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Subunit-Subunit Interactions Play a Key Role in The Heme-Degradation Reaction of HutZ from *Vibrio cholerae*

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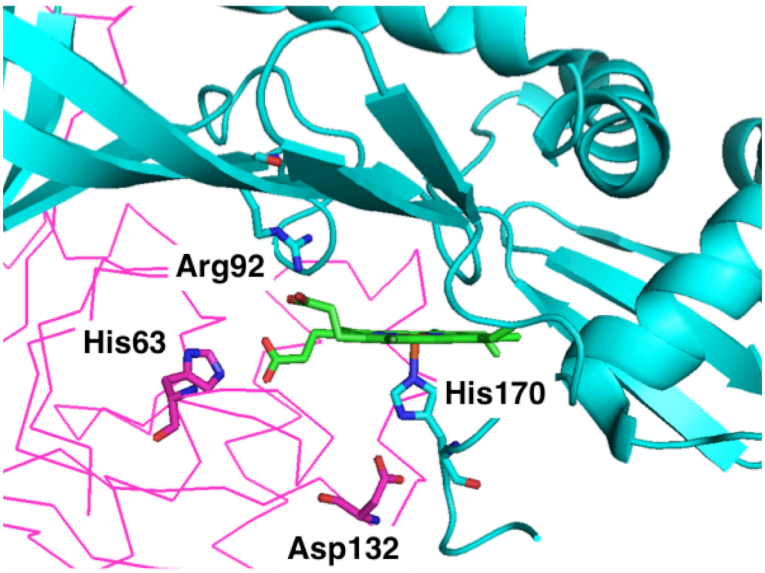

Fig. S1 Crystal structure of HugZ from *Helicobacter pylori* (PDB 3GAS) at the heme-binding site. Residues are numbered according to the amino acid sequence of HutZ from *V. cholerae*.

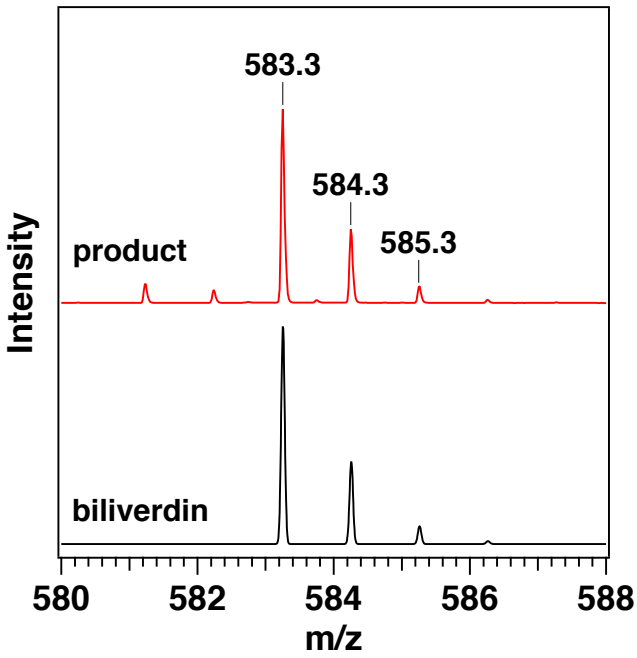

Fig. S2 Mass spectrum of final product in the reaction of HutZ with H_2O_2.

