Supporting Information

for

Crystallographic and Spectroscopic Insights into Heme Degradation by *Mycobacterium tuberculosis* MhuD

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Contents

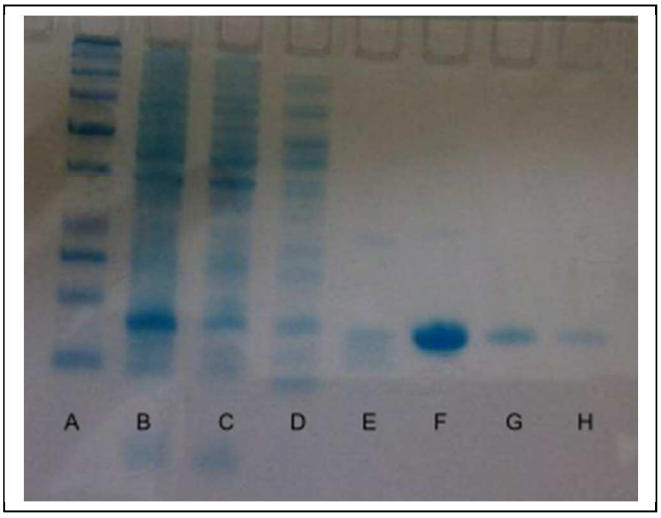

Figure S1. SDS-PAGE gel for the modified apo-MhuD purification. From left to right, the lanes represent: (A) PageRuler Plus prestained protein ladder (Pierce), (B) MhuD lysate, (C) Ni-NTA column flow through, (D) 25 mM imidazole wash, (E) 75 mM imidazole wash, (F) purified MhuD, (G) 1/10 dilution of purified MhuD, and (H) 1/100 dilution of purified MhuD.

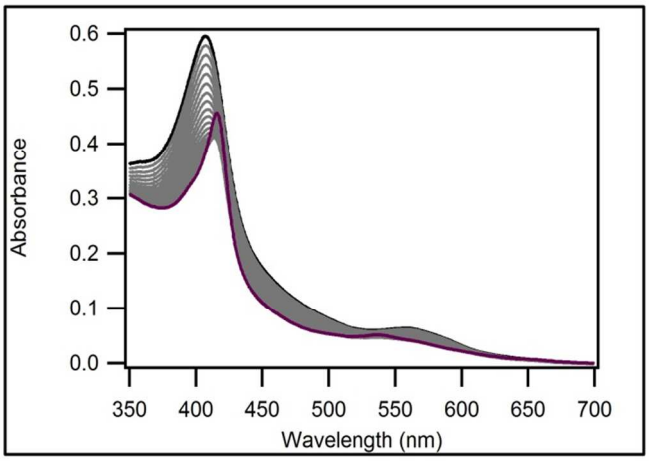

Figure S2. Heme/ascorbate degradation assay for MhuD–heme in 20 mM NaPi pH 7.8.[1] 10 mM ascorbate was used as a reductant and an Abs spectrum was measured every 5 minutes for 1 hour. The solid black line represents initial spectrum, the solid purple line is the final recorded spectrum, and the grey lines are the intermediate readings.

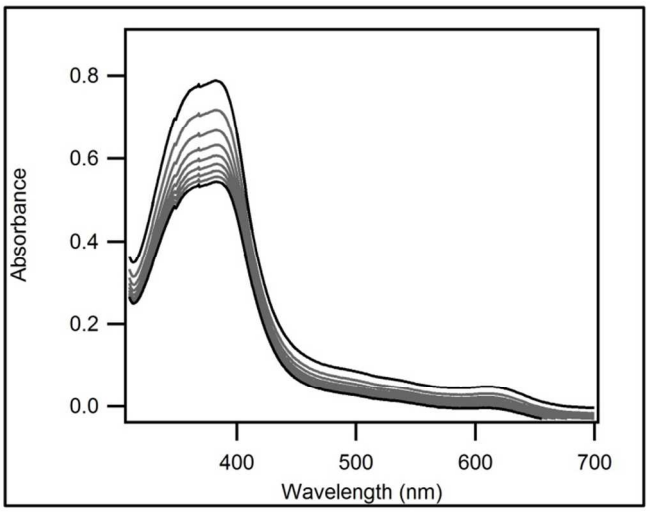

Figure S3. Heme/acsorbate degradation assay for heme without MhuD in 20 mM Tris pH 7.4, 50 mM NaCl. Ascorbate was titrated in to the heme solution over a 2 hour period until a final concentration of 50 mM ascorbate was reached.

