Supplementary information for

Selective oxidation of aromatic sulfide catalysed by an artificial metalloenzyme : New activity of hemozymes

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Organic and Biomolecular chemistry

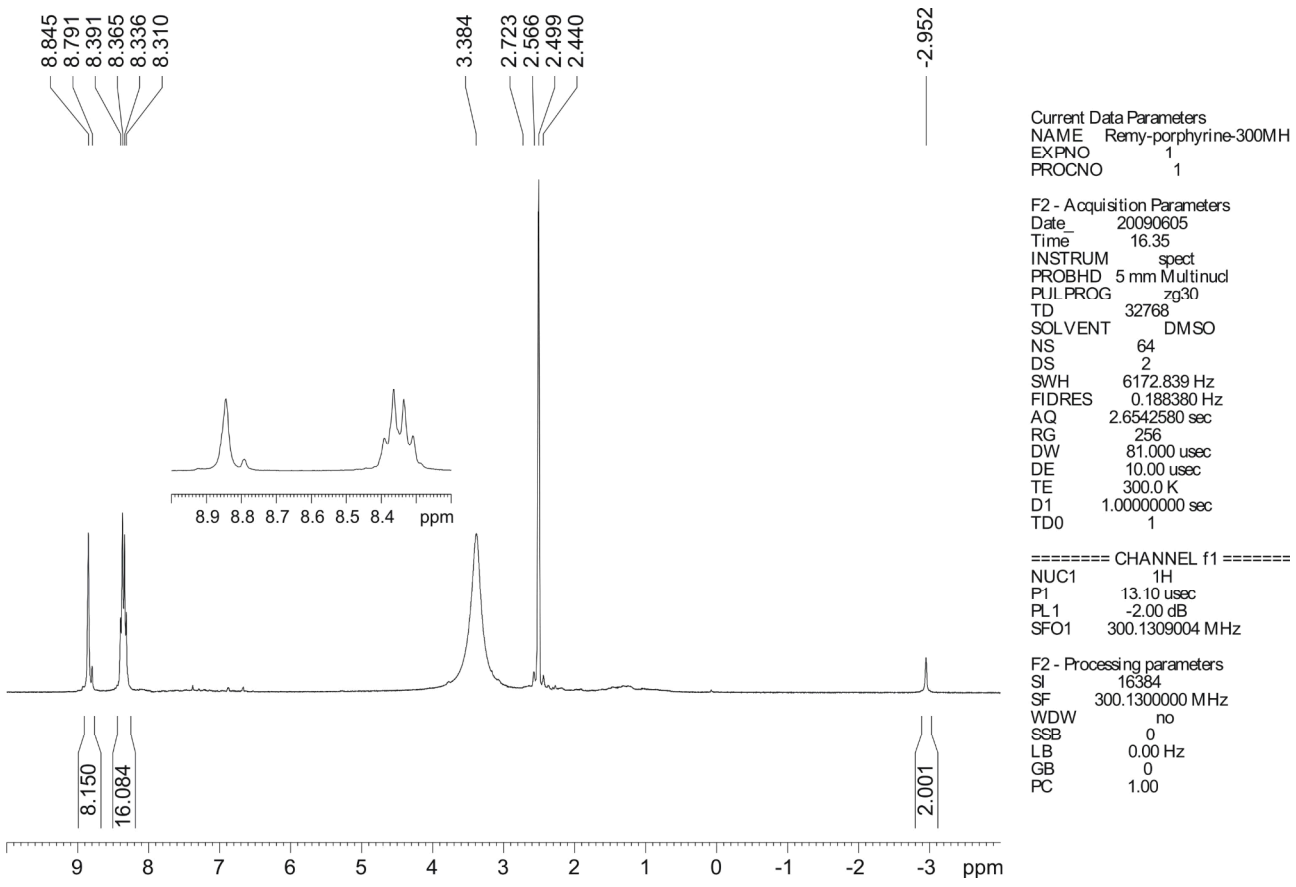
I. Synthesis of Meso-Tetrakis(4-carboxyphenyl)porphyrinatoiron(III) [Fe(III)TpCPP] 1-Fe and Meso-Tetrakis(4-carboxyphenyl)porphyrinatoiron(III) [Fe(III)TpCPP] 1-Fe

The tetra-arylporphyrins were synthetized according to literature procedures. **1** was obtained with a 10.5 % yield by condensation of p-carboxybenzaldehyde with pyrrole in propionic acid according to the procedure of Adler et al.[16] **2** was prepared in a quantitative yield by sulfonation of tetraphenyl porphyrin by concentrated sulfuric acid at 110 C for 5 h as described by Fleischer et al.[17]

1-Fe was prepared by refluxing **1** for 1 h with excess $FeCl_2$ in glacial acetic acid in the presence of sodium acetate followed by recristallization from 0.5 N NaOH in EtOH according to Harada et al.[18] **2-Fe** was obtained in a 90 % yield by treatment of 2 with 1.1 eq. $FeCl_2.4H_2O$ in 1M acetate buffer pH 4 under reflux for 4 h, followed by precipitation in acetone.[19]

All the new compounds gave satisfactory spectrometric data (^{1}H NMR, UV-visible) and consistent high-resolution mass spectrometry data. The chemical shifts are given in ppm versus TMS.