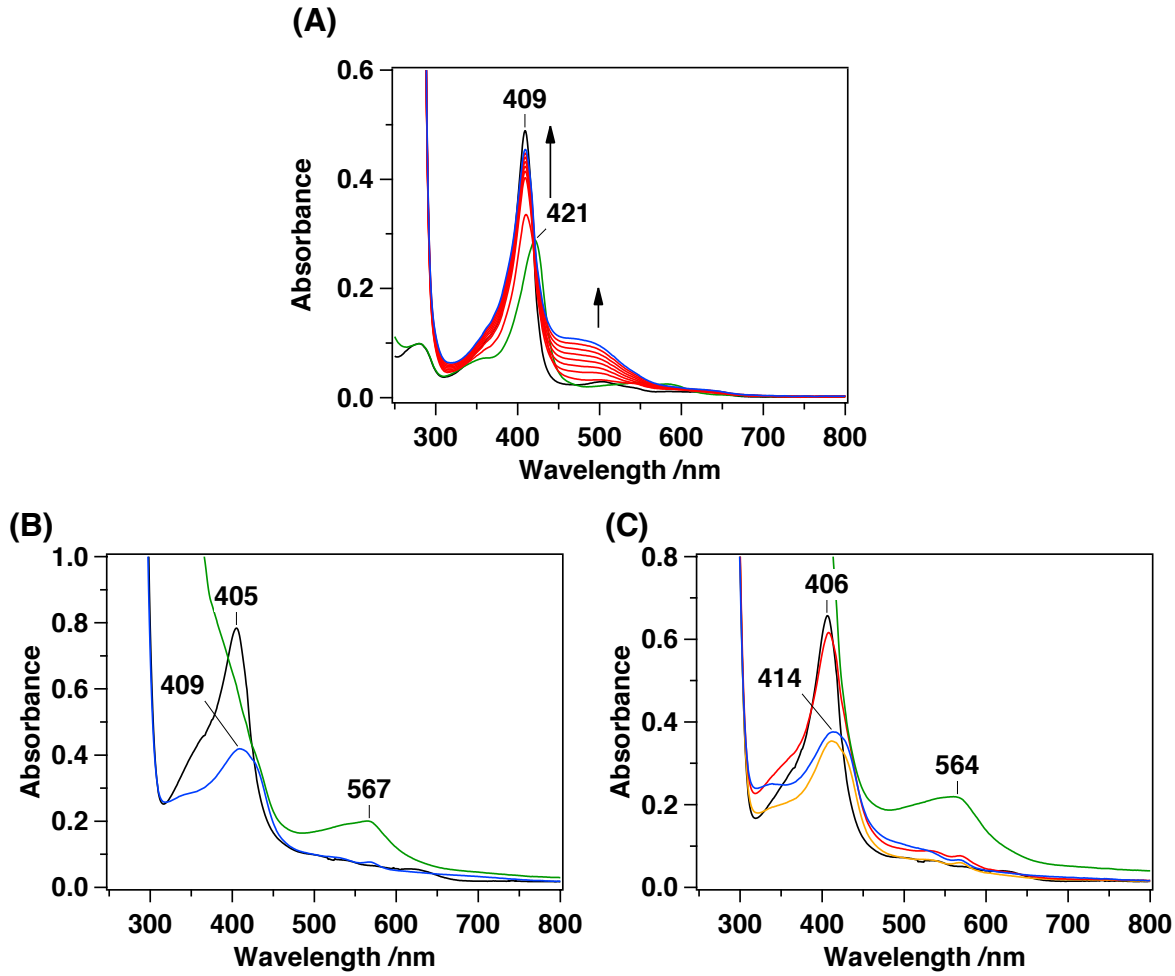

Fig. S3 (A) Reaction of myoglobin (3 μM) with H_2O_2 (0.2 mM) before (black) and after (green) the addition of H_2O_2. Guaiacol was added to the H_2O_2-treated myoglobin. Spectra were recorded at 4-min intervals for 28 min after addition of guaiacol (red). (B) Heme-degradation reaction of HutZ with ascorbic acid in the presence of catalase at pH 6.0. The concentrations of the protein, heme and ascorbic acid were 3 μM, 6 μM and 1 mM, respectively. The black line showed the spectrum immediately after the initiation of the reaction by adding HutZ to the solution containing heme, ascorbic acid, and catalase, and the blue line showed the spectrum 2 hours after the initiation of the reaction. (C) Heme-degradation reaction of 1 equivalent of heme-bound HutZ with ascorbic acid in the presence of catalase at pH 6.0. The orange line showed the spectrum 1 hour after the reaction. Then, 1 equivalent of heme was added (red) and incubated for 1 hour (blue). The green line showed the spectra after addition of ferrozine 60 min after initiation of the reaction.

