Supporting Information

Redox cofactor rotates during its stepwise decarboxylation – molecular mechanism of conversion of coproheme to heme b

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4.1 Generation of LmChdC variants

Site-directed mutagenesis to obtain LmChdC M149A, Q187A and M149A/Q187A variants using the QuikChange Lightning Kit (Agilent Technologies) has been described previously. Generation of LmChdC Y147A, Y147H, R133A, R179A, K151A, Y113A and Y113A/K151A, Y147A/R220A/S225A variants were produced following the same protocol as described in detail previously.

4.2 Expression and purification of LmChdC wild-type and variants.

LmChdC wild-type and all variants were subcloned into a modified version of the pET21b(+) expression vector with an N-terminal StreplI-tag, cleavable TEV protease, or into a pETM11 expression vector with an N-terminal, TEV cleavable 6×His-tag, expressed in E. coli Tuner (DE3) cells (Merck/Novagen) and purified via a StrepTrap HP or a HisTrap HP 5 mL column (GE Healthcare) as described in detail previously. The enzymes were reconstituted by the addition of equimolar concentrations of free Fe(III) coproporphyrin III chloride (coproheme; Frontiers Scientific) (in 50 mM phosphate buffer, pH 7.0, predissolved in 0.5 M NaOH, \( \varepsilon_{390} = 128,800 \text{ M}^{-1} \text{ cm}^{-1} \)) to apo-LmChdC dissolved in 50 mM phosphate buffer, pH 7.0.

4.3 Coproheme decarboxylase activity

In order to test coproheme decarboxylase activity of the LmChdC Y147A variant, titrations were performed where small (sub-equimolar) aliquots of H\(_2\)O\(_2\) were added to coproheme-ChdCs. In a typical experiment H\(_2\)O\(_2\) was added in 1 µM aliquots to 5 µM coproheme bound to excess ChdC (20 µM). To ensure complete reaction of the H\(_2\)O\(_2\), an interval of at least 15 min was set between each peroxide addition. The titrations were performed in a stirred 1 mL cuvette at room temperature and monitored by UV-vis absorption spectroscopy between 250 and 700 nm, using a scanning spectrophotometer (Cary60).

4.4 Coproheme decarboxylase activity triggered with chlorite

In order to determine the stoichiometry of chlorite to coproheme, titrations were performed where small (sub-equimolar) aliquots of chlorite were added to coproheme-LmChdC. In a typical
experiment chlorite was added in 1 µM aliquots to 7 µM coproheme-LmChdC. The reaction was completed rapidly and, in contrast to titration with hydrogen peroxide, there was no need to wait for 15 min between additions of the oxidant. The titrations were performed in a stirred cuvette (1 mL sample volume) at room temperature and monitored by UV-vis absorption spectroscopy between 250 and 700 nm, using a scanning spectrophotometer (Cary60).

4.5 Pre-steady-state kinetics

Transient pre-steady-state kinetics were measured with a stopped-flow apparatus (SX-18MV or pi-star fitted equipped with diode array detector or a monochromator) from Applied Photophysics. The optical quartz cell has a path length of 10 mm and a volume of 20 µL. The fastest time for mixing was 0.68 ms. Measurements were performed at room temperature. For single wavelength measurements a minimum of three reactions were monitored for each substrate concentration. Hydrogen peroxide, hypochlorous acid, chlorite, peroxoacetic acid, dicumyl peroxide, and tert-butyl hydroperoxide were used as substrate in the concentration range from 1 µM to 1 mM. 1 - 5 µM LmChdC variant Y147H was mixed with substrates at pH 7, pH 8.5 (50 mM HEPES) and pH 10 (50 mM borate buffer) and reactions were followed with the diode array detector for screening of the reactivity. For the determination of $k_{\text{app}}$ of Compound I formation of LmChdC Y147H, 5 µM enzyme were mixed with 250, 500, 750, 1000, 1250, 1500 µM chlorite at pH 7 and the reaction was followed at 388 nm. The rate was determined from the slope of the plot of $k_{\text{obs}}$ versus the chlorite concentration. Simulated spectra were obtained with Pro-Kineticists software (Applied Photophysics).

