

Supporting Information

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**Experimental Documentation of the Structural Consequences of
Hydrogen-Bonding Interactions to the Proximal Cysteine of
a Cytochrome P450****

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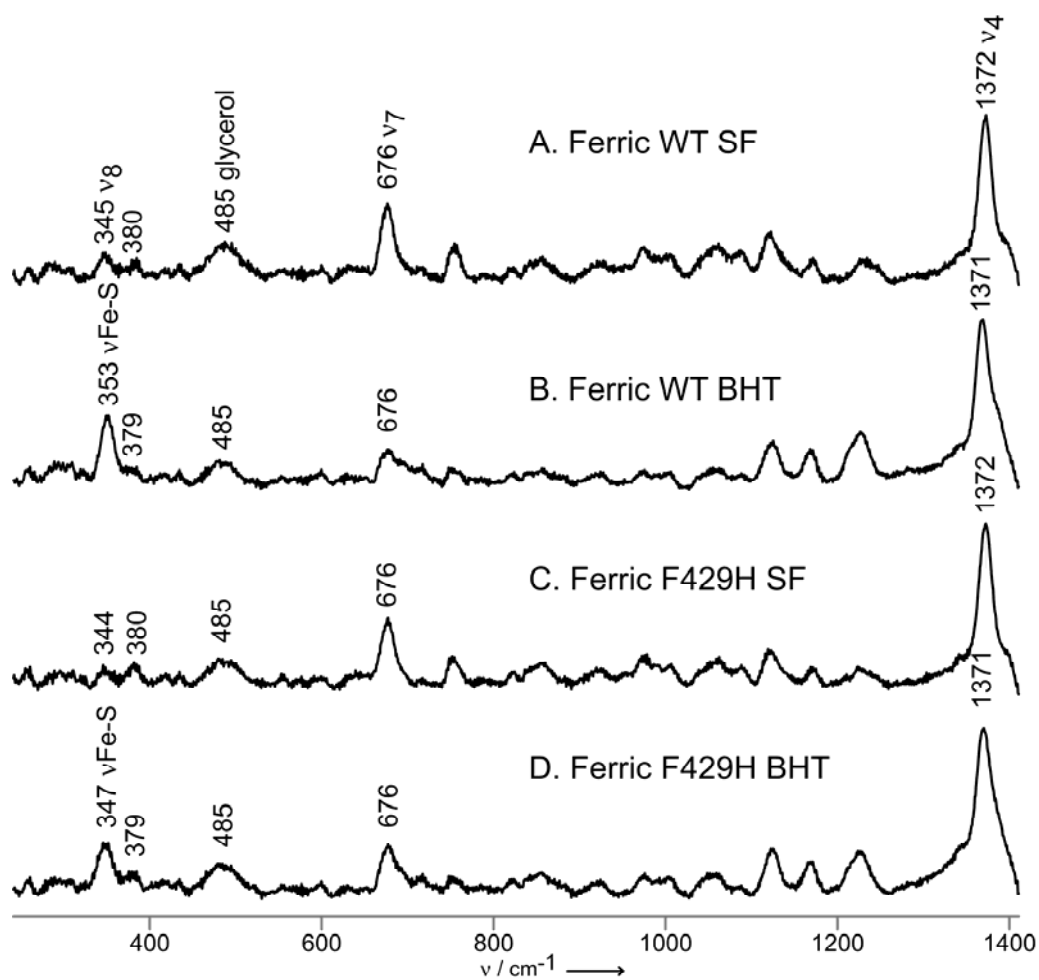


Figure S1. The RR spectra of ferric CYP2B4, wild-type substrate-free (A), wild-type BHT-bound (B), F429H mutant substrate-free (C) and F429H mutant BHT-bound. Spectra measured with 356.4 nm excitation line and normalized to the ν_4 mode.

