

Supporting Information:

Putidaredoxin Binds to a Similar Site on Cytochrome P450cam in the Open and Closed Conformation

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Table S1: Summary of DEER analysis parameters for P450cam:Pdx pairs in both redox state: initial point of the background/ cut off time point/ regulation parameter (α)/dimensionality.

	C73 Pdx	S29C Pdx	E14C Pdx
S48C P450cam	3016/4416/100/3.4	3016/3984/100/3.45	3016/3768/100/3.1
S190C P450cam	2564/3384/100/3.4	2836/3696/100/3.6	3016/3960/100/3.5
Q227C P450cam	3000/3744/100/3.7	2990/3916/100/3.65	3000/3720/100/3.75
Q272C P450cam	3200/4272/100/3.2	2500/3400/1/3.7	3200/4128/100/3.6

Table S2. De-convoluted ESI Mass spectra results for P450cam and Pdx mutants, with and without MTSL attachment.

	Label	Predicted (Da)	Actual (Da)
P450cam S48C	MTSL	46612	46610
	-	46428	46426
P450cam S190C	MTSL	46612	46609
	-	46428	46426
P450cam Q227C	MTSL	46565	46574
	-	46381	46384
P450cam Q272C	MTSL	46565	46573
	-	46381	46384
Pdx C73	MTSL	11587	11590
	-	11403	11401
Pdx S29C	MTSL	11587	11589
	-	11403	11401
Pdx E14C	MTSL	11551	11548
	-	11367	11359

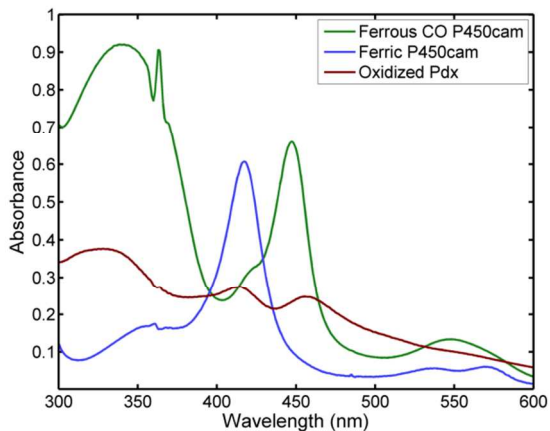


Figure S1: UV/Vis spectra of ferrous CO bound P450cam (green), ferric P450cam (blue) and oxidized Pdx (red).

