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Title	A heme degradation enzyme, HutZ, from Vibrio cholerae
Author(s)	Uchida, Takeshi; Sekine, Yukari; Matsui, Toshitaka et al.
Citation	Chemical Communications, 48(53), 6741-6743 https://doi.org/10.1039/c2cc31147j
Issue Date	2012-06
Doc URL	https://hdl.handle.net/2115/68577
Type	journal article
File Information	supporting information.pdf

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Supporting Information for

A Heme Degradation Enzyme, HutZ from, *Vibrio cholerae*[*]

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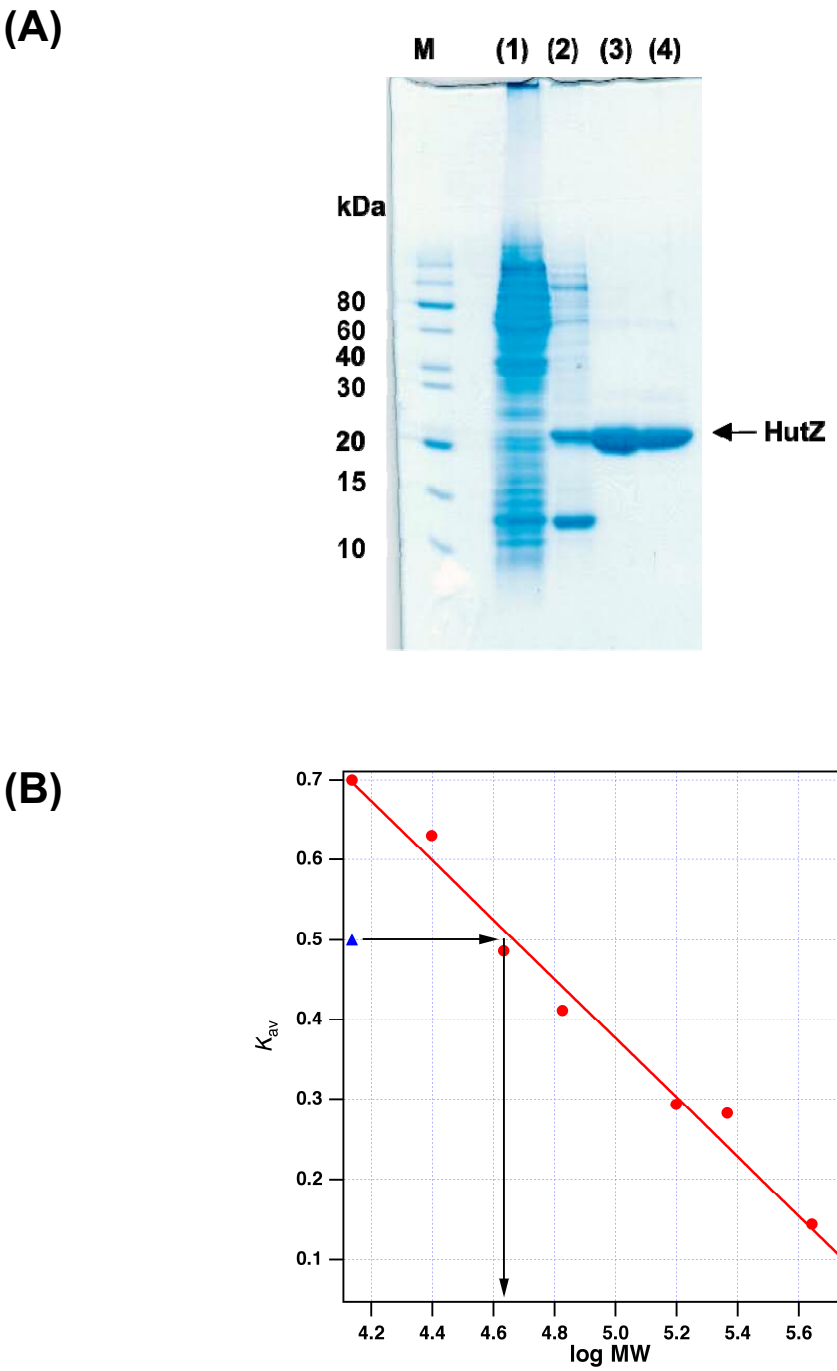