4.6 Crystallization

Crystallization trials of LmChdC wild-type were carried out in SWISSCI MRC three-well crystallization plates (Molecular Dimensions) using the vapor diffusion method. Crystallization drops were set using the mosquito LCP crystallization robot (TTP LabTech). The reservoir was filled with 35 µL of crystallant solution. In the sample wells drops containing proteins of 175 µM (5.25 mg mL$^{-1}$) for protein : crystallant-ratios of 100 : 150 nL, 150: 150 nL, 200 : 150 nL were set up. The protein was dissolved in 50 mM phosphate buffer, pH 7.0 and supplemented with 10 mM KCN, in order to minimize residual activity. Commercially available crystallization screens were used for initial screening. Crystallization plates were stored at 22°C. Diffracting crystals grew in condition A6 of the ShotGun screen (Molecular dimensions), 0.1 M HEPES, pH 7.5, 20 % (w/v) PEG 4000, 10 % (v/v) 2-propanol, which was a suitable condition for freezing the
crystals without any further cryo-protectants. Addition of 2 µL of the mother liquor to the drop prior to freezing diluted the cyanide concentration significantly.

4.7 X-ray data collection, structure determination, and refinement

Datasets were collected at beamline ID29 of the European Synchrotron Radiation Facility (ESRF, Grenoble, France) at 100 K using a DECTRIS PILATUS 6M detector. The data set was processed with XDS and symmetry equivalent reflections merged with XDSCONV. Intensities were not converted to amplitudes. The high-resolution cutoff was based on a CC1/2* criterion. The phase problem was solved by molecular replacement using Phaser-MR taking pdb structure 5LOQ, ChdC from Listeria monocytogenes. The model was further improved by iterative cycles of manual model building using COOT and maximum likelihood refinement using PHENIX-Refine. PHENIX-refine converted intensities into amplitudes using the French and Wilson algorithm. Final stages of refinement included Translation Liberation Screw (TLS) parameters, isotropic B-factor model, automated addition of hydrogens and water molecules, optimization of X-ray/ADP weight and optimization of X-ray/stereochemistry weight. The model was validated with MolProbity. Figures were prepared with PyMOL (http://www.pymol.org).

Reevaluation and further refinement of the structure of LmChdC, originally deposited in the pdb-data bank as 5LOQ, was performed as above using COOT and PHENIX-Refine. Restraints for monovinyl, monopropionate deuteroheme (Ligand ID VOV) were generated using eLBOW taking an sdf file as input and applying the final-geometry option.

4.8 Detection of tyrosyl radicals in LmChdC

Spin trapping experiments of LmChdC, reacting with H2O2, were performed using MNP (2-methyl-2-nitrosopropane). 5 mg MNP were dissolved in 100 µL H2O and heated up to 60°C in the dark. 100 µL assays were set up containing 60 µM ChdC, 30 µM coproheme in 50 mM phosphate buffer. Hydrogen peroxide concentration in the assays was 0 to 2.5 mM. The final MNP concentration in all assays was 5 mg mL⁻¹.

20 µg of each sample in 1×PBS were S-alkylated with iodoacetamide and further digested with sequencing grade modified trypsin (Promega). 5 µg of the peptide mixture were analyzed using a Dionex Ultimate 3000 HPLC-system (Thermo Scientific) directly linked to a QTOF instrument (maXis 4G ETD, Bruker) equipped with the standard ESI source in the positive ion, DDA mode (=switching to MSMS mode for eluting peaks). MS-scans were recorded (range:
150-2200 m/z, spectra rate: 1.0 Hz) and the 5 highest peaks were selected for fragmentation. Instrument calibration was performed using ESI tuning mix (Agilent).

