Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Spectroelectrochemistry of Fe^{III} and Co^{III} mimochrome VI artificial enzymes immobilized in mesoporous ITO electrodes.

R. Vitale,[a] L. Lista,[a] S. Lau-Truong,[b] R.T. Tucker,[c] M.J. Brett,[c,d] B. Limoges,[e] V. Pavone,[a] A. Lombardi,*[a] and V. Balland*[e]

[a] Department of Chemical Sciences, Complesso Universitario Monte S. Angelo, University of Naples "Federico II", Via Cintia, 80126 Naples, Italy. E-mail: alombard@unina.it
[b] Laboratoire ITODYS, UMR CNRS 7086, Université Paris Diderot, Sorbonne Paris Cité, 15, rue Jean-Antoine de Baïf, 75205 Paris Cedex 13, France.
[c] Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta, Canada T6G 2V4.
[d] NRC National Institute for Nanotechnology, Edmonton, Alberta, Canada T6G 2M9.
[e] Laboratoire d'Electrochimie Moléculaire, Université Paris Diderot, UMR CNRS 7591, 15, rue Jean-Antoine de Baïf, 75205 Paris Cedex 13, France. E-mail: veronique.balland@univ-paris-diderot.fr

1. Experimental section

All chemicals were purchased from Sigma-Aldrich and used without further purification. Aqueous solutions were prepared with milli-Q water obtained from a Millipore purification system. Mimochrome VI (MC-6) was chemically synthesized by Fmoc- solid phase peptide synthesis, and its metal complexes were prepared as described elsewhere [12].

UV-vis characterization of MC-6 in solution (~ 50 μM) was achieved using 1 mm path length cells. MC6 concentration in solution was determined by UV-visible spectroscopy, using ε_{390} = 63 mM cm^{-1}, for the Fe^{III} complex and ε_{413} = 113 mM cm^{-1}, for the Co^{III} complex, in 1 mm path length cell.

Preparation of ITO electrodes. The ITO substrates were deposited by electron beam evaporation in a glancing angle deposition (GLAD) process. [10] Mesoporous ITO nanopillars of 1 μm thickness were deposited while the substrates were maintained at a constant glancing angle of α = 75° relative to the source material. With the aim to have a well-defined working electrode area during spectroelectrochemical experiments, an electroactive area of 0.5 cm^2, for Fe^{III}-MC-6-ITO experiments, and 0.3 cm^2, for Co^{III}-MC-6-ITO experiments was delimited at the extremity of the ITO rectangular piece with an insulating layer of varnish.

Resonance Raman. rR spectra were excited at 405 nm with a single frequency collimated laser Module (Ondax, Inc) and recorded with a Labram HR 800 microspectrometer (Jobin Yvon). Spectra were recorded at room temperature as described elsewhere.[9] The reported spectra

correspond to the averaging of 10 scans recorded with a 30s and 60s acquisition time on 50 µM Fe^{III}- or Co^{III}- mimochrome VI solutions, respectively (10 mM MES, pH 6.5). For the measurements on the GLAD ITO electrodes, the reported spectra are the result of the average of 6 single spectra, each recorded with a 1 s integration time.

UV-visible spectroelectrochemistry. Spectroelectrochemistry was performed in a homemade one-compartment three-electrode cell. A DRIREF Ag/AgCl, KCl (3 M) reference electrode (World Precision Instrument) and a platinum wire (1 mm diameter) were used as reference and auxiliary electrode, respectively. The three electrodes were inserted into a 1 cm path length quartz cell through a silicon cap that hermetically closes the cell. An additional tygon tube for degassing was introduced. The spectroelectrochemical cell was filled with 1.5 mL buffer, continuously purged with argon during the entire experiment and thermostated to 20 °C. All experiments were carried out in an aqueous medium buffered with 90 mM MES, 10 mM KPF_6 at pH 6.5.
Cyclic voltammetry measurements were performed with an Autolab potentiostat (EcoChemie) interfaced to a PC computer (GPES software) with ohmic drop compensation (ca. 100 Ω). UV-visible absorption spectroscopy was carried out on a TORUS-50 diode array spectrometer (Ocean Optics) equipped with a balanced deuterium tungsten source (Micropack). The cell was thermostated to 20 °C using a Peltier-controlled cuvette holder (Quantum Northwest). Integration time was fixed to 10 ms except for rapid scan measurements (e.g. 10 V s^{-1}), for which an integration time of 4 ms was used.