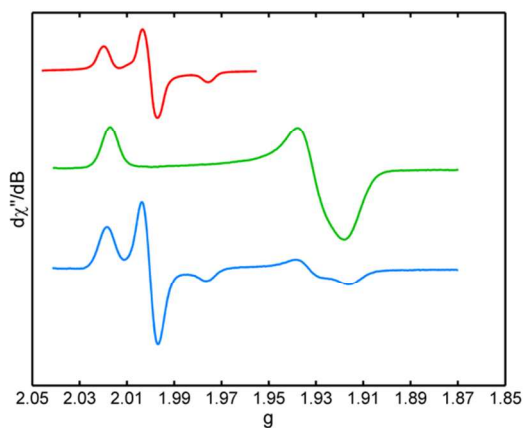
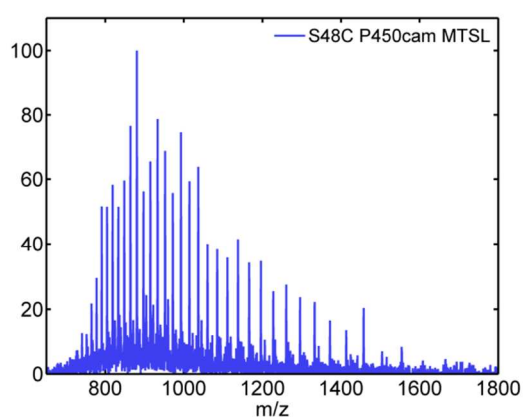
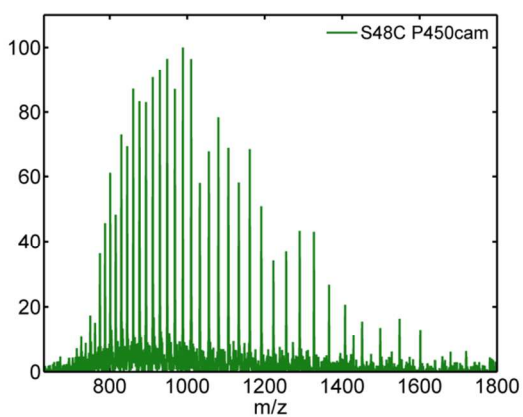
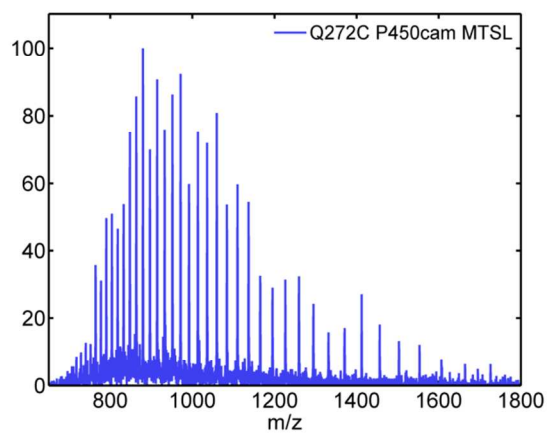
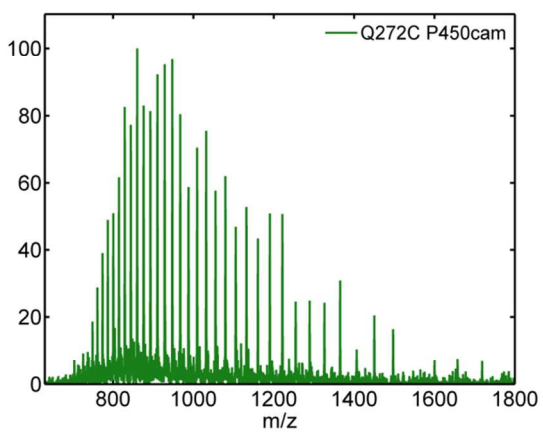
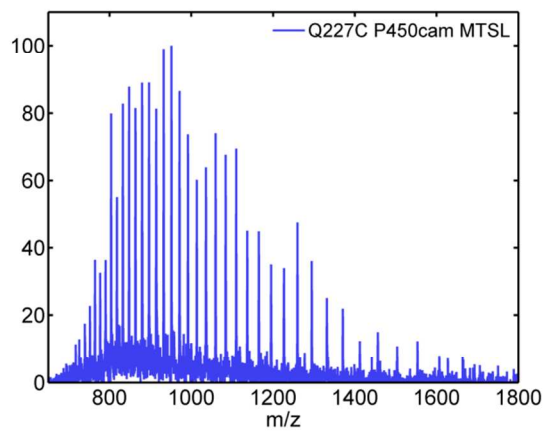
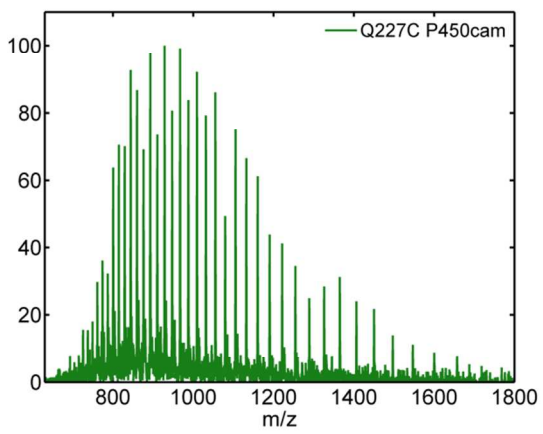
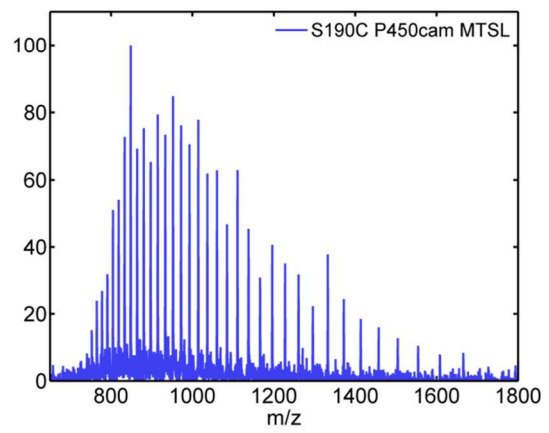
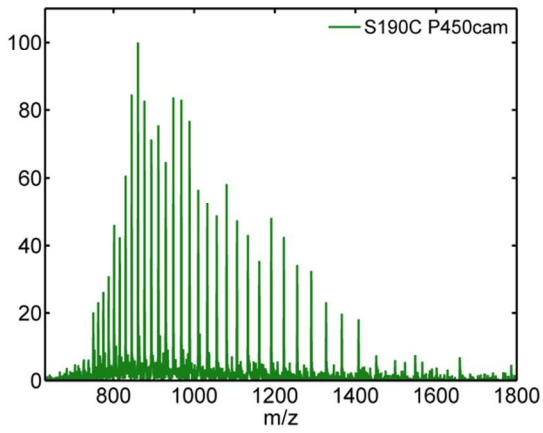


Figure S2: EPR spectra for MTSL-labeled P450cam_{S190C} at 140K and 0.2mW (red), reduced Pdx at 90K and 0.2mW (green) and reduced Pdx bound to MTSL-labeled P450cam_{S190C} at 90K and 0.2mW (blue). The modulation amplitude and conversion time are 10G and 40.96s, respectively.