Peptides were separated using a Thermo BioBasic C18 separation column (5 µm particle size, 150×0.320 mm. A gradient from 96% solvent A and 4% solvent B (Solvent A: 65 mM ammonium formiate buffer, pH 3.0; Solvent B: 80% ACN and 20% A) to 40% B in 35 min was applied, followed by a 15 min gradient from 40% B to 94% B, at a flow rate of 6 µL/min at 32 °C. Manual glycopeptide searches were done using DataAnalysis 4.0 (Bruker).

4.9 Electronic absorption

Electronic absorption spectra were recorded using a 5 mm NMR tube (300 nm min⁻¹ scan rate) or a 1 mm cuvette (600 nm min⁻¹ scan rate) at 25 °C by means of a Cary 60 spectrophotometer (Agilent Technologies) with a resolution of 1.5 nm. For the differentiation process, the Savitzky–Golay method was applied using 15 data points (LabCalc, Galactic Industries, Salem, NH). No changes in the wavelength or in the bandwidth were observed when the number of points was increased or decreased.

4.10 Resonance Raman (RR)

The resonance Raman (RR) spectra were obtained at 25 °C using a 5 mm NMR tube by excitation with the 406.7 and 413.1 nm lines of a Kr⁺ laser (Coherent, Innova300 C, Coherent, Santa Clara, CA, USA). Backscattered light from a slowly rotating NMR tube was collected and focused into a triple spectrometer (consisting of two Acton Research SpectraPro 2300i instruments and a SpectraPro 2500i instrument in the final stage with gratings of 3600 grooves/mm and 1800 grooves/mm) working in the subtractive mode, equipped with a liquid nitrogen-cooled CCD detector.

For the low temperature experiments, a 50 µL drop of the sample was put in a 1.5 cm diameter quartz crucible that was positioned in a THMS600 cryostat (Linkam Scientific Instruments, Surrey, UK) and frozen. After freezing the sample, the cryostat was positioned vertically in front of the triple spectrometer and the laser light was directed onto the quartz window. To avoid sample denaturation or photo-reduction, the laser position was changed frequently. The sample temperature was maintained at 80 K. With the low temperature setup, the plasma laser lines of the 406.7 nm excitation wavelength in the low frequency region are very intense. In particular those at 381 and 398 cm⁻¹ overlap with the propionate bending modes,
preventing their assignment. Hence, the RR spectra in the low frequency region of the coproheme complexes were obtained with the 413.1 nm excitation wavelength to avoid plasma lines in the propionate bending region. It should be noted that the spectra of the coproheme complexes obtained with \( \lambda_{\text{exc}} \) 406.7 nm (in resonance with the Soret maximum) and \( \lambda_{\text{exc}} \) 413.1 nm are identical, as shown in Figure S5 where, as an example, the spectra of the coproheme-WT and –Y113A obtained with the two excitation wavelengths, are compared.

A spectral resolution of 1.2 cm\(^{-1}\) and spectral dispersion of 0.40 cm\(^{-1}\)/pixel were calculated theoretically on the basis of the optical properties of the spectrometer for the 3600 grating. The RR spectra were calibrated with indene and carbon tetrachloride as standards to an accuracy of 1 cm\(^{-1}\) for intense isolated bands. All RR measurements were repeated several times under the same conditions to ensure reproducibility. To improve the signal-to-noise ratio, a number of spectra were accumulated and summed only if no spectral differences were noted. Absorption spectra were measured both prior and after RR measurements to ensure that no degradation occurred under the experimental conditions used. All spectra were baseline-corrected.

4.11 Sample preparation for UV-Vis and RR

Ferric coproheme was purchased from Frontier Scientific, Inc. (Logan, Utah, USA) as lyophilized powder. A coproheme solution at pH 7.0 in 50 mM Hepes buffer was prepared by dissolving the coproheme powder in a 0.5 M NaOH solution and then diluting a small aliquot of this concentrated alkaline solution with an appropriate volume of 50 mM Hepes buffer, pH 7.0.

