

## Insight into copper coordination in O<sub>2</sub> reduction by water-soluble cytochrome *c* oxidase models

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## Methods and materials

**General.** Fe<sup>III</sup>TPPS<sup>1</sup> and 2,3-monoepoxy-per-O-methylated- $\beta$ -CD<sup>2</sup> were synthesized as previously reported. Column chromatography was performed with silica gel from Merck (Kieselgel 60; 63-200  $\mu$ m). Size exclusion chromatography was performed with Bio-beads S-X1 beads from Bio-Rad Laboratories, Phosphate buffer (0.05 M, pH 7.0) was prepared by dissolving the appropriate quantities of NaH<sub>2</sub>PO<sub>4</sub> and NaHPO<sub>4</sub> in Q-milli water (18.2 M $\Omega$ .cm). Aqueous NaClO<sub>4</sub> buffer (0.1 M) was prepared by dissolving the appropriated quantities of NaClO<sub>4</sub> in Q-milli water (18.2 M $\Omega$ .cm). Other chemical substances were purchased and used as received. <sup>1</sup>H NMR spectra were recorded on a Bruker Advance 500 MHz spectrometer. Chemical shifts are given in ppm and were determined by taking the solvent as a reference. All coupling constants are in Hz. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were obtained on a Bruker Autoflex II TOF-TOF spectrometer or Bruker Daltonics Autoflex speed spectrometer. Electrospray ionization time-of-flight (ESI-TOF) mass spectra were recorded on a Bruker Daltonics MicroTOF mass spectrometer. Mass spectra measurements and elemental analysis were performed at the "Service de Spectrométrie de Masse de la Fédération de Chimie Le Bel".

**UV-visible spectroscopy.** UV-visible spectra were recorded on a Shimadzu 1500 spectrometer, a Shimadzu 3600 double-beam spectrometer, an Agilent 8453E spectrometer, an Agilent Cary 60 or an Agilent Cary 5000 double-beam spectrometer. For all titration experiments monitored by UV-vis spectroscopy the changes in concentrations were negligible (<5%) and the UV-vis spectra shown are uncorrected.

**General procedure for pH titration.** In a 100 mL beaker equipped with a magnetic stir bar and a pH-meter, the pH of Fe<sup>III</sup>TPPS/BipyCD<sub>2</sub> (20  $\mu$ M, 50 mL) or Fe<sup>III</sup>TPPS/BipyCD<sub>2</sub> (14  $\mu$ M, 50 mL) in an NaClO<sub>4</sub> (0.1 M) solution was firstly adjusted to 2 with HClO<sub>4</sub>, and 3 mL of the solution were transferred to a quartz optical cell (1 cm). The corresponding UV-visible spectrum was recorded from 200 nm to 800 nm. Then the pH was adjusted with NaOH or HClO<sub>4</sub> solutions and the same process was repeated for every 0.2 or 0.5 increment of pH from 2 to 12.

**EPR spectroscopy.** EPR spectra were recorded on a Bruker EMX spectrometer operating at X-band (9.31 GHz) with a standard rectangular cavity (TE 102). An ESR900 cryostat (Oxford Instruments) was used for the low temperature measurements. Sample solutions (0.4-0.5 mM) in 0.025 M phosphate buffer at pH 7 or Q-milli water (18.2 M $\Omega$ .cm) at pH 7 mixed or not with 33% HPLC grade MeOH in quartz tubes (4 mm of diameter) were degassed with helium by three freeze-thaw cycles in liquid N<sub>2</sub>.

**Preparation of Fe<sup>II</sup> and Fe<sup>II</sup>/Cu<sup>I</sup> reduced complexes.** Reduced Fe<sup>II</sup> or Fe<sup>II</sup>/Cu<sup>I</sup> complexes were prepared by adding an excess of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to a dilute solution of Fe<sup>III</sup> or Cu<sup>I</sup>/Fe<sup>III</sup> in argon-degassed phosphate buffer (0.05 M) at pH 7.0 at room temperature.

**Preparation of Fe<sup>II</sup>-CO and Fe<sup>II</sup>-CO/Cu<sup>I</sup> carboxylated complexes.** Carboxylated Fe<sup>II</sup>-CO or Fe<sup>II</sup>-CO/Cu<sup>I</sup> complexes were prepared by bubbling CO for 2 min in the reduced Fe<sup>II</sup> or Cu<sup>I</sup>/Fe<sup>II</sup> solutions at room temperature.

