Supporting Information

Calculated and Experimental Spin State of Seleno Cytochrome P450

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Part I: Experimental Details

1. Materials

Buffer salts and chemicals were purchased from Fisher Scientific. *L*-selenocystine and amino acids were purchased from Sigma-Aldrich. Unless otherwise specified, all other reagents were purchased from Sigma-Aldrich (St. Louis, MO). BSTFA was purchased from Fisher (Thermo Scientific). Water was purified with a Milli-Q® purification system (Millipore). All buffers and supplements were prepared using Millipore water and the supplements were filter sterilized using a 0.22 μm filter.

2. Construction of CYP119 expression vector

The expression vector used for the expression of a C-terminal His_6-tagged SeCYP119 was constructed as follows. The forward primer 5'-CA GGAAACA**GGATCC**ATCGATGCTTAGGAGGTCATATGTATGACTGGTTTAGTG-3' and the reverse primer 5'-ATTATT**T C T A G A**CCGGAAGCT TTTAGTGATGGTGATGGTGATGTTCATTACTCTTCAACCTG-3' were used to amplify the CYP119 coding gene sequence from an existing pCW/*cyp119* vector as a template. The bold letters in the forward and reverse primers indicate engineered *BamH* I and *Xba* I restriction cloning sites, respectively. The underlined letters in the reverse primer encode a C-terminal His_6 tag. The PCR product was purified by 1% *(w/v)* agarose gel electrophoresis, digested directly using *Xba*I and *BamH*I (New England Biolabs), and ligated into a similarly cut pCWori(+) vector to provide the expression vector pCW/*CYP119*-CHis_6. The resulting plasmid was transformed into *E. coli* DH5α cells for ampicillin screening and the resulting construct was sequenced.

3. Preparation of minimal media

The cysteine auxotrophic *E. coli* host cell BL21(DE3) *selB::kan cys51E* (referred to as BL21(DE3)*cys*) was kindly provided by Dr. Marie-Paule Strub (National Institute of Health, Bethesda, MD). Competent cells were prepared by a modified RbCl method.

The cell growth medium (**A**) was prepared as follows (1 L): A mixture of NaOAc (1 g/L), NH_4Cl (2 g/L), K_2HPO_4 (10 g/L), sodium succinate (2.75 g/L), glycerol (0.8 %) and Milli-Q® water (900 mL) was autoclaved and cooled to room temperature. In a separate flask, the amino acid (–Cys) solution containing Ala, Arg, Gln, Glu, Gly, Ser (400 mg/L ea.), Asp, Met (250 mg/L ea.), and Asn, His, Ile, Leu, Lys, Pro, Thr, Tyr, Phe, Trp, Val (100 mg/L, ea.) was prepared in pre-autoclaved water. Typically, a 10x solution of the amino acid mixture (100 mL) was sterile filtered (0.22 μm) and added to the above mentioned buffer to make the cell growth medium. The medium was then supplemented with the sterile filtered stock solutions of the following components (the amounts provided are per 1L of media): Cys (60 mg), $Mg(OAc)_2$ (0.96 g), $CaCl_2$ (14.7 mg), biotin (0.5 mg), nicotinamide (100 mg), thiamine (50 mg), ampicillin (100 mg), kanamycin (50 mg) and a trace element solution (0.25 mL, containing $FeCl_3 \cdot 6H_2O$

(2.7 g/100 mL), $ZnCl_2 \cdot 4H_2O$ (0.2 g/100 mL), $CoCl_2 \cdot 6H_2O$ (0.2 g/100 mL), $CaCl_2.6H_2O$ (0.1 g/100 mL), $Na_2MoO_4 \cdot H_2O$ (0.2 g/100 mL), $CuCl_2$ (0.1 g/100 mL), H_3BO_3 (0.05 g/100 mL) and 10 mL con. HCl).

The cell expression medium, (**B**, 1 L) contained all ingredients except the cysteine.

The minimal media buffer to wash cells, (**C,** 1 L) contained NaOAc (1 g/L), K_2HPO_4 (10 g/L), NH_4Cl (2 g/L), sodium succinate (2.75 g/L), glycerol (0.8%) and Milli-Q® water (1000 mL). The wash buffer was autoclaved and pre-chilled to 4 ºC before use.

4. Expression and purification of seleno-CYP119

A pCW/*CYP119*-$CHis_6$ plasmid was freshly transformed into the BL21(DE3)c*ys* competent cells and plated on an LB/AMP plate containing cysteine (20 mg/L). The resulting plate was incubated in an oven at 37 °C for overnight. A single colony was picked and grown overnight in LB medium containing ampicillin (100 mg/L), kanamycin (50 mg/L), and Cys (60 mg/L) at 37 ºC with shaking at 250 rpm. 50 mL of this culture was centrifuged at 4,000 rpm for 15 min at 4 ºC and the pellet was re-suspended in 1 L of the cell growth medium **A**. The resulting culture was grown at 37 ºC with shaking at 250 rpm until the OD_{600} reached 1.2 – 1.3 (after 5 - 6 h). The cells were then harvested under sterile conditions at 4200 rpm for 15 min at 4 ºC. The resulting cell pellet was washed twice with pre-chilled minimal media buffer solution **C** (2 x 1 L) and re-suspended in 1 L of the cell expression medium **B**. The cells were then incubated at 30 °C with shaking at 220 rpm for 30 min followed by the addition of *L*-selenocystine (~100 mg/L), δ-aminolevulinic acid (1 mM) and IPTG (1 mM). The resulting pale yellow culture was further incubated at 30 ºC and 220 rpm for 14-16 h. The brick red colored cells were then harvested (by centrifugation at 4 °C, 4200 rpm for 12 min), washed with cold phosphate buffer (20 mM, pH 7.5 containing 1 mM EDTA), and stored at –80 ºC until further use. The wet cell pellet weighed ~7 g/L of culture.