2. Adsorption kinetics of Fe^{III} MC-6 on 1 μM GLAD ITO electrode

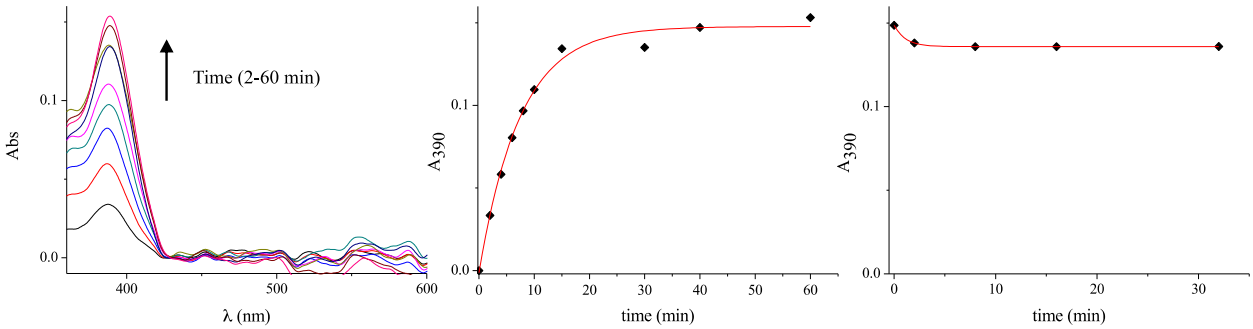

Figure S1. Left: UV-visible absorption spectra recorded at Fe^{III}-MC-6-ITO electrode, soaked into a 50 μM MC-6 solution (10 mM MES, pH 6.5) for different incubation time (ranging from 2 to 60 min). Each reported spectra is the averaging of 256 spectra recorded each with an integration time of 10 ms. Right: Plots of the absorbance monitored at 390 nm as a function of adsorption and desorption times (i.e., in a protein-free buffer of 90 mM HEPES, 10 mM KPF_6 pH 6.5). The adsorption kinetic was fitted (red line) to a pseudo first-order kinetic with an apparent rate constant k_{ads} = 0.14 min^{-1}. T = 20 °C.

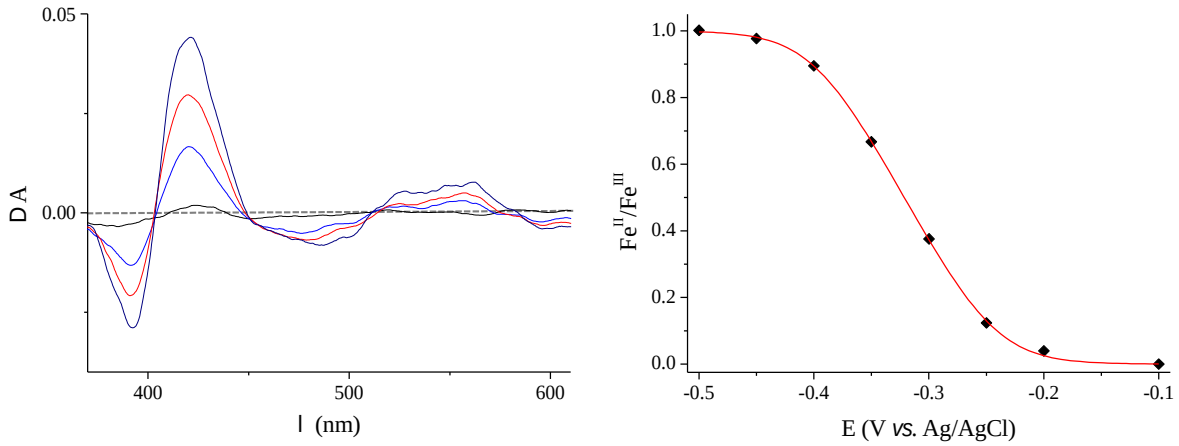

Figure S2. Left: difference spectra recorded during the spectroelectrochemical redox titration of Fe^{III}-MC-6 adsorbed within the ITO thin film held at an applied potential of (black) -0.1, (blue) -0.25, (red) -0.35, (navy) -0.5 V vs. Ag/AgCl. Each reported spectrum is the averaging of 100 spectra recorded each with an integration time of 10 ms. Right: fraction of ferrous-MC-6 determined from the absorbance at 420 nm during reductive titrations. Red line: Nernst fits using n_{app} = 0.7 and $E^{0'}$ = -0.32 V. For all experiments the buffer was a 90 mM MES, 10 mM KPF_6 (pH 6.5; T = 20 °C).

