SUPPORTING INFORMATION

for

A Bacterial Hemerythrin Domain Regulates Activity of a *Vibrio cholerae* Di-Guanylate Cyclase

Ruth A. Schaller,[‡] Syed Khalid Ali,[§,†] Karl E. Klose,[§,†] and Donald M. Kurtz, Jr.*[,‡]

[‡]*Department of Chemistry,* [§]*Department of Biology, and* [†]*South Texas Center for Emerging and Infections Diseases, University of Texas at San Antonio, San Antonio, TX 78249*

Corresponding Author

*Tel 210-458-7060; Fax 210-458-7428; e-mail donald.kurtz@utsa.edu

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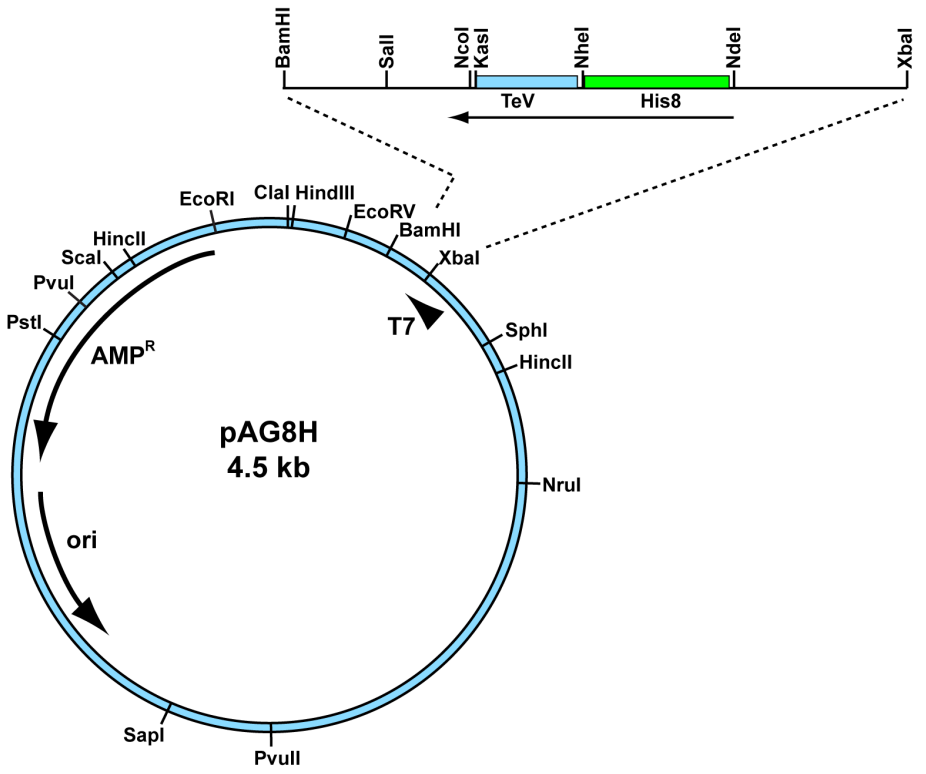
TEV Protease Expression, Isolation and Purification

A glycerol stock of *E. coli* BL21(DE3)-RIL cells containing the plasmid pRK793, encoding a TeV protease S219V variant, which self cleaves to form containing a His-tag at the N-terminus, was obtained from Addgene (addgene.org). Cells were plated on LB agar containing 100 micrograms amp and 35 micrograms Cm per mL. Individual colonies were used to inoculate 100-mL LB/amp/Cm starter cultures, which were incubated overnight at 37 ^{o}C. The starter cultures were used to inoculate 1-L volumes of LB/amp/Cm, and these cultures were grown to an O.D. of 1.0 with shaking at 37 ^{o}C. Expression of the TeV protease was induced by the addition isopropyl-beta-D-thiogalactoside to a concentration of 1 mM to the 1-L cultures. These cultures were incubated with shaking at 30 ^{o}C for 6 more hours, and cells were then harvested by centrifugation at 4 ^{o}C. The cell paste was frozen and stored at -80 ^{o}C. The TEV protease was purified using a HisPure column as described for Vc Bhr-DGC. The purified TeV protease was stored at -80 ^{o}C in 250 mM Tris, 5mM citrate, 5 mM beta-mercaptoethanol, 50% glycerol, 0.1% Triton X100 pH 7.5 at a concentration of 1 mg/ml.



