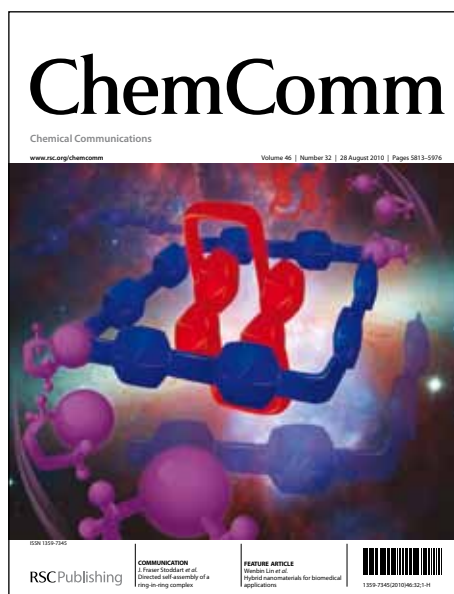


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## COMMUNICATION

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## Spectroelectrochemistry of Fe<sup>III</sup>- and Co<sup>III</sup>-mimochrome VI artificial enzymes immobilized in mesoporous ITO electrodes

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**UV-visible absorption spectroelectrochemistry elucidated the different redox behaviours of Fe<sup>III</sup>- and Co<sup>III</sup>-mimochrome VI artificial enzymes, adsorbed within mesoporous conductive films of ITO. The reduction of the ferric complex was rapid and reversible, while the cobaltic complex exhibited irreversible processes probably related to multiple coordination states.**

The design of peptide-based synthetic metalloenzymes requires the adequate combination of both metal cofactors and protein chemistries, which can mutually modulate their functional properties.<sup>1</sup> This idea was the basis for the construction of the protein mimetic mimochrome VI (MC-6), a covalent deuteroporphyrin-peptide system, with two  $\alpha$ -helical peptides embracing the porphyrin in a sandwiched structure (Fig. 1).<sup>1-3</sup>

MC-6 peptide chains were designed to (i) impart an asymmetry, in the first and second coordination shells, and (ii) to stabilize the five-coordination. The spectroscopic and functional characterization of the Fe<sup>III</sup>-MC-6 complex confirmed the correctness of the design. Fe<sup>III</sup>-MC-6 exhibits a peroxidase-like catalytic activity with high stability under turnover conditions.<sup>3</sup>

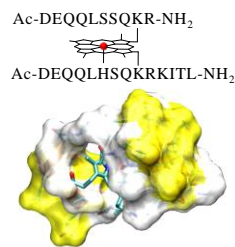
More recently, we explored Co<sup>III</sup>-MC-6 coordination by UV-visible (UV-vis) spectroscopy, highlighting similarities with coboglobins, cobalt reconstituted cytochrome c, and N-acetyl-Co<sup>III</sup>-MP-8 (Tab. S2 ESI).<sup>4-7</sup> However, based on its absorption spectra, the Co<sup>III</sup>-MC-6 coordination mode is not completely clear and further studies are needed.

According to their catalytic properties, Fe<sup>III</sup>- and Co<sup>III</sup>-MC-6 complexes offer the chance to construct new electrocatalytic active surfaces, with potential applications in the field of sensors or heterogeneous catalysis. Their development requires

the (i) immobilization of MC-6 catalysts on conductive surfaces without significant structural perturbation, and (ii) the characterization of the resulting redox- and electrocatalytically- active surface by appropriate electroanalytical techniques. In this respect, the selection of advanced conductive matrices is crucial. Nanostructured films of tin-doped indium oxide (ITO) are very promising materials both for sensing and catalytic applications, thanks to their high surface area and excellent electrical and optical properties.<sup>8</sup> Their high transparency in the visible range allows characterization of immobilized redox biomolecules by various techniques (i.e. UV-vis or resonance Raman spectroscopies), deepening insight into the catalytic centre at the electrode surface. Furthermore, they can be exploited to develop innovative real-time spectroelectrochemical methods to study redox proteins or enzymes, but also biomimetic catalysts or artificial enzymes.<sup>9-11</sup>

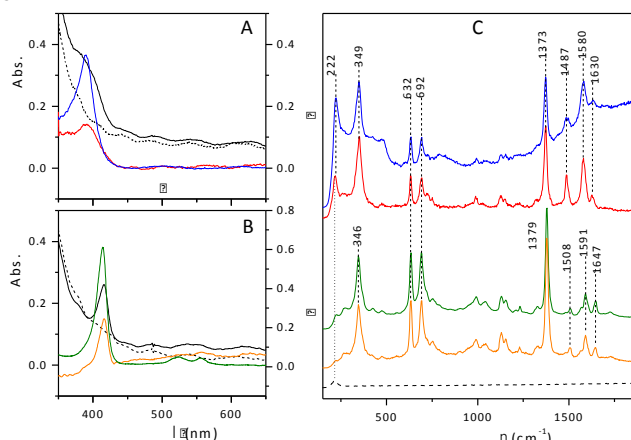