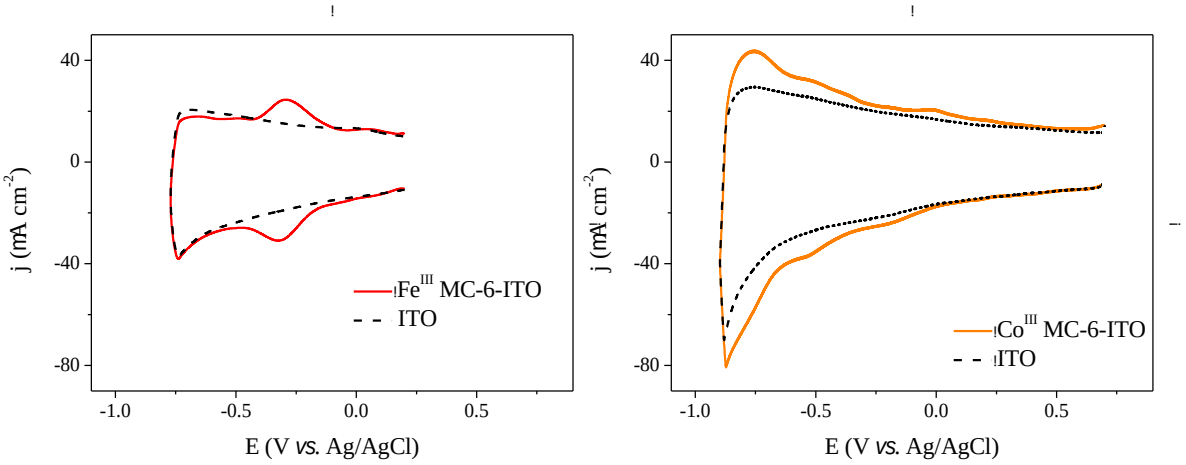

Figure S3. Cyclic voltammograms recorded before (dotted) and after absorption of MC-6 (red and orange are respectively for Fe^{III}- and Co^{III}-MC6) in the GLAD ITO electrode (CVs were normalized to the geometric electrode surface area). Scan rate: 0.02 Vs^{-1}; ohmic drop compensation: 100 Ω; buffer: 90 mM MES buffer, 10 mM KPF_6 (pH 6.5; T = 20 °C).

