supplementary Fig. 4 Comparison of central metal signals of (B)Chl cofactors in the electron transfer chain.
Supplementary Fig. 5 Multiple sequence alignment of PscA sequences from chloroacidobacterial species.
Supplementary Table 1 List of (B)Chl types sorted by local resolution.
Supplementary Data 1 Files for generation of cone scan data (external).
Supplementary Data 2 Coordinate file for altered CabRC structure where the three acetyl moieties of the BChl *a* molecules in sites A902, A906, and a908 are flipped.



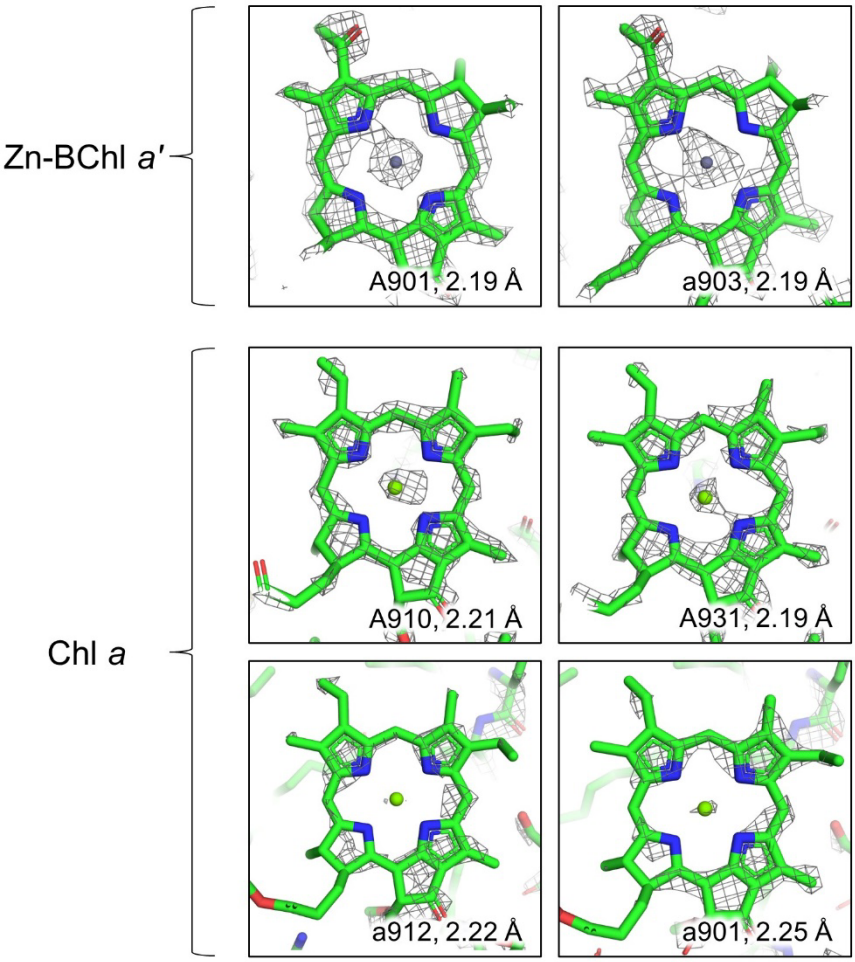
Supplementary Fig. 1 Heat maps of Chl *a* and BChl *a* C3 cone scans moving away from the C3[1] carbon atom derived from calculated cryo-EM maps at 2.0-, 2.2-, 2.5-, 3.0, and 3.5-Å resolutions. The top images show the part of the structure being scanned.



Supplementary Fig. 2 Cone scans of Chl *a* and BChl *a* C3 at expected bond distances derived from calculated cryo-EM maps at 2.0-, 2.2-, 2.5-, 3.0, and 3.5-Å resolutions. The top images show the part of the structure being scanned.



Supplementary Fig. 3 Perspective of C7 and C8 carbons in Chl *a* and BChl *a* molecules of theoretical ESP maps. Chl *a* and BChl *a* are shown in green and yellow, respectively.



Supplementary Fig. 4 Comparison of central metal signals of (B)Chl cofactors in the electron transfer chain. For each panel, the site name and local resolution are shown in the bottom right. All maps are shown at 12.7σ.

