Supplementary Materials for

Intramolecular Heme Ligation of the CYP2C9-R108H Mutant Demonstrates Pronounced Conformational Flexibility of the B-C Loop Region: Implications for Substrate Binding

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RUNNING TITLE: Conformational flexibility of CYP2C9.

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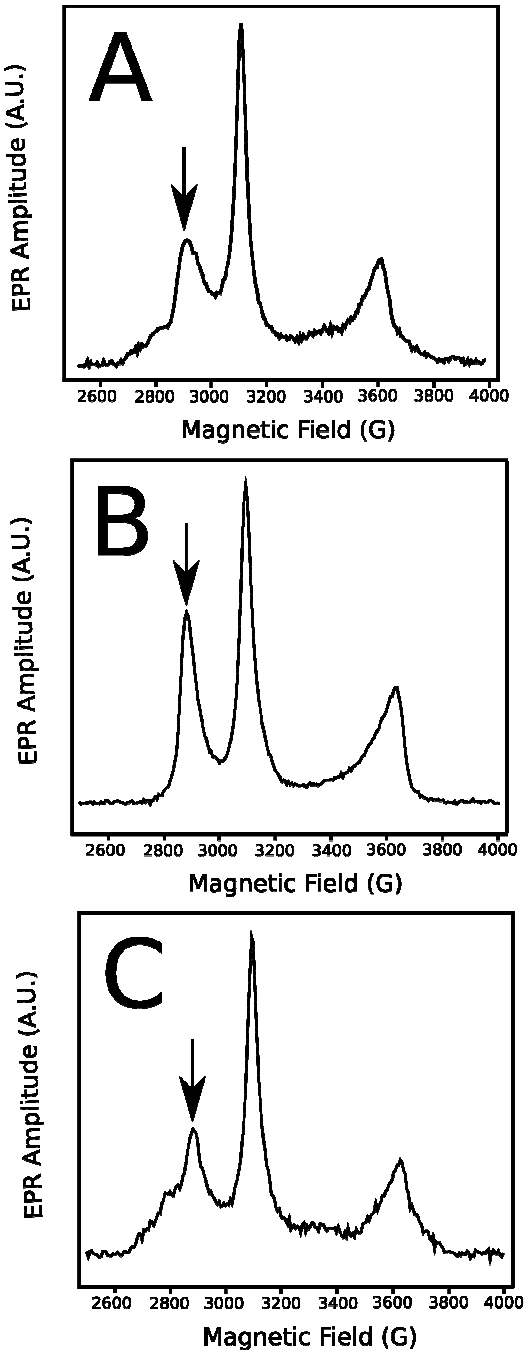

Figure S1. *Spin echo-detected EPR spectra are shown for A) CYP2C9, B) the R108H mutant and C) CYP2C9 with 100 mM imidazole.* The spectra were measured with the spacing between the two microwave pulses at τ=160 ns in order to emphasize the principal g-values as a result of the orientation dependence of the ESEEM and the phase memory relaxation of the sample. The g-values of the EPR peaks under the various conditions were similar. The arrow denotes the position where the HYSCORE spectra were measured.

Table S1. *Additional Force field parameters used for CYP2C9*[a]

Bond	Energy (kcal mol^{-1} $Å^{-2}$)	Distance (Å)[b]	Reference
Fe-S	87.589	2.377	(*1*)

Angle[c]	Energy (kcal $mol^{-1}rad^{-2}$)	Reference
Fe-S-C_β	21.646	(*1*)
N_P-Fe-S	13.277	(*1*)

Dihedral[c]	Energy (kcal mol^{-1})	Reference
C_β-S-Fe-N_P	0.034[d]	(*1*)

[a] Fe, heme iron; S, sulfur of C435; C_β, C_β of C435; N_P, pyrrole heme nitrogen.
[b] Equilibrium distance value from (*1*).
[c] The angles and dihedrals, used in the simulations, were calculated using the values measured from the 1R9O CYP2C9 X-ray crystal structure (*2*).
[d] Autenrieth et al., 2004 obtained energy values for cytochrome c of 0.04 kcal mol^{-1}(*3*)

Table S2. Additional force field parameters for R108H CYP2C9[a]

Bond	Energy (kcal mol^{-1} $Å^{-2}$)	Distance (Å)	Reference
Fe-N_ε	65[b]	2.05[c]	(*3*)

Angle	Energy (kcal $mol^{-1}rad^{-2}$)	Angle (degrees)	Reference
N_P-Fe-N_ε	50	90^o	(*4*)

Improper Dihedral	Energy (kcal $mol^{-1}rad^{-2}$)	Angle (degrees)	Reference
N_ε-C_δ -C_ε -Fe	29.4	0^o	(*3*)

[a] N_ε, C_δ and C_ε correspond to atoms on H108 of the mutant.
[b] Henry et al., 1985 obtained similar values for hemoglobin of 60 kcal mol^{-1} $Å^{-2}$.
[c] Intermediate value between 2-2.1 Å that was used previously in (*1, 3, 4*).

References

1. Oda, A., Yamaotsu, N., and Hirono, S. (2005) New AMBER force field parameters of heme iron for cytochrome P450s determined by quantum chemical calculations of simplified models, *J. Comput. Chem. 26*, 818-826.
2. Wester, M. R., Yano, J. K., Schoch, G. A., Yang, C., Griffin, K. J., Stout, C. D., and Johnson, E. F. (2004) The structure of human cytochrome P450 2C9 complexed with flurbiprofen at 2.0-A resolution, *J. Biol. Chem. 279*, 35630-35637.
3. Autenrieth, F., Tajkhorshid, E., Baudry, J., and Luthey-Schulten, Z. (2004) Classical force field parameters for the heme prosthetic group of cytochrome c, *J. Comput. Chem. 25*, 1613-1622.
4. Henry, E. R., Levitt, M., and Eaton, W. A. (1985) Molecular dynamics simulation of photodissociation of carbon monoxide from hemoglobin, *Proc. Natl. Acad. Sci. U. S. A. 82*, 2034-2038.