The frozen cell pellet was thawed on ice and re-suspended in buffer **X** (50 mM sodium phosphate, pH 7.4, 500 mM NaCl, 5% (*v/v*) glycerol and 20 mM imidazole) along with 1 mM PMSF and 1 mg/mL lysozyme. The cell suspension was stirred at 4 ºC for 0.5 – 1 h followed by

sonication using a Branson sonicator (5 x 1-min bursts at 50% power, with 2 min cooling on ice between each burst). Cell debris was removed by centrifugation at 35000 rpm for 1 h at 4 ºC. The supernatant solution was loaded onto a 5 mL HisTrap™ HP nickel column (GE Healthcare) pre-equilibrated with buffer **X**. The column was then washed with buffer **X** containing 40 mM imidazole for approximately 10 column volumes. Finally, the seleno protein was eluted with buffer **X** solution containing 400 mM imidazole. Fractions of deep reddish color were collected and dialyzed overnight in 25 mM KPi buffer (pH 7.4) containing 5% glycerol (*v/v*) and 0.1 mM EDTA at 4 ºC. The resulting protein was further purified using a HiPrep Q-Sepharose™ (GE) fast flow column on FPLC (Amersham Bioscience). The column was washed with approx. 10 column volumes of buffer **Y** (50 mM Tris-HCl, pH 7.4 at 4 °C) and the protein was eluted using a gradient of buffer **Y** containing 250 mM NaCl (0-250 mM NaCl over 50 min). The red colored fractions containing the heme protein (Rz ≥ 1.2) were pooled and concentrated using an Amicon Ultra centrifugal filter (Millipore, 30,000 MWCO). The concentrated protein was buffer exchanged by dialyzing against KPi buffer (50 mM, pH 7.4) containing 5% glycerol (*v/v*) and 0.1 mM EDTA before storage. The concentration of SeCYP119 was estimated using a Soret absorption maximum at 454 nm obtained from a typical CO spectrum (assumed $\varepsilon_{454\ nm} = 91\ mM^{-1}cm^{-1}$). The estimated yield of seleno-CYP119 was ~2.6 mg/L of culture.

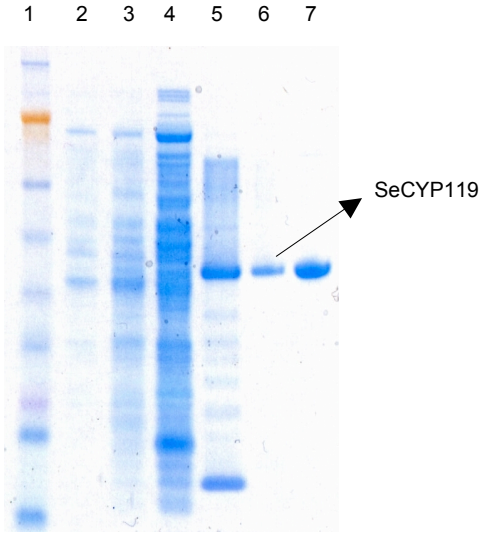


Figure S1. SDS-PAGE gel. Lane 1, protein marker; lane 2, cells after the initial growth phase; lane 3, cells after the expression in presence of SeCys; lane 4, supernatant before Ni column; lane 5, SeCYP119 after the Ni column; lane 6, SeCYP119 after the Q-Sepharose purification and lane 7, WT-CYP119

5. Optical absorption spectroscopy

Optical spectra were recorded on a CARY 1E UV-Vis scanning spectrophotometer (Varian) in 100 mM potassium phosphate buffer (pH 7.4) at 24 ºC (room temperature). The instrument was initially blanked with buffer solutions in both sample and reference cuvettes. The resting state spectrum of the protein (ferric) was recorded by adding the SeCYP119 (final concentration, 1.5 μM) to the sample cuvette and the resulting spectrum was scanned between 700 and 250 nm. Formation of the ferrous carbon monoxide complex was achieved by flushing the ferric enzyme solution with CO gas (Airgas, CA) for approximately 30 s through a septum-sealed cuvette followed by the injection of freshly prepared sodium dithionite solution (final concentration, 1 mM) using a gas tight syringe (Hamilton, Reno, NV). The reduction of ferric and simultaneous formation of ferrous-CO complex was monitored by recording the absolute UV-vis spectrum of the enzyme in a kinetic mode at 1 min interval. The spectrum for the wild type CYP119 was recorded in a similar manner.

Table S1. UV-vis absorption frequencies of the WT and SeCYP119

CYP119	WT	Se Mutant
Ferric	280, 360, 416, 536, 567 nm	280, 363, 417, 534, 568 nm
Ferrous-CO	449, 547 nm	454, 558 nm

6. Whole protein ESI/MS of SeCYP119

SeCYP119 sample was reconstituted to a final concentration of ~5 μM in a mixture of aqueous acetonitrile solution (1:1) containing 1% formic acid. ~5-10 pmol of protein from this solution was loaded onto a 1 x 150 mm C4 Vydac column and the contents were separated using a gradient elution with increase in concentration of acetonitrile (ACN) from 10-50 % over 40 min (mobile phase A: 0.1% TFA in H_2O and B: 0.1% TFA in acetonitrile). The sample was monitored at 214 nm using an UV detector. Protein fractions from the column were collected, concentrated and injected onto a self packed 0.1 x 100 mm C4 column held at 5 % ACN followed by a 5 – 50% gradient of ACN over 30 minutes in an LC/ESI-MS coupled to a MDS Sciex QSTAR hybrid quadrupole OA-TOF mass spectrometer. The resulting raw data was

collected, analyzed and protein spectra deconvoluted using the standard Analyst and BioAnalyst software of the QSTAR system.