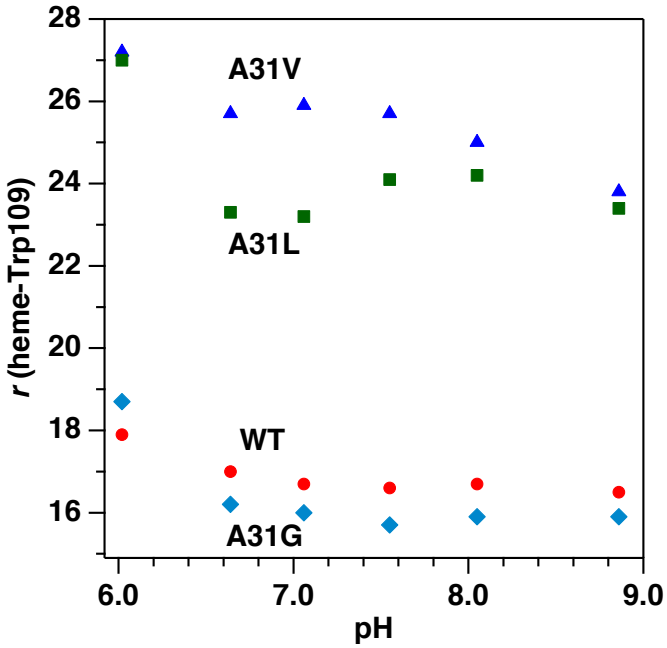

Fig. S4 pH dependence of the distance between heme and Trp109 estimated by quenching of tryptophan fluorescence by energy transfer.

```
VcHutZ          ------------------------------------------------------------
PjHutZ          ------------------------------------------------------------
HsHutZ          ------------------------------------------------------------
SaHutZ          ------------------------------------------------------------
HpHugZ          -MLNRIIEHXNAHHVEDXKGLLKKFGQVHHAENVAFKSVDSQGIVIGYNNNQTLRIEFNH
CjHugZ          MNFESIISHMNDHHKSNLVDLCKKFGGIEQVQDVFLKSVDFNGLDLVYNDKENLRVEFPK

VcHutZ          --------------MDQQVKQERLQGRLEPEIKEFRQERKTLQLATVDAQGRPNVSYAPF
PjHutZ          ---------------MSQVKQERLQNRLGPEIKEFRESCLTLQLATVDADGKPNVSYAPF
HsHutZ          --------------MTETNRQEVLQNRLGPEIQDLKAQSKTIMLATVGKDGTPNVSYAPF
SaHutZ          ----------------MTSENQRLQEKLLPEIEEFKQGRTTLQLATQDANGIPNASYAPF
HpHugZ          EVKDPKDYKNATIELCQSVEKTHDLKGVEEEVKAFKEGFDSVCLATLHPNGHVVCSYAPL
CjHugZ          KADEN-TIKDTIISLCMSAKSEQNFSGVEKELNEFMLSFNSVALATLNVNGEVVCSYAPF
                                    ..      :  *:: :     :: ***   :*    ****:

VcHutZ          VQNQEGYFVLISHIARHARNLEVNP-QVSIMMIEDETEAKQLFARKRLTFDAVASMVERD
PjHutZ          ALLDDGYYVLISQIARHARNLLENP-QVSLMMIEDEGTAKTLYARKRLTFEADVSVVERE
HsHutZ          AISNGEYQVFISTIARHARNLQEVP-KVSLMLIEDESKSRQIFARRRLSFDATSRVIERE
SaHutZ          ALADDGFYILVSDLARHGINLKQSP-RVSVMLVEDEAEARSVFARRRLTFDAAAELIARD
HpHugZ          XSDGKQYYIYVSEVAEHFAGLKNNPHNVEVXFLEDESKAKSAILRKRLRYKTNTRFIERG
CjHugZ          VSTQWGNYIYISEVSEHFNNIKANPNNIEIMFLEDESKAASVILRKRLRYRVDASFLERD
                       : :* ::.*  .:   * .:.: ::***  :     *:** : .   .: *

VcHutZ          SELWCQVIAQMGERFG-EIIDGLSQLQDFMLFRLQPEQGLFVKGFGQAYQVSGDDLVDFV
PjHutZ          TERWAEAVAGLKTRFG-EIIDGLSGLEDFKMFRLAPTQGLFVKGFGQAFQVSGDDLVDFV
HsHutZ          TEEWKASIAVLKERHG-ALIDEISTYKDFYLFSFKPIQGLFVKGFGQAFQVSNEDLVSFV
SaHutZ          SQGFAKGVQVLSGRFG-EMIDNLAALTDFNLFKLVPERGLYVKGFGQAFSLSGAELLDVN
HpHugZ          AEFDKAFDSFIEKTGGAGGIKTIRAXQDFHLIALDFKEGRFVKGFGQAYDILGDKIAYVG
CjHugZ          ERFDQIYDEFEKQTGGEGGIKTIRKMLDFHLVKLEFKKGRFVKGFGQAYDIENGNVAHVG
                 .             *   *. :    ** :. :   .* :*******:.: . .:  .

VcHutZ          HLEEGHRKISNG----------
PjHutZ          HLTEGHRRIKDGSEVESPTEKL
HsHutZ          HLTESHQDN-------------
SaHutZ          WMRDGHHGTPKAVPA-------
HpHugZ          DKGNPHNFAHKKLE--------
CjHugZ          ASGNRHKH--------------
                   : *.
```