**Reduction of Fe<sup>III</sup> and Fe<sup>III</sup>/Cu<sup>I</sup> complexes and preparation of oxygenated complexes.** Reduced Fe<sup>II</sup> or Cu<sup>I</sup>/Fe<sup>II</sup> complexes were prepared by adding an excess of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> to a concentrated solution of Fe<sup>III</sup> or Cu<sup>I</sup>/Fe<sup>III</sup> (1 mM) in phosphate buffer (0.05 M) at pH 7.0 in an N<sub>2</sub> atmosphere UNILab glovebox (MBRAUN). Excess Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> was removed by a Sephadex G-25 desalting column to give the pure Fe<sup>II</sup> or Cu<sup>I</sup>/Fe<sup>II</sup> complexes inside of the glove box and then the appropriate amount of concentrated solutions

<sup>1</sup> K. Kano, H. Kitagishi, S. Tamura, A. Yamada, *J. Am. Chem. Soc.*, 2004, 126, 15202.

<sup>2</sup> K. Kano, H. Kitagishi, M. Kodera, S. Hirota, *Angew. Chem. Int. Ed.*, 2005, 44, 435.

of the  $\text{Fe}^{\text{II}}$  or  $\text{Cu}^{\text{I}}/\text{Fe}^{\text{II}}$  complexes was transferred to a quartz cuvette and sealed with a septum and the cuvette was removed from the glovebox. To obtain final oxygenated complexes, a concentrated solution of  $\text{Fe}^{\text{II}}$  or  $\text{Cu}^{\text{I}}/\text{Fe}^{\text{II}}$  was diluted in  $\text{O}_2$ -saturated phosphate buffer (0.05 M) at pH 7.0 at 10 °C.

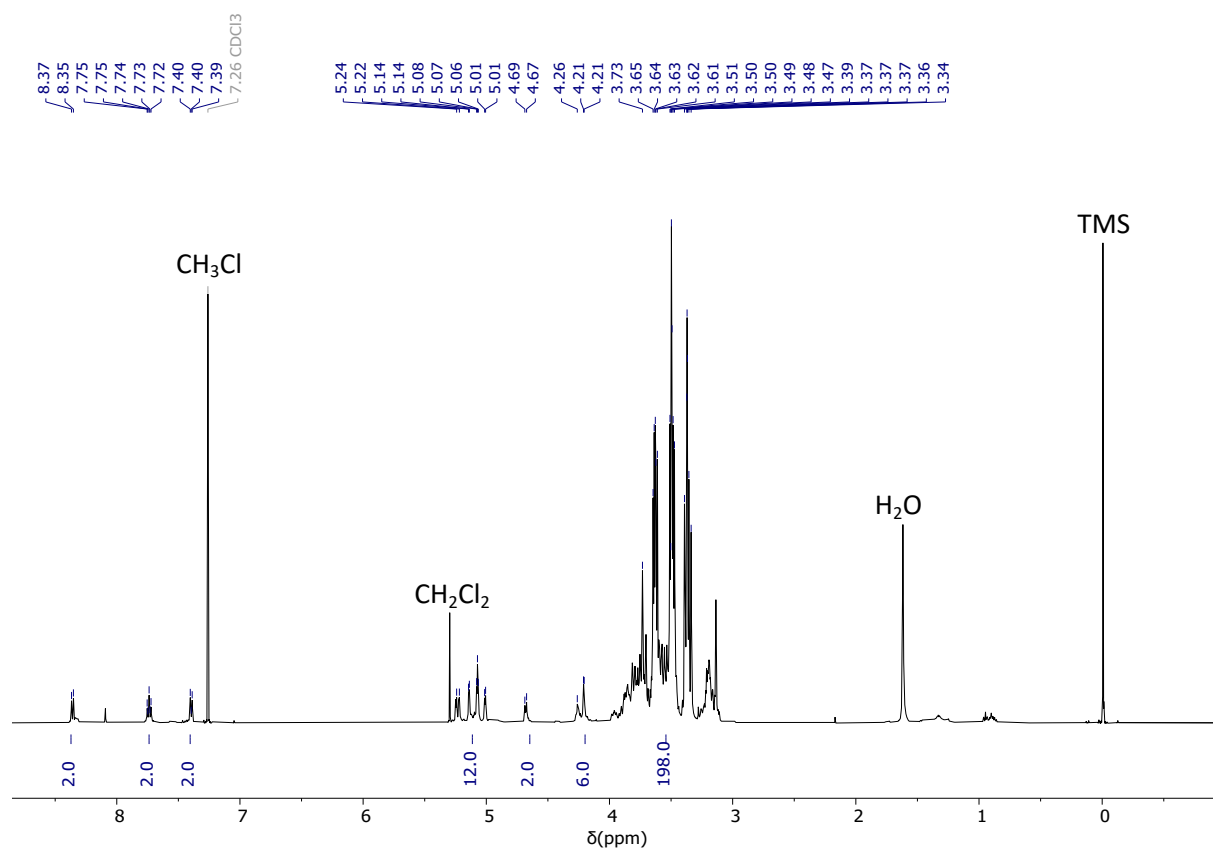
**Cyclic voltammetry.** Cyclic voltammetry experiments were carried out in 0.05 M phosphate buffer at pH 7 with 0.5 M  $\text{Na}_2\text{SO}_4$  as electrolyte in a classical three-electrode cell. The working electrode was a glassy carbon disk electrode (3 mm in diameter) used for cyclic voltammetry at  $0.1 \text{ V}\cdot\text{s}^{-1}$  scan rate, from 0.5 V to -1.2 V, and then from -1.2 V to 0.5 V, unless otherwise specified. The auxiliary electrode was a Pt wire, and the reference electrode was a saturated calomel electrode (SCE). The cell was connected to an Autolab PGSTAT30 potentiostat (Eco Chemie B.V., Utrecht, The Netherlands) driven by a GPSE software running on a personal computer.