Table S2. Whole protein ESI/MS data for the selenium incorporated CYP119

	Calculated MW (Da)	Observed MW (Da)
WT-CYP119	43685.9	43689 ± 5
SeCYP119	43734.9	43733 ± 5

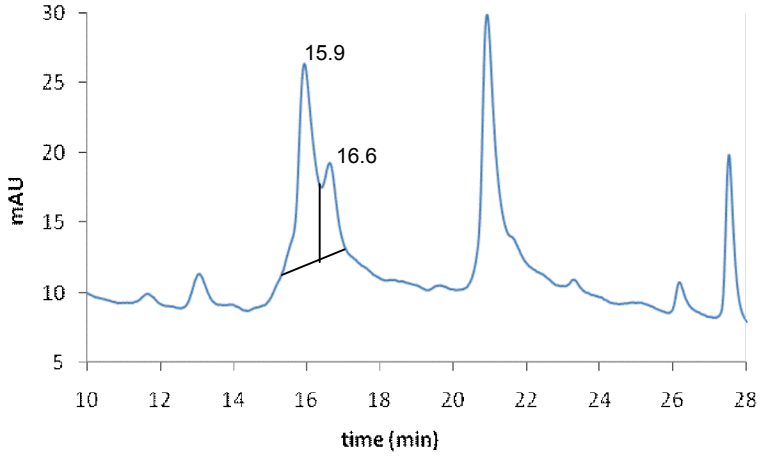


Figure S2. HPLC spectrum of the denatured SeCYP119 protein (integrated peaks indicate the presence of CYP119 at 16.6 min)

7. Enzyme activity assays

In a typical assay, SeCYP119 (1.5 µM) was pre-incubated in a mixture of potassium phosphate buffer (final concentration: 50 mM, pH 7.4) and lauric acid (100 µM) containing 1% ethanol by final volume for 5 min at 50 °C. To this was added hydrogen peroxide to a final concentration of 10 mM and the resulting mixture was incubated at 50 °C for 40 min (final volume, 500 µL). After which the reaction was quenched with 1 N hydrochloric acid and then extracted with diethyl ether (2 x 3 mL). The combined organic extracts were dried over anhydrous $MgCl_2$, filtered and concentrated under a gentle stream of nitrogen gas at ambient temperature. The resulting residue was derivatized by the addition of BSTFA (100 µL) and incubated at room

temperature (24 °C) for 1 h in a sealed screw cap vial. The *O*-silylated hydroxylated products were then analyzed by GC (Agilent Technologies, CA) coupled to a mass selective detector (MSD) operating in EI mode at a voltage of 70 eV and set to scan from *m/z* 40 to 600. The separation of products was achieved on a HP-5MS column (30 m x 0.25 mm i.d.) heated at 70 °C initially for 1 min followed by ramping at 10 °C per min up to 300 °C and held finally at 300 °C for 1 min. The ω-2, ω-1 and ω hydroxylated products appeared at the retention times of 15.6, 15.74 and 16.43 min respectively. The corresponding mass and fragmentation pattern of the products were matched with the appropriate standards. A control reaction with no P450 or with no hydrogen peroxide failed to generate any products, indicating that both enzyme and peroxide were necessary for the oxidation of the substrate. The activity was further confirmed by a time-course assay in which the above reaction was scaled up to a final volume of 1 mL and 0.25 mL of the reaction was quenched and analyzed as mentioned above at 15 min intervals. Furthermore, assuming a similar activity for both WT and seleno mutant, a control reaction containing 0.45 µM (30% of 1.5 µM used originally) of WT protein resulted in a specific activity of 24.6 pmoles min^{-1} $nmol^{-1}$ of enzyme. This indicates that the major portion of the activity observed in case of SeCYP119 stems from the reaction of the mutant protein and not from the WT contamination.

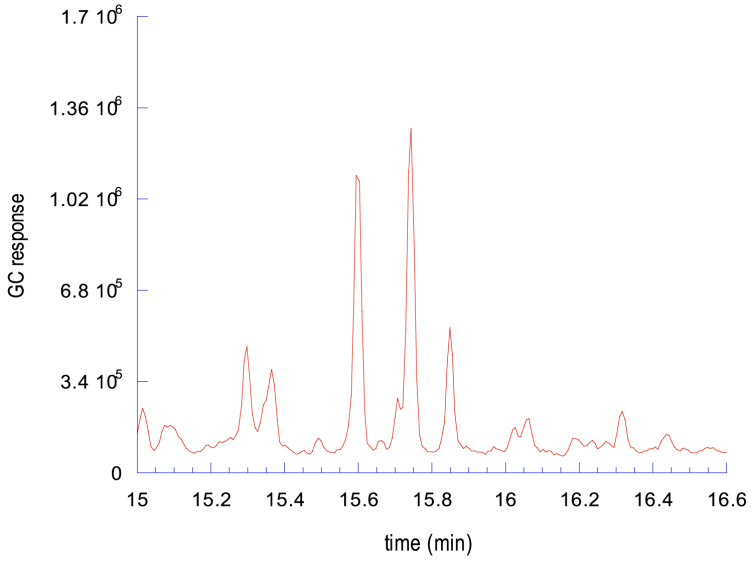


Figure S3. A representative GC trace of the BSTFA treated reaction mixture showing the separation of hydroxylated products.

Table S3. Regiospecificity in lauric acid hydroxylation by WT and SeCYP119 (values shown are the average of at least 3 independent measurements)

P450	% ω	% ω-1	% ω-2
WT-CYP119	1.5	53.1	45.4
SeCYP119	2.6	54.6	42.8

8. EPR and resonance Raman spectroscopy

The EPR and resonance Raman analyses were carried out at 40 and 90 μM enzyme concentrations, respectively. The ferric-nitrosyl complexes were prepared after prolonged purging with argon on a Schlenk line, and exposure to excess NO gas (^{14}NO from Airgas and ^{15}NO from ICON, previously treated with 1 M KOH) to reach approximately 1 mM NO concentration. The ferrous-CO complexes were prepared by addition of excess dithionite to protein samples saturated with CO. EPR spectra were obtained on a Bruker E500 X-band EPR spectrometer equipped with a superX microwave bridge and a dual mode cavity with a helium flow cryostat (ESR900, Oxford Instruments, Inc.). The microwave power, modulation amplitude, magnetic field sweep, and the sample temperature were varied to optimize the detection of all potential EPR active species. The resonance Raman spectra were obtained using a custom McPherson 2061/207 spectrograph (set at 0.67 m with variable gratings) equipped with a Princeton Instruments liquid-N_2-cooled CCD detector (LN-1100PB). Spectra were collected using a 90^o scattering geometry on room-temperature samples mounted on a reciprocating translation stage. The 413-nm excitation was obtained from an Innova 302 krypton laser (Coherent, Santa Clara CA), the 442-nm excitation from a He/Cd laser (Liconix 4240NB), and the 458-nm excitation from an Innova 90 argon laser (Coherent, Santa Clara CA). Optical supernotch filters (Kaiser Optical Systems, Ann Arbor MI) or long-pass filters (Edge filters, Semrock, Rochester NY) were used to attenuate Rayleigh scattering. Frequencies were calibrated relative to indene and aspirin standards and are accurate to ±1 cm^{-1}. Polarization conditions were optimized using CCl_4. The integrity of the RR samples, before and after illumination, was confirmed by direct monitoring of their UV-vis spectra in Raman capillaries.