Fig. S5 Sequence alignment of HutZ and member of HugZ family. Proteins used in the alignment are (from top to bottom): *Vc*HutZ, HutZ from *Vibrio cholerae*, *Pj*HutZ, HutZ from *Photobacterium jeanii*, *Hs*HutZ, HutZ from *Histophilus somni*, *Sa*HutZ, HutZ from *Shewanella algae*, *Hp*HugZ, HugZ from *Helicobacter pylori*, *Cj*HugZ, HugZ from *Campylobacter jejuni*.

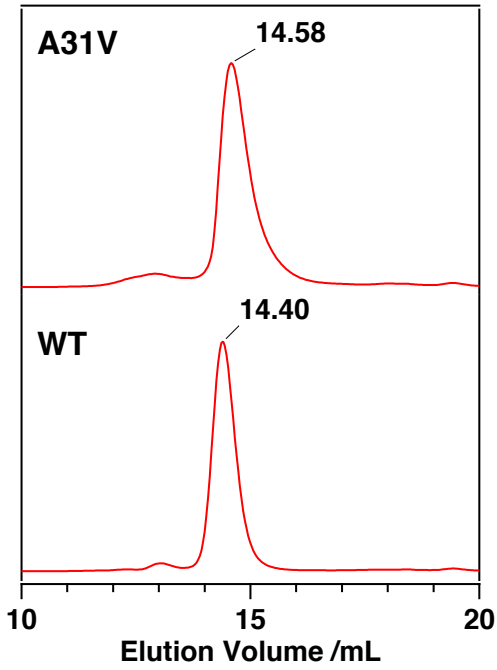

Fig. S6 Analytical gel filtration was performed using an ENrich SEC 650 10/300 column (Bio-Rad) column equilibrated with 50 mM Tris-HCl, 150 mM NaCl (pH 8.0) with a flow rate of 1 mL min^{-1}.

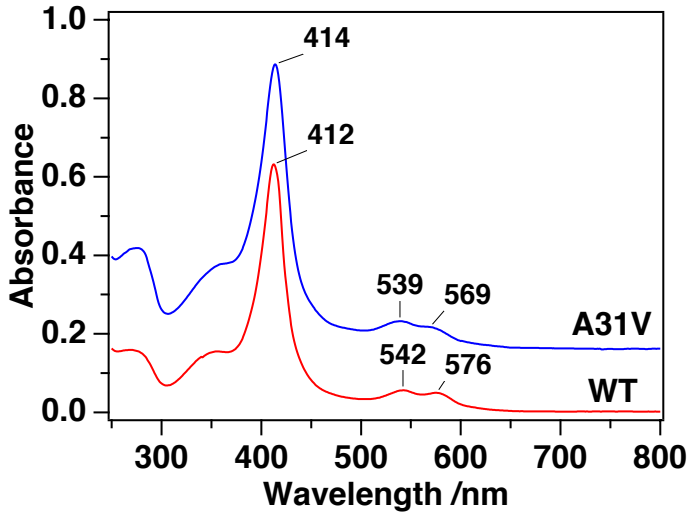

Fig. S7 Absorption spectra of the ferric heme-WT and heme-A31V mutant in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0).

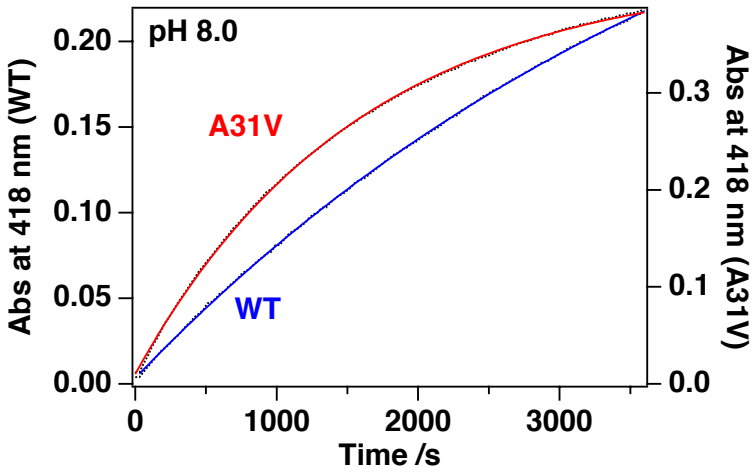

Fig. S8 Reduction of ferric heme-HutZ. Time course of absorbance changes at 419 nm at pH 8.0 after the addition of ascorbic acid (final concentration, 1 mM) to the heme-A31V mutant under a CO atmosphere is compared with that of heme-WT HutZ.