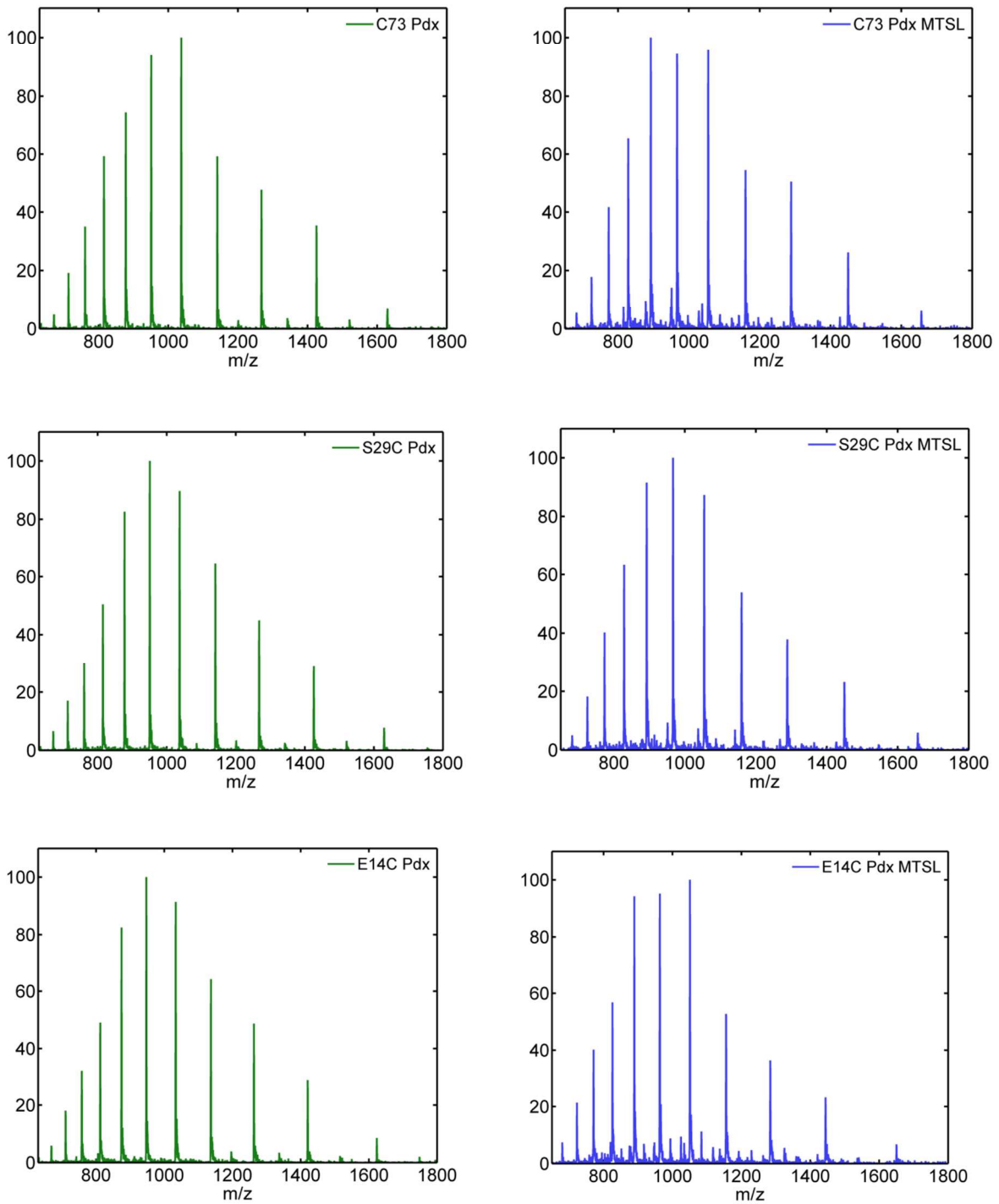
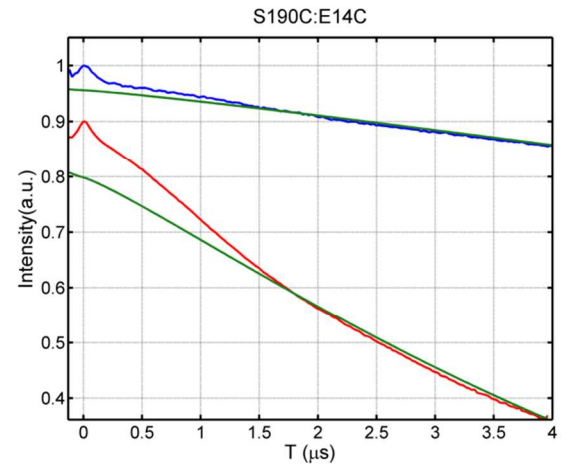
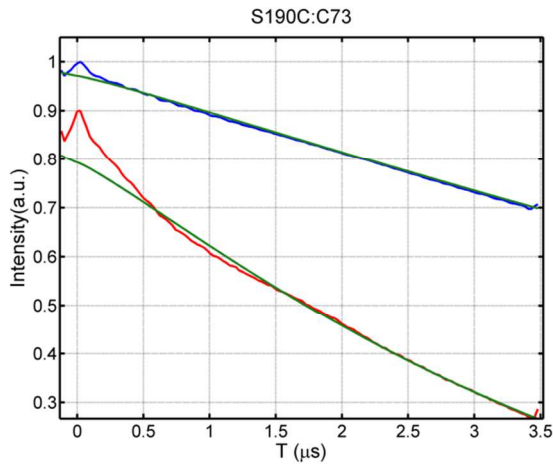
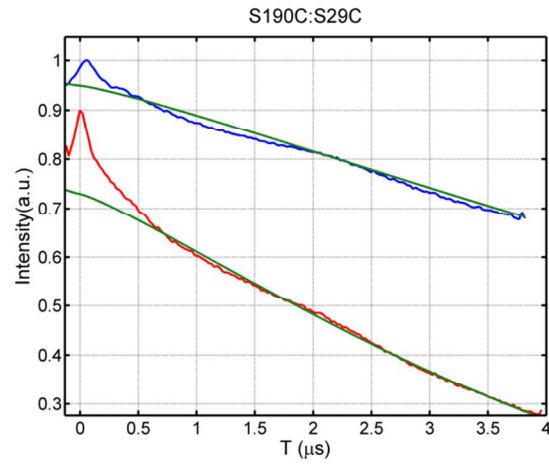