**Rotating ring-disk electrode and linear sweep voltammetry.** The rotating ring-disk assembly (AFMT29TGEPT from Pine Instruments Company) consisted of edge-oriented pyrolytic graphite disk (EOPG diameter 5.6 mm) surrounded by a platinum ring (inner diameter 6.2 mm, outer diameter 7.9 mm). The EOPG disk and the ring are separated by an insulating ring of Teflon. A bipotentiostat Autolab PGSTAT30 (Eco Chemie B.V., Utrecht, The Netherlands) was used to control the potential of the disk and to maintain the ring potential at 0.8 V vs. SCE. A platinum wire was used as the counter-electrode. The reference electrode was an SCE saturated electrode. Prior to depositing a catalyst, the electrode was cleaned with a wet 600 grit SiC paper and sonicated for 1 min in  $\text{H}_2\text{SO}_4$ , and then rinsed with water. For deposition of the catalyst, 10  $\mu\text{L}$  of a 0.25 mM aqueous solution of catalyst were coated with Nafion 5 wt% dispersion (10  $\mu\text{L}$ ) on the EOPG disk electrode. Measurements were carried out in  $\text{O}_2$ -saturated 0.05 M phosphate buffer at pH 7 containing 0.5 M  $\text{Na}_2\text{SO}_4$  as electrolyte.