8.1. EPR Spectrum.

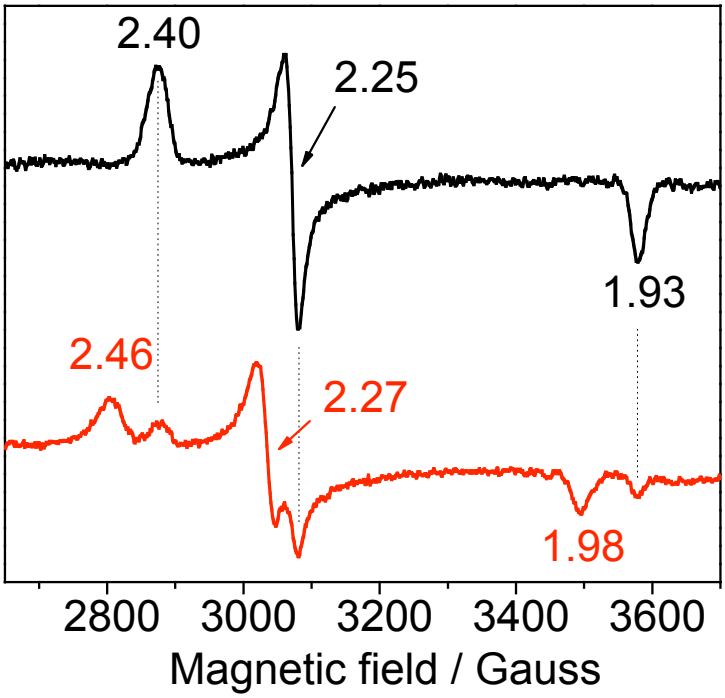


Figure S4. EPR spectra of ferric wild-type CYP119 (black) and SeCYP119 (red) at 10 K. conditions: microwave power, 0.1 mW; modulation amplitude, 10 G; microwave frequency, 9.66 GHz.

8.2. Resonance Raman spectra of ferric-nitrosyl complexes of WT and SeCYP119 proteins.

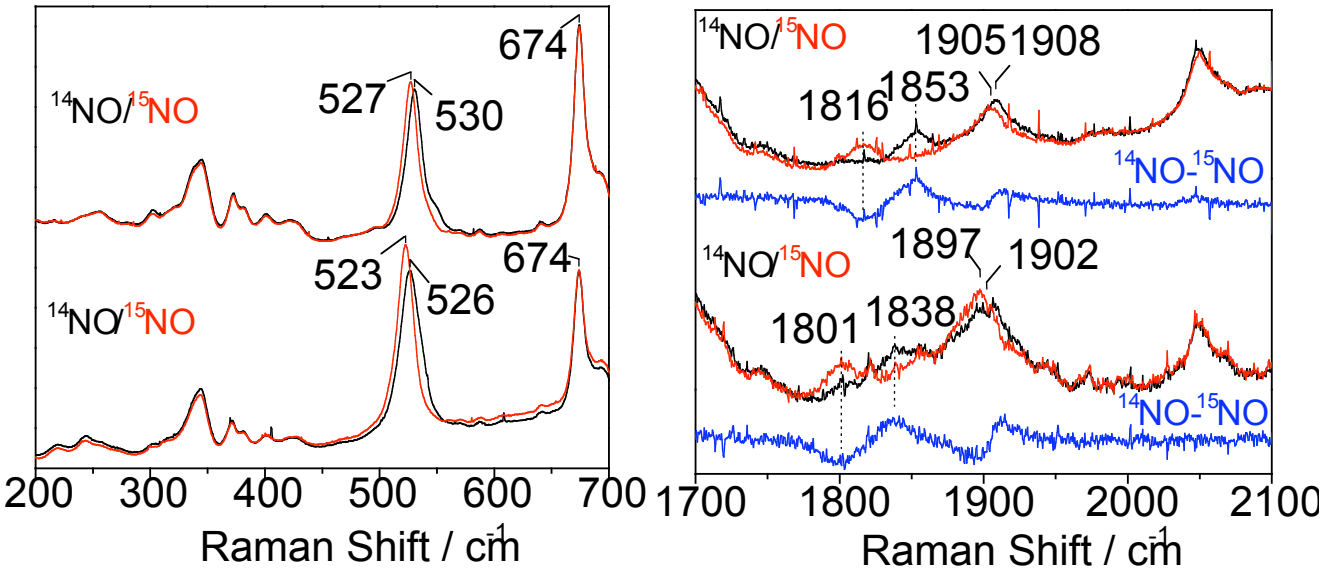


Figure S5. Resonance Raman spectra of ferric-nitrosyl complexes in WT CYP119 (top traces) and SeCYP119 (bottom traces) at room temperature (excitation wavelength, 442 nm; isotope-sensitive signals near 1900 cm^{-1} are assigned to [ν_4 + ν(Fe-NO)] combination bands).

