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Analysis of the residual alignment of a paramagnetic multiheme cytochrome by NMR

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Supporting Information

D-ALA synthesis

The method reported by Wang et al[1] was used to synthesize 5-^{13}C-δ-ALA. The nature of the final product was confirmed by 1D-NMR (supplementary Figure S2), and the overall yield of the reaction was 67%, in agreement with the literature. Analytical data of 5-^{13}C-δ-ALA, ^{1}H-NMR (D_2O) δ: 2.66 (m, 2H), 2.83 (m, 2H), 4.06 (dd, 2H, J= 143 Hz); 13C-NMR (D_2O) δ: 47.0 (s).

Cell growth and protein purification

Cells of *E. coli* LS542 strain were grown in Lysogeny Broth (LB) supplemented with 5-^{13}C-δ-ALA 50 mg/L to ensure the biosynthesis of STC labeled in the heme rings. 10 mg/L ampicilin and 34 mg/L chloramphenicol, were added to the growth medium to maintain selective pressure for the pET21a-STC (D2N) and pEC86 plasmids, respectively. Cells were allowed to grow for 20 hours at 30 ˚C and 180 rpm, in 500 mL *Erlenmeyer* flasks filled with one-fifth of the total volume and were harvested by centrifugation at 4 ˚C, 10000 X *g*, for 10 min. Cell pellet was ressuspended in a extraction solution (30 mL/L of culture), containing 500 mM sucrose, 200 mM Tris pH 8.0, 0.5 mM EDTA and 100 mg/L lysozyme, incubated in ice for 30 min with gentle stirring, and ultra centrifuged at 4 ˚C, 138000 x *g*, for 1 hour. The supernatant was dialyzed against 10 mM Tris-HCl pH 7.6, over-night, and concentrated. Protein purification was performed as described by Fonseca et al[2]. The final yield of pure protein was 1.3 mg/L.

NMR experiments

Protein samples (0.5 mM) were freeze-dried and dissolved in 500 μL of D_2O (99.9 atom %) and contained 10 mM potassium phosphate buffer (pH 7.6). The pH value reported is a direct reading without correction for the isotope effect. The assignment of the meso carbons and protons was made using a set of NMR experiments performed at 25 °C on a Bruker Avance II 500 MHz NMR spectrometer equipped with a QXI or a TXI probe, and at a Avance III 800 MHz spectrometer, equipped with a QXI probe . 2D-^{1}H-^{13}C heteronuclear multiple quantum correlation (HMQC) spectra were obtained without proton-carbon decoupling. The spectra collected at 500 MHz were acquired with 2048 points covering a spectral width of 20 kHz in the ^{1}H dimension and 256 increments with time-proportional phase incrementation (TPPI) to give a spectral width of 37.7 kHz in the ^{13}C dimension, with 64 scans. The spectra collected at 800 MHz were acquired with 2048 points covering a spectral width of 32 kHz in the ^{1}H dimension and 512 increments with time-proportional phase incrementation (TPPI) to give a spectral width of 80.5 kHz in the ^{13}C dimension. ^{1}H nuclear Overhauser effect spectroscopy (NOESY) spectra were acquired with 25 ms of mixing time, 2048 points in the direct dimension and 400 increments with time-proportional phase incrementation (TPPI) in the indirect dimension, both covering a spectral width of 39.7 kHz with 400 scans. All NMR experiments were calibrated using the water signal as an internal reference. The Bruker TopSpin program (Bruker BioSpin, Wissembourg, France) and Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco) were used to visualize and analyze the NMR spectra. The splitting of the signals was measured using TopSpin and was considered to have an associated uncertainty of ±1 Hz.

Heme plane determination

In order to determine the normal to each of the hemes of STC, the average plane for each heme was defined by minimizing the distance to the coordinates of the iron and the pyrrole nitrogen atoms extracted from the pdb file (PDB ID: 1M1Q). The normal to this plane was considered the normal of the heme.

Determination of the alignment tensor of STC

For the determination of the overall alignment tensor it was considered that the zz axis of the magnetic susceptibility tensor of the individual hemes is a vector perpendicular to the heme plane. We calculated the vector sum of these four vectors. For the case where all hemes are considered to have equal contribution they were considered all of unit length. For the case where the hemes were considered to have different contributions the length was weighted by the value of the energy splitting of the frontier molecular orbitals of each heme reported in the literature. [3].