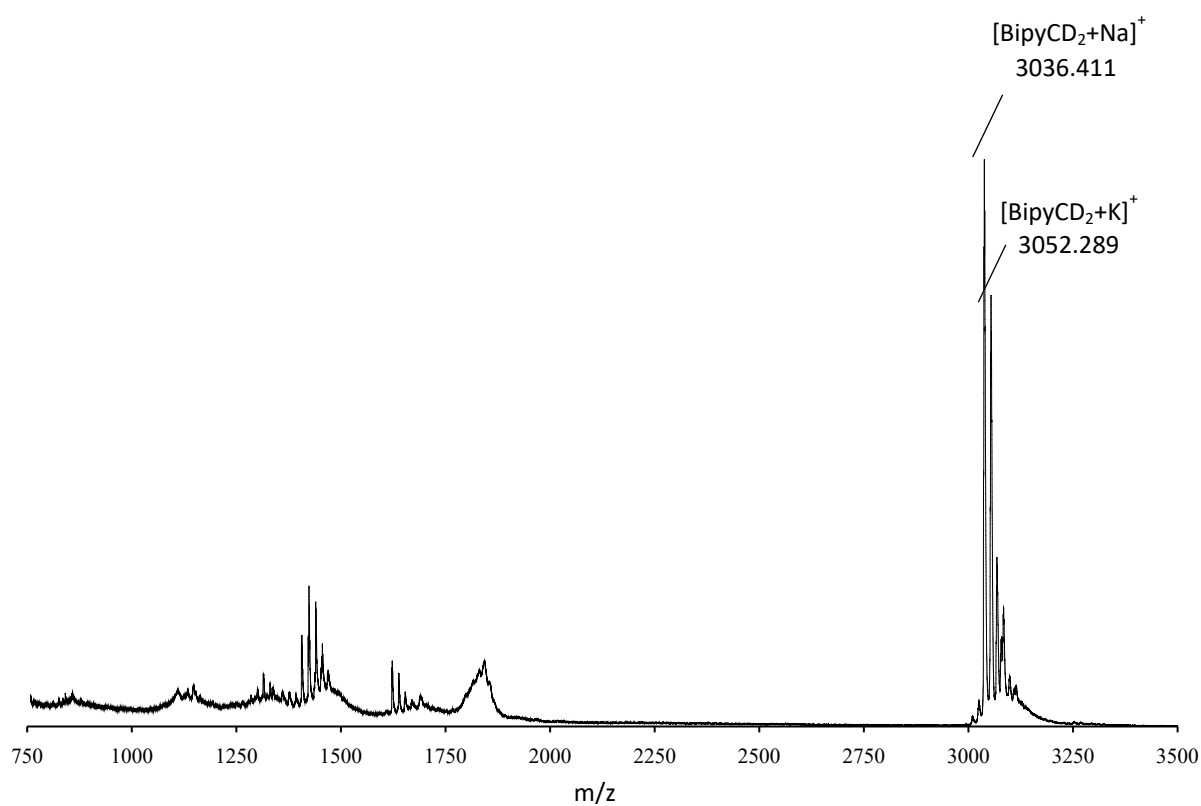
**Synthesis of Bipy- $\text{CD}_2$ .** To a degassed solution of 2,3-monoepoxy-per-O-methylated- $\beta$ - $\text{CD}^2$  (2.00 g, 1.45 mmol) in 0.1 M aqueous  $\text{NaHCO}_3$  (50 mL), a suspension of 6,6'-bis(mercaptomethyl)-2,2'-bipyridine<sup>3</sup> **1** (195 mg, 0.79 mmol) in methanol (20 mL) was added. The mixture was refluxed under argon for 16 h. After cooling to room temperature, the solution was extracted with  $\text{CHCl}_3$  (3 x 100 mL). The organic layers were combined, dried over  $\text{Na}_2\text{SO}_4$ , and filtered. After removal of the solvent, the crude product was purified by column chromatography ( $\text{SiO}_2$ ,  $\text{CHCl}_3$ /acetone (5/2) to  $\text{CHCl}_3$  to  $\text{CHCl}_3$ /methanol (95/5)). The last fraction was further purified by size exclusion chromatography (methanol) to yield **Bipy- $\text{CD}_2$**  (925 mg, 0.31 mmol, 43%) as a white powder. UV-visible  $\lambda_{\text{max}}$  ( $\text{H}_2\text{O}$ )/nm ( $\epsilon/\text{M}^{-1}\text{cm}^{-1}$ ): 240 (11 000), 248sh (9 400), 295 (17 000), 298sh (12 000).  $^1\text{H}$  NMR  $\delta_{\text{H}}$  (500 MHz;  $\text{CHCl}_3$ ) 8.36 (2 H, d,  $J = 7.7$ ), 7.73 (2 H, t,  $J = 7.7$ ), 7.39 (2 H, d,  $J = 7.7$ ) 5.25-4.99 (12 H, m), 4.68 (2 H, d,  $J = 7.1$ ), 4.21 (6 H, m) 3.73-3.05 (198 H, m). MS (MALDI-TOF):  $m/z$  3036.411 ( $[\text{M}+\text{Na}]^+$ ),  $m/z$  3052.289 ( $[\text{M}+\text{K}]^+$ ). Elemental analysis: Found: C, 52.9; H, 7.5; N, 1.0.  $\text{C}_{134}\text{H}_{224}\text{N}_2\text{O}_{68}\text{S}_2 + \text{H}_2\text{O}$  requires C, 53.1; H, 7.5; N, 0.9%.