Meso-Tetrakis(4-carboxyphenyl)porphyrin TpCPPH$_2$ 1: ^{1}H NMR (d_6-DMSO) 8.85 (8H, s), 8.38 (8H, d, J = 8. Hz), 8.32 (8H, d, J = 8 Hz), -2.95 (2H, s, NH).

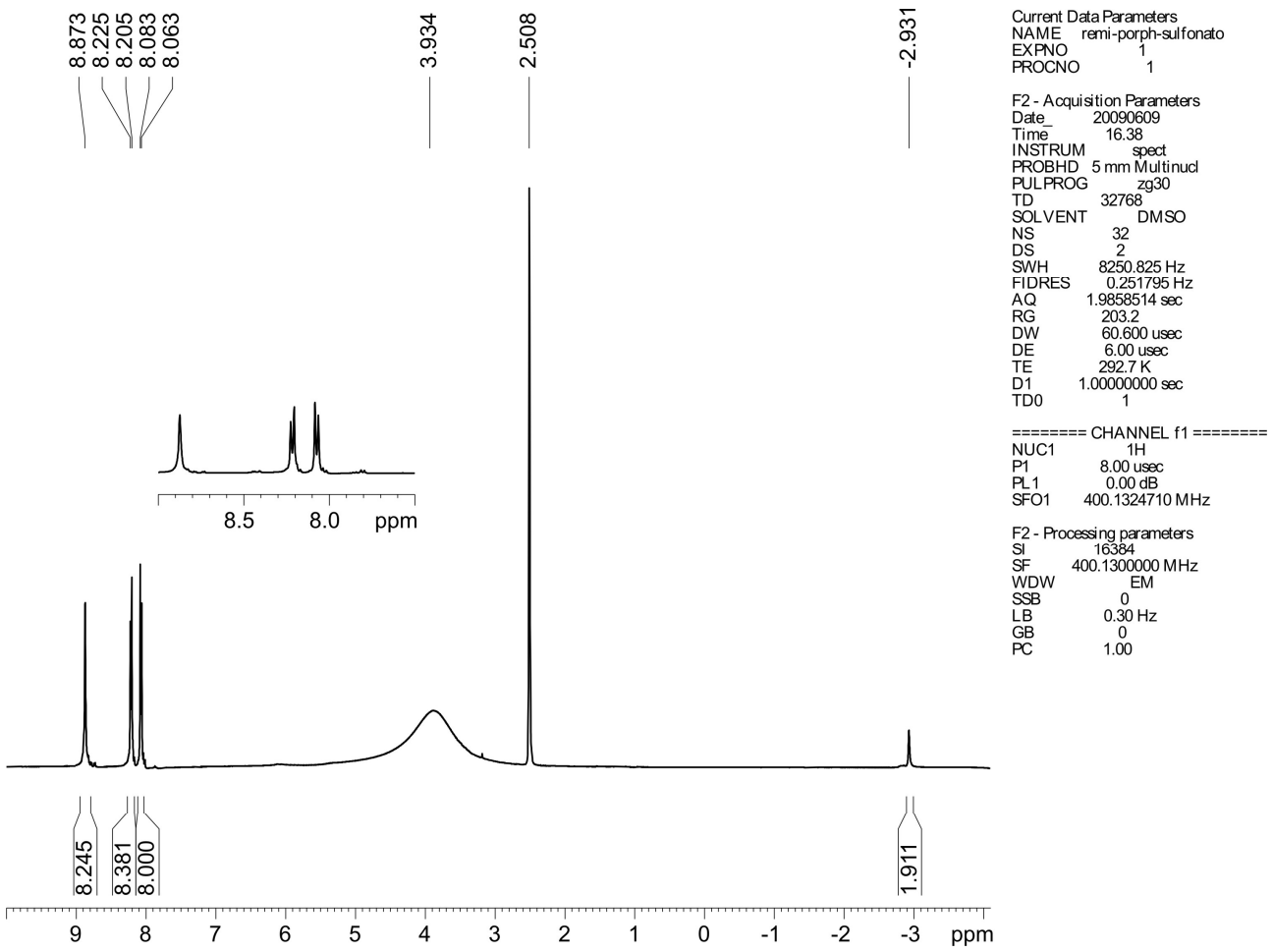


ESI-MS (70V): m/z 845.4 (M + H$^+$, 100%).

Meso-Tetrakis(4-carboxyphenyl)porphyrinatoiron(III) [Fe(III)TpCPP] 1-Fe: (λ_{max}(nm) : 419 (ε_{DMSO}: 1.25 $M^{-1}cm^{-1}$), 527.

