SUPPORTING INFORMATION FOR:

Zinc substituted cytochrome $P450_{cam}$: characterization of protein conformers F450 and F420 by photoinduced electron transfer.

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Table S1. Absorption maxima of metal porphyrins and metal-substituted cytochromes P450 in the presence of thiol ligands.

	Wavelength [nm]			
	hyper	Soret	β	α
FePP+$C_6H_5CH_2S^-$ [a]	376	470	561	
Fe-P450-cys^- (Fe III-6cls species)[b, c]		392	509	540
Fe-P450-cys^- (Fe II-5chs species)[b]		409	544	
Fe-P450-cys^-/H_2O(Fe III-6cls species) [b]		417	535	571
Fe-P450-cys^-/CO (Fe II-6cls species) [b]	363	446	550	
Fe-P450-cys^-/DMS^- (Fe II-6cls species) [b, d]		446	541	568
ZnTPP in benzene, [e]		422	549	588
ZnPP in DMF/H_2O buffer [f, g]		414	544	583
Zn-F420[g]	**334**	**424**	**550**	**584**
ZnTPP+ C_4H_9SH[eh]		426	558	599
ZnTPP+ $C_4H_9S^-$ [e]	378	449	587	633
Zn-F450[g]	**367**	**449**	**568**	**602**

[a] Ruf, H. H., and Wende, P. *JACS* **1977**, *99*, 5499-5500.
[b] Egawa, T., Hishiki, T., Ichikawa, T., Kanamori, Y., Shimada, H., Takahashi, S., Kitagawa, T., and Ishimura, Y. *J.Biol. Chem.* **2004**, *279*, 32008–32017.
* 5chs -five-coordinated high spin; 6cls -six-coordinated low-spin
[c] Additional band at 645nm
[d] DMS, dimethyl sulfide
[e] ZnTPP - Zinc tetraphenylporphyrin
[f] This work
[g] ZnPP – Zinc protoporphyrin IX
[h] Nappa M., and Valentine, J. S. *JACS* **1978**, *16*, 5075-5080.