All the protein-coproheme complexes were prepared by adding the coproheme solution at pH 7.0 to the apo-proteins dissolved in 50 mM Hepes buffer, pH 7.0. The ferric heme b-LmChdC complexes were prepared by adding small aliquots (3-10 \( \mu \)L) of a concentrated solution of \( \text{H}_2\text{O}_2 \) in 50 mM Hepes buffer, pH 7.0 to the corresponding coproheme-LmChdC complex.

Sample concentrations, in the range of 40-100 \( \mu \)M for UV-vis and RR measurements, were determined using an extinction coefficient (\( \varepsilon \)) of 68000 M\(^{-1}\) cm\(^{-1}\) at 395 nm (coproheme-WT and mutants) and 76600 M\(^{-1}\) cm\(^{-1}\) at 410 nm (heme b-LmChdC WT and mutants)\(^{13}\).

4.12 Titration of the coproheme-M149A complex with hydrogen peroxide

The UV-Vis titration was performed in a 1 mm cuvette: small aliquots (1-10 \( \mu \)L) of a diluted solution of \( \text{H}_2\text{O}_2 \) in 50 mM Hepes buffer, pH 7.0 were added to 50 \( \mu \)L of a 100 \( \mu \)M
coproheme-M149A complex buffered solution. When variations in the UV-Vis spectrum were observed, the sample was transferred to the THMS600 cryostat (see above), frozen at 80 K and RR spectra taken. After the RR spectra, the sample was warmed to 25 °C and the UV-Vis spectrum recorded and compared with that obtained before freezing to ensure that no degradation had occurred. The titration with H$_2$O$_2$ was then continued until new variations in the UV-Vis spectrum appeared and the sample was frozen again. This experiment was repeated several times to ensure reproducibility.