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Supplementary Figures

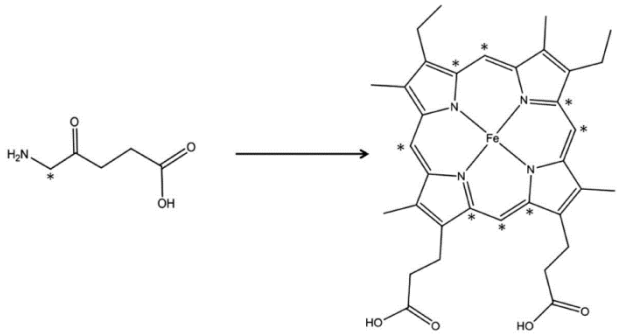

Figure S1. Outcome of heme biosynthesis using 5-^{13}C-δ-ALA showing the position of the labeled carbon atoms in the porphyrin ring.

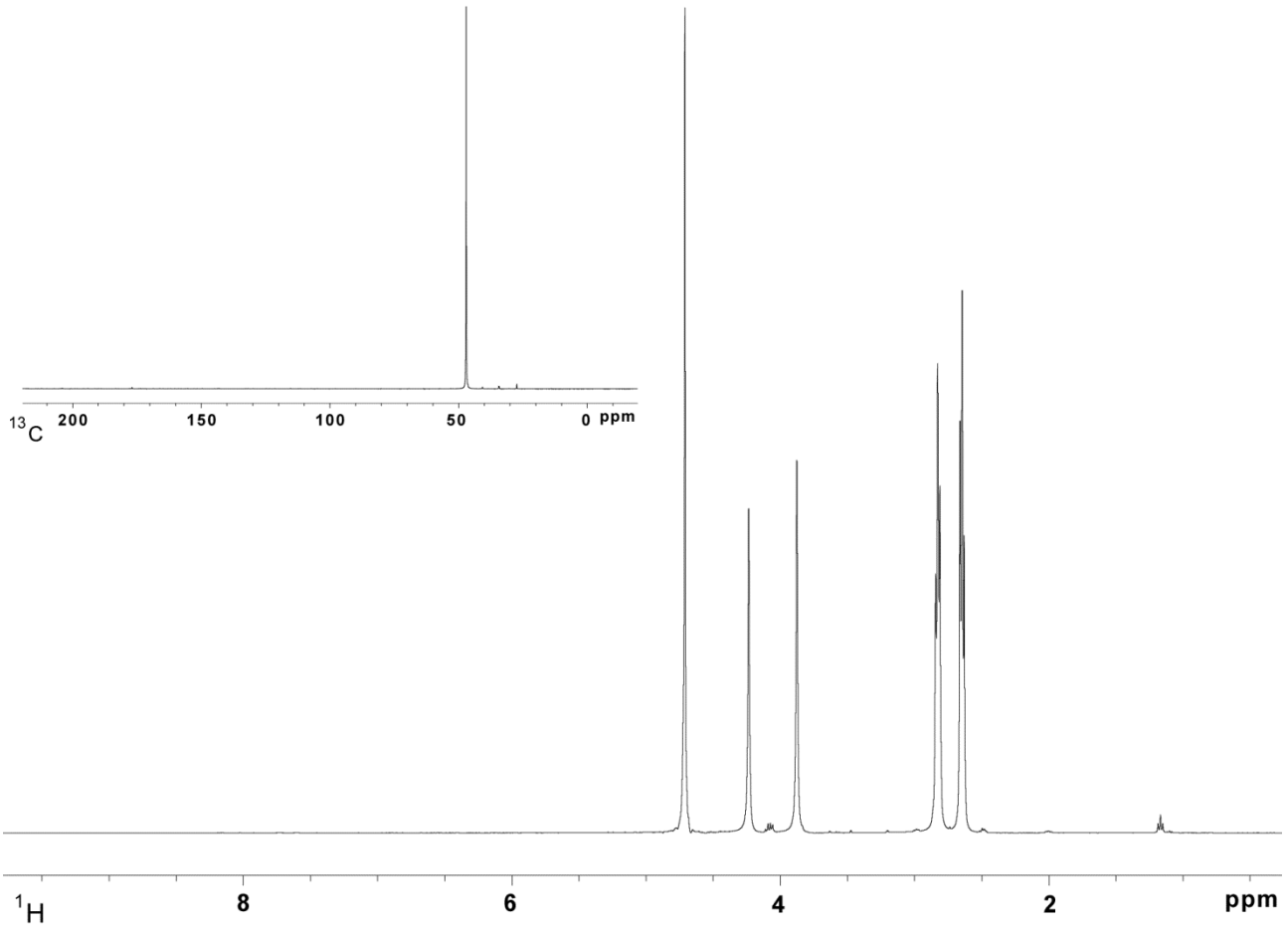

Figure S2. ^{1}H-NMR spectrum from synthesized ^{13}C-5-D-ALA. The inset shows the ^{13}C-NMR spectrum with the single peak corresponding to the labelled carbon of the molecule.