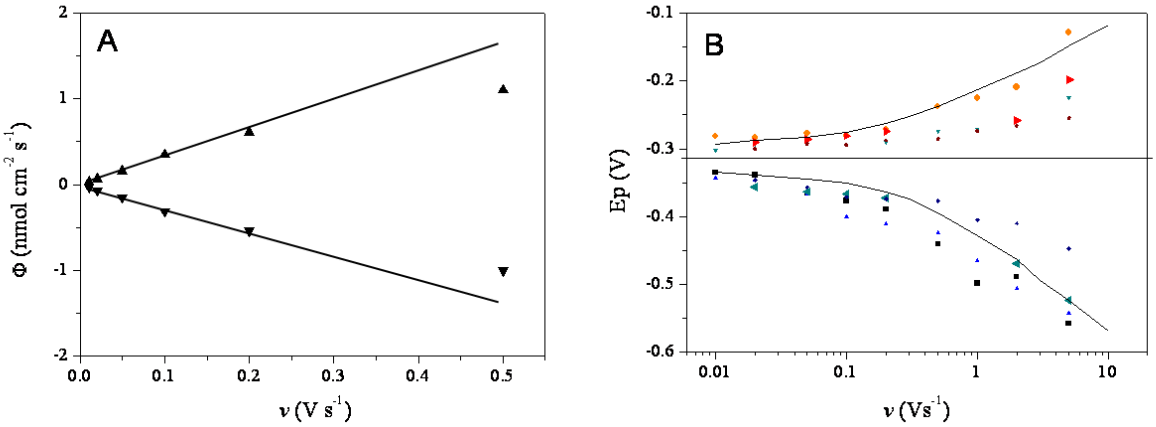

Figure S4. Plots of (A) anodic and cathodic peak flux densities (determined from DCVAs) and (B) anodic and cathodic peak potentials (obtained from both CVs and DCVAs recorded at two different modified electrodes) as a function of v, relative to Fe^{III}-MC-6-ITO electrodes. The plain lines in A are the linear fits to the flux densities recorded at $v < 0.2$ V/s. The plain curves in B are the theoretical plots resulting from simulations of the Butler–Volmer equations under thin-layer conditions and using a heterogeneous electron transfer rate constant of $k^0 = 4$ s^{-1} and the charge transfer coefficient $\alpha = 0.4$. At $v > 0.2$ V s^{-1}, the loss of linearity in A and the increased ΔE_p in B reflect the progressive kinetic control by the heterogeneous electron transfer rate.

3. Spectroscopic characterization of Fe^{III}-MC-6: UV-vis absorption and rR

The absorption spectra of ferric porphyrin model compounds, *e.g.* Fe^{III}-OEP (SbF_6) (OEP: octaethylporphyrin), with well-characterized spin-admixed ground states, are similar to those of high-spin ferric porphyrin complexes. On the contrary, differences in spin states are evident in rR spectra.[11, 18]

It was previously reported that the charge transfer (CT1) band position is highly indicative of the heme proteins spin state, because it depends on the vinyl conjugation to the porphyrin and the axial ligand type. In fact, in 5-coordinated HS proteins, containing an imidazole as axial ligand, the CT1 typically ranges from 640 to 652 nm. In 6-coordinated HS systems, the CT1 is found from 600 to 637 nm, depending on the nature of the sixth ligand. Smulevich and co-workers suggested that in QS hemes, the CT1 band is located at 630-635 nm.[15]

Table S1. Parameters of UV-Visible spectra corresponding to ferric and ferrous MC-6 with the corresponding molecule adsorbed in mesoporous ITO film, and with typically LS, HS/QS mixed and purely QS systems.

Compound	pH	Spin state	Coordination mode	Soret	Visible Region λ (nm)		
Fe^{III}-MC-6 [a]	6.5	5-c	HS/QS	391	491	530 (sh) 570 (sh)	614
Fe^{II}-MC-6 [a]	6.5	5-c	HS	419		544	
Fe^{III}-MC-6-ITO	6.5	5-c	HS/QS	390			
Fe^{II}-MC-6-ITO	6.5	5-c	HS	420			
Fe^{III}-MC-1 [b]	7.0	6-c	LS	402	525	560 (sh)	630 (sh)
Fe^{II}-MC1 [b]	7.0	6-c	LS	411	518	547	
Fe^{III}-BP1 [c, d]	6.5	5-c	HS/QS	398	496	530 (sh)	640
Fe^{III}-BP1 [c]	6.5*	5-c	QS	403	490	530 (sh)	632
Fe^{II}-BP1 [c]	6.5	5-c	HS	437	526 (sh)	557 585 (sh)	
Fe^{III}-HRPA2 [d]	7.3	5-c	HS/QS	403	500	530 (sh)	639
Fe^{III}-SBP [d]	7.0	5-c	HS/QS	402	496	530 635	
Fe^{III}-Cyt c' [e]	1-10	5-c	QS	396-408	500	535 (sh)	635
Fe^{III}-Cyt c [f]	6.5	6-c	LS	410		528	

Table S2. Comparison of the high-frequency resonance Raman modes (ν, cm^{-1}) of ferric MC-6 with the corresponding molecule adsorbed within mesoporous ITO film, and with typically LS and QS systems.

Compound	pH	Spin state	Coordination mode	ν_4	ν_3	ν_2	ν_{10}
MC-6 [a]	6.5	5-c	HS/QS	1373	1487	1580	1630
MC-6-ITO	6.5	5-c	HS/QS	1373	1487	1580	1630
MC-1 [b]	7.0	6-c	LS	1377	1502	1587	1640
BP1 [c, d]	6.5	5-c	HS/QS		1504	1578	1632
BP1 [c]	6.5*	5-c	QS		1515	1588	1648
HRPA2 [d]	7.3	5-c	HS/QS		1503	1572	1631
SBP [d]	7.0	5-c	HS/QS		1504	1575	1632
Cyt c' [e]	7.0	5-c	QS	1371	1502	1580	1637
Cyt c [f]	7.0	6-c	HS/QS	1374	1502	1582	1636
OEP(SbF_6) [e]		5-c	QS	1377	1513	1581	1646