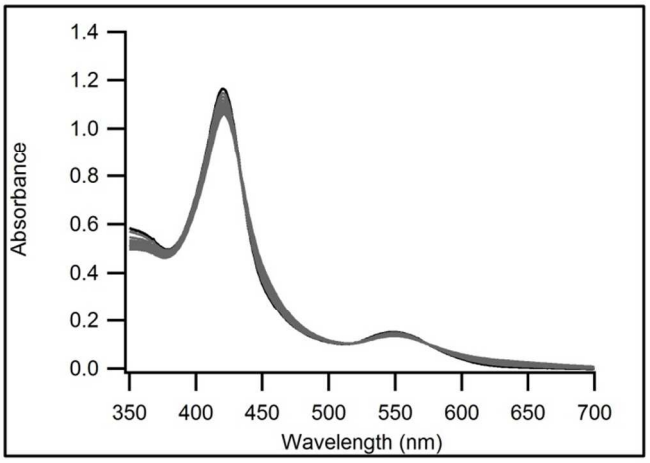

Figure S4. Heme/ascorbate degradation assay for MhuD–heme–CN in 20 mM NaPi pH 7.4. 100 mM ascorbate was used as a reductant and an Abs spectrum was measured every 5 minutes for 2 hours.

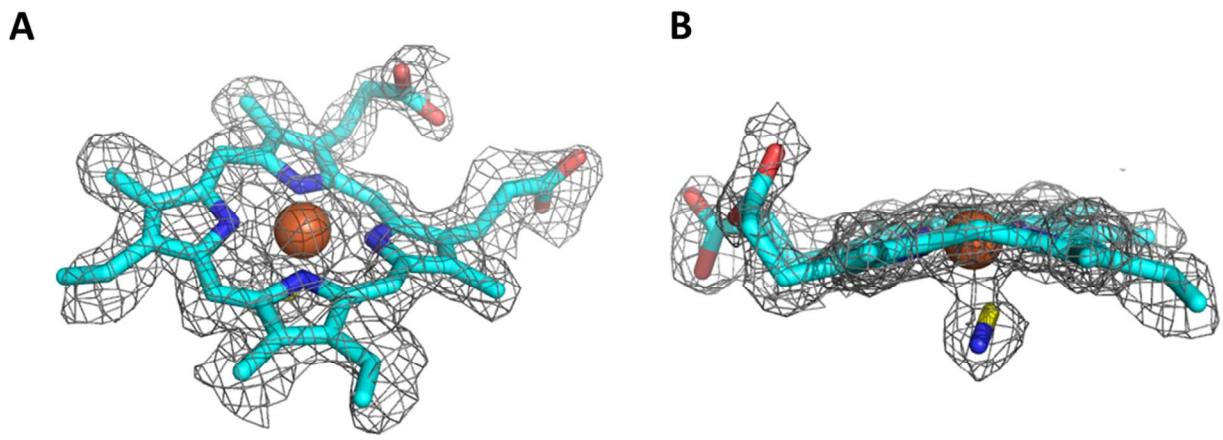

Figure S5. $2mF_o$-ΔF_c composite omit electron density map for bound heme–CN calculated with the heme and cyanide ligands omitted. The electron density mesh contoured at 1.0σ is colored in grey. Heme–CN is represented as a stick model, where nitrogen, oxygen, heme carbon, and cyano carbon atoms are in blue, red, cyan, and yellow, respectively. Iron is depicted as on orange sphere. Heme is shown in two confirmations to demonstrate that the (A) vinyl groups fit the density well and that the (B) porphyrin ring is distorted.