Fig. S1. Purification of HutZ. A, analysis by SDS-PAGE gel electrophoresis of different fractions. For electrophoresis, a 12.5% polyacrylamide gel was used, and the gel was stained with CBB Stain One (Nacalai Tesque). Lane M, molecular weight markers; lane 1, whole cell protein extracts; lane 2, partially purified HutZ after the first HisTrap (GE Healthcare) affinity chromatography; lane 3, His_6-tag cleaved HutZ by PreScission protease (GE Healthcare), followed by the second HisTrap affinity chromatography; lane 4, purified His_6-tag cleaved HutZ after the gel-filtration chromatography (HiLoad 16/600 Superdex 200 pg, GE Healthcare). B, Determination of molecular weight of HutZ by gel-filtration column chromatography. Analytical gel filtration was performed using a Superdex 200pg column (GE Healthcare) equilibrated with 50 mM Tris-HCl/150 mM NaCl, pH 8.0 with a flow rate of 1 mL/min. The plot shows K_{av} *versus* the log of molecular weight standards including the following protein standards: chymotrypsinogen, albumin, aldolase, catalase, ferritin, and blue dextran (GE Healthcare). K_{av} of HutZ was 0.497, which corresponds to logMW of 4.62.

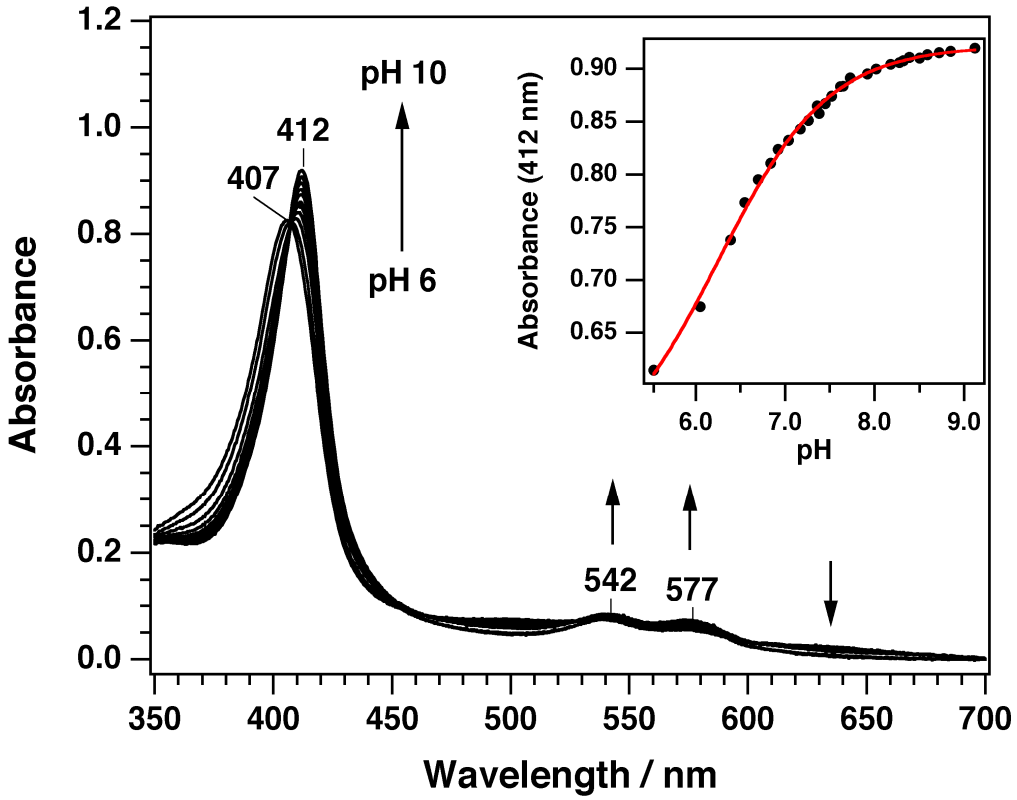

Fig. S2. Absorption spectra of the ferric HutZ-heme complex as a function of pH. The pH of the solution was adjusted by incremental addition of HCl to the HutZ sample in 50 mM Tris-Cl/150 mM NaCl. Inset, pH-dependence of the absorbance at 412 nm. The pK_a for the transition from the high-spin Fe^{3+}-H_2O complex to the low-spin Fe^{3+}-OH^- complex was measured by following the absorption at 412 nm and by fitting the data to the Henderson-Hasselbach equation.