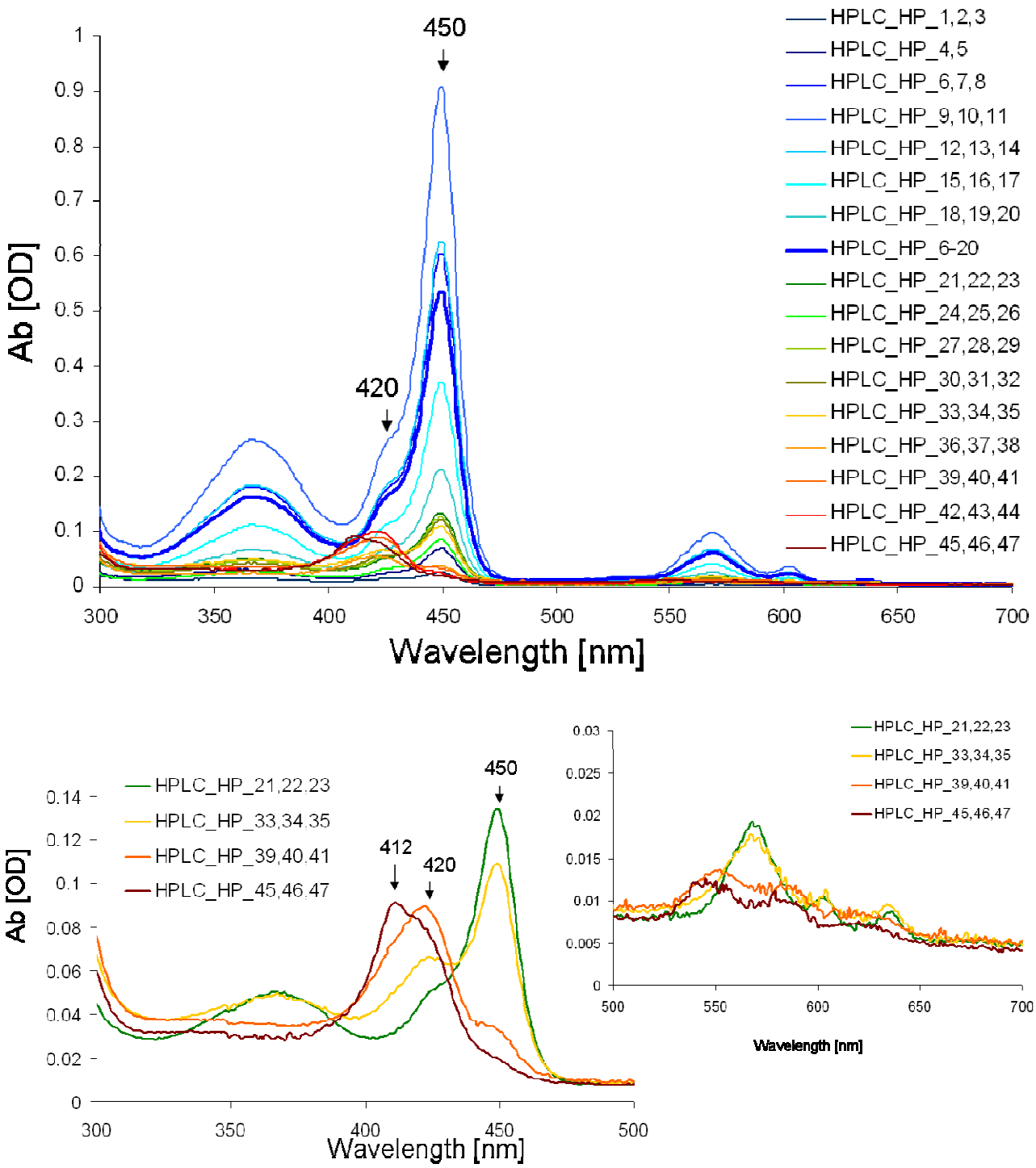

Figure S1. UV-vis spectra of the HPLC fractions result after protein substitution perform as described in material and methods.

Figure S1 shows the complete set of the UV-vis spectra acquired during a typical HPLC separation of the products of the zinc substitution of $P450_{cam}$. The main fractions collected for measurements contain the mixture of F420 and F450 (HPLC_HP_6-20), where the F450 dominates. Repeated attempts to completely separate these two forms from were not successful.

In all cases both conformers were present, however, it was possible to collect fractions which contained primarily F420 (HPLC_HP_36-41), which were followed by the unfolded protein fractions with the characteristic Soret band at 412nm. Because the fractions which contain primarily F420 are present in the elution only in low concentration and since they may contain the unfolded protein with a closely lying Soret band at 412 nm, this part of protein was not used for the electron transfer studies.

Figures 2 and 3 in the main text show the spectra of ZnP450 samples which were collected at different elution times. It was found to be more reliable to selectively excite the mixed samples of F420 and F450 rather than to perform experiments of F420 containing the unfolded form absorbing at 412 nm.

Fitting gaussian functions to the UV-Vis spectra reveals in detail the spectral contribution of the conformers to the overall spectra of fractions rich in F420 and F450, respectively (Figure S2). It was found that gaussian functions reproduce the experimental line shapes significantly better than lorentzians. The peaks at 449 and 370 nm belong to form F450 and the peak at 424 nm to form F420. The features at 440 and 635 nm appear when the sample was exposed to oxygen. This phenomenon was previously described by Morishima *et al.* The presence of oxygen disturbs the fluorescence spectrum of the protein as well and leads to increased intensity at 640 nm (Figure S3). As described in the main text, all samples used in the electron transfer measurements were thoroughly degassed.