Polylinker and Cleavage Site

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CGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATA
           T7 promotor                        XbaI

    NdeI                                NheI                    KasI NcoI
TACCATATGGACCACCATCACCATCACCATCACCATGCTAGCGAGAATCTTTATTTTCAGGGCGCCATGGTCTAGCATGAC
      MetAspHisHisHisHisHisHisHisHisAlaSerGluAsnLeuTyrPheGlnGlyAlaMetValter
                His-tag                      TeV binding     |
                                                          TeV cut

TGGTCGACAGAATGGGCGGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA
   SalI           BamHI                                                T7 Terminator

ACTAGCATAACCCC
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Sequencing Primers

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T7 promotor and terminator
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Expressed Sequence

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MDHHHHHHHHHASENLYFQGAMV
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Figure S1. pAG8H plasmid map. We are grateful to Drs. P. John Hart and Stephen Holloway for providing this diagram.

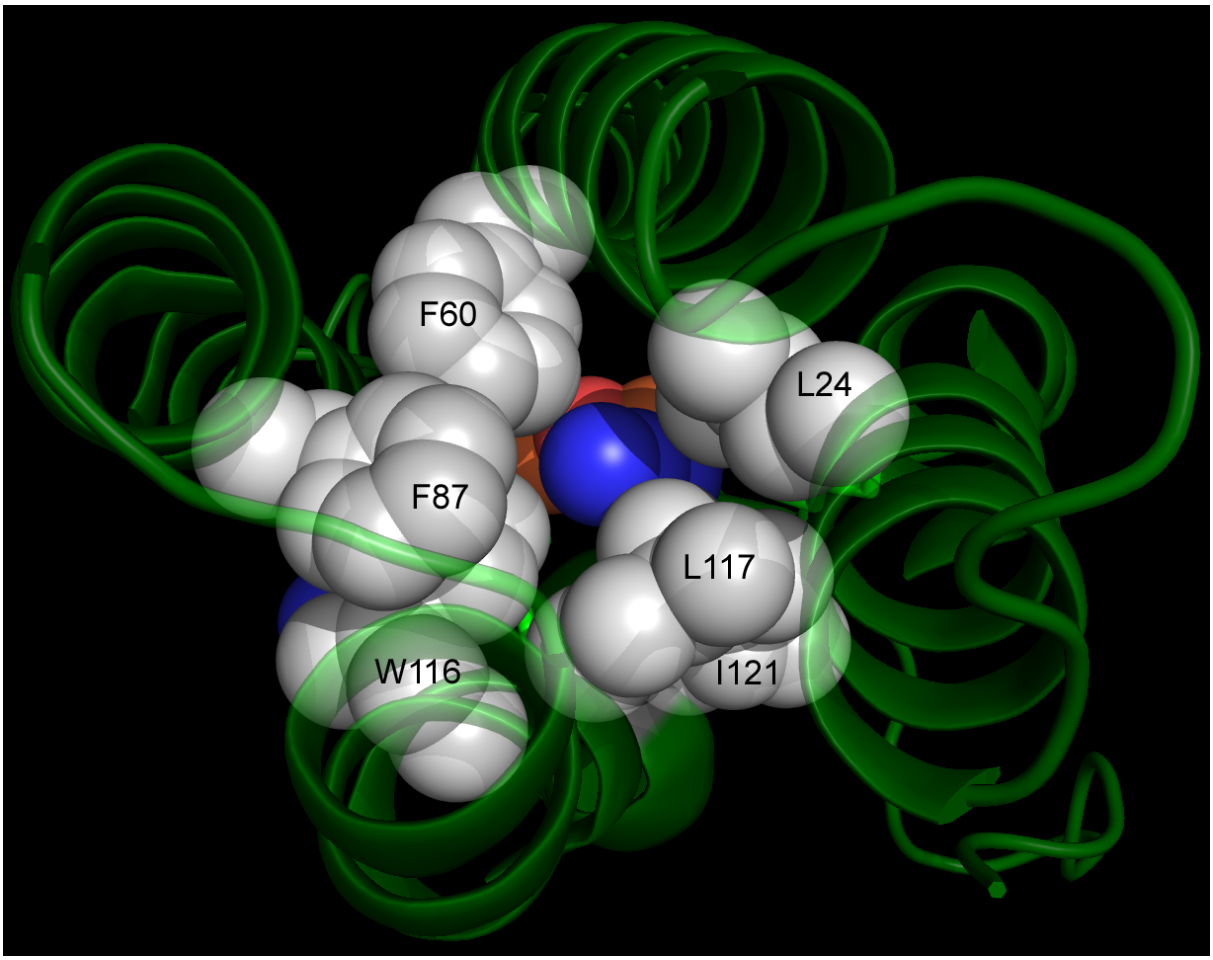

Figure S2. Bhr domain model of Vc Bhr-DGC (obtained as described in the text) showing the conserved hydrophobic residue side chains (gray spheres, hydrogens omitted) surrounding the azide (blue spheres) and Fe-O-Fe (orange-red spheres). No adjustments in either the server-generated SWISS-MODEL or 2avk coordinates were made. View is down the long axis of the four-helix bundle (green shadow cartoon) with the N-terminal loop at the lower right. Image was generated using PyMOL.

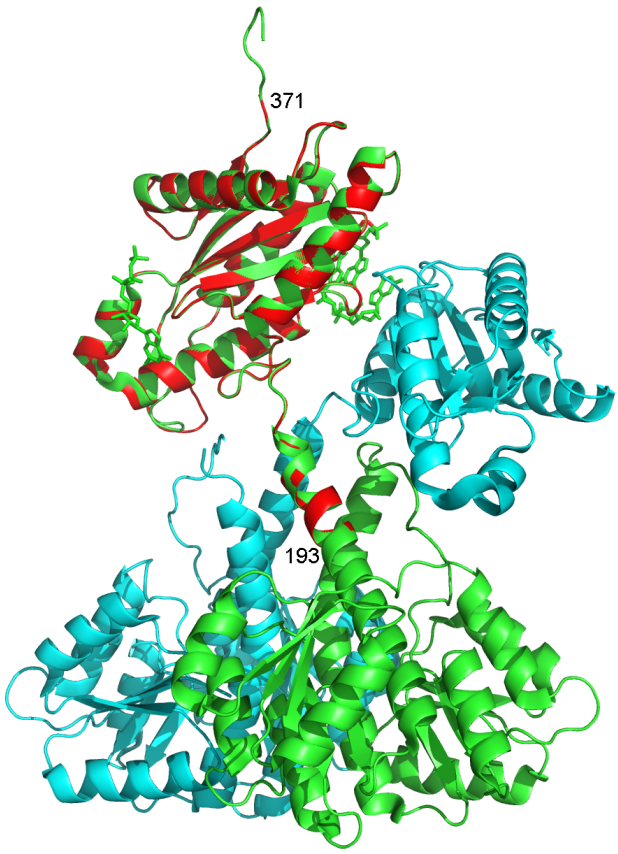

Figure S3. Cartoon representation of the Vc DGC domain model (colored red) superimposed on the structure of the subunit (colored green) of the PleD homodimer (PDB entry 2v0n), which was chosen by the SWISS-MODEL web server as the template. The other subunit of the PleD homodimer (colored cyan) is also shown. Locations of residues 193 and 371, representing the N- and C-terminal ends of the Vc DGC domain model, are indicated. Two c-di-GMP (between the subunits) and one GTP molecules in the PleD structure are also shown in stick representation.