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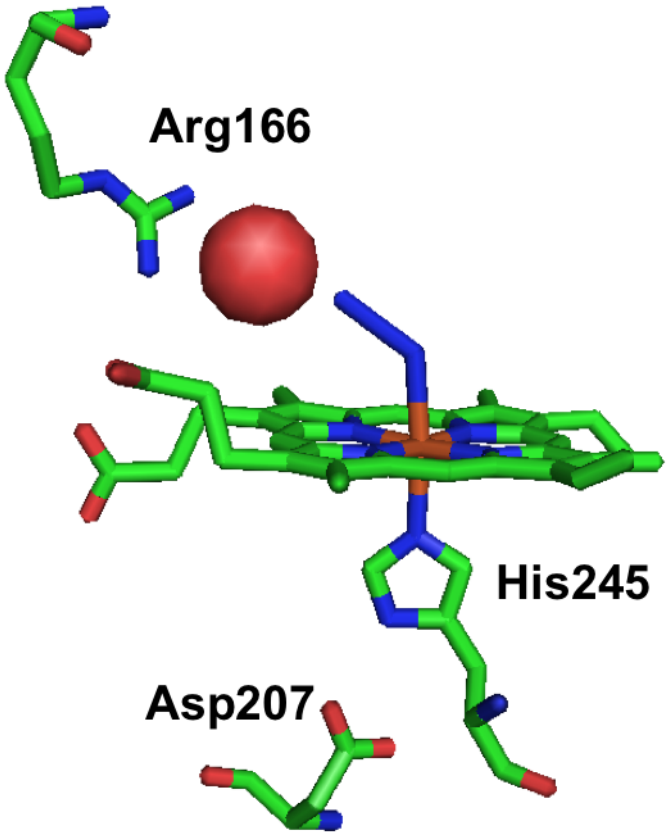

Fig. S3. Heme environmental structure of HutZ homologous protein, HugZ from *H. pylori* (PDB accession number 3GAS; Y. Hu, F. Jiang, Y. Guo, X. Shen, Y. Zhang, R. Zhang, G. Guo, X. Mao, Q. Zou and D. C. Wang, *J. Biol. Chem.*, 2011, **286**, 1537.)

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HutZ  ------------------------------------------------------------
HugZ  MLNRIIEHMNAHHVEDMKGLLKKFGQVHHAENVAFKSVDSQGIVIGYNNNQTLRIEFNHE 60


HutZ  -------------MDQQVKQERLQGRLEPEIKEFRQERKTLQLATVDAQGRPNVSYAPFV 47
HugZ  VKDPKDYKNATIELCQSVEKTHDLKGVEEEVKAFKEGFDSVCLATLHPNGHVVCSYAPLM 120
                   : *.*:: :    :* *:* *::  .:: ***:..:*:   *****::

HutZ  QNQEGYFVLISHIARHARNLEVNPQ-VSIMMIEDETEAKQLFARKRLTFDAVASMVERDS 106
HugZ  SDGKQYYIYVSEVAEHFAGLKNNPHNVEVMFLEDESKAKSAILRKRLRYKTNTRFIERGA 180
      .: : *:: :*.:*.*  .*: **: *.:*::***::**. : **** :.: : ::**.:

HutZ  ELWCQVIAQMGERFG-EIIDGLSQLQDFMLFRLQPEQGLFVKGFGQAYQVSGDDLVDFVH 165
HugZ  EFDKAFDSFIEKTGGAGGIKTIRAMQDFHLIALDFKEGRFVKGFGQAYDILGDKIAYVGD 240
      *:   . : : :  *   *. :  :*** *: *: ::* *********:: **.:. . .

HutZ  LEEGHRKISNG 176
HugZ  KGNPHNFAHKK 251
        : *.   :
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Fig. S4. Amino acid sequence alignment of HutZ with *H. pylori* HugZ. The alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).[1] The histidine heme axial ligand in bold type and in red and active site arginine are shown in bold type and in blue.