8.3. Resonance Raman spectra of ferrous-carbonyl complexes of WT and SeCYP119.

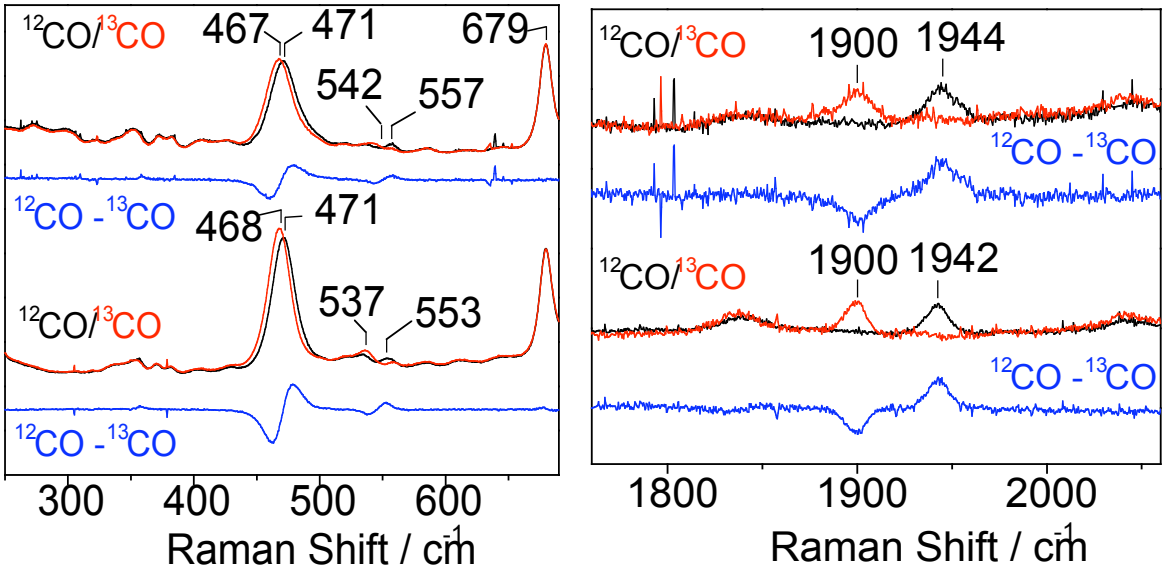


Figure S6. Resonance Raman spectra of ferrous-carbonyl complexes in WT CYP119 (top traces) and SeCYP119 (bottom traces) at room temperature (excitation wavelength, 458 nm).

Part II: Theoretical calculations

9. Se-Resting state Mutant of P450cam

9.1 Setup of the system

Since the structure of the selenocysteine mutant is not known, we derived an initial geometry of Se-OH_2 the resting state with a selenocysteine ligand (Se-OH_2) from a previously investigated model system for the wild type resting state of CYP101 (S-OH_2). This model system was built from the experimental X-ray structure of the Resting state species of CYP101 (PDB code 1DZ4, Brookhaven Protein Database) (Schlichting, I.; Berendzen, J. ;Chu, K.; Stock, A.M.; Maves, S.A.; Benson, D.A.; Sweet, R.M.; Ringe, D.; Petsko, G.A.; Sligar, S.G. *Science* **2000**, *287*, 1615) The construction of the model system and the preparatory force field calculations were described in details in Schöneboom, J.C; Thiel, W. J. Phys. Chem. B **2004**, 108, 7468-7478. The protonation state of the protein was considered, as Prot1 in Schöneboom, J.C; Thiel, W. *J. Phys. Chem. B* **2004**, 108, 7468-7478. The complete system consists of 28,498 atoms, including 21,117 atoms in the solvent.

We selected three snapshots from a molecular dynamics (MD) trajectory of S-OH_2 which were previously fully optimized by means of QM/MM geometry optimization. The cysteine was mutated to selenocysteine and a full QM/MM optimization was performed for the three snapshots corresponding to 60, 120 and 200ps from the equilibrium trajectory of the WT species. Three basis sets were used abbreviated as B1, B2 and B_L (see definitions in section F3.3). All snapshots were fully optimized with B1 and B2. Additional single point energy calculations were carried out with B_L on the fully optimized snapshot with B1 abbreviated as B//B1. The largest basis set, B_L, was used for the full optimization of snapshot 200ps. Details on the QM/MM method and software are described in details in Cohen, S.; Kumar, D.;Shaik S., *J. Am. Chem. Soc*.,**2006**,128, 2649-2653.

9.2 Force Field Parameters

The force field parameters for the axial water ligand were taken from the standard TIP3P parameter set (oxygen: type OT, charge -0.834e; hydrogen: type HT, charge 0.417e).The

selenium atom of the proximal cysteine was assigned an atomic charge of -0.07e. All other parameters were used as obtained from the CHARMM22 library.

9.3 Basis set notations

B1: LACVP for iron, 6-31G for the rest.

B2: Wachters' all-electron basis set with an additional diffuse d function and a set of f polarization functions in the contraction [8s7p4d1f] for Fe, LACVP+* for selenium, 6-31+G* for five coordinating atoms around Fe (four pyrrole nitrogens, and the O atom of the axial water ligand), 6-31++G** for the two protons of the axial water ligand and 6-31G for others.

$\mathbf{B}_L$: valence triple-_ basis set with polarization on all atoms (TZVP).

(A. Schafer, C. Huber and R. Ahlrichs, *J. Chem. Phys.* **1994**, *100***,** 5829)

9.4 QM region notations

R1: iron- porphine, axial water ligand, SeCys357, CO group of Leu356 and NH-C^aH_2 unit of Leu358

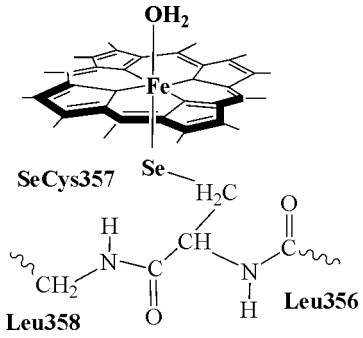


Table S4. Summary of QM and QM/MM energies for Se-OH_2 species (UB3LYP/MM)