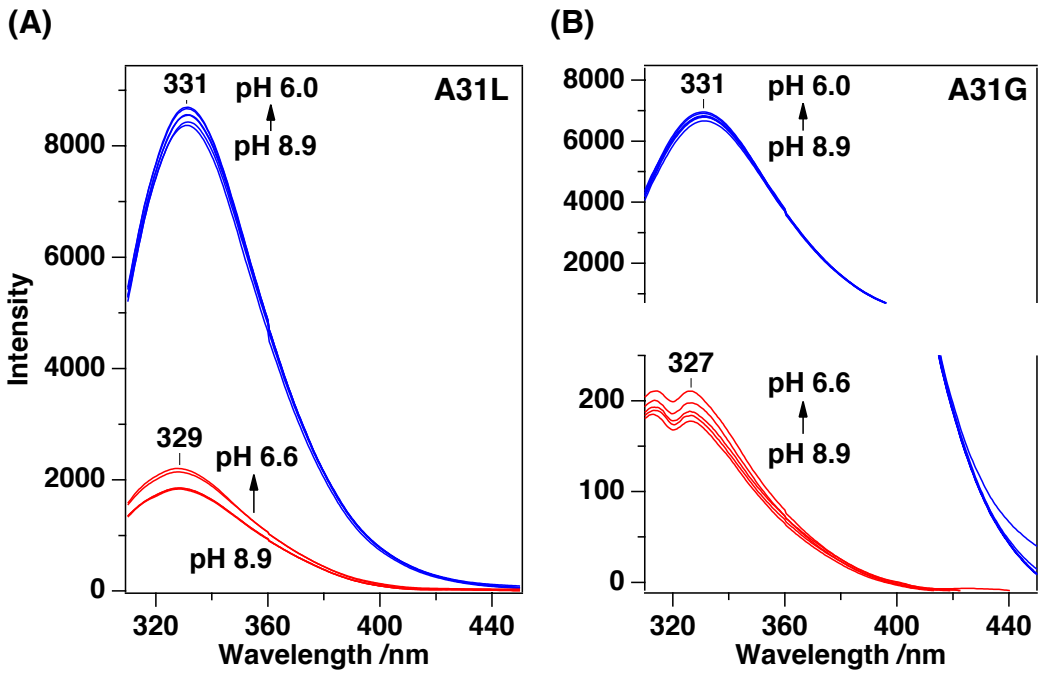

Fig. S9 Fluorescence spectra of (A) heme-A31L and (B) heme-A31G mutant. (C) pH-dependence of the fluorescence intensity at 331 nm. The spectra were recorded with excitation at 295 nm. The sample concentration was 3 μM.

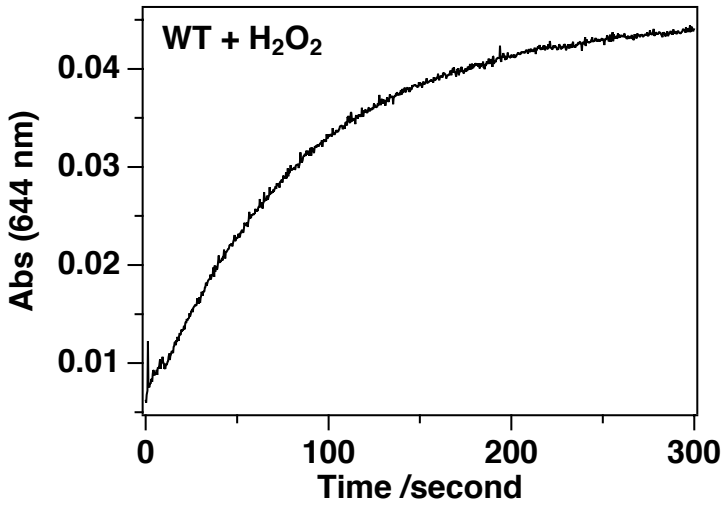

Fig. S10 Time course of absorbance at 644 nm after the reaction of heme-WT HutZ with H_2O_2 in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0).