1. M. A. Larkin, G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson and D. G. Higgins, *Bioinformatics*, 2007, **23**, 2947

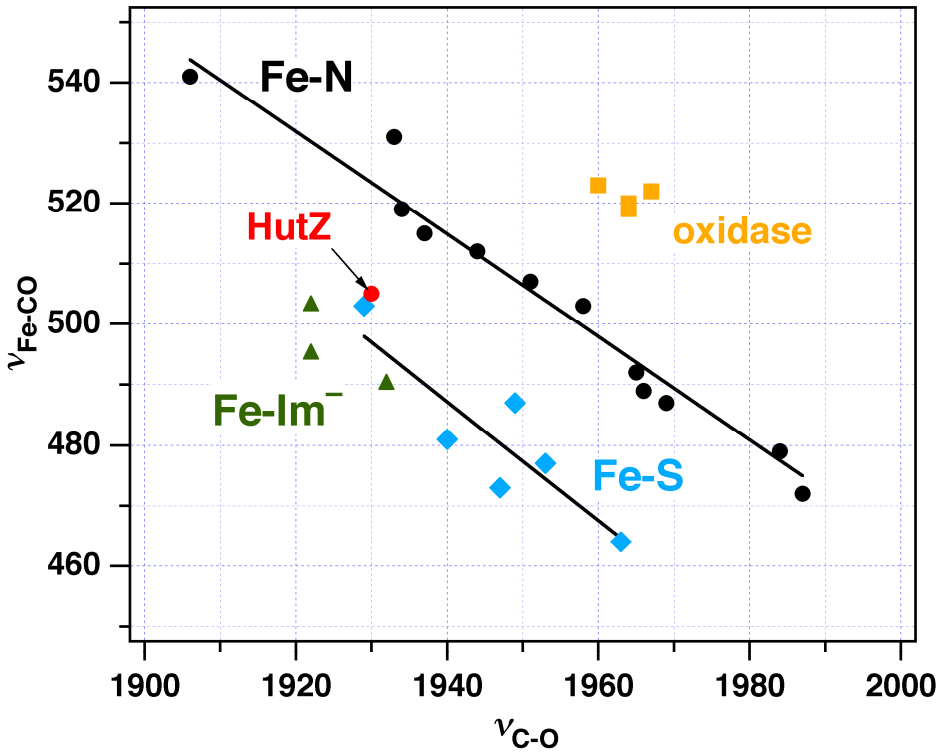

Fig. S5. Correlation plot between frequencies of the $\nu_{Fe\text{-}CO}$ and $\nu_{C\text{-}O}$ stretching modes. The two solid lines correspond to correlations for proximal imidazoles (solid circles in black), proximal imidazolates (solid triangles in green) and thiolate-ligated hemoproteins (solid diamonds). The data points for the oxidase superfamilies are depicted as solid squares in oranges. The data point for HutZ is presented as a solid circle in red. The data shown in the $\nu_{Fe\text{-}CO}$ versus $\nu_{C\text{-}O}$ inverse correlation plot are taken from refs 2-4.

2. G. B. Ray, X. Y. Li, J. A. Ibers, J. L. Sessler and T. G. Spiro, *J. Am. Chem. Soc.*, 1994, **116**, 162.

3. B. S. Lou, J. K. Snyder, P. Marshall, J. S. Wang, G. Wu, R. J. Kulmacz, A. L. Tsai and J. L. Wang, *Biochemistry*, 2000, **39**, 12424.