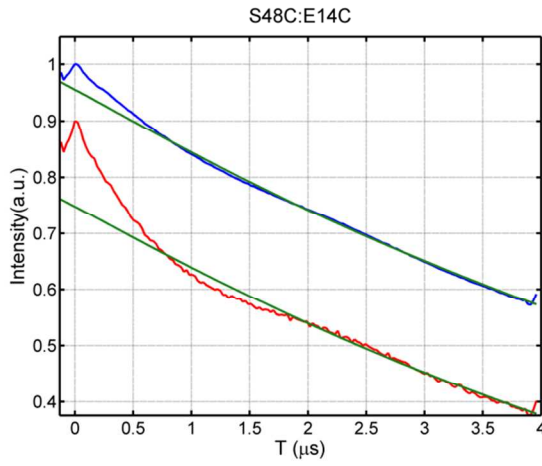
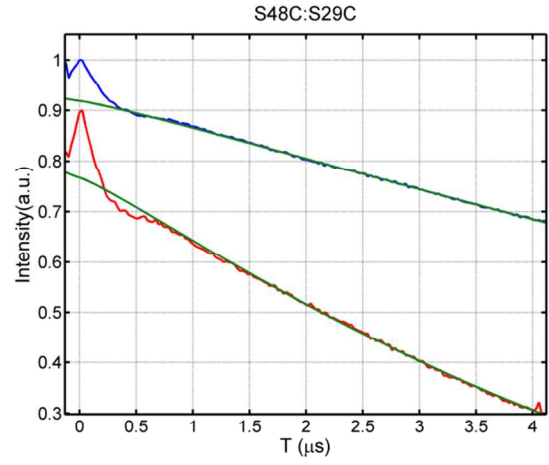
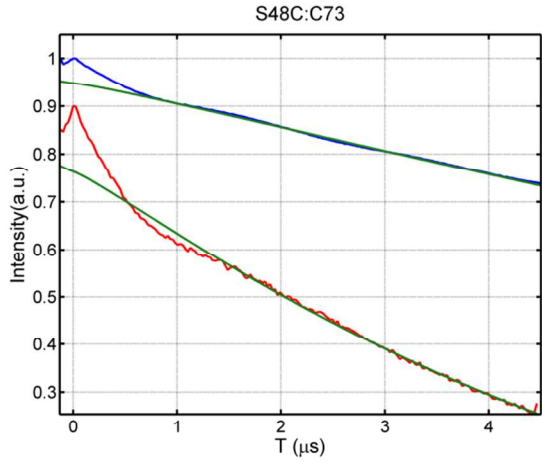