Basis B1		Absolute Energy (a.u.)			Relative Energy (kcal/mol)		
		E_{QM}	E_{MM}	$E_{QM/MM}$	DE_{QM}	DE_{MM}	$DE_{QM/MM}$
Snap 60ps	^2A	-1653.286992	-143.712292	-1796.999284	0.00	0.00	0.00
	^4A	-1653.286157	-143.707130	-1796.993288	0.52	3.24	3.76
	^6A	-1653.27759	-143.71107	-1796.98866	5.90	0.77	6.67
Snap 120ps	^2A	-1653.283345	-143.598310	-1796.881655	0.00	0.00	0.00
	^4A	-1653.280364	-143.595731	-1796.876095	1.87	1.62	3.49
	^6A	-1653.278044	-143.595073	-1796.873118	3.33	2.03	5.36
Snap 200ps	^2A	-1653.285688	-143.693749	-1796.979438	0.00	0.00	0.00
	^4A	-1653.284809	-143.691761	-1796.976569	0.55	1.25	1.80
	^6A	-1653.275842	-143.695381	-1796.971223	6.18	-1.02	5.16
Basis B2		E_{QM}	E_{MM}	$E_{QM/MM}$	DE_{QM}	DE_{MM}	$DE_{QM/MM}$
Snap 60ps	^2A	-2793.669157	-143.718249	-2937.387405	0.00	0.00	0.00
	^4A	-2793.671290	-143.713388	-2937.384678	-1.34	3.05	1.71
	^6A	-2793.667230	-143.716786	-2937.384016	1.21	0.92	2.13
Snap 120ps	^2A	-2793.662844	-143.600206	-2937.263050	0.00	0.00	0.00
	^4A	-2793.679197	-143.591522	-2937.270719	-10.26	5.45	-4.81
	^6A	-2793.676986	-143.593610	-2937.270596	-8.87	4.14	-4.74
Snap 200ps	^2A	-2793.665376	-143.695890	-2937.361266	0.00	0.00	0.00
	^4A	-2793.667338	-143.694050	-2937.361388	-1.23	1.15	-0.08
	^6A	-2793.662048	-143.696986	-2937.359034	2.09	-0.69	1.40
Basis B_L//B1		E_{QM}	E_{MM}	$E_{QM/MM}$	DE_{QM}	DE_{MM}	$DE_{QM/MM}$
Snap 60ps	^2A	-5186.899633	-143.554699	-5330.454332	0.00	0.00	0.00
	^4A	-5186.901936	-143.549626	-5330.451563	-1.45	3.18	1.74
	^6A	-5186.897321	-143.553578	-5330.450900	1.45	0.70	2.15
Snap 120ps	^2A	-5186.896646	-143.438700	-5330.335346	0.00	0.00	0.00
	^4A	-5186.896989	-143.436171	-5330.333159	-0.22	1.59	1.37
	^6A	-5186.897074	-143.435656	-5330.332730	-0.27	1.91	1.64
Snap 200ps	^2A	-5186.900036	-143.535960	-5330.435996	0.00	0.00	0.00
	^4A	-5186.902171	-143.534183	-5330.436355	-1.34	1.12	-0.22
	^6A	-5186.896315	-143.537822	-5330.434137	2.33	-1.17	1.17
Basis B_L		E_{QM}	E_{MM}	$E_{QM/MM}$	DE_{QM}	DE_{MM}	$DE_{QM/MM}$
Snap 200ps	^2A	-5186.904892	-143.544337	-5330.449230	0.00	0.00	0.00
	^4A	-5186.907683	-143.542193	-5330.449877	-1.75	1.35	-0.41
	^6A	-5186.903112	-143.545604	-5330.448716	1.12	-0.79	0.32

Table S5. Geometries for the Se-OH$_2$ species (UB3LYP/MM)

Basis B1	Distances[Å]			Angles[^{o}]				
	Fe-O	Fe-Se	Av(Fe-N)	O-Fe-Se	Fe-Se-C_β	Fe-O-H1	Fe-O-H2	O-H1-H2-Fe
Snap 60ps								
^{2}A	2.123	2.440	2.031	172.3	127.5	107.3	113.3	-41.2
^{4}A	2.424	2.643	2.030	169.4	124.2	100.7	107.5	-53.4
^{6}A	2.367	2.586	2.086	169.7	124.6	101.7	109.2	-50.9
Snap 120ps								
^{2}A	2.123	2.455	2.030	171.7	129.5	108.5	111.6	-41.8
^{4}A	2.424	2.679	2.028	165.2	126.3	105.5	104.9	-51.6
^{6}A	2.448	2.655	2.083	161.5	126.5	111.8	107.0	-44.2
Snap 200ps								
^{2}A	2.137	2.469	2.032	169.2	128.7	105.9	120.9	-36.1
^{4}A	2.420	2.712	2.030	164.8	125.7	105.6	119.2	-38.8
^{6}A	2.362	2.667	2.083	165.0	125.8	106.1	119.3	-38.1
Basis B2	Fe-O	Fe-Se	Av(Fe-N)	O-Fe-Se	Fe-Se-C^β	Fe-O-H1	Fe-O-H2	O-H1-H2-Fe
Snap 60ps								
^{2}A	2.215	2.362	2.033	170.1	128.9	104.1	112.8	-46.2
^{4}A	2.649	2.558	2.034	165.9	125.4	99.1	108.0	-55.8
^{6}A	2.700	2.485	2.093	166.6	125.0	94.8	108.1	-59.4
Snap 120ps								
^{2}A	2.220	2.371	2.032	171.9	129.7	108.6	110.9	-44.0
^{4}A	4.364	2.548	2.029	156.0	126.2	82.1	41.8	-139.2
^{6}A	4.526	2.476	2.097	156.6	125.5	80.5	42.6	-140.5
Snap 200ps								
^{2}A	2.257	2.382	2.034	169.3	129.1	106.4	121.4	-37.5
^{4}A	2.762	2.602	2.031	164.5	125.7	110.7	121.6	-34.5
^{6}A	2.685	2.527	2.090	166.5	125.5	107.2	122.0	-37.3
Basis B_L	Fe-O	Fe-Se	Av(Fe-N)	O-Fe-Se	Fe-Se-C^β	Fe-O-H1	Fe-O-H2	O-H1-H2-Fe
Snap 200ps								
^{2}A	2.234	2.396	2.033	169.7	129.4	107.6	121.8	-36.1
^{4}A	2.598	2.617	2.032	165.5	126.5	109.8	121.3	-35.5
^{6}A	2.556	2.534	2.090	166.7	126.1	109.0	121.9	-35.6

Table S6. Group spin and charge densities for the Se- OH_2 species (UB3LYP/MM)

