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Supporting Information

Use of Apomyoglobin to Gently Remove the Heme from a H_2O_2-Dependent Cytochrome P450 and Allow Its Reconstitution

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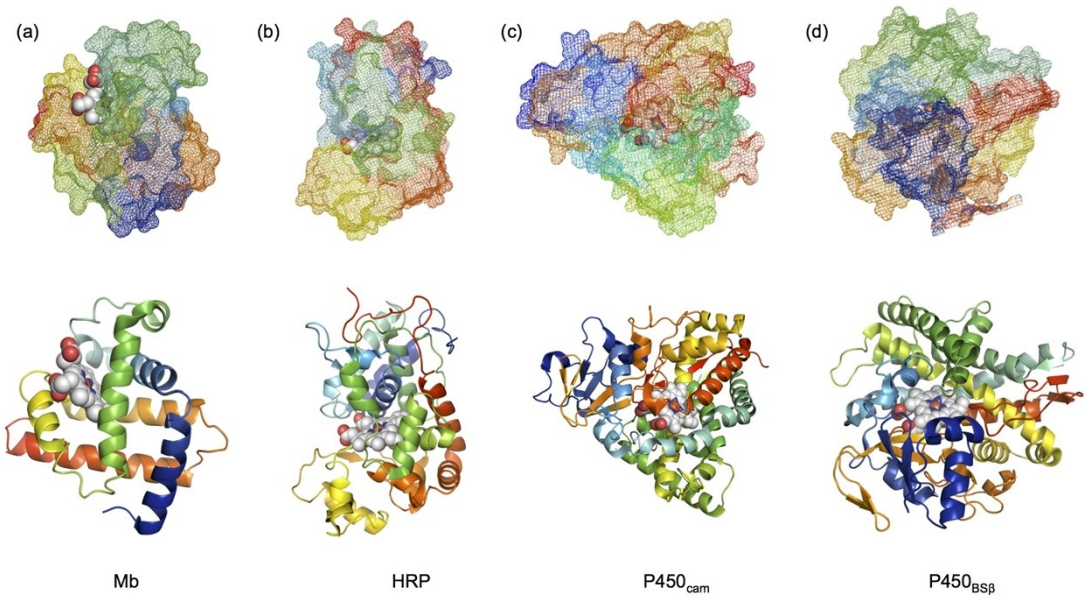
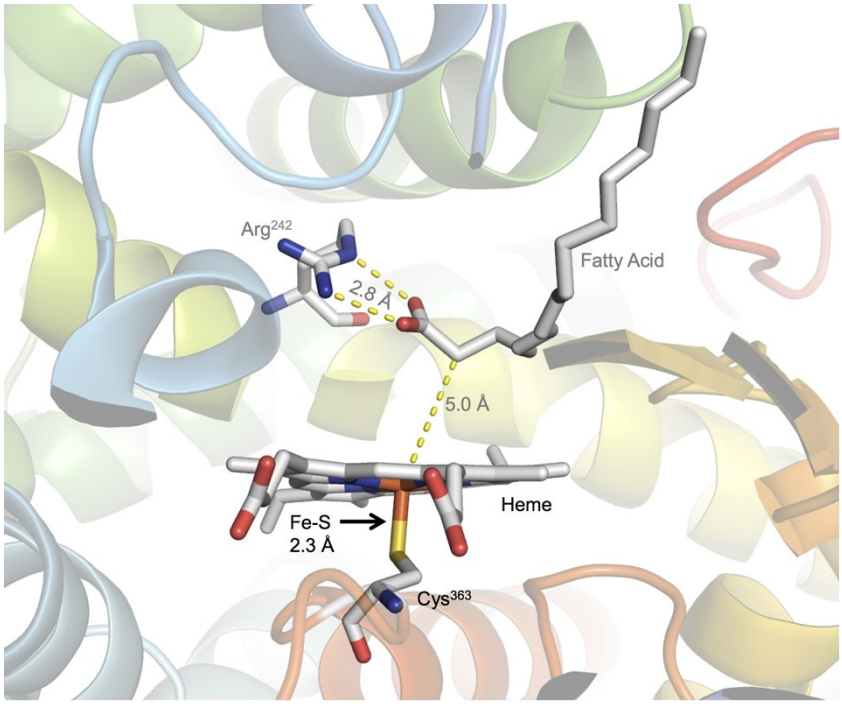
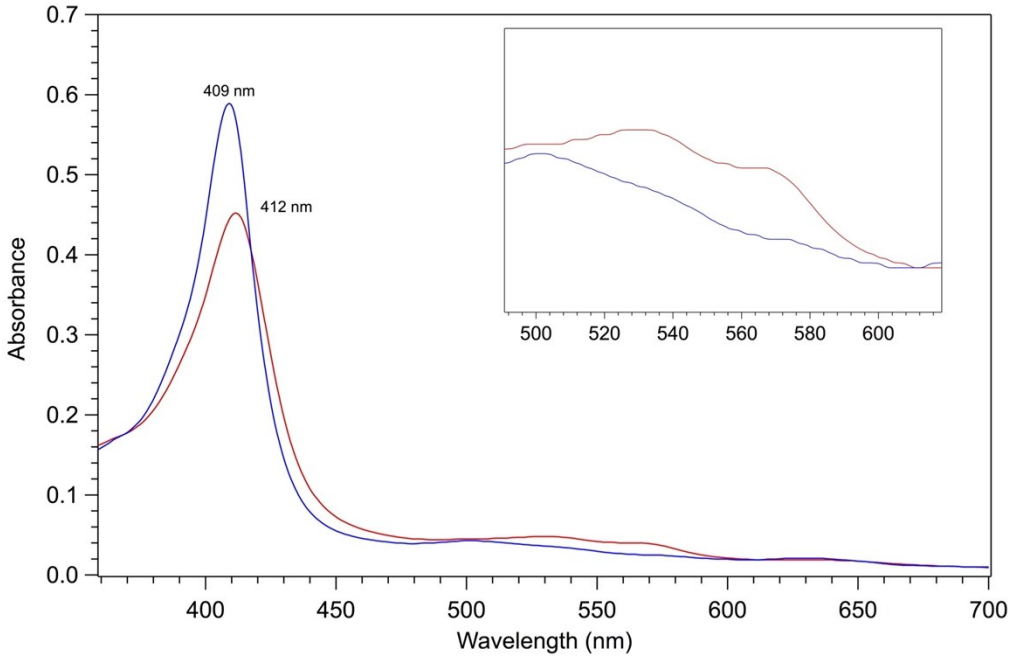
This file contains **Supporting Figures S1-S7 and Scheme S1-S2.**