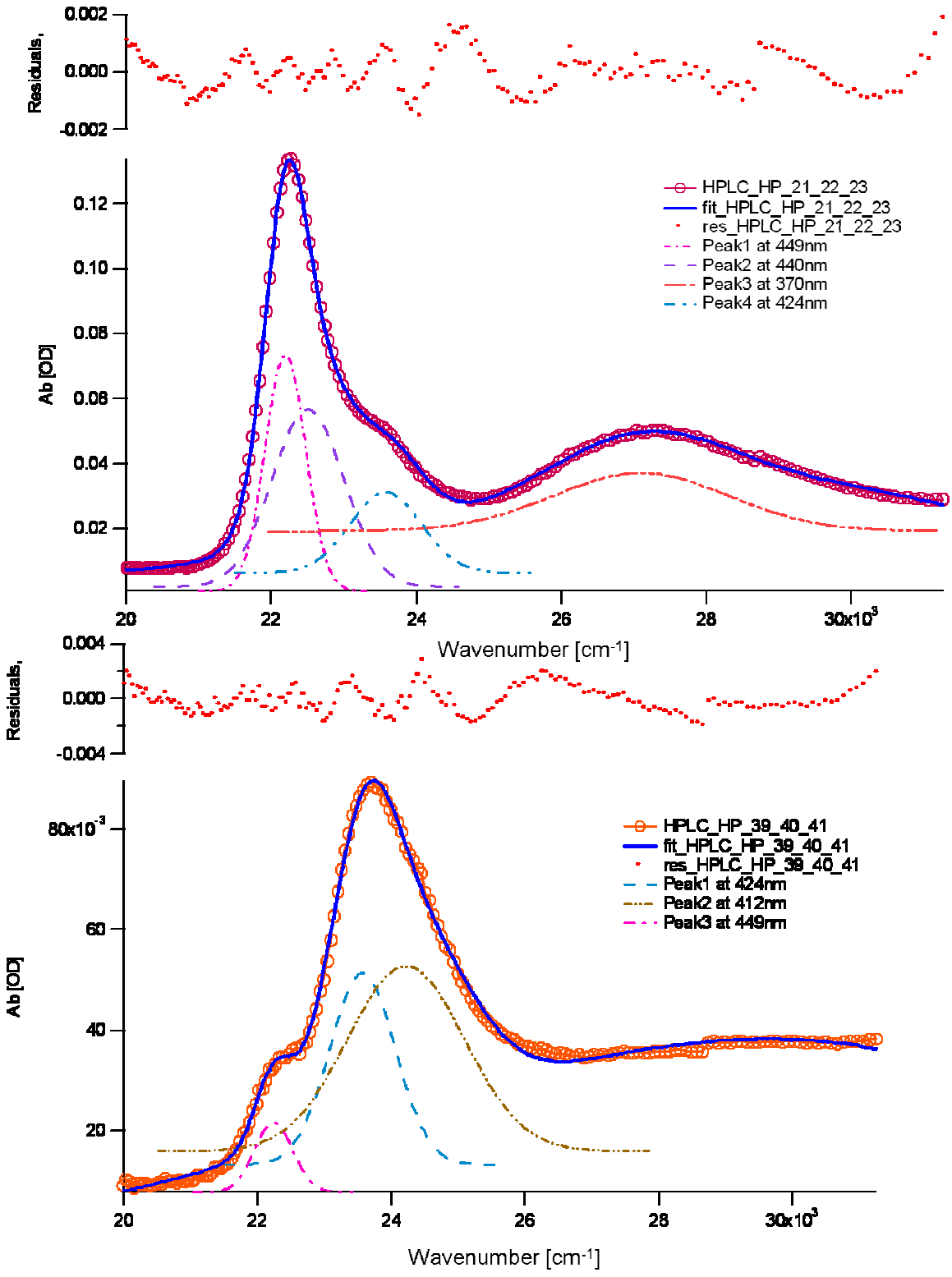

Figure S2. Gaussian line shape analysis of the contributions of conformers F420 and F450 to the overall spectra of HPLC fractions of ZnP450 rich in F450 (top) and F420 (bottom).

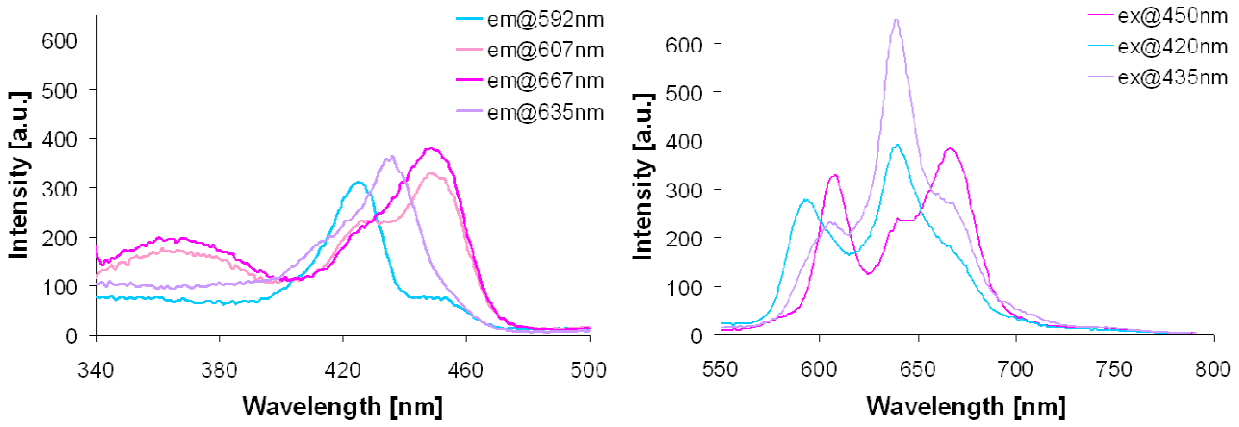

Figure S3. Fluorescence excitation and emission spectra of ZnP450 colelcted under aerobic conditions.