Figure S3. ESI Mass Spectra of four P450cam mutants and three Pdx mutants, before and after MTSL labeling.



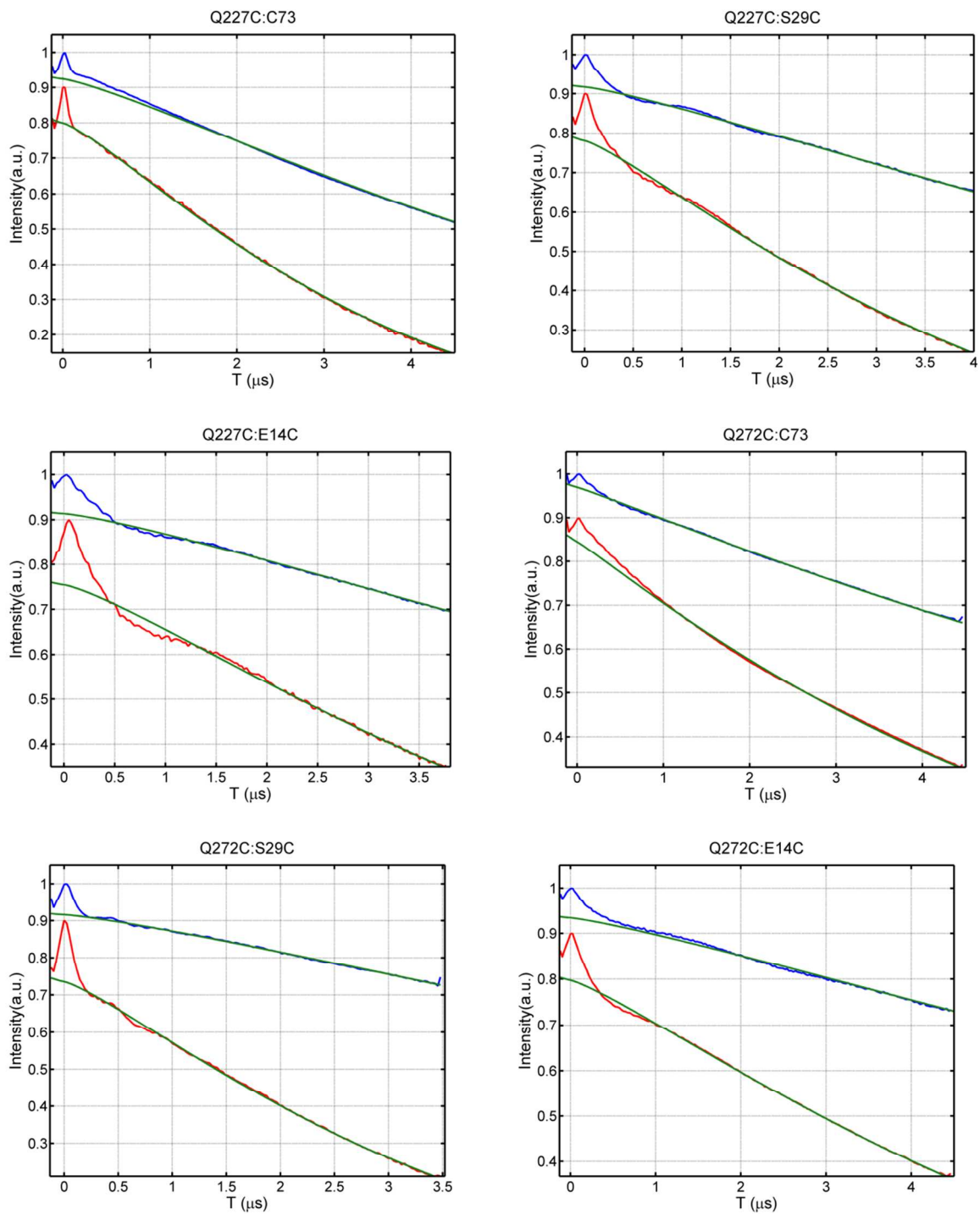


Figure S4. Raw DEER data and background subtraction fits for $(\text{Fe}^{3+})\text{P450cam/Pdx}_{\text{ox}}$ and $(\text{Fe}^{2+}\text{CO-S})\text{P450cam/Pdx}_{\text{re}}$ complexes. Blue and red traces are the spectra of $(\text{Fe}^{3+})\text{P450cam/Pdx}_{\text{ox}}$ and $(\text{Fe}^{2+}\text{CO-S})\text{P450cam/Pdx}_{\text{re}}$ complexes before background subtraction. The green traces are the background curves.

