Supporting Information

Heme Binding to Porphobilinogen Deaminase from *Vibrio cholerae* Decelerates the Formation of 1-Hydroxymethylbilane

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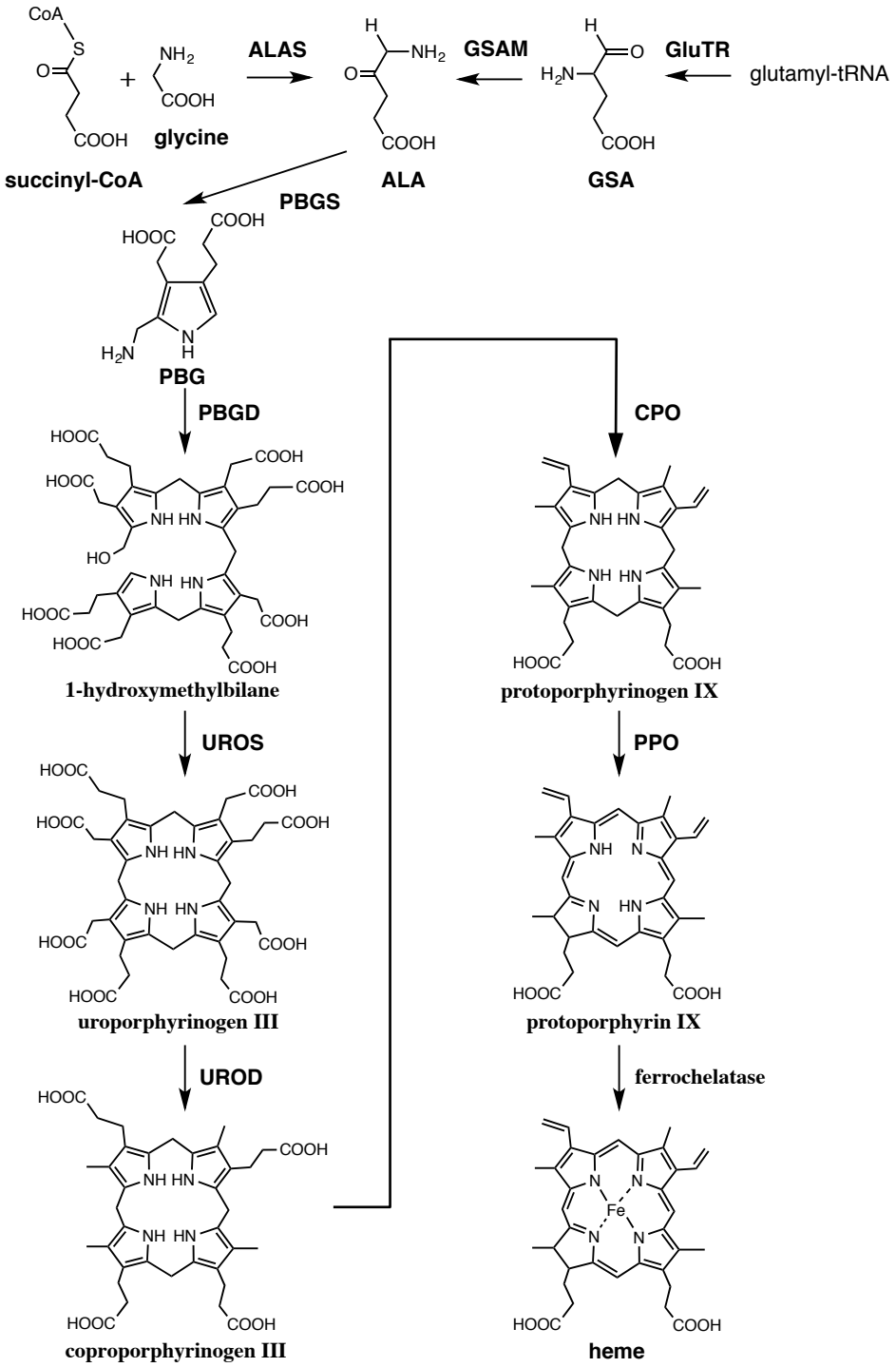
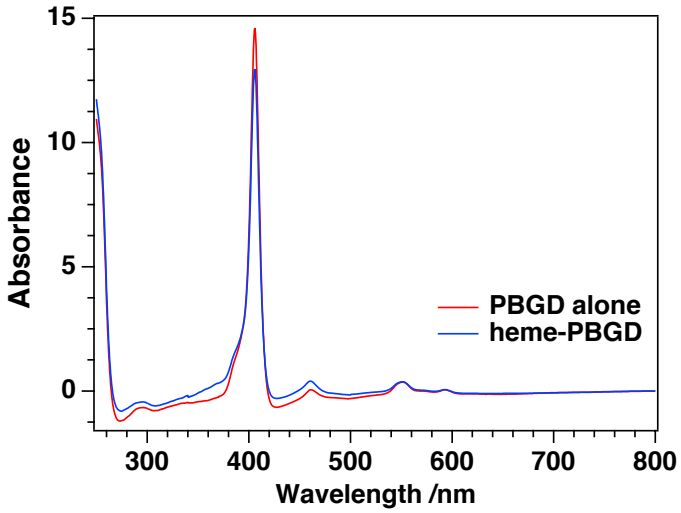
Supplementary Table 1: Oligonucleotides used for construction of expression vectors for mutants. The underlined bases signify the introduced mutations.