Basis B1		Spin Densities				Charge Densities			
		ρ_{Fe}	ρ_{OH2}	ρ_{SeL}	ρ_{Por}	Q_{Fe}	Q_{OH2}	Q_{SeL}	Q_{Por}
Snap 60ps	^{2}A	1.231	0.005	-0.137	-0.098	0.365	0.192	-0.142	-0.415
	^{4}A	2.579	0.021	0.418	-0.017	0.529	0.155	-0.332	-0.352
	^{6}A	3.989	0.024	0.456	0.530	0.679	0.163	-0.256	-0.586
Snap 120ps	^{2}A	1.253	0.004	-0.157	-0.100	0.383	0.200	-0.165	-0.419
	^{4}A	2.589	0.022	0.409	-0.019	0.548	0.158	-0.355	-0.352
	^{6}A	3.986	0.021	0.466	0.527	0.700	0.163	-0.281	-0.583
Snap 200ps	^{2}A	1.279	0.004	-0.179	-0.104	0.408	0.203	-0.185	-0.427
	^{4}A	2.599	0.023	0.402	-0.024	0.583	0.160	-0.378	-0.365
	^{6}A	4.003	0.025	0.445	0.527	0.737	0.171	-0.308	-0.600
Basis B2		ρ_{Fe}	ρ_{OH2}	ρ_{SeL}	ρ_{Por}	Q_{Fe}	Q_{OH2}	Q_{SeL}	Q_{Por}
Snap 60ps	^{2}A	1.139	0.002	-0.062	-0.079	1.225	0.160	-0.586	-0.800
	^{4}A	2.573	0.019	0.416	-0.009	1.399	0.008	-0.669	-0.738
	^{6}A	4.046	0.019	0.439	0.496	1.542	0.011	-0.632	-0.921
Snap 120ps	^{2}A	1.157	0.001	-0.077	-0.081	1.186	0.159	-0.597	-0.747
	^{4}A	2.547	-0.001	0.426	0.027	1.067	0.009	-0.644	-0.432
	^{6}A	4.021	-0.001	0.421	0.559	1.093	0.009	-0.605	-0.497
Snap 200ps	^{2}A	1.187	0.001	-0.103	-0.085	1.186	0.122	-0.607	-0.701
	^{4}A	2.588	0.013	0.404	-0.005	1.368	-0.035	-0.678	-0.656
	^{6}A	4.059	0.018	0.423	0.500	1.488	-0.015	-0.647	-0.826
Basis B_L//B1		ρ_{Fe}	ρ_{OH2}	ρ_{SeL}	ρ_{Por}	Q_{Fe}	Q_{OH2}	Q_{SeL}	Q_{Por}
Snap 60ps	^{2}A	1.212	0.002	-0.110	-0.104	0.350	0.176	-0.212	-0.314
	^{4}A	2.678	0.017	0.364	-0.059	0.551	0.090	-0.426	-0.215
	^{6}A	4.166	0.020	0.392	0.423	0.734	0.107	-0.353	-0.489
Snap 120ps	^{2}A	1.236	0.001	-0.130	-0.106	0.351	0.187	-0.227	-0.311
	^{4}A	2.684	0.018	0.357	-0.058	0.560	0.093	-0.440	-0.213
	^{6}A	4.155	0.017	0.403	0.425	0.776	0.097	-0.364	-0.509
Snap 200ps	^{2}A	1.258	0.001	-0.149	-0.110	0.371	0.180	-0.240	-0.311
	^{4}A	2.692	0.018	0.352	-0.062	0.581	0.093	-0.463	-0.211
	^{6}A	4.172	0.020	0.382	0.426	0.765	0.112	-0.395	-0.481
Basis B_L		ρ_{Fe}	ρ_{OH2}	ρ_{SeL}	ρ_{Por}	Q_{Fe}	Q_{OH2}	Q_{SeL}	Q_{Por}
Snap 200ps	^{2}A	1.222	0.000	-0.111	-0.110	0.352	0.145	-0.197	-0.300
	^{4}A	2.692	0.010	0.355	-0.057	0.559	0.062	-0.422	-0.200
	^{6}A	4.170	0.011	0.379	0.439	0.729	0.073	-0.335	-0.467

Table S7. Hydrogen bonds to Se- OH_2 species (UB3LYP/MM)

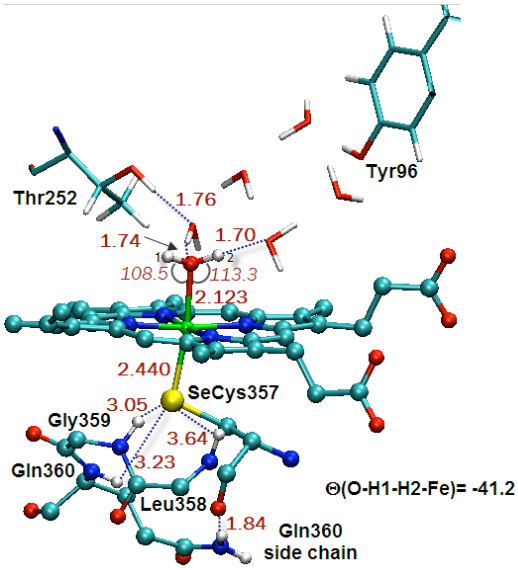
Basis B1	Backbone N-H---Se(Cys)						Side chain Gln360 amid N-H----O=C(Cys357)	
	Distances [Å]			Angles [°]			Distance [Å]	Angle [°]
	Leu358	Gly359	Gln360	Leu358	Gly359	Gln360		
Snap 60ps								
^{2}A	3.64	3.05	3.23	78.4	108.4	95.7	1.84	169.2
^{4}A	3.62	2.96	3.02	77.4	109.2	99.9	1.83	169.6
^{6}A	3.64	3.03	3.04	76.5	106.2	98.9	1.84	168.7
Snap 120ps								
^{2}A	3.67	2.69	3.39	77.9	128.7	93.1	1.85	163.2
^{4}A	3.66	2.62	3.21	77.1	129.8	95.0	1.85	162.8
^{6}A	3.69	2.68	3.28	76.4	126.9	93.1	1.85	163.4
Snap 200ps								
^{2}A	3.63	2.48	3.36	81.2	139.3	104.9	3.84	72.4
^{4}A	3.66	2.45	3.05	79.1	138.0	108.5	3.74	73.5
^{6}A	3.69	2.48	3.09	77.7	135.8	106.0	3.77	72.9
Basis B2								
Snap 60ps								
^{2}A	3.64	3.06	3.28	78.3	109.3	96.0	1.84	169.4
^{4}A	3.64	2.96	3.01	76.6	109.6	101.2	1.84	169.2
^{6}A	3.66	3.05	3.06	105.6	98.3	40.0	1.85	168.6
Snap 120ps								
^{2}A	3.65	2.68	3.40	78.1	129.3	93.4	1.86	162.7
^{4}A	3.70	2.65	3.23	75.3	128.6	94.5	1.85	162.8
^{6}A	3.75	2.73	3.23	73.3	124.2	92.4	1.85	163.2
Snap 200ps								
^{2}A	3.63	2.48	3.36	81.2	139.3	104.9	3.84	72.4
^{4}A	3.66	2.45	3.05	79.1	138.0	108.5	3.74	73.5
^{6}A	3.69	2.48	3.09	77.7	135.8	106.0	3.77	72.9
Basis B_L								
Snap 200ps								
^{2}A	3.62	2.49	3.36	82.1	139.1	104.7	3.86	71.9
^{4}A	3.63	2.44	3.08	80.7	138.5	108.0	3.76	72.9
^{6}A	3.65	2.47	3.12	79.5	136.4	105.9	3.792	72.5