4.13 MD simulations

As a starting configuration for molecular dynamics simulations, we used the apo ChdC crystal structure (PDB ID:4WWS) at 2 Å resolution. Missing residues (V5, K6, and N113–D123) of chains B–D were remodelled by superimposing chain A on them using the molecular visualisation package Pymol. The coproheme molecule was built by adding two propionate groups to the porphyrin system taken from the crystal structure of chlorite dismutase (Cld) (PDB entry 3NN1). The coproheme and monovinyl, monopropionate deuteroheme molecules were inserted into the active sites mimicking the corresponding binding pose of heme b in Cld and subsequently manually adjusted to facilitate coordination of iron to the Nε atom of H174. The positions of coproheme and monovinyl, monopropionate deuteroheme that were rotated by 90°, i.e. with their p2 towards Tyr147, were obtained by manual rotation of coproheme and monovinyl, monopropionate deuteroheme to resemble the binding pose of coproheme in Ref. (PDB entry 5T2K, pose 0). All MD simulations were carried out using the GROMOS11 software simulation package, employing the 54a8 forcefield. Proteins were energy-minimized in vacuum using the steepest-descent algorithm and subsequently solvated in a rectangular, periodic and pre-equilibrated box of single point charge (SPC) water. Minimum solute to box-wall distances were set to 0.75 in all dimensions. This led to systems containing about 104 thousand atoms for the pentamer. Another minimization in water was performed using the steepest descent algorithm. To achieve electroneutrality of the system, 65 sodium ions were added. For the equilibration, the following protocol was used: initial velocities were randomly assigned according to a Maxwell–Boltzmann distribution at 60 K. All solute atoms were positionally restrained with a harmonic potential using a force constant of $2.5 \times 10^4$ kJmol$^{-1}$nm$^{-2}$. In each of the four subsequent 20 ps MD simulations, the force constant of the positional restraints was reduced by one order of magnitude and the temperature was increased by 60 K. Subsequently, the positional restraints were removed and rototranslational constraints were introduced on all solute atoms. The last step of equilibration was performed at a constant
pressure of 1 atm for 300 ps. After equilibration production runs of 15-35 ns, depending on the system, were performed with a constant number of particles, constant temperature (300 K) and constant pressure (1 atm). To sustain a constant temperature, we used the weak-coupling thermostat \(^{21}\) with a coupling time of 0.1 ps. The pressure was maintained using a weak coupling barostat with a coupling time of 0.5 ps and an isothermal compressibility of \(4.575 \times 10^{-4}\) kJ\(^{-1}\)·mol·nm\(^{-3}\). Solute and solvent were coupled to separate temperature baths. Implementation of the SHAKE algorithm \(^{22}\) to constrain bond lengths of solute and solvent to their optimal values allowed for a 2-fs time step. Nonbonded interactions were calculated using a triple range scheme. Interactions within a short-range cutoff of 0.8 nm were calculated at every time step from a pair list that was updated every fifth step. At these points, interactions between 0.8 and 1.4 nm were also calculated explicitly and kept constant between updates. A reaction field \(^{23}\) contribution was added to the electrostatic interactions and forces to account for a homogenous medium outside the long-range cutoff using a relative dielectric constant of 61 as appropriate for the SPC water model \(^{24}\). Coordinate and energy trajectories were stored every 0.5 ps for subsequent analysis. To examine the properties of coproheme and monovinyl, monopropionyl deuteroheme bound to ChdC, four independent simulations were performed, coproheme with p2 and p4 facing Tyr 147 as well as monovinyl, monopropionyl deuteroheme with p2 and p4 facing Tyr 147 (see figure 4 of the main manuscript).
Figure S1. Reaction of LmChdC wild-type with chlorite. (A) UV-vis absorption spectra recorded following the stepwise titration of 7 µM coproheme-LmChdC wild-type with chlorite (0-60 µM) at pH 7.0 (50 mM HEPES). (B) Plot of normalized absorbance changes at 410 nm (cyan) after each titration step versus the chlorite/coproheme-LmChdC ratio (including sigmoidal fit). Points obtained after the reaction was completed at higher chlorite concentrations, which represent heme bleaching are depicted in grey.
Figure S2. Formation of Compound I and heme bleaching of LmChdC Y147H mediated by chlorite. Spectral transitions upon mixing of 5 µM LmChdC with 250 µM chlorite at pH 7.0 (50 mM HEPES) recorded for 5 s. The black spectrum was obtained after 0.68 ms. Grey spectra were taken at 34.5, 68.7, 102.7, 136.7, 216.2, 774.5, 1450.4, and 2709.1 ms; the spectrum at 411.4 ms is depicted in red and the final depicted spectrum is shown in green (after 5000.0 ms). The inset shows the time trace of this measurement at 388 nm. The time points of the highlighted spectra are shown as circles in the respective colors.
Figure S3. RR spectra in the high frequency region of the coproheme-LmChdC complexes of the WT and mutants obtained at 80 K ($\lambda_{exc}$ 406.7 nm). The band wavenumbers in magenta indicate the 6cLS species, while those in olive green and orange are assigned to minor 5cQS and 5cHS species, respectively. The mutants label colour, beige, green, brown and light blue, indicate the position 2, 4, 6, and 7, respectively, of the propionate group/s with which the mutated residues interact. In the spectra of the coproheme-R133A, –R179A, and –M149A/Q187A complexes there is a small amount of the reduced form ($\nu_4$ band at 1355 cm$^{-1}$). Due to the very high fluorescent background, it was not possible to obtain the spectrum of the coproheme-Q187A complex. The spectra have been shifted along the ordinate axis to allow better visualization. Experimental conditions: laser power at the sample 5-10 mW, average of 6 spectra with a 60 min integration time (M149A/Q187A and R179A); average of 2 spectra with a 20 min integration time (R133A); average of 8 spectra with 160 min integration time (WT); average of 9 spectra with a 90 min integration time (Y113A); average of 12 spectra with a 120 min integration time (M149A); average of 6 spectra with a 60 min integration time (K151A); average of 8 spectra with a 40 min integration time (Y113A/K151A); average of 6 spectra with a 30 min integration time (Y147A/R220A/S225A).
Figure S4. Comparison of the RR spectra in the low frequency region of the coproheme-LmChdC complexes of the WT and mutants obtained at 80 K (λ_{exc} 413.1 nm). The bands tentatively assigned to the bending mode $\delta(C_\beta C_\delta)$ of the propionate groups in positions 2, 4, 6 and 7 are reported in beige, green, brown and light blue, respectively. Accordingly, the mutant label colours indicate the position of the propionate group/s with which the mutated residues interact. In magenta is reported the band due to the 6cLS species. The spectrum of the coproheme-WT complex is reported in red. The spectra have been shifted along the ordinate axis to allow better visualization. Experimental conditions: laser power at the sample 5-10 mW, average of 6 spectra with a 120 min integration time (Q187A); average of 10 spectra with a 200 min integration time (M149A/Q187A); average of 24 spectra with a 8 hours integration time (R179A); average of 15 spectra with a 5 hours integration time (R133A); average of 5 spectra with a 100 min integration time (WT); average of 9 spectra with a 180 min integration time (Y113A); average of 6 spectra with a 120 min integration time (M149A/Q187A); average of 7 spectra with a 140 min integration time (K151A); average of 4 spectra with a 80 min integration time (Y113A/K151A); average of 10 spectra with a 200 min integration time (Y147A/R220A/S225A).
Figure S5. Comparison of the RR spectra in the low frequency region of the coproheme-LmChdC complexes of the WT and Y113A mutant obtained at 80 K with $\lambda_{exc}$ 413.1 nm (black) and $\lambda_{exc}$ 406.7 nm (red). The plasma laser lines of the 406.7 nm excitation wavelength (red) are marked with an asterisk. Except for the plasma lines, the spectra obtained with the two excitation wavelengths are identical. Experimental conditions: $\lambda_{exc}$ 413.1 nm, laser power at the sample 10 mW, average of 5 spectra with 100 min integration time (WT); average of 9 spectra with a 180 min integration time (Y113A). $\lambda_{exc}$ 406.7 nm, laser power at the sample 5-10 mW, average of 4 spectra with 80 min integration time (WT); average of 6 spectra with a 120 min integration time (Y113A).
# S3 Supporting Tables