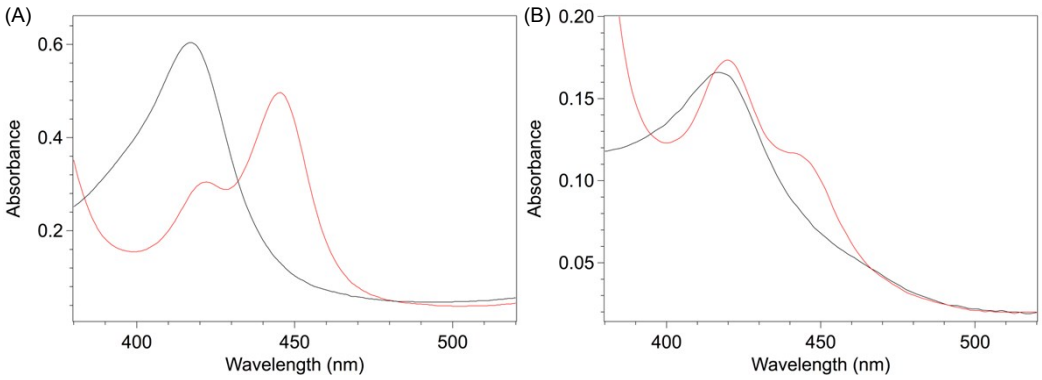
Supporting Figures S1. Contrast of four different heme enzyme structures, with the heme cofactor drew in sphere models (C: white, N: blue, O: red, and Fe: orange color). (a) The heme of Mb (PDB ID: 1MBN) is positioned close to the surface of whole structure. (b) The heme of HRP (PDB ID: 1W4W) is an example of a partially buried heme which the position between theirs in Mb and P450. (c) P450cam (PDB ID: 3WRH) the reaction pocket is buried deeply in the structure of the protein. (d) The heme pocket of $P450_{BS\beta}$ (PDB ID: 1IZO) is also in the deep of the structure with a channel for the pathway of fatty acid. Note that these differences in the protein shell and the positioning of the cofactor account for the differences in the facility of cofactor removal and reconstitution.



