Supplementary Information

Green and efficient biosynthesis of indigo from indole by engineered myoglobins

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Fig. S1 Absorbance at 670 nm upon oxidation of (A) indole (0.5-4 mM) by addition of the same equivalent of H2O2 (0.5-4 mM), and (B) indole (4 mM) by addition of different amounts of H2O2 (1-10 mM), respectively, catalyzed by F43Y Mb (10 μM) for 15 min at 37 °C.
**Fig. S2** ESI-MS spectra of the oxidation product of indole in precipitation catalyzed by F43Y Mb. (A) Indigo (C₁₆H₁₀N₂O₂), Calculated: 262.1 Da; Observed: 261.07 Da ([M-H]⁻), and the side product (B) 6H-oxazolo[3,2-a:4,5-b']diindole (C₁₆H₁₀N₂O), Calculated: 246.1 Da; Observed: 245.07 Da ([M-H]⁻).

**Fig. S3** TLC analysis of the precipitates after centrifugation. As: Authentic sample of Indigo. The photo was taken directly after the expansion (A), and under the UV illumination (λ= 254 nm) (B).
Fig. S4 (A) Color changes of the oxidation of indole (1 mM) catalyzed by F43Y/H64D Mb (20 μM) with H₂O₂ (1 mM) as an oxidant. (B) UV-visible spectral changes of the oxidation of indole catalyzed by F43Y/H64D Mb. The change of the absorbance at 670 nm was shown as an inset.
Fig. S5 (A) Color changes of the oxidation of indole (1 mM) catalyzed by F43Y Mb (20 μM) with H₂O₂ (1 mM) as an oxidant. (B) UV-visible spectral changes of the oxidation of indole catalyzed by WT Mb.
**Fig. S6** HPLC analysis of (A) the components extracted from the liquid supernatant of reaction mixture and monitored at 280 nm, and (B) the precipitate monitored at 250 nm catalyzed by F43Y/H64D Mb. HPLC traces of authentic samples of oxindole, isatin and indigo were shown for comparison.

**Scheme S1.** Proposed reaction mechanism for indigo formation from indole catalyzed by Mb mutants (Xu et al., Catal. Sci. Technol., 2012, 2: 739-744).