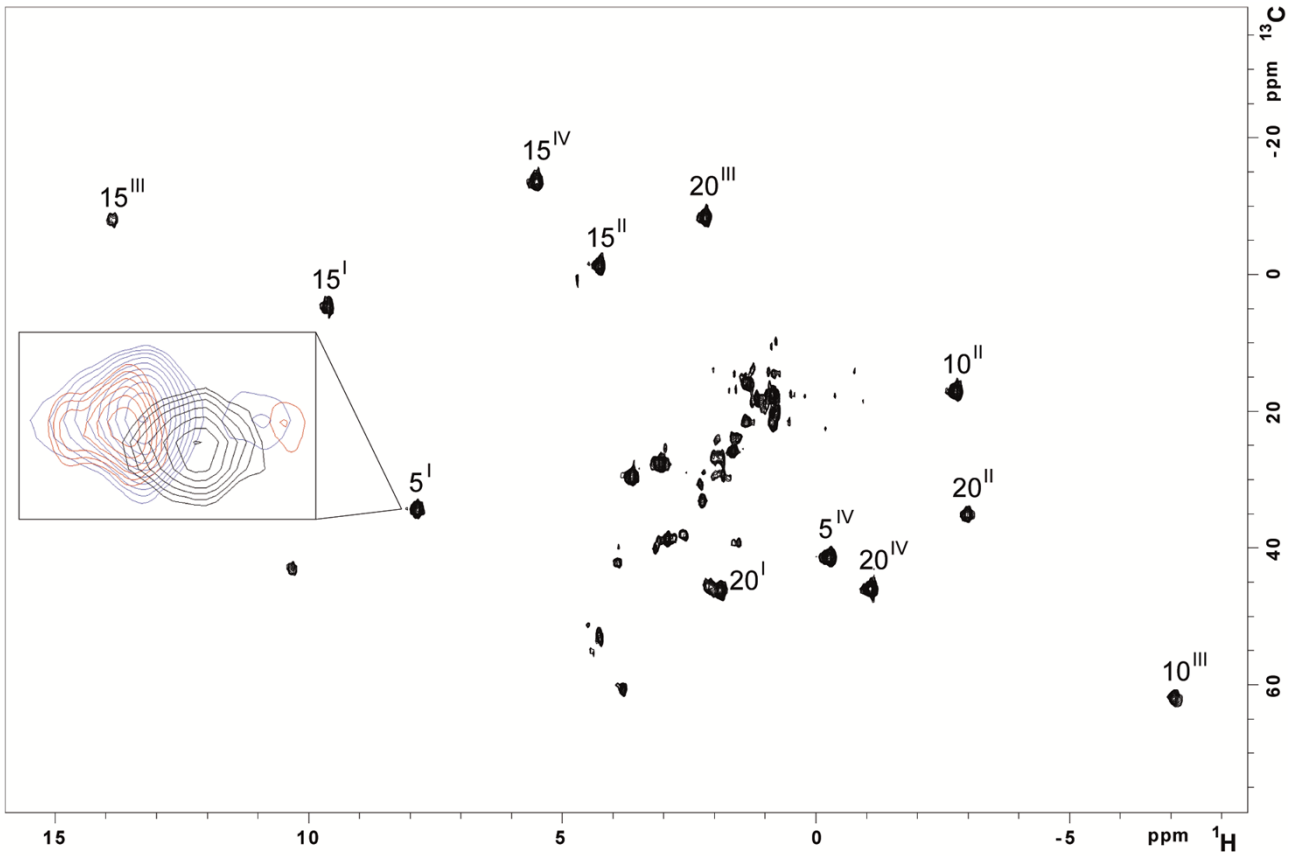

Figure S3. 2D ^{1}H-^{13}C HMQC from STC of *S. oneidensis* MR-1 at pH 7.6 and 25°C, acquired with proton-carbon decoupling, with the assigned mesos labelled using the IUPAC-IUBMB nomenclature with superscripts to designate the heme in roman numbers, ordered according to the sequence of attachment to the polypeptide chain. The inset zooms in the signal of the spectrum (black) and superimposed with the signals of 2D ^{1}H-^{13}C HMQC spectra from the same sample acquired at 500 MHz (red) and 800 MHz (blue), without decoupling. Split peaks in this paramagnetic protein have asymmetric intensity as reported in the literature[4].

Notes and references

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