Table S8. Summary of QM and QM/MM single-point energies for Se-OH_2 species (UB-LYP/MM)

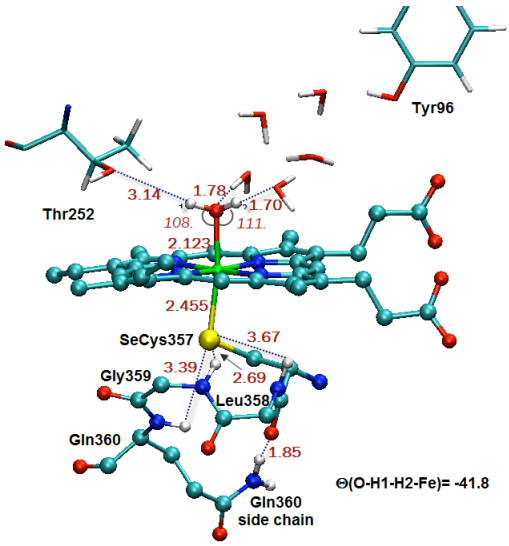
Basis B1		Absolute Energy (a.u.)			Relative Energy (kcal/mol)		
		E_{QM}	E_{MM}	$E_{QM/MM}$	ΔE_{QM}	ΔE_{MM}	$\Delta E_{QM/MM}$
Snap 60ps	^{2}A	-1652.723131	-143.712292	-1796.435423	0.00	0.00	0.00
	^{4}A	-1652.713089	-143.707054	-1796.420143	6.30	3.29	9.59
	^{6}A	-1652.690744	-143.710982	-1796.401726	20.32	0.82	21.14
Snap 120ps	^{2}A	-1652.719227	-143.598292	-1796.317519	0.00	0.00	0.00
	^{4}A	-1652.707402	-143.595647	-1796.303049	7.42	1.66	9.08
	^{6}A	-1652.691185	-143.595019	-1796.286204	17.60	2.05	19.65
Snap 200ps	^{2}A	-1652.721780	-143.693628	-1796.415408	0.00	0.00	0.00
	^{4}A	-1652.711766	-143.691741	-1796.403507	6.28	1.18	7.47
	^{6}A	-1652.688844	-143.695279	-1796.384123	20.67	-1.04	19.63

Table S9. Group spin and charge densities for the Se- OH_2 species (UB-LYP/MM)

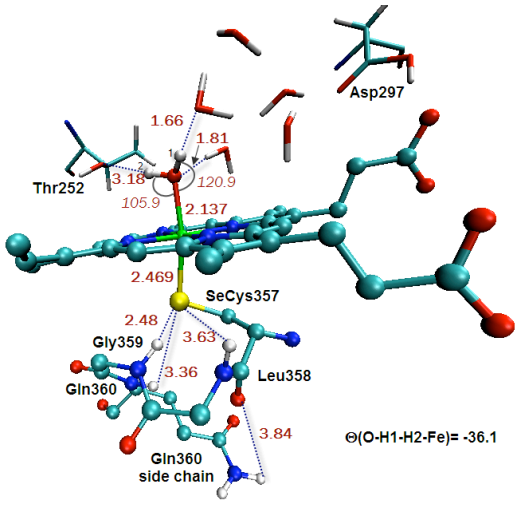
Basis B1	Spin Densities				Charge Densities			
	ρ_{Fe}	ρ_{OH2}	ρ_{SeL}	ρ_{Por}	Q_{Fe}	Q_{OH2}	Q_{SeL}	Q_{Por}
Snap 60ps								
^{2}A	0.964	0.003	0.076	-0.043	0.197	0.219	-0.115	-0.302
^{4}A	2.431	0.027	0.477	0.064	0.355	0.174	-0.300	-0.229
^{6}A	3.845	0.031	0.515	0.609	0.488	0.182	-0.232	-0.437
Snap 120ps								
^{2}A	1.006	0.002	0.036	-0.045	0.215	0.228	-0.143	-0.300
^{4}A	2.449	0.028	0.457	0.066	0.375	0.179	-0.329	-0.225
^{6}A	3.853	0.028	0.505	0.613	0.510	0.184	-0.267	-0.428
Snap 200ps								
^{2}A	1.032	0.002	0.011	-0.045	0.241	0.232	-0.166	-0.307
^{4}A	2.465	0.029	0.443	0.063	0.411	0.182	-0.356	-0.237
^{6}A	3.868	0.033	0.485	0.614	0.546	0.193	-0.292	-0.447



Snapshot 60 ps



Snapshot 120 ps



Snapshot 200 ps

Figure S7. Detailed structure of the Se-OH_2 species in the ^{2}A state (B3LYP/MM, B1 basis).