4. T. G. Spiro and I. H. Wasbotten, *J. Inorg. Biochem.*, 2005, **99**, 34.

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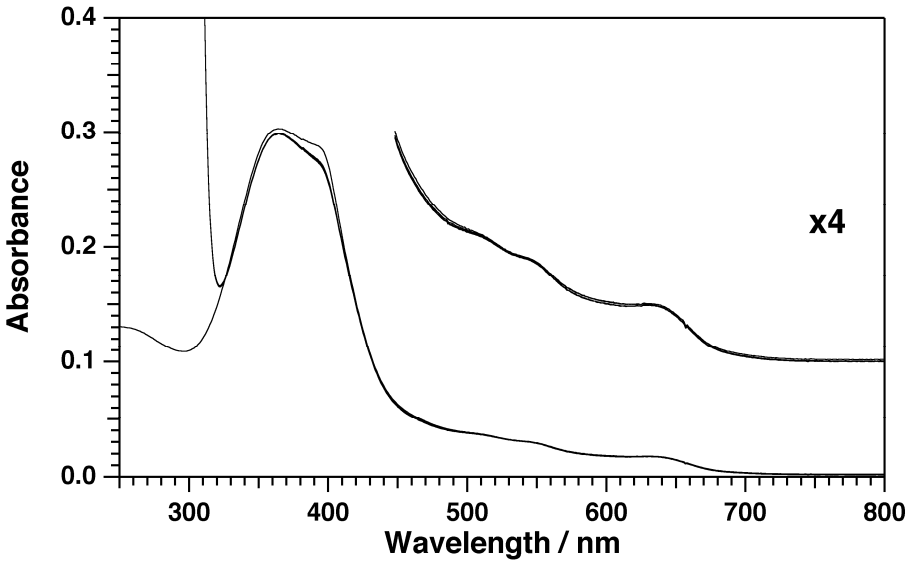

Fig. S6. Reaction of hemin with ascorbic acid. The spectra were taken before and at 0.5, 1, 2, 3, and 4 min after addition of ascorbic acid (1 mM) to hemin (5 μM) in 50 mM MES, pH 6.0 at 25 °C. The progression of the spectra is indicated by the arrows.

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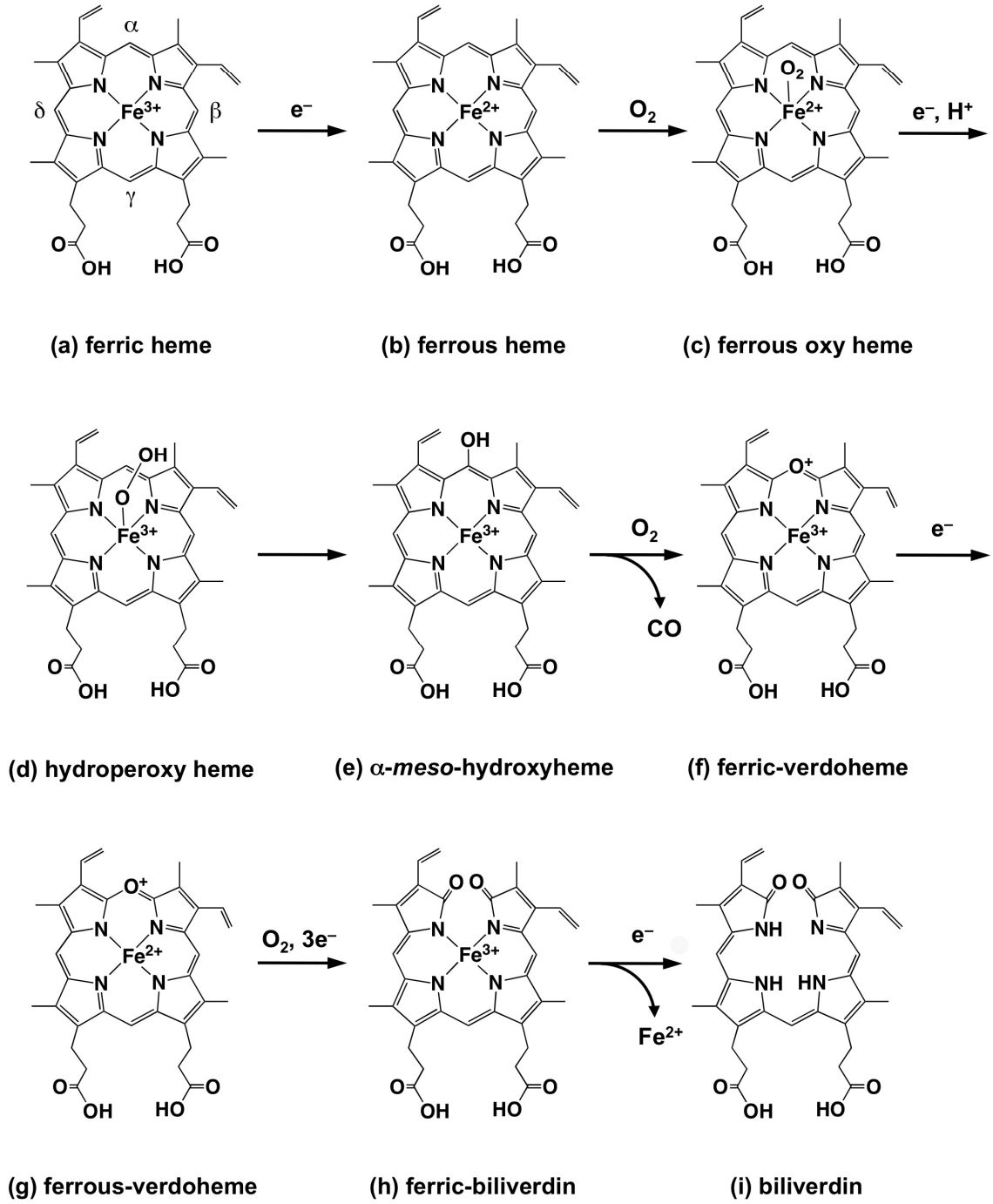