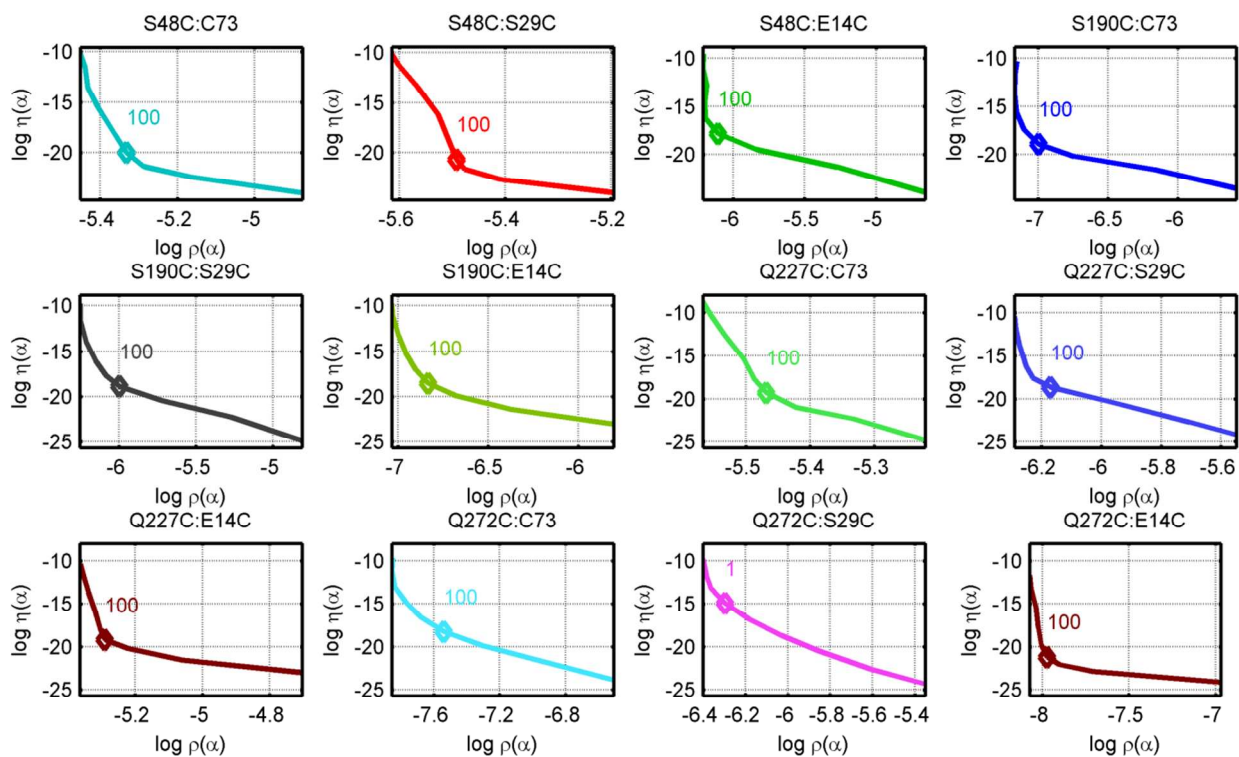


Figure S5. L-curve analysis of DEER spectra in the $(\text{Fe}^{3+})\text{P450cam}/\text{Pdx}_{\text{ox}}$ complexes.