The initial F420 to F450 ratio depends on the camphor concentration during the exchange of the prosthetic group (Figure S4). The substitution was performed in the presence of different camphor concentration (0 – 1 mM) and under all conditions that we have explored, the F450 is more abundant. The F420 content was higher at lower camphor concentrations. The ratio F420:F450 changed from 1:2.8 to 1:1.4 when camphor was eliminated from all buffers used in substitution. The F420:F450 ratio in fractions used for the electron transfer studies was kept close to 1:2. In this case substitution was performed in the presence of 0.1 mM camphor. The amount of form F450 changed if additional camphor was introduced to the protein solution which was substituted and processed without camphor (Figure S5). On the other hand, F450 to F420 conversion was observed when imidazole was present in the protein solution. The structure of the wile-type $P450_{cam}$ crystallized with imidazole is very similar to the substrate-free conformation,[1] which confirms that the F420 is the open, substrate free analog of native $P450_{cam}$ (Figure S5).

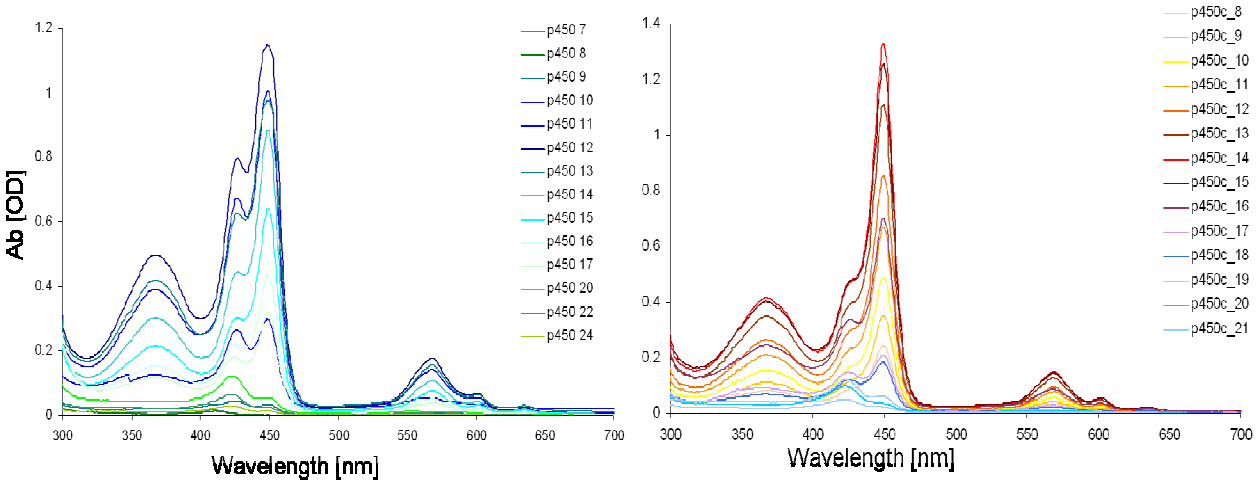

Figure S4. HPLC results for ZnP450 substituted in the absence (left) and presence (right) of camphor.