Fig. S7. Heme degradation mechanism of heme oxygenase (M. Unno, T. Matsui and M. Ikeda-Saito, *Nat. Prod. Rep.*, 2007, **24**, 553).

(A)

(B)

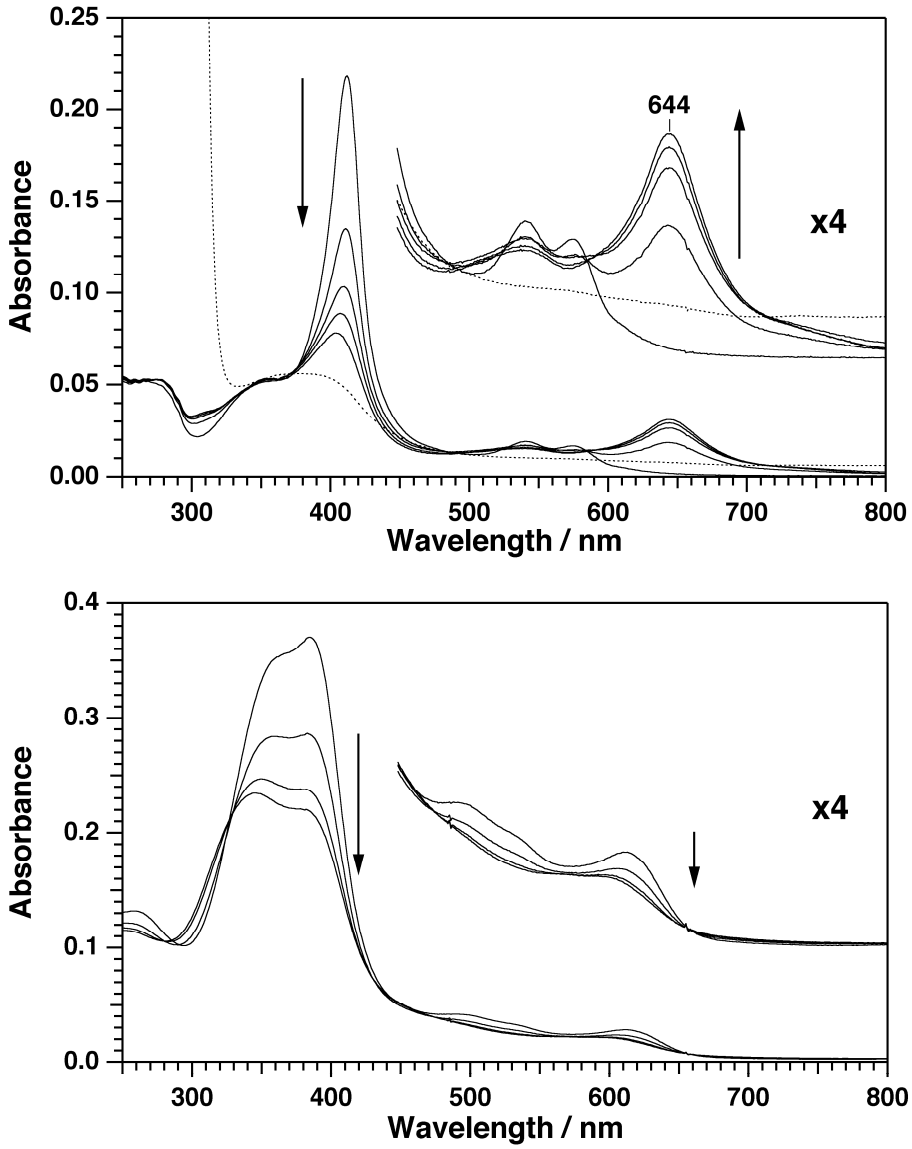

Fig. S8. Reaction of HutZ or hemin with H_2O_2. A, the spectra were taken before and at 0.5, 1, 1.5, and 3 min after addition of H_2O_2 (1 mM) to ferric heme-HutZ (1 μM) in 50 mM Tris-Cl/150 mM NaCl, pH 8.0 at 25 °C. The progression of the spectra is indicated by the arrows. At 3 min after the addition of H_2O_2, ascorbic acid (10 mM) was added to the H_2O_2-treated HutZ. The spectrum at 30 s