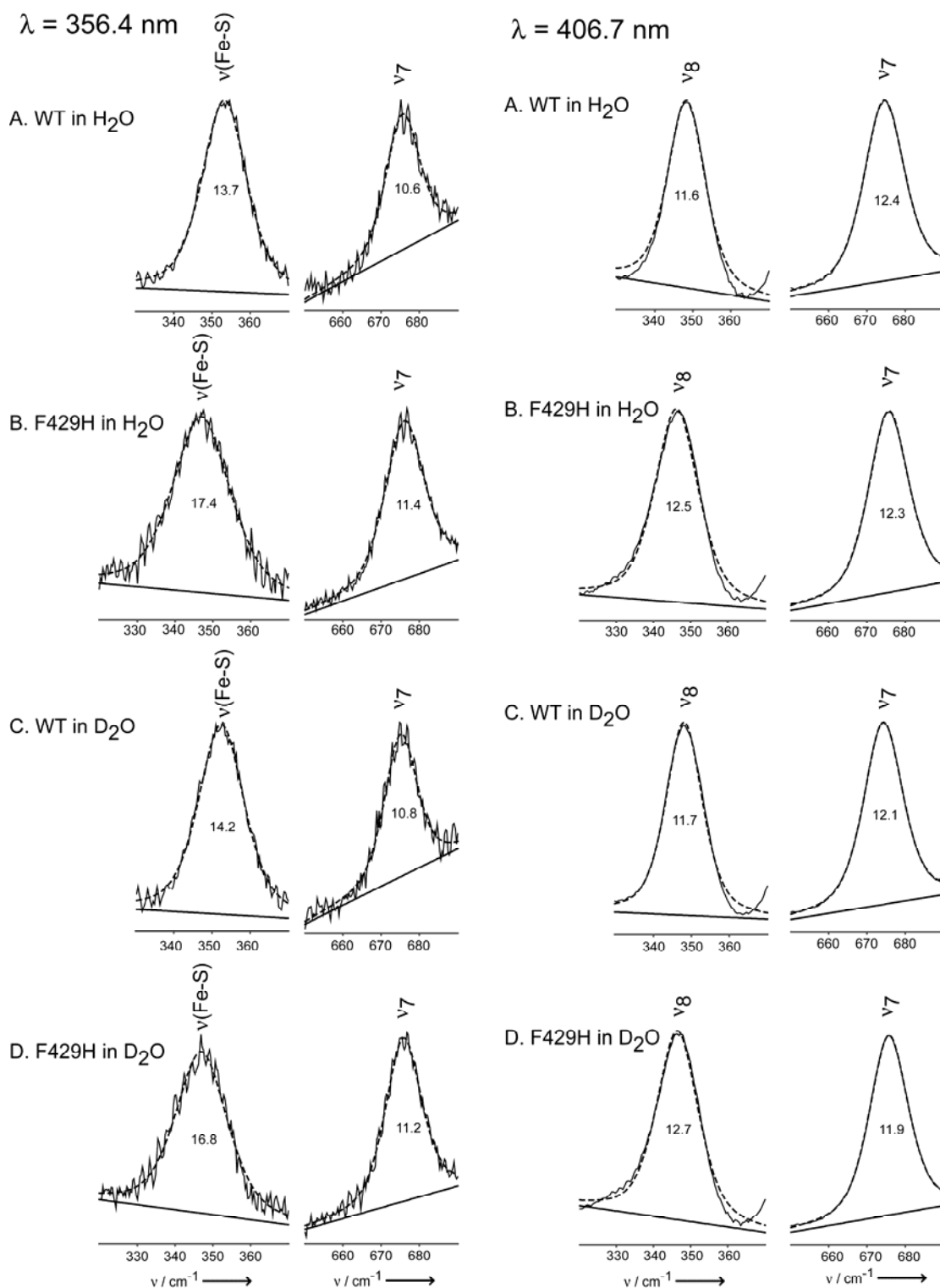


Figure S2. Deconvolution of the $\nu(\text{Fe-S})$, the ν_7 and the ν_8 bands of the ferric CYP2B4 BHT-bound spectra of wild type (WT) in H₂O buffer (traces A), F429H mutants in H₂O buffer (traces B), WT in D₂O buffer (traces C), F429H in D₂O buffer (traces D) measured with 356.4 nm (left panel) and 406.7 nm (right panel) excitation lines. The experimental data (in black) were fitted (dashed line) with 50%/50% Gaussian/Lorentzian functions. The numbers at $\frac{1}{2}$ half of the peaks indicate their bandwidths. It is noted that the experiments in D₂O were performed in order to investigate the (admittedly unlikely) possibility that change to deuterated medium might lead to slight frequency shifts. Though none were seen, the data is included here, since they provide additional evidence for selective broadening of the $\nu(\text{Fe-S})$ modes.