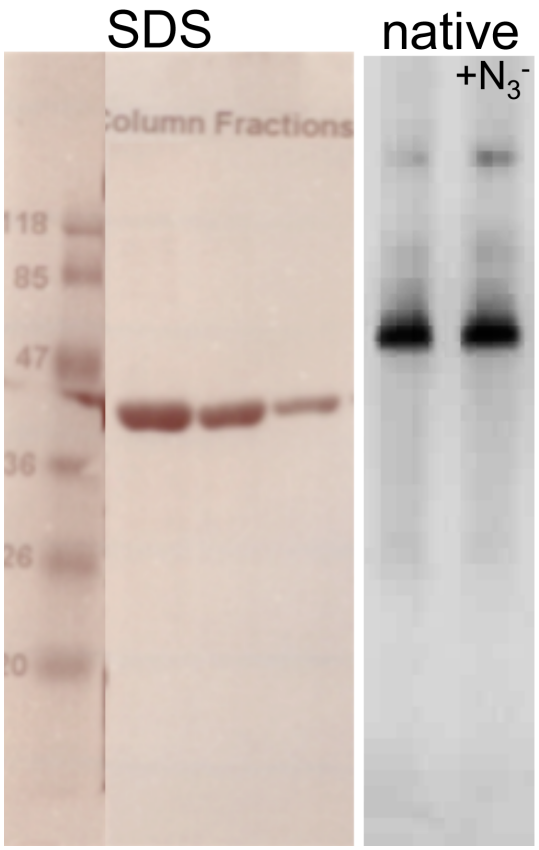

Figure S4. Coomassie Blue-stained SDS- and native-polyacrylamide gel electrophoresis of His-tagged Vc Bhr-DGC. "+N_3^-" indicates a protein sample that was incubated with excess sodium azide prior to electrophoresis.

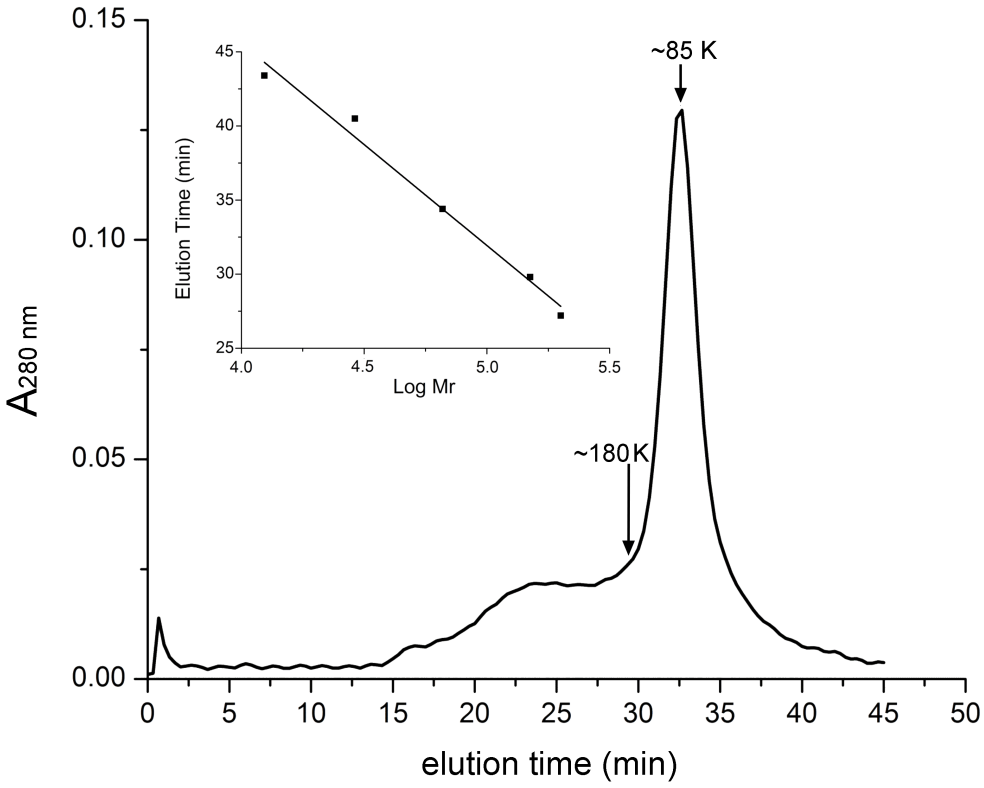

Figure S5. Analytical size exclusion chromatography of as-isolated Vc Bhr-DGC on a Superose 6 10/30 column in 50 mM MOPS, 250 mM NaCl pH 7.3. Molecular weight calibration of the same column under the same conditions is shown as an inset. Data points represent those of M_r standards (GE Healthcare): cytochrome *c* (12,400), carbonic anhydrase (29,000), serum albumin (66,000), alcohol dehydrogenase (150,000) and amylase (200,000). The calibration plot does not include Blue Dextran (2,000,000 M_r), which eluted at ~15 minutes under these conditions, and represents the column void volume. In multiple preparations the broad feature centered at ~23 min had variable intensity relative to the peak labeled ~85 K (85,000) but was never more intense than shown in this chromatogram. The ~180 K (180000) arrow represents the calibrated elution volume that would correspond to a Vc Bhr-DGC tetramer.