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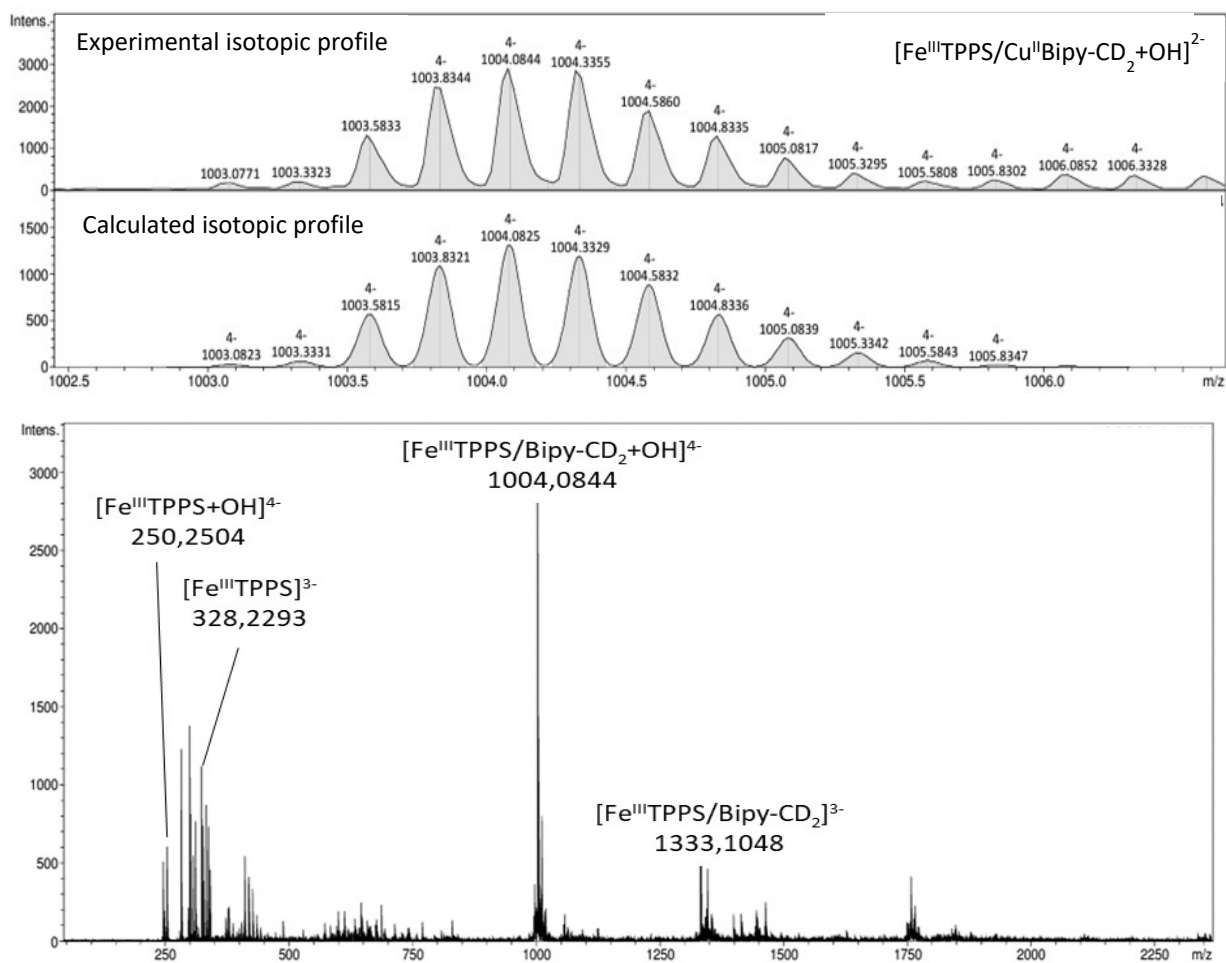
<sup>3</sup> a) G. R. Newkome and D. K. Kohli, *Heterocycles*, 1981, 15, 739; b) A. P. Smith, J. S. Lamba and C. L. Fraser, *Org. Synth.*, 2002, 10, 107.