[a] Data from reference 3, [b] data from reference 13, [c] data from reference 16, [d] data from reference 15, [e] cytochrome c' from *Chromatium vinosum* data from reference 11, [f] E. Margoliash and N. Frohwirt, *Biochem. J.* , 1959, 71, 570–572.
* T=20 K. MC1: mimochrome I, BP1: Barley peroxidase 1, SBP: Soybean peroxidase, HRPA2: Horseradish peroxidase isoenzyme A2, Cyt c': cytochrome c', Cyt: cytochrome c, OEP: octaethylporphyrin.

4. Spectroscopic characterization of Co^{III}-MC-6: UV-visible absorption and resonance Raman spectroscopies

It was previously reported that the optical spectra of cobaltic reconstituted hemoglobin ($CoHb^{+}$) and the oxygenated cobaltous hemoglobin (Oxy CoHb) were closely similar. In particular, the CT bands were found at nearly identical wavelengths, with almost the same extinction coefficients. Moreover, oxygenation resulted in no evident changes in the EPR spectrum, since oxy and deoxy CoHb showed identical spin label EPR spectra.
It was proposed that in the low-spin oxy CoHb, the unpaired electron is largely transferred to the oxygen, supporting the idea that the oxygen ligand could be a superoxide ion. Finally, the optical spectrum of the oxy cobalt hemoglobin suggested that the oxidation state of the metal ion could be closer to +3 than +2, due to the large delocalization of the unpaired electron largely transferred to the oxygen.[4]

Table S3. Parameters of UV-Visible spectra corresponding to cobaltic and cobaltous MC-6 with the corresponding molecule adsorbed within ITO network, and with typically cobalt-reconstituted heme proteins and porphyrins.

Compound	pH	Soret	Visible Region λ (nm)	
Co^{III}-MC-6	6.5	414	524	556
Co^{III}-MC-6-ITO	6.5	416		
Co^{II}-MC-6	6.5	390	515 (sh)	545
Co^{II}-MC-6-ITO	6.5	393		
Co^{III}-Cyt-c [g]	7.0	426	530	567
Co^{II}-Cyt -c [g]	7.0	416	520	549
Co^{III}-Hb [h]	7.0	427	535	568
Co^{II}-Oxy Hb [h]	7.0	428	538	571
Co^{II}-Deoxy Hb [h]	7.0	402	510 (sh)	552
Co^{II}-Oxy-DP-Hb [h]	7.0	412	528	559
Co^{II}-Deoxy-DP-Hb [h]	7.0	393	510 (sh)	541
Co^{II}-Oxy Mb [h]	7.0	426	539	577
Co^{II}-Deoxy Mb [h]	7.0	406	510 (sh)	558
Co^{II}-Oxy-DP- Mb [h]	7.0	413	528	558
Co^{II}-Deoxy-DP-Mb [h]	7.0	396	510 (sh)	540
Co^{III}-DP [h]	*	415	525	556
Co^{III}-PP [h]	*	424	535	569

Table S4. Comparison of high-frequency resonance Raman modes (ν, cm^{-1}) of cobaltic MC-6 with the corresponding molecule adsorbed in mesoporous ITO film, and with typically cobaltous heme proteins and cobaltic porphyrins.

Compound	pH	ν_4	ν_3	ν_2	ν_{10}
MC-6	6.5	1379	1508	1591	1647
MC-6-ITO	6.5	1379	1508	1591	1647
Oxy Hb [i]	7.0	1380	1512	1596	1649
Oxy Mb [i]	7.0	1380	1512	1600	1652
Oxy- DP- Mb [i]	7.0	1387		1603	1651
PP [i]	*	1379	1509	1596	1645
DP [i]	*	1383	1510	1600	1644

([g]) Data from reference 6, ([h]) data from reference 5, ([i]) data from reference 19
Hb: Hemoglobin; Mb: Myoglobin; DP: Deuteroporphyrin; PP: Protoporphyrin.
* 0.1 M NaOH-pyridine-H_2O (3:10:17)

Note: For bibliographic numbers, please, refer to the main text.