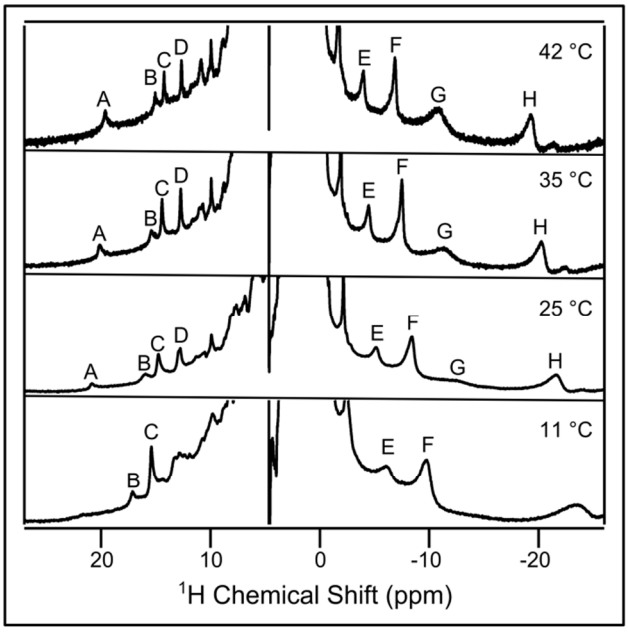

Figure S6. ^{1}H Super-WEFT spectra of 1.5 mM MhuD–heme–CN in 20 mM NaPi pH 7.4 at 11°, 25°, 35°, and 42°C.

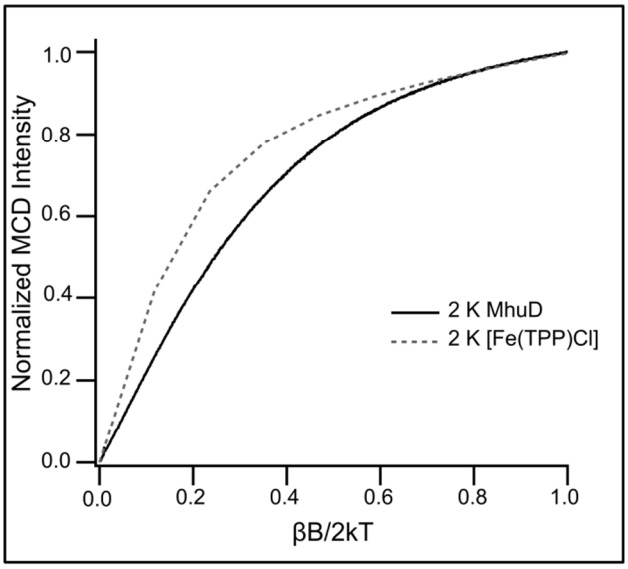

Figure S7. VTVH MCD saturation magnetization curves of MhuD–heme–CN (experiment) and high-spin chloro(*meso*-tetraphenylporphinato)iron(III) at 2 K (simulation) with $D = 6.9$ cm^{-1}.[2]