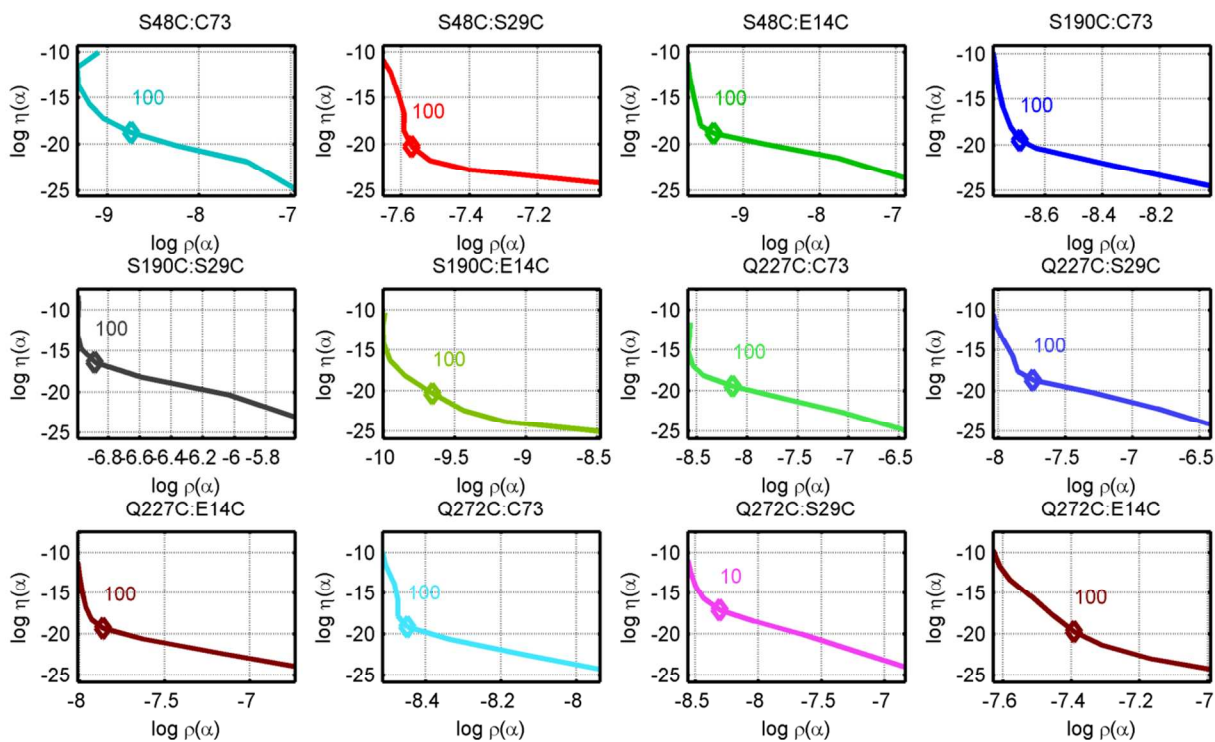


Figure S6. L-curve analysis of DEER spectra in the $(\text{Fe}^{2+}\text{CO-S})\text{P450cam}/\text{Pdx}_{\text{re}}$ complexes.

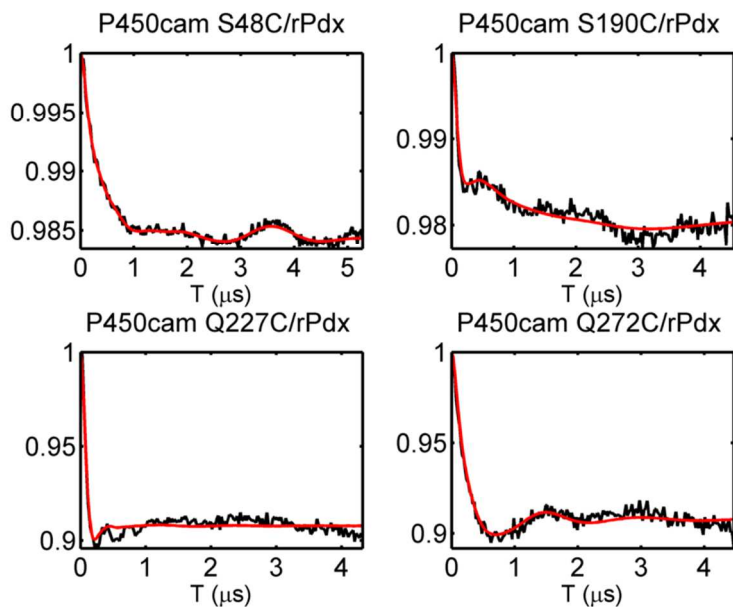


Figure S7. DEER spectra of four P450cam mutants with MTSL attached bound to reduced unlabeled wild-type Pdx.

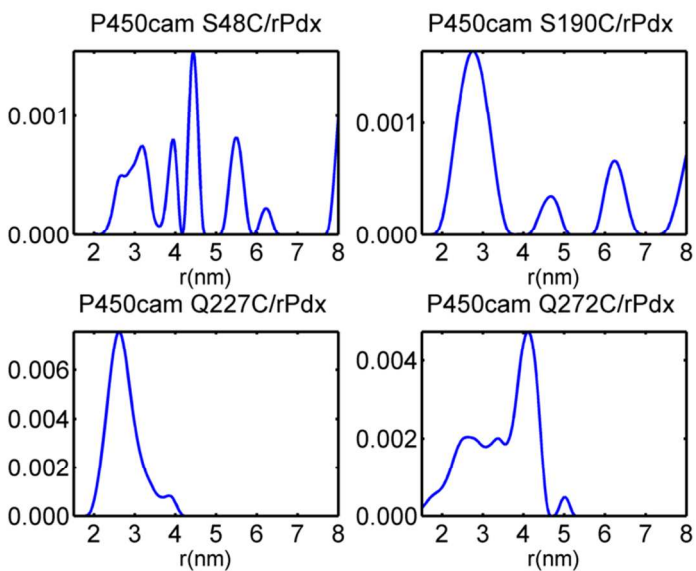


Figure S8. Distance distributions of four P450cam mutants with MTSL attached bound to reduced unlabeled wild-type Pdx.