**Table S1.** Distances (Å) between the oxygen atoms of the propionate groups in positions 2, 4, 6 and 7 in the subunits A, B, C, D, E and the studied mutated residues of coproheme-LmChdC (6FXJ). Hydrogen bonding networks are marked in bold.

|        | Propionate 2 |           | Propionate 4 |           |           |           |           |           |           |
|--------|--------------|-----------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|
|        | A  | B  | C  | D  | E  | A  | B  | C  | D  | E  | A  | B  | C  | D  | E  |
| Q187/1 | 11.1 | 11.7 | 10.6 | 11.3 | 11.1 | 11.4 | 11.4 | 11.4 | 11.5 | 13.6 |     |     |     |     |     |
| Q187/2 | 7.0  | -   | -   | 7.3  | -   | 8.8  | -   | -   | 9.0  | -   |     |     |     |     |     |
| R133   | 14.6 | 14.7 | 13.3 | 14.6 | 14.6 | 11.4 | 10.7 | 10.7 | 13.0 | 11.8 |     |     |     |     |     |
| R179   | 13.0 | 18.5 | 15.1 | 14.6 | 16.4 | 13.5 | 17.1 | 16.3 | 13.6 | 13.4 |     |     |     |     |     |
| Y113   | 13.2 | -   | 14.6 | 13.1 | 14.5 | 2.6H2.8 | - | 2.6H3.0 | 2.6H2.6 | 2.6H2.7 |     |     |     |     |     |
| M149   | 4.0  | 4.7  | 3.8  | 4.0  | 4.6  | 8.7  | 8.4  | 7.7  | 8.8  | 7.9  |     |     |     |     |     |
| K151   | 2.9H2.5 | 8.0  | 7.4  | 3.0H2.6 | 2.9 |     | 2.9  | 3.4  | 4.2  | 3.0  | 2.9  |     |     |     |     |     |
| Y147   | 4.9 (2.5 to β-carbon) | 2.6  | 2.9  | 5.3 (2.5 to β-carbon) | 2.5 |     | 12.3 | 12.8 | 12.4 | 12.4 |     |     |     |     |     |
| R220   | 2.9H2.5 | 2.8H3.1 | 2.9H2.8 | 2.6H2.9 | 2.9H3.0 | 14.8 | 15.7 | 15.0 | 15.1 | 15.3 |     |     |     |     |     |
| S225   | 2.6  | 2.8  | 2.7H2.7 | 2.8  | 2.8  | 9.2  | 9.7  | 9.1  | 9.5  | 9.3  |     |     |     |     |     |