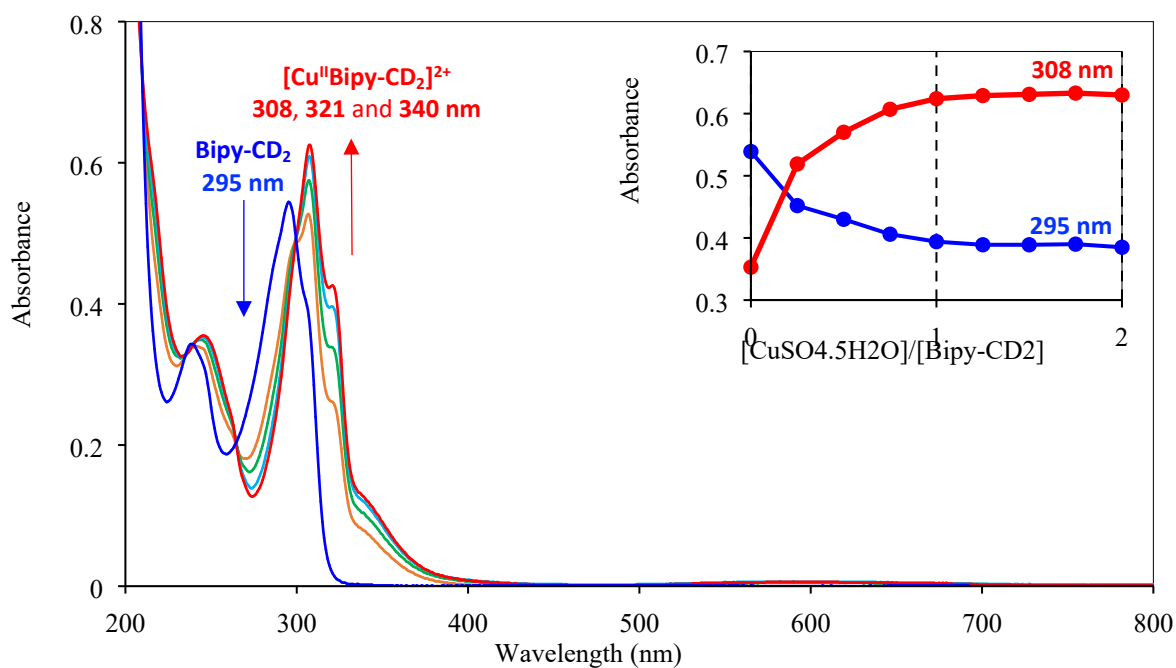
**Figure S1.** <sup>1</sup>H NMR spectrum (500 MHz, CDCl<sub>3</sub>) of Bipy-CD<sub>2</sub>.



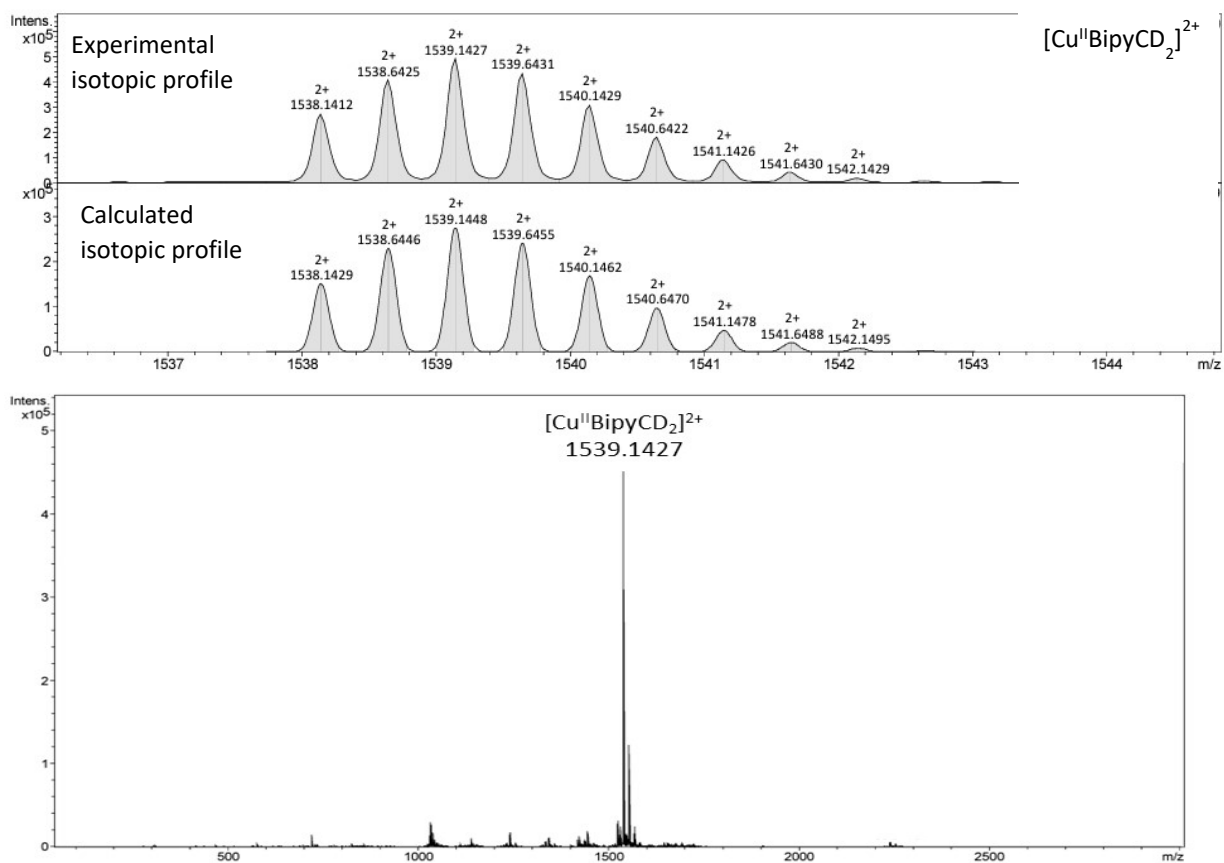
**Figure S2.** MALDI-TOF mass spectrum of Bipy-CD<sub>2</sub> with a subsequent addition of  $\alpha$ -cyano-4-hydroxycinnamic acid matrix (positive mode).



