Supplementary Information

Artificial heme-proteins: determination of axial ligand orientations through paramagnetic NMR shift.

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Materials

Water (LC-MS grade) was purchased from Romil. Deuterated solvents, 2,2,2-Trifluoroethanol-d3 (TFE) and Dimethyl sulfoxide-d6 (DMSO), were purchased from Eurisotop.

Fe(III)-mimochrome IV complex was synthesized as previously described.[1]

NMR characterization

NMR analysis was performed in phosphate buffer 10 mM, pH = 6.5, and in phosphate buffer (10 mM, pH 6.5)/TFE/DMSO solution (60/20/20, v/v/v) at 298 K. NMR samples of Fe(III)-mimochrome IV were prepared by dissolving weighted amounts of the compound in the solvent system (V = 0.600 ml) for a final concentration of 0.6 mM. Proton 1D and 2D nuclear overhauser spectra were collected on a Bruker Avance II 500 MHz spectrometer with a 5 mm QXI probe at "Centro de Ressonância Magnética António Xavier" (CERMAX), hosted at the Instituto de Tecnologia Química e Biológica (ITQB), Oeiras, Portugal.

Proton ^{1}H nmr spectra were acquired with a spectral width of 30 kHz, collecting 4 K data points. The water signal was suppressed by using a 500 ms selective pulse.

WEFT-NOESY spectrum was performed as described in the literature[2] with an acquisition time of 50 ms, an interpulse delay (t) of 50 ms and a mixing time of 50 ms. The spectral width was of 30 kHz in both dimension. The data file consisted of 4K and 512 data points in F2 and F1, respectively.

A preliminary NMR analysis was carried out in aqueous solution (phosphate buffer 10 mM, pH = 6.5. The spectra showed broad resonances and no dipolar connectivities, probably due to chemical

exchange phenomena occurring in this solvent system. However, four signals (at 21.5 ppm, 19.9 ppm, 14.5 ppm and 10.7 ppm) could be reasonably assigned to the heme methyl protons (see Fig. S1). The spread of these resonances is 10.8 ppm. Although this value is smaller than the one observed in phosphate buffer (10 mM, pH 6.5)/TFE/DMSO solution (20 ppm), it is still indicative of a preferred orientation of axial ligands (~5 ppm is the spread observed for freely rotating axial ligands).[3]

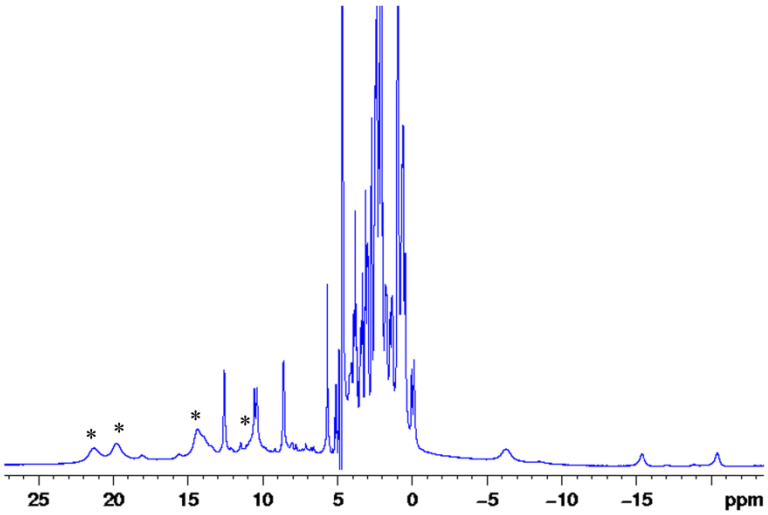

Fig. S1. Fe(III)-mimochrome IV 1D ^{1}H spectrum in phosphate buffer (10 mM, pH 6.5)

Determination of the axial ligand orientations

The axial ligand orientations of Fe(III)-mimochrome IV in 10 mM, pH 6.5)/TFE/DMSO solution (60/20/20, v/v/v) was determined by using the empirical equation proposed by Turner:[4]

$$\delta_i(\text{ppm}) = \cos\beta[38.0\ \sin^2(\theta_i-\phi)-4.1\ \cos^2(\theta_i+\phi)-15.9]+13.8 \qquad (1)$$

This equation contains two unknown variables, independent from each other, that define the axial ligand orientations: β, the acute angle between the two histidine planes, and ϕ, the average orientation of the His planes, projected on the heme plane, with respect to the N_{21}-N_{23} direction (see figure 3 in the main text).

δ_i is the hyperfine shift of the *i*th methyl, experimentally determine for each methyl from NMR spectra. θ_i, is the angle between the metal-*i*th methyl direction and the metal-N_{23} axis; it represents a

parameter which is proper to each methyl, and can be deduced from the heme geometry. In this paper, we used the θ_i values proposed by Turner (**Table S1**).[4]

The equation (1) was implemented in Microsoft office excel and, for each methyl, the experimental δ value and the θ value, as derived from Turner (see **Table S1**), were inserted. The β and ϕ values were optimized to obtain the best fit to the four experimental δ_i values (see **Table S2**).

Table S1 θ_i for the heme methyl groups

methyl	θ_i
7-CH_3	108
12-CH_3	18
17-CH_3	-80
3-CH_3	162

Table S2 ^{1}H chemical shifts of the heme methyl groups

methyl	δ (ppm) experimental	δ (ppm) calculated
7-CH_3	26.1	23.4
12-CH_3	6.7	4.1
17-CH_3	24.5	24.8
3-CH_3	16.9	17.0

References

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