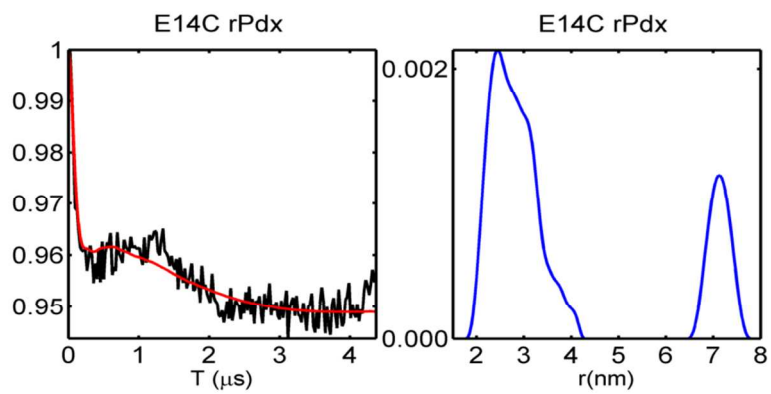


Figure S9. DEER spectrum and distance distribution of reduced and labeled E14C Pdx with MTSL attached.

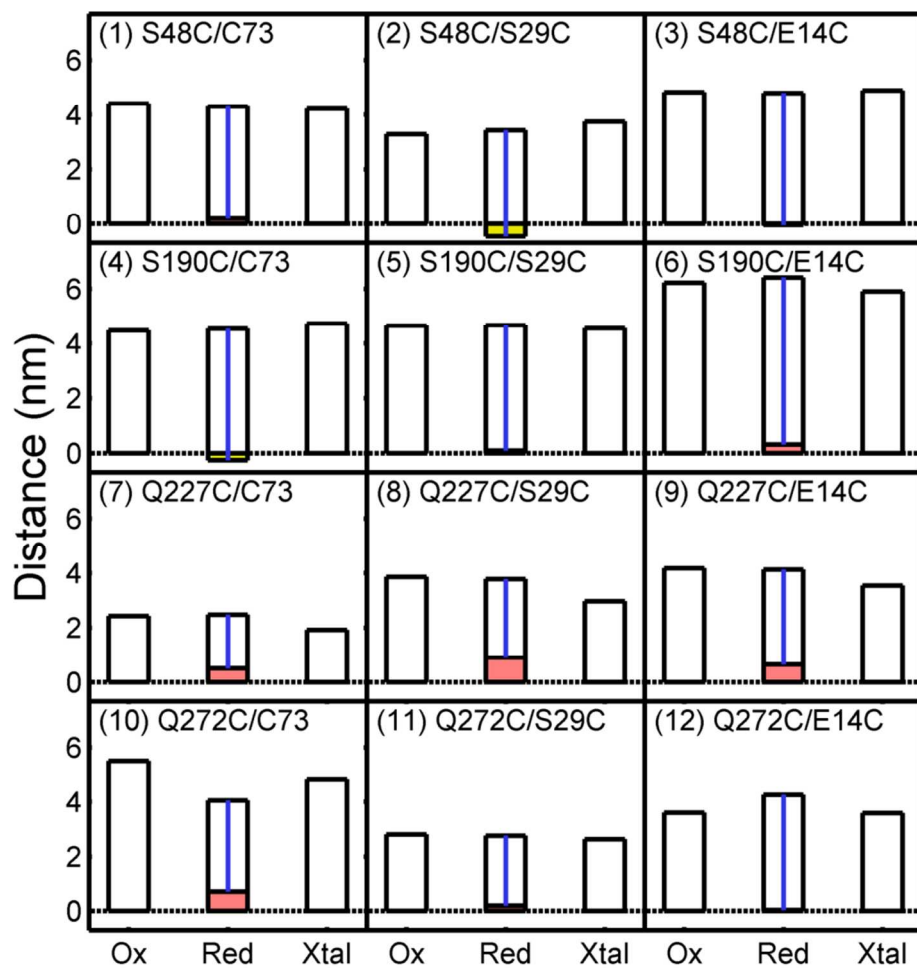


Figure S10. Bar diagram of distance distributions. The empty bars represent the distances from the P450cam(Fe^{3+})/Pd x_{ox} (Ox), P450cam($\text{Fe}^{2+}\text{CO}\cdot\text{S}$)/Pd x_{re} (Red) and the crystal structure (pdb entry 4JWS) (Xtal). The yellow or orange bars are the negative or positive distance differences (Δ) between P450cam(Fe^{3+})/Pd x_{ox} and crystal structure. The blue lines are $R_{\text{xtal,red}}$ after application of Δ to the $R_{\text{DEER,red}}$.