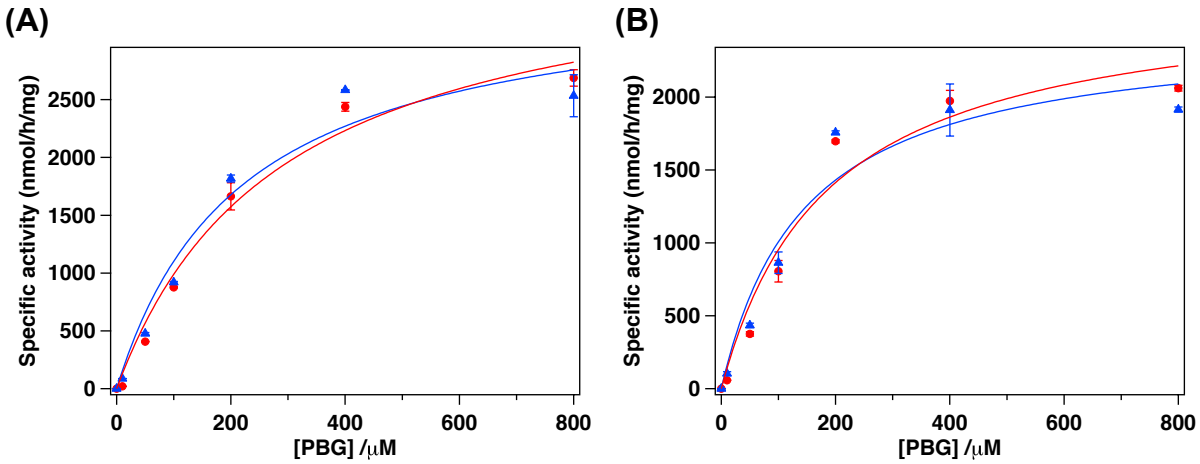
Mutants	Primers (up, sense; bottom, anti-sense)
C105S	5'-ACCATCTCTGAACGGGAAGATCCGCGT-3'
	5'-CCGTTCAGAGATGGTGACTAAGCCCA-3'
C105H	5'-ACCATCCATGAACGGGAAGATCCGCGT-3'
	5'-CCGTTCATGGATGGTGACTAAGCCCA-3'
C211S	5'-ATTGAATCTCGCGTGAATGATCAACG-3'
	5'-CACGCGAGATTCAATACCAACGGCAC-3'
C248S	5'-GGTGGCTCTCAGGTGCCGATTGGTAG-3'
	5'-CACCTGAGAGCCACCTTGTAACGTGA-3'
H227D	5'-TCTTAACGATGCTGATACCGCCGATC-3'
	5'-TCAGCATCGTTAAGAGGTGCGAGTAG-3'