**Figure S3.** High resolution ESI mass spectrum negative mode of  $[\text{Fe}^{\text{III}}\text{TPPS}/\text{Bipy-CD}_2]^{3-}$  in water.



**Figure S4.** UV-visible titration of **Bipy-CD<sub>2</sub>** (32  $\mu\text{M}$ ) in water with the stepwise addition of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  at 25  $^\circ\text{C}$ . The inset shows changes in absorbances as a function of  $[\text{CuSO}_4 \cdot 5\text{H}_2\text{O}]/[\text{Bipy-CD}_2]$  concentrations.



**Figure S5.** High resolution ESI mass spectrum positive mode of [Cu<sup>II</sup>BipyCD<sub>2</sub>]<sup>2+</sup> in water.

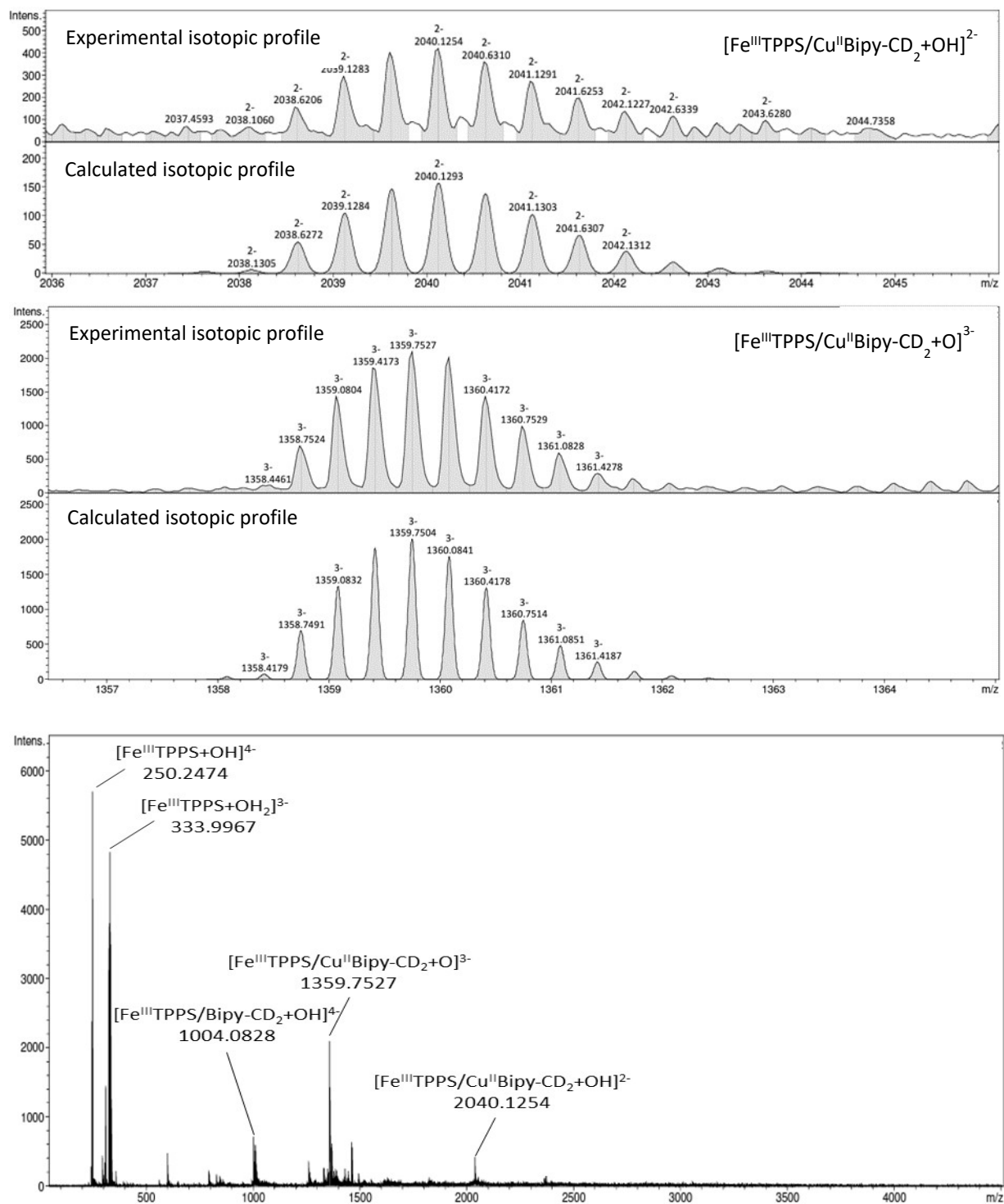
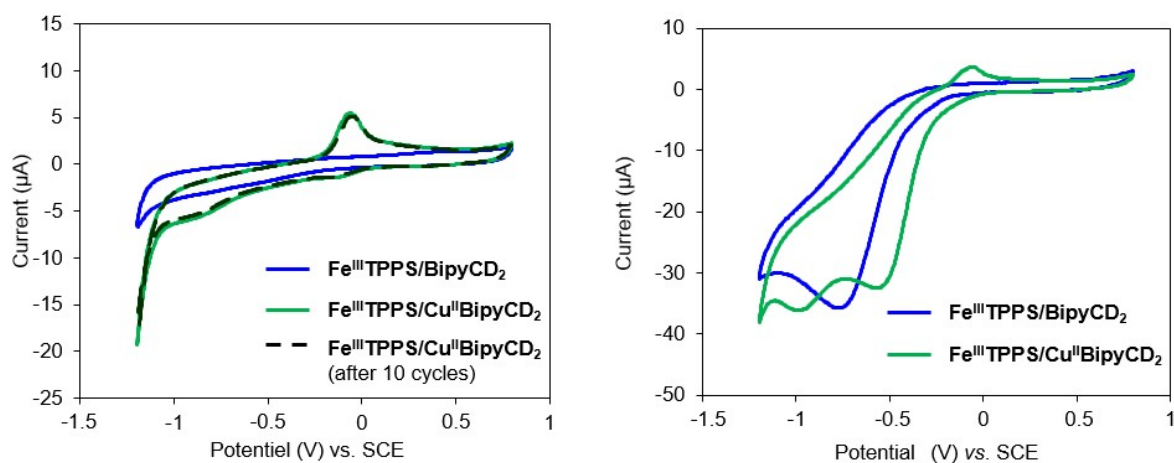