after the addition of ascorbic acid to the H_2O_2-treated HutZ is shown in a dotted line. B, the reaction of hemin (6 μM) with H_2O_2 under the same reaction condition as in Fig. S7 (A).

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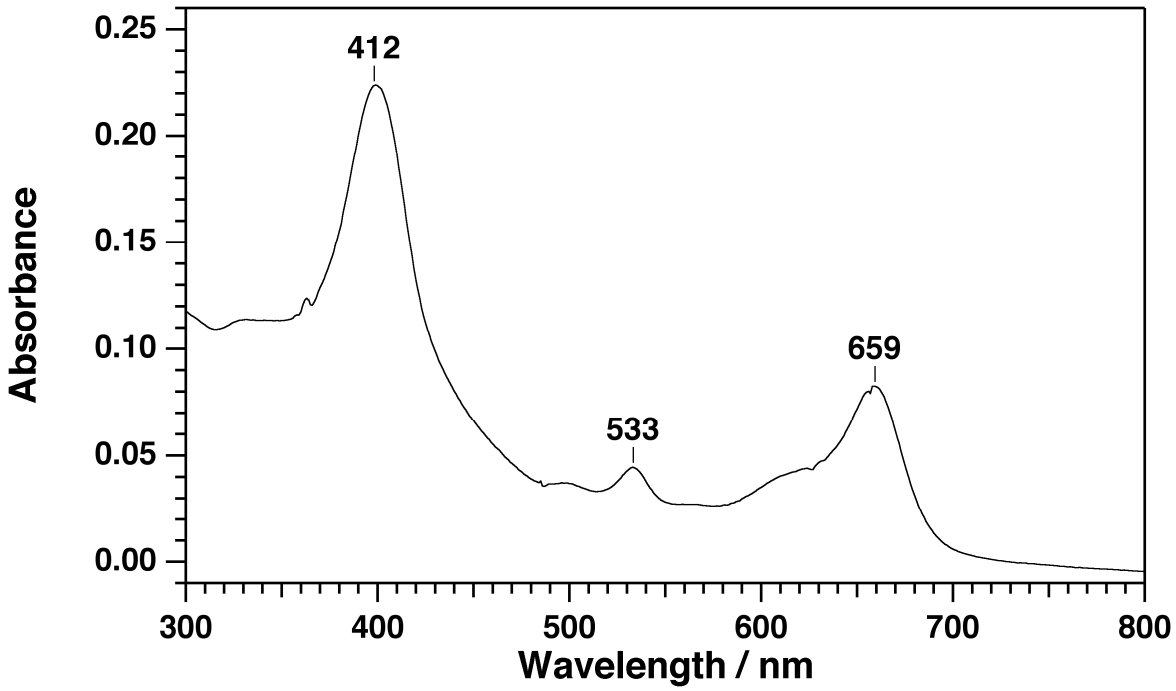

Fig. S9. Absorption spectrum of verdoheme in aqueous pyridine. Ferric heme-bound HutZ was reacted with H_2O_2 under the same condition as that in Figure S7. 10 min after the reaction, pyridine was added to the reaction mixture to the final concentration of 33% to liberate the product from the protein.