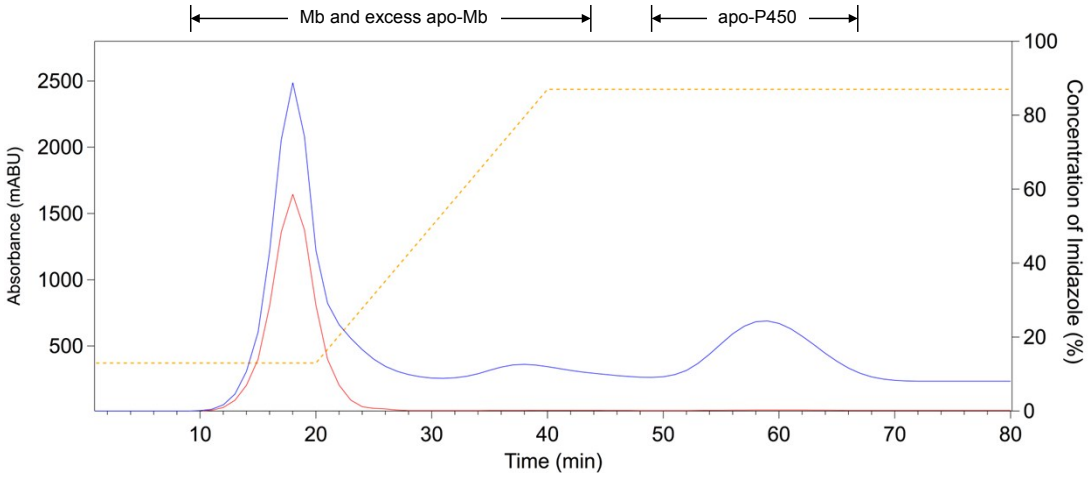
Supporting Figures S2. The active site of $P450_{BS\beta}$ composed by a heme, Arg^{242}, and fatty acid. Atoms with colors are shown as, C: white, N: blue, O: red, Fe: orange and S: wheat color. A coordinated bonding between a thiolate of cysteine residues (Cys^{363}) and the prosthetic group, heme, and the weak bondings between arginine residues Arg^{242} and fatty acid are drawn in yellow color sticks. The distances from atom to atom are showing in numbers.



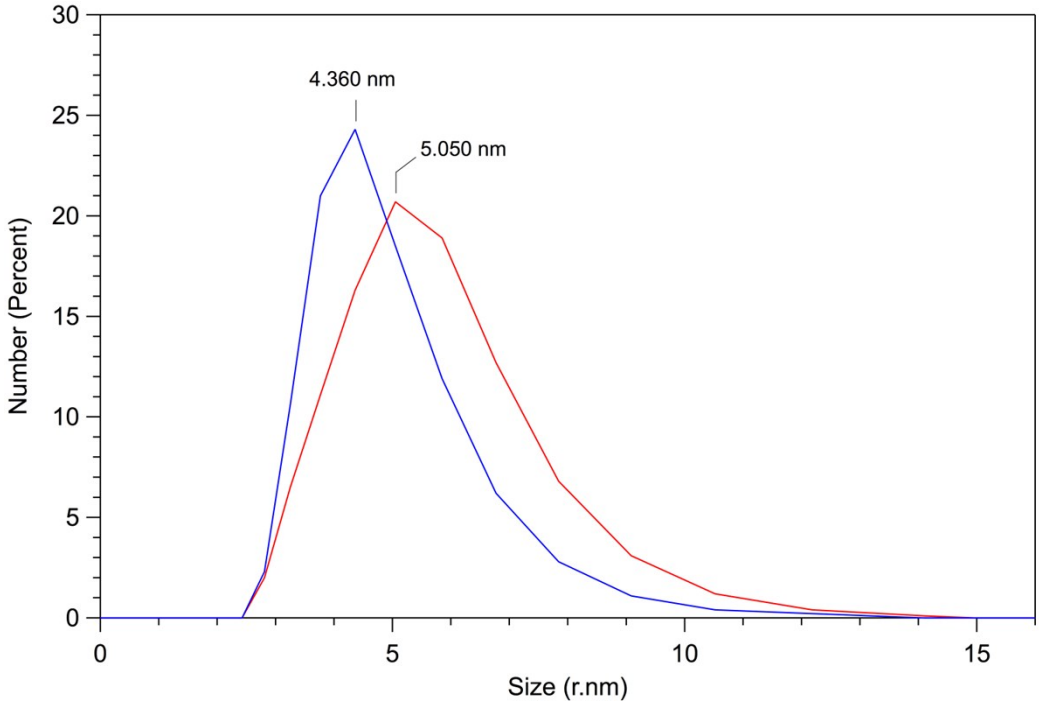
Supporting Figures S3. UV-Vis absorption spectra of $P450_{BS\beta}$ with apo-Mb at 4 °C vs. 25 ° C after 24 hours. Comparison result between 25 °C experiment (blue line) was observed with the Soret band from heme of Mb at 409 nm, and 4 °C experiment (red line) was observed the Soret band shifted to 412 nm only with an apparent Q band of heme of P450 (expanded part). The result showed that the rate of heme transfer at 4 °C was slower then that at 25 °C.