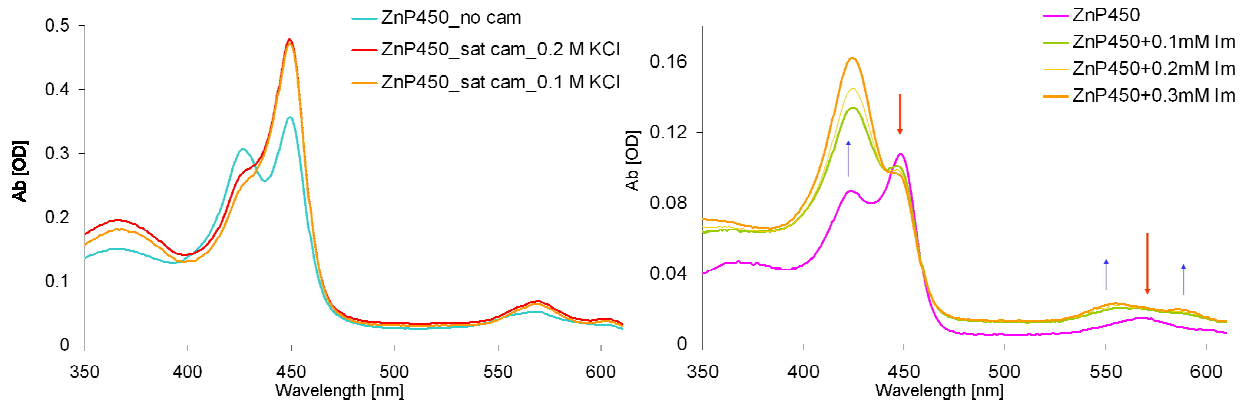

Figure S5. Left: absorption spectrum of ZnP450 after equilibration for 3 hours without or with camphor and K^+ ions. Right: F450 to F420 conversion upon binding with imidazole.

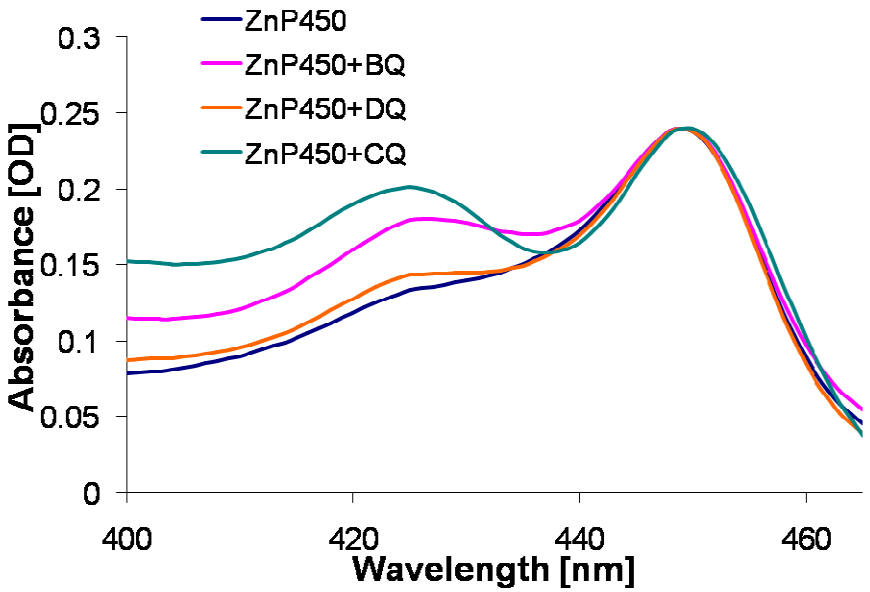

Figure S6. Changes in the appearance of the UV-Vis spectra upon addition of quinones (5×10^{-5} M) to ZnP450 (1×10^{-6} M).