ESI-MS (70V): m/z 845.4 (M + H$^+$, 100%).

Tetrasodium 5,10,15,20-Tetra(4-sulfophenyl)porphyrin $TpSPPH_2$ 2: ^1H NMR (d_6-DMSO) 8.87 (8H, s), 8.21 (8H, d, J = 8.1 Hz), 8.07 (8H, d, J = 8.1 Hz), -2.93 (2H, s, NH).



ESI-MS (20V) m/z 935.0 ($M + H^+$, 100%), 957.3 ($M + H^+ + Na^+$, 56%), 979.2 ($M + H^+ + 2\ Na^+$, 45%), 1001.1 ($M + H^+ + 3\ Na^+$, 28%), 1024.3 ($M + H^+ + 4\ Na^+$, 13%).

Tetrasodium 5,10,15,20-Tetra(4-sulfophenyl)porphyrinatoiron(III) [Fe(III)TpSPP] 2-Fe: UV-vis (λ_{max} (nm): 392 ($\varepsilon_{H2O,\ pH\ 3}$: 1.52 $M^{-1}cm^{-1}$), 528.

ESI-MS (70V): m/z 988.2 ($M + H^+$, 100%)

Production and purification of xylanase A (Xln10A).

Seven-day-old cultures of *S. lividans* from Bennett-thiostrepton plates were used as initial inoculum. The spores were scraped from the plates and inoculated into 12.5 mL minimal M14 medium (composition per liter: glucose 10 g; K_2HPO_4, 5.0 g; $(NH_4)_2SO_4$, 1,4 g; KH_2PO_4, 1.0 g; $CaCl_2{\cdot}2H_2O$, 300 mg; $MgSO_4{\cdot}7H_2O$, 300 mg; $FeSO_4.H_2O$, 5.0 mg; $CoCl_2.\ 6H_2O$, 2.0 mg; $MnSO_4.H_2O$, 1.6 mg; $ZnSO_4.7H_2O$, 1.4 mg; Tween 80, 2.0 mL; pH 7.4) and incubated for 18 hours at 34 °C with agitation. Bacteria were recovered by centrifugation, used to inoculate 500 mL of the same medium and allowed to grow for 72 hours under the same conditions. Proteins contained in the supernatant of *S. lividans* culture were first concentrated by ultrafiltration with a 3 kDa cut-off membrane (Omega). The concentrated proteins were then loaded at 4 °C on a Sephacryl S100 beaded column (2.6 x 60 cm; Pharmacia) with 100 mM sodium phosphate pH 6.0, as the eluant. Purified Xln10A containing fractions were pooled, dialysed and freeze-dried.

Molecular modeling.

The atomic coordinates of Xln 10A were downloaded from the Protein Data Bank [29] (PDB code 1V0L). [30] The exploration of the protein cavity volumes and shapes were performed with CASTp [31] and Q-sitefinder [32] webserver.

The structures of the different *meso*-tetraphenyl-porphyrins have been generated using the chem3D package (CambridgeSoft Corporation). A short minimization was performed on each compound using the MM2 force field [33] implemented in Chem3D. For the MP8 system, the environment of the heme has been generated from one of the crystal structures available of the human cytochrome C (PDB code 1CRI [34]). The minimization was followed by a short molecular dynamic run to better relax the system. All the volumes of the ligands have been calculated using the Connolly solvent excluded volume package included in Chem3D.

Docking studies were performed using the GOLD v3 program [35] with the Chemscore [36] fitness function. The accessible docking space was defined as a 20 Å sphere around one of the residues at the core of the cleft. Fifty solutions were generated for each porphyrin macrocycle and ranked according to their Chemscore values. We selected this approach since it has been applied with success in predicting protein-ligand interactions for metal containing systems like cytochrome P450. [37] Since the ligand remains quite exposed to the solvent, a large number of low energy solutions were analyzed in detail. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco. [38]

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Oxidation of thioanisole

Standard incubations (total volume, 0.5 mL) were performed at room temperature in phosphate buffer (0.1 M, pH 7.4) or phosphate/citrate buffer (0.05 M, pH 3) containing thioanisole (8.5 mM) and the catalyst, either 20 µM **1-Fe** or **2-Fe** alone, or **1-Fe** or **2-Fe** Xln10A prepared by preincubation of 20 µM Fe-porphyrin with 25 µM XlnA for 2 h at 4°C. The oxidant, H_2O_2 (final concentration, 3.5 mM), was then added dropwise to the solution at a rate of 10 x 5 µL drops over a period of 2.3 h. The reaction was quenched by the addition of an excess of Na_2SO_3. The organic products were then extracted with ethyl-acetate and analyzed by GC using benzophenone as the internal standard to determine the degree of conversion of the sulfide, and by HPLC on a Chiracel OD-H column (iso-hexane/propan-2 ol; 95/5; v/v) to determine the enantiomeric excess of the sulfoxide thus obtained.