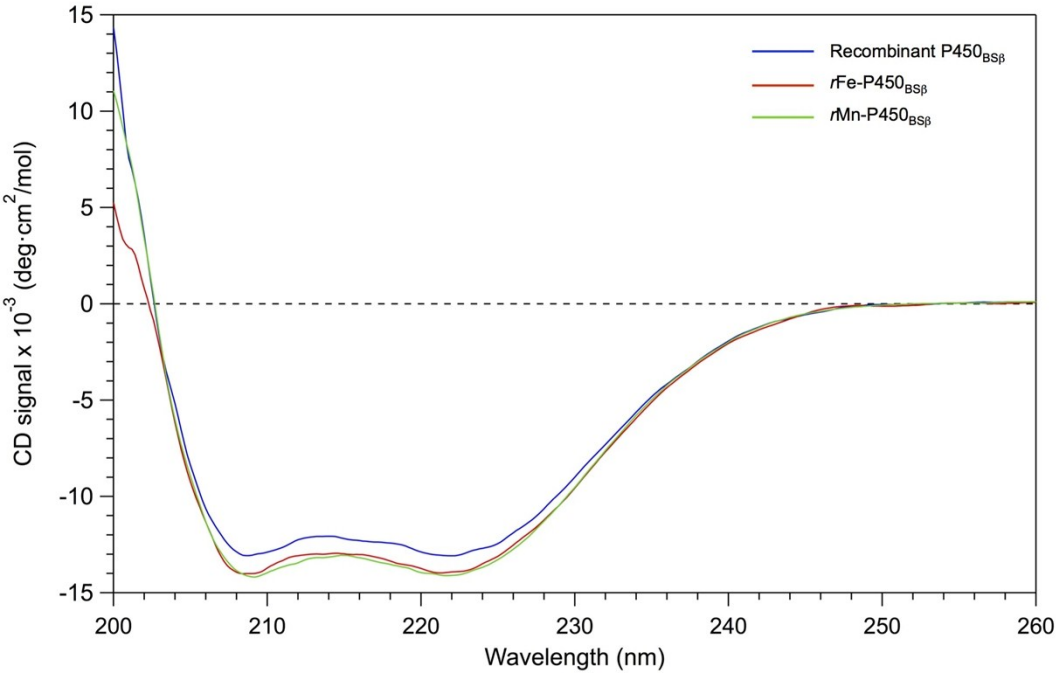
Supporting Figures S4. UV-Visible absorption spectra of ferric and ferrous-CO bound form of (A) recombinant $P450_{BS\beta}$ and (B) *r*Fe-$P450_{BS\beta}$ in 0.1 M potassium phosphate buffer (pH 7.0) containing 0.3 M KCl and 20% (v/v) glycerol. The resting state of P450 (black line) and the Fe^{2+}–CO state (red line). *r*Fe-P450 led to a higher content of the P420 isoform to 60% (Abs_{420nm} = 0.174 and Abs_{445nm} = 0.117) than recombinant $P450_{BS\beta}$.