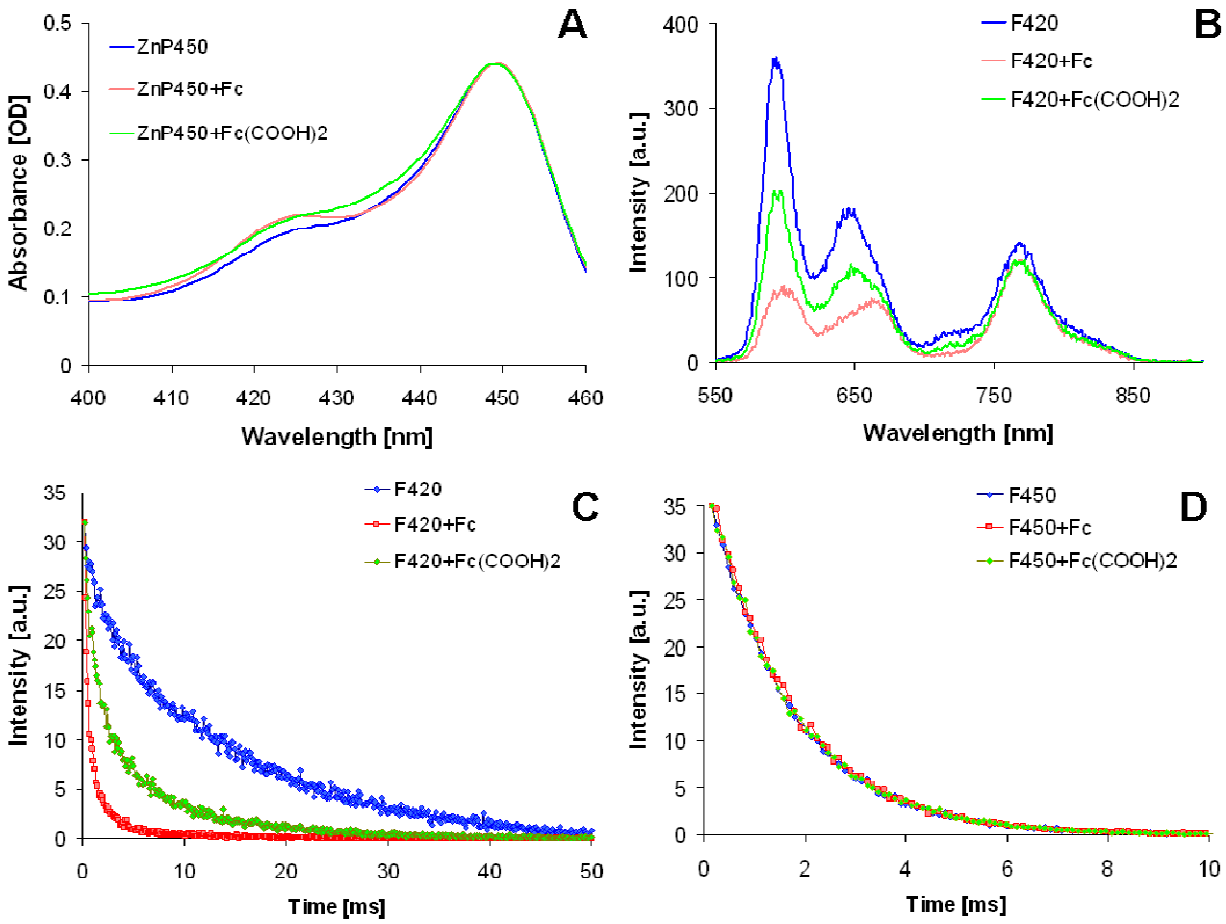

Figure S7. The results of reductive quenching of the excited state of $ZnP450_{cam}$ (1×10^{-6} M) in the presence of electron donors (saturated solution of ferrocene and 1.5×10^{-4} M of $Fc(COOH)_2$ in 40 mM KPi, 0.1 mM camphor at pH = 7.4): (A) Absorption spectrum of $ZnP450_{cam}$; (B) phosphorescence spectrum of $ZnP450_{cam}$ excited at 420 nm; (C) phosphorescence decay of $ZnP450_{cam}$ exited at 420 nm and monitored at 590 nm; (D) phosphorescence decay of $ZnP450_{cam}$ excited at 450nm and monitored at 768 nm.

The approximate redox potentials of the ZnP450 triplet state were calculated using the ground state potentials of zinc substituted cytochrome c (E^{red} = -1.3eV and E^{ox} = 0.8) and the 0-0 transition energies obtained from the phosphorescence spectra of F450 and F420 (768 nm for F450 and 723 nm for F420 (Table S2).[2,3]

Table S2. Calculated triplet excited state redox potentials of the F450 and F420 forms of $ZnP450_{cam}$.

	$E^{0-0}(^3ZnPP^*)$	$E^{ox}(^3ZnPP^+/ZnPP^*)$	$E^{red}(^3ZnPP^*/ZnPP^-)$
F450	1.61 V	-0.81 V	0.31 V
F420	1.72 V	-0.92 V	0.42 V

[1] A. Verras, A. Alian and P. R.Ortiz de Montellano (2006) Cytochrome P450 active site plasticity: attenuation of imidazole binding in cytochrome $P450_{cam}$ by an L244A mutation *Protein Engineering, Design & Selection 19*, 491–496.

[2] Shen C., and Kostić, N. M. (1996) Reductive quenching of the triplet state of zinc cytochrome *c* by the hexacyanoferrate (II) anion and by conjugate bases of ethylenediaminetetraacetic acid, *Inorg. Chem. 35,* 2780-2784.

[3] Furukawa, Y., Ishimori, K., and Morishima, I. (2000) Electron transfer reactions in Zn-substituted cytochrome $P450_{cam}$, *Biochemistry 39*, 10996 -11004.