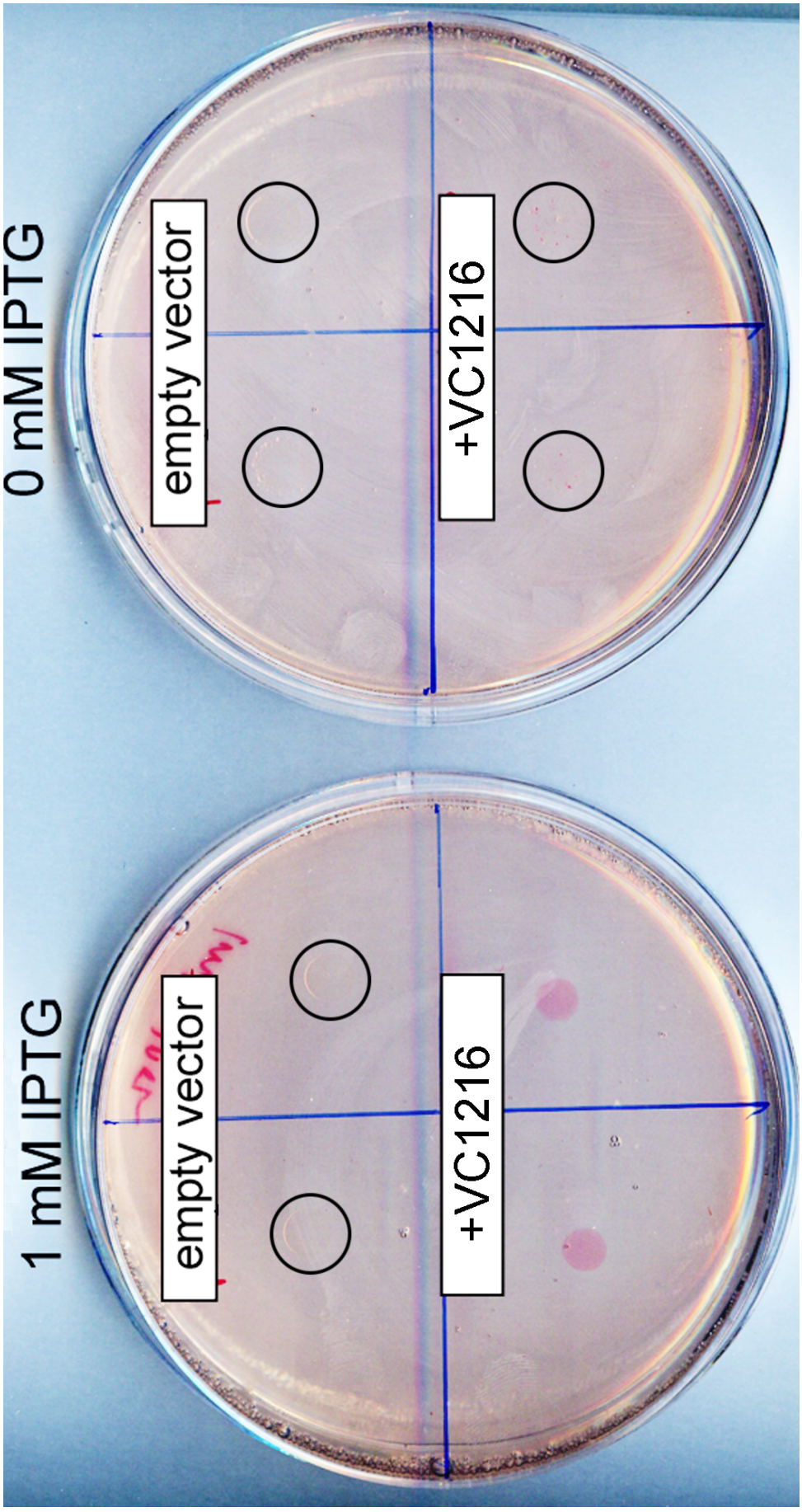

Figure S6. Magnified and 90-degree-rotated view of the Congo Red plate image in Figure 6.