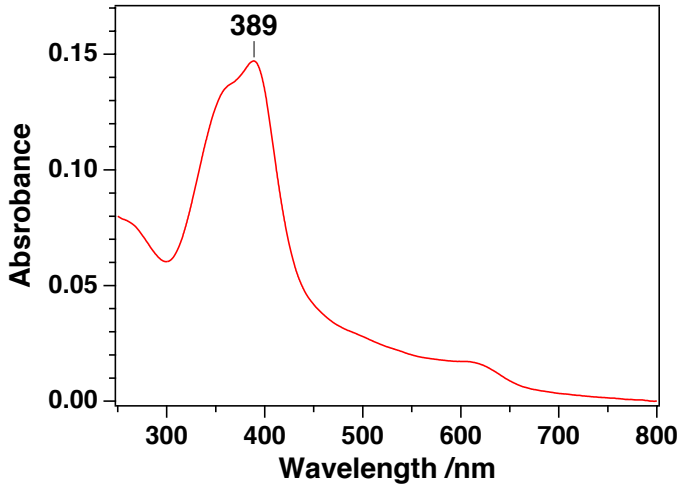
Supplementary Figure 1 Heme biosynthesis. ALA, 5-aminolevulinic acid; ALAS, ALA synthase; GSA, glutamate-1-semialdehyde; GSAM, glutamate-1-semialdehyde-2, 1-aminomutase; GluTR, glutamyl-tRNA reductase; PBG, porphobilinogen; PBGD, porphobilinogen deaminase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen III decarboxylase; CPO, coproporphyrinogen oxidase; PPO, protoporphyrin IX oxidase. Layer, G., Reichelt, J., Jahn, D., and Heinz, D. W. (2010) Structure and function of enzymes in heme biosynthesis. *Protein Sci.* **19**, 1137–1161.



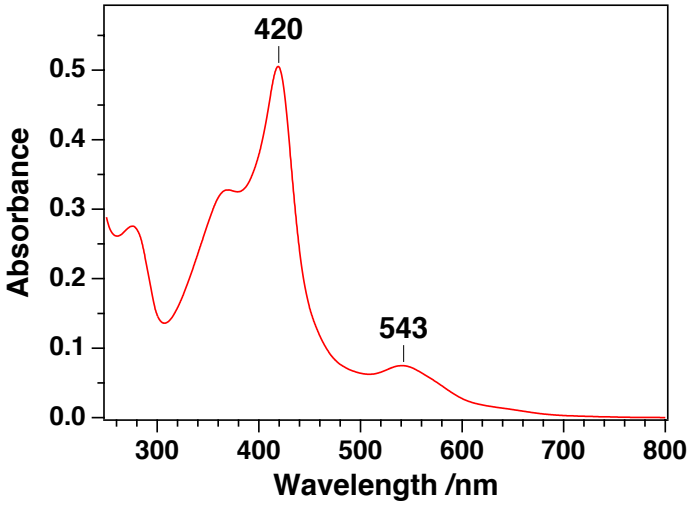
Supplementary Figure 2 Absorption spectra of uroporphyrinogen I, reaction product of PBGD alone (red) and heme-PBGD (blue).



Supplementary Figure 3 The catalytic activity of the (A) C105S and (B) H227D mutants as a function of the substrate concentration. The activity was measured at 30 °C in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0) in the absence (●) and presence (▲) of heme. The plot in the presence of heme was measured by the addition of 3 equivalents of heme to PBGD.



Supplementary Figure 4 Absorption spectra of heme-C105H mutant PBGD. PBGD (10 mM) in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0) was reconstituted with an equimolar of heme.



Supplementary Figure 5 Absorption spectra of CN-bound form of heme-PBGD in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0).