|        | Propionate 6 |           | Propionate 7 |           |           |           |           |           |           |
|--------|--------------|-----------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|
|        | A  | B  | C  | D  | E  | A  | B  | C  | D  | E  | A  | B  | C  | D  | E  |
| Q187/1 | 7.3  | 5.5  | 8.2  | 7.2  | 7.1  | 2.8  | 4.7  | 4.2  | 2.6  | 5.4  |     |     |     |     |     |
| Q187/2 | 8.0  | -   | -   | 8.0  | -   | 5.1  | -   | -   | 3.8  | -   |     |     |     |     |     |
| R133   | 3.2H2.5 | 3.0  | 4.3  | 2.7H2.9 | 3.6 |     | 5.7  | 3.8  | 4.0  | 4.3  | 4.6  |     |     |     |     |     |
| R179   | 2.9  | 8.9  | 8.3  | 2.7  | 3.6  | 5.4  | 4.7  | 5.9  | 3.2  | 3.5  |     |     |     |     |     |
| Y113   | 13.8 | -   | 11.4 | 12.7 | 14.5 | 17.8 | -   | 18.0 | 14.8 | 16.2 |     |     |     |     |     |
| M149   | 15.1 | 13.1 | 15.1 | 15.2 | 15.5 | 15.6 | 15.1 | 16.1 | 13.1 | 15.7 |     |     |     |     |     |
| K151   | 11.7 | 11.6 | 13.6 | 11.7 | 13.3 | 15.5 | 14.3 | 16.6 | 12.8 | 14.5 |     |     |     |     |     |
| Y147   | 15.1 | 13.9 | 16.4 | 15.2 | 15.9 | 12.6 | 14.2 | 14.1 | 13.5 | 14.7 |     |     |     |     |     |
| R220   | 19.7 | 19.7 | 21.6 | 18.9 | 21.4 | 18.3 | 19.9 | 19.9 | 19.0 | 19.8 |     |     |     |     |     |
| S225   | 13.0 | 15.2 | 15.7 | 13.2 | 15.8 | 15.0 | 15.1 | 15.8 | 14.3 | 14.9 |     |     |     |     |     |
Table S2. Probabilities of an electron transfer for propionates Cβ from the terminal oxygen of Tyr147.

|                      | p2  | p4  |
|----------------------|-----|-----|
| Coproheme (pose 90)  | 0.001 | 0.131 |
| Coproheme (pose 0)   | 0.160 | 0.002 |
| monovinyl, monopropionyl deuteroheme (pose 90) | 0.120 |
| monovinyl, monopropionyl deuteroheme (pose 0) | 0.001 |

Table S3. H-bonding environment of p6 and p7 of the monovinyl, monopropionyl deuteroheme LmChdC subunit

| LmChdC subunit | p6                                      | p7                                      |
|----------------|-----------------------------------------|-----------------------------------------|
| Chain A        | K151 (2.6 Å)                            | -                                       |
| Chain B        | -                                       | R133 (2.5 Å)                            |
| Chain C        | H₂O (2.5 Å) – Y113 (3.0 Å)              | -                                       |
| Chain D        | K151 (3.0 Å)                            | H₂O (2.5 Å) – Y113 (2.5 Å)              | -                                       |
| Chain E        | H₂O (2.4 Å) – Y113 (3.1 Å)              | R133 (2.7 Å)                            |
S4 References

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