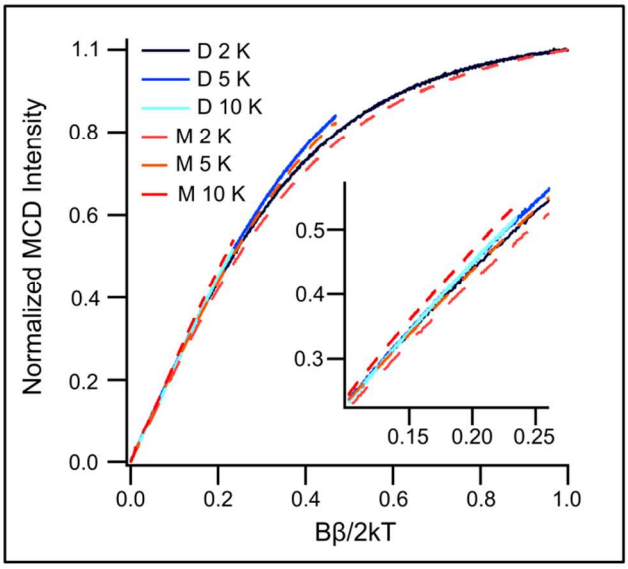

Figure S8. VTVH MCD saturation magnetization curves for MhuD-heme-CN at 2 K (M 2 K), 5 K (M 5 K), 10 K (M 10 K); and MhuD-diheme-CN at 2 K (D 2 K), 5 K (D 5 K), and 10 K (D 10 K). All MhuD–diheme–CN curves fall between the 2 K and 10 K curves of MhuD–heme–CN.

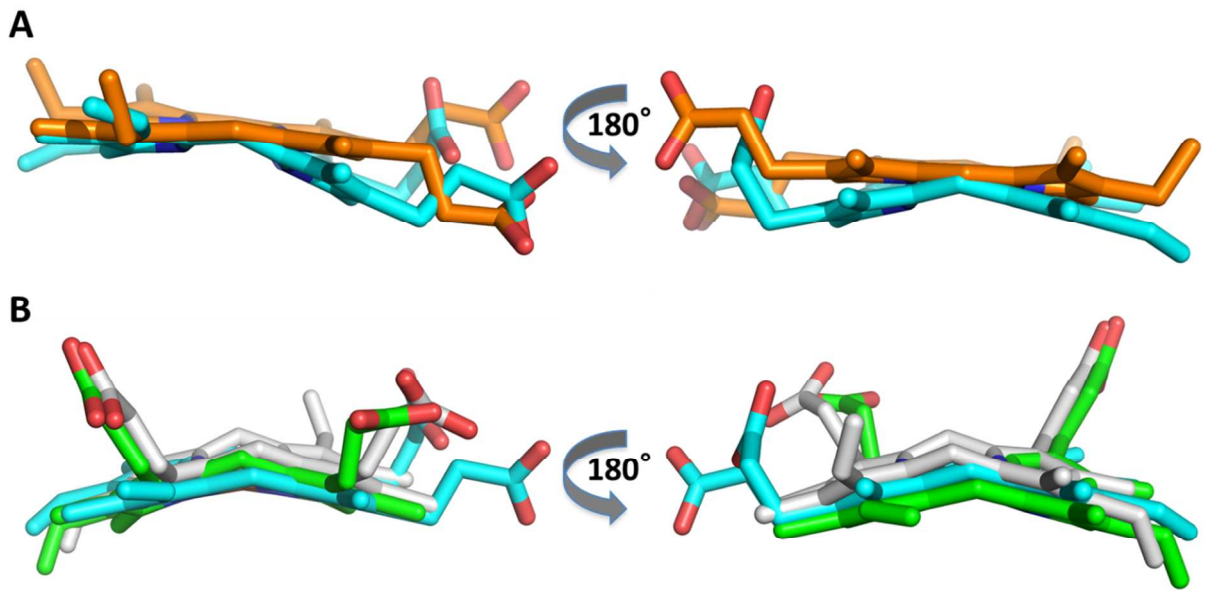

Figure S9. Degree of heme ruffling within heme degrading enzymes. Panel A: Overlay of MhuD–heme–CN (cyan) and rHO–heme–CN (orange, PDB ID 2E7E). Both panels show rHO heme being mostly planar. Overlay of hemes from MhuD–heme–CN (cyan, PDB ID 4NL5), N7A IsdG (white, PDB ID 2ZDO) and IsdI–heme–CN (green, PDB ID 3QGP) following structural overlay.

REFERENCES

(1) Chim, N.; Iniguez, A.; Nguyen, T. Q.; Goulding, C. W. *J. Mol. Biol.* **2010**, *395*, 595-608.
(2) Browett, W. R.; Fucaloro, A. F.; Morgan, T. V.; Stephens, P. J. *J. Am. Chem. Soc.* **1983**, *105*, 1868-1872.