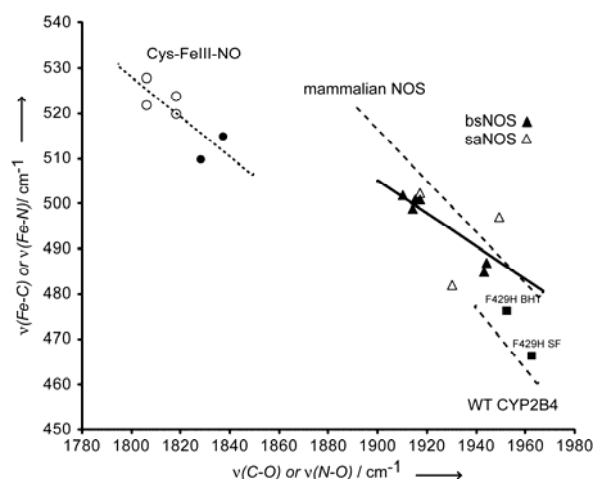


Figure S3. Linear correlation between $\nu(\text{Fe-C})$ or $\nu(\text{Fe-N})$ and $\nu(\text{C-O})$ or $\nu(\text{N-O})$ frequencies. The dashed lines represent the $\nu(\text{Fe-C})/\nu(\text{C-O})$ correlation lines (from Figure 3 of main text) for wild type CYP2B4 and mammalian NOSs.^[37-40] The solid squares represent data for the F429H mutant of P4502B4. The triangles represent the points for bsNOS and saNOS and the solid line represents the $\nu(\text{Fe-C})/\nu(\text{C-O})$ linear correlation for those two sets of bacterial NOSs.^[18,S1,S2] In the left upper corner are shown points for thiolate ferric NO adducts of P450cam (o) and model compounds (•) and their correlation line (dotted line).^[S3-S5]

Experimental Section

Preparation of samples for rR measurements: The truncated wild type 2B4 and F429H mutant samples for rR measurements were in 100 mM phosphate buffer pH 7.4 containing 100-fold molar excess of BHT relative to protein, 1 mM dilauroylphosphatidylcholine (DLPC), 0.3 M NaCl and 20 % of glycerol (deuterated glycerol for samples in D_2O buffer). Final protein concentration was ~ 0.4 mM in heme. The ferrous CO adducts were prepared by saturating the ferric samples enclosed in 5 mm NMR tubes with CO gas and reducing them with ~ 2 molar equivalents of sodium dithionite solution.

The rR measurements: The ferric samples were measured using the 406.7 nm and 356.4 nm excitation lines from a Kr^+ laser (Coherent Innova Sabre Ion Laser) and the Fe(II)-CO adducts were excited by 441.6 nm line provided by a He-Cd laser (IK Series He-Cd laser, Kimmon Koha CO., LTD.). The RR spectra of all samples were measured using a Spex 1269 spectrometer equipped with an Andor Newton EMCCD detector (Model DU971, Andor Technologies). The slit width was set at 150 μm and the 1200 or 2400 g/mm grating were used. The laser power at the ferric sample was adjusted to ~ 10 mW while for ferrous CO adducts was kept at ~ 1 mW. Spectra were calibrated with fenchone (Sigma-Aldrich, WI), toluene- D_6 and acetone- D_6 (Cambridge Isotope Laboratories, Inc., MA) and processed with Grams/32 AI software (Galactic Industries, Salem, NH).

References

- [S1] A. Brunel, A. Wilson, L. Henry, P. Dorlet, J. Santolini, *J. Biol. Chem.* **2011**, *286*, 11997-12005.
 [S2] J. Santolini, M. Roman, D. J. Stuehr, T. A. Mattioli, *Biochemistry* **2006**, *45*, 1480-1489.
 [S3] S. Hu, J. R. Kincaid, *J. Am. Chem. Soc.* **1991**, *113*, 2843-2850.
 [S4] E. Obayashi, K. Tsukamoto, S. Adachi, S. Takahashi, M. Nomura, T. Iizuka, H. Shoun, Y. Shiro, *J. Am. Chem. Soc.* **1997**, *119*, 7807-7816.
 [S5] N. Suzuki, T. Higuchi, Y. Urano, K. Kikuchi, T. Uchida, M. Mukai, T. Kitagawa, T. Nagano, *J. Am. Chem. Soc.* **2000**, *122*, 12059-12060.