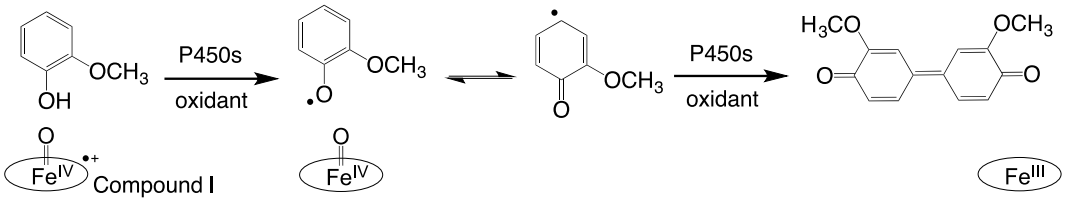
Supporting Figures S5. Spectra of purification by TOSOH equipped with a Ni-chelate affinity chromatography column to separate Mb and 6 × His-tagged apo-P450$_{BS\beta}$. The blue line represented absorption at 280 nm and the red line was the absorption at 417 nm. The first peak and weak second peak contained holo-Mb and excess apo-Mb. After 40 min, the concentration of elution buffer was increased to 87% and a third peak around 60 min was eluted with absorption only at 280 nm, which showed as a pure apo-P450$_{BS\beta}$ without the absorption of heme.



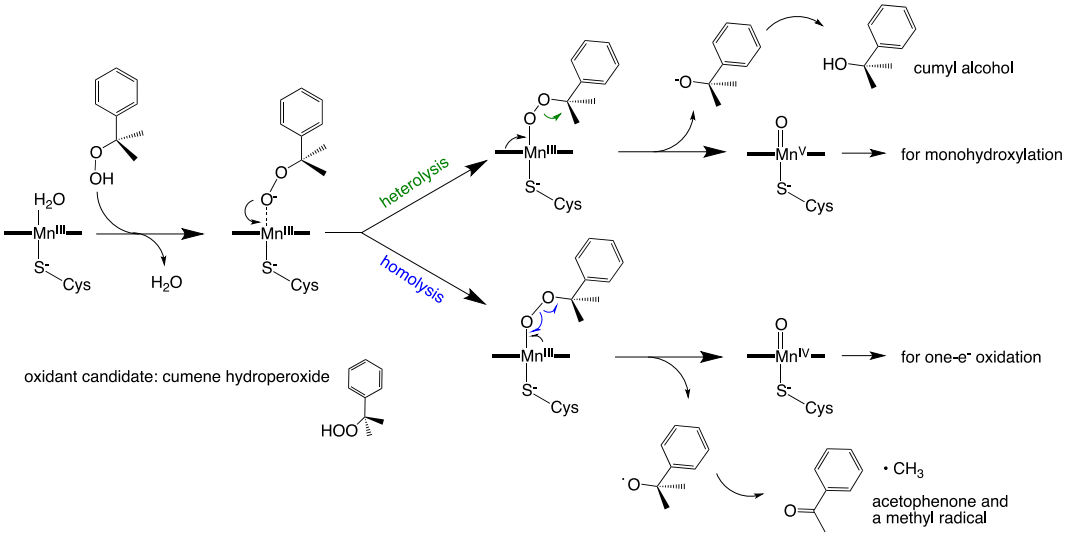
Supporting Figures S6. DLS spectra of holo- and apo-P450$_{BS\beta}$ in linear scale; holo-P450$_{BS\beta}$ is shown as a blue solid line and apo-P450$_{BS\beta}$ as a red solid line. The average protein size radiuses of holo- and apo-P450$_{BS\beta}$ were 7.84 ± 3.35 and 7.84 ± 3.76 nm, respectively.



Supporting Figures S7. CD spectra (0.0025 M) of recombinant (blue line), *r*Fe- (red line) and *r*Mn-$P450_{BS\beta}$ (green line) in 0.1 M potassium phosphate buffer (pH 7.0) containing 0.3 M KCl and 20% (v/v) glycerol. The spectra showed that two of reconstituted $P450_{BS\beta}$ were stayed in analogous α-helical structures as the recombinant $P450_{BS\beta}$.



Supporting Scheme S1. Proposed oxidation mechanism of guaiacol with P450.



Supporting Scheme S2. Proposed mechanisms in Mn-P450$_{BS\beta}$ to active species by heterolytic or homolytic O-O bond cleavages.