Figure S6. High resolution ESI mass spectrum negative mode of  $[\text{Fe}^{\text{III}}\text{TPPS}/\text{Cu}^{\text{II}}\text{Bipy-CD}_2]^{1-}$  in water.



**Figure S7.** Cyclic voltammograms of the Fe<sup>III</sup> and Fe<sup>III</sup>/Cu<sup>II</sup> catalysts in degassed (left) and in O<sub>2</sub>-saturated (right) phosphate buffer (0.05 M) at pH 7 with 0.5 M Na<sub>2</sub>SO<sub>4</sub> as supporting electrolyte.

**Table S1.** Graphical parameters, number of exchanged electrons and kinetic rate constants determined from Koutecky-Levich plots, and equations 2 and 3.

Catalyst	Slope at -0.6 V $B_L$ (rad <sup>1/2</sup> .s <sup>-1/2</sup> .mA <sup>-1</sup> )	Number of exchanged electrons <sup>a</sup> at -0.6 V $n_{KL}$	Intercept at -1 V $1/I_k$ (mA <sup>-1</sup> )	Kinetic rate constant <sup>b</sup> at -1 V $K_{app}$ (in M <sup>-1</sup> .s <sup>-1</sup> )
2 e- reference (HOPG)	27.261	2	-	-
4 e- reference (simulated)	13.630	4	-	-
Fe <sup>III</sup> TPPS/BipyCD <sub>2</sub>	15.719	3.5	5.47	5.1 × 10 <sup>-4</sup>
Fe <sup>III</sup> TPPS/Cu <sup>II</sup> BipyCD <sub>2</sub>	15.377	3.6	3.88	1.2 × 10 <sup>-5</sup>

<sup>a</sup> Number of electron exchange during the reduction of O<sub>2</sub> by the catalyst at -1 V determined from equation 2.

<sup>b</sup> Determined from equation 3.