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C. thermophilum     MASFSSYANGVKRWYQKLELPMPPERIFGAHMMLIGGLACLIGTYFFASMTMWNDGYVNL 60
C. aggregatum       MASFSSYANGVKRWYQKLELPMPPERIFGAHMMLIGGLACLIGTYFFASMTMWNDGYVNL 60
C. sp. CP2_5A       MASFSSYANGVKRWYQKLELPMPPERIFGAHMMLIGGLACLIGTYFFASMTMWNDGYVNI 60
C. validum          MASFSSYANGVKRWYQKLELPMPPERIFGAHMMLIGGLACLIGTYFFASMTMWNDGYVNI 60
                    ************************************************************:

C. thermophilum     TLRPRLISLGIYDPYDTEQIQRVWLPLIGEFSTSKLPFFGQYPLTMTDFRLFGWGCFHIG 120
C. aggregatum       TLRPRLISLGIYDPYDTEQIQRVWLPLIGEFSTSKLPFFGQYPLTMTDFRLFGWGCFHIG 120
C. sp. CP2_5A       TLRPRLISLGIYDPYDTEQIQKVWLPLIGEFSAKHLPFFGQYPLTMTDFRLFGWGSFHIG 120
C. validum          TLRPRLISLGIYDPYDTEQIQRVWLPLIGEFSTKHLPFFGQYPLTMTDFRLFGWGSFHIG 120
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C. thermophilum     LGLWLVYAGAAHYYGARGGATIGEIFWLLPYVPGLKGLCQIKWFTPEGPWYKVGLPWGSF 180
C. aggregatum       LGLWLVYAGAAHYYGARGGATIGEIFWLLPYVPGLKGLCQIKWFTPEGPWYKVGLPWGSF 180
C. sp. CP2_5A       LGLWLIYAGAAHYYGARGGATIGEIFWLLPYVPGLKGLCQIKWFTPDGPWYKVGLPWGSF 180
C. validum          LGLWLIYAGAAHYYGARGGATIGEIFWLLPYVPGLKGLCQIKWFTPEGPWYKVGLPWGSF 180
                    *****:****************************************:*************

C. thermophilum     ANTPWPILRRTYADALSPHTIYIGLLFFIWGFVLWFVLDKPPVPLQPAQVMTPNGLMPLE 240
C. aggregatum       ANTPWPILRRTYADALSPHTVYIGLLFFIWGFVLWFVLDKPPVPLQPAQVMTPNGLMPIE 240
C. sp. CP2_5A       ANTPWKILRRTYADALSPHTIYIGLLFFIWGLVLWLVLDKPPVPLQPAQVMTPNGMMPIE 240
C. validum          ANTPWRILRRTYADALSPHTIYIGLLFFIWGAVLWFVLDKPPVPLQPAQVMTPNGMLPIE 240
                    ***** **************:********** ***:********************::*:*

C. thermophilum     QAPFPYGWFDPYLNQVMHPMNTINGETTMCFVWGVLFVALGAYWWYRPPRSINITHLEDT 300
C. aggregatum       QAQFPYGWFDPYLNQVMHPMNTINGETTMCFVWGVLFVALGAYWWYRPPRSINITHLEDT 300
C. sp. CP2_5A       QAQFPYGWYDPYLRQVMHPMNTINGETTMCFVWGVLFVALGAYWWYRPPRSVNITHLEDT 300
C. validum          QAQFPYGWYDPYLQQVMHPMNTINGETTMCFVWGVLFVALGAYWWYRPPRSVNITHLEDT 300
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C. thermophilum     KAVFHVHLTAIGYVSFALAIVGFLALRNHPSYLMLNDMNVIIYGKKIVNPGRMIHNMITF 360
C. aggregatum       KAVFHVHLTAIGYVSFALAIVGFLALRNHPSYLMLNDMNVIIYGKKIVNPGRMIHNMITF 360
C. sp. CP2_5A       KAVFHVHLTAIGYVSFALAIVGFLALRNHPSYLMLNDMNVIIYGKKIVNPGRMIHNMITF 360
C. validum          KAVFHVHLTAIGYVSFALAIVGFLALRNHPSYLMLNDMNVIIYGKKIVNPGRMIHNMITF 360
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C. thermophilum     NHVQVGLLYVAAGVFHGGQYLHGLNISGAYKQARSKFITWFQNPDLQTKIVGTTMFVSFV 420
C. aggregatum       NHVQVGLLYVAAGVFHGGQYLHGLNISGAYKQARSKFITWFQNPDLQTKIVGTTMFVSFV 420
C. sp. CP2_5A       NHVQVGLIYVAAGVFHGGQYLHGLNISGAYRQARSKFITWFQNPDLQTKIVGTTMFVSFV 420
C. validum          NHVQVGLIYVAAGVFHGGQYLHGLNISGAYKQARSKFITWFQNPDLQTKIVGTTMFVSFV 420
                    *******:**********************:****************************

C. thermophilum     TVVFGYGMICWNTGAELDLNFGIYQFRSFRAIQMDGEAGNIGYRVFRPKNPWDPTAGGDW 480
C. aggregatum       TVVFGYGMICWNTGSELDLNFGIYQFRSYRAIQMDGEAGNVGYRVFRPKNPWDPTAGGDW 480
C. sp. CP2_5A       TVVFGYGMICWNTGSELDLNFGIYQFRSYRAIQMDGEAGNVGYRVFRPKNPWDPTAGGAW 480
C. validum          TVVFGYGMICWNTGSELDLNFGIYQFRSYRAIQMDGEAGNVGYRVFRPKNAWDPTAGGDW 480
                    **************:*************:***********:********* ******* *

C. thermophilum     VKNPDGTAKLVKARNLQVGDRILNEELGIGSSPTYSFTTIEEINYKPEWGQPKLYAVQWG 540
C. aggregatum       VKNPDGTAKLVKARNLQVGDRILNEELGIGSSPTYSFTTIEEINYKPEWGQPKLYAVQWG 540
C. sp. CP2_5A       VTNPDGTAKLVKARNLQVGDRILNEELGIGSSPTYSFTTIEEINYKPEWGQSKLYAVQWG 540
C. validum          VTNPDGTAKLVKARNLQVGDRILNEELGIGSSPTYSFTTIEEINYKPEWGQSKLYAVQWG 540
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C. thermophilum     SWTHFLRKVNPLFWVDKGIWYLQNQKTFEATRKADEAYLAAHLKAVSLLNQIDDAQTEEA 600
C. aggregatum       SWTHFLRKVNPLFWVDKGIWYLQNQKTFEAARKADEAYLAAHLKAVSLLNQLDEAQTEDA 600
C. sp. CP2_5A       SWTHFLRKVNPLFWVDKGIWYLQNQKTFEASRKADEAYLAAHLKAVSLLNQLDEAKSDED 600
C. validum          SWTHFLRKVNPLFWVDKGIWYLQNQKTFEASRKADETYLAAHLKAVSLLNQLDDAKTDDD 600
                    ******************************:*****:**************:*:*::::

C. thermophilum     KQKAQAELDKFRPELEKAHANMLEWNERLASTPAVLYSNLRDQHRDGEINDAIFFWLMIG 660
C. aggregatum       KNKAQAELDKFRPELEKVHASMLEWNERLASTPAVLYSNLRDQHRDGEINDAIFFWLMIG 660
C. sp. CP2_5A       KKKAQEAVDQFRPDLEKAHASMLEWNERLASTPAVLYSNLRDQHRDGEINDAIFFWLMIG 660
C. validum          KKKAQEALDKFRPDLEKAHAAMLEWNERLASTPAVLYSNLRDQHRDGEINDAIFFWLMIG 660
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C. thermophilum     GWLFGFIPLLRIAFHNYQSPWYRDFEWRKQSPDFPCIGPVKGGTCGVSIQDQLWFCILFS 720
C. aggregatum       GWLFGFIPLLRIAFHNYQSPWYRDFEWRKQSPDFPCIGPVKGGTCGVSIQDQLWFCILFS 720
C. sp. CP2_5A       GWLFGFIPLLRIAFHNYQSPWYRDFEWRKQSPDFPCIGPVKGGTCGVSIQDQLWFCILFS 720
C. validum          GWLFGFIPLLRIAFHNYQSPWYRDFEWRKQSPDFPCIGPVKGGTCGVSIQDQLWFCILFS 720
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C. thermophilum     IKPLSAIAWYLDGGWIATMMARGNEAYYLTHNISHTGGVFLYMWNETTWIWTDNHLTAML 780
C. aggregatum       IKPLSAIAWYLDGGWIATMMARGNEAYYLTHNISHTGGVFLYMWNETTWIWTDNHLTAML 780
C. sp. CP2_5A       IKPLSAIAWYLDGGWIATMMARGNEAYYLTHNISHTGGVFLYMWNETTWIWTDNHLTAML 780
C. validum          IKPLSAIAWYLDGGWIATMMARGNEAYYLTHNISHTGGVFLYMWNETTWIWTDNHLTAML 780
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C. thermophilum     LLGHLIWFVSFALWFKDRGSRAEGGDIQSRWVRLMGKRLGIKTLQEVRFPVSNLATAKLW 840
C. aggregatum       LLGHLIWFVSFALWFKDRGSRAEGGDIQSRWVRLVGKRLGIKTLQEVRFPVSNLATAKLW 840
C. sp. CP2_5A       LLGHLIWFVSFALWFKDRGSRAEGGDIQSRWVRLMGKRLGIKTLQEVRFPVSNLATAKLW 840
C. validum          LLGHLIWFVSFALWFKDRGSRAEGGDIQSRWVRLMGKRLGIKTLQEVRFPVSNLATAKLW 840
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C. thermophilum     GTVFFYTGTFVLVFLYFADGFFQNR 865
C. aggregatum       GTVFFYTGTFVLVFLYFADGFFQNR 865
C. sp. CP2_5A       GTVFFYTGTFVLVFLYFADGFFQNR 865
C. validum          GTVFFYTGTFVLVFLYFADGFFQNR 865
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Supplementary Fig. 5 Multiple sequence alignment of PscA from chloroacidobacterial species. Highlighted residues are involved in H-bonding with the C3 acetyl moiety of BChl *a* molecules in the CabRC structure (PDB 7VZR). The sequences used in the alignment are as follows: *C. thermophilum*, NCBI reference number WP_014100663.1; *Chloracidobacterium aggregatum*, WP_211422155.1; *Chloracidobacterium* sp. CP2_5A, GenBank reference number OYT70531.1; *Chloracidobacterium validum*, NCBI reference number WP_211428666.1.

Supplementary Table 1 List of (B)Chl types sorted by local resolution. The residue name, residue number, and chain correspond to those annotated in PDB 7VZR.

Zn-BChl *a′* molecules			
Residue Name	**Residue Number**	**Chain**	**Resolution (Å)**
2GO	901	A	2.185
2GO	903	a	2.186
BChl *a* molecules			
Residue Name	**Residue Number**	**Chain**	**Resolution (Å)**
BChl *a*	908	A	2.228
BChl *a*	910	a	2.231
BChl *a*	909	A	2.259
BChl *a*	911	a	2.259
BChl *a*	903	A	2.303
BChl *a*	904	a	2.312
BChl *a*	908	a	2.317
BChl *a*	906	A	2.318
BChl *a*	902	A	2.325
BChl *a*	905	a	2.341
BChl *a*	909	a	2.361
BChl *a*	907	A	2.367
BChl *a*	905	A	2.403
BChl *a*	907	a	2.418
BChl *a*	904	A	2.495
BChl *a*	906	a	2.499
Chl *a* molecules			
Residue Name	**Residue Number**	**Chain**	**Resolution (Å)**
Chl *a*	931	A	2.193
Chl *a*	910	A	2.205
Chl *a*	912	a	2.218
Chl *a*	915	a	2.227
Chl *a*	901	a	2.245
Chl *a*	933	A	2.246
Chl *a*	912	A	2.260
Chl *a*	914	a	2.285
Chl *a*	913	a	2.323
Chl *a*	911	A	2.348

Supplementary Data 1 Files for generation of cone scan data (external). These files can be found at https://github.com/DavidAFlesher/CabRC_ConeScans_2023.

Supplementary Data 2 Coordinate file for altered CabRC structure where the three acetyl moieties of the BChl *a* molecules in sites A902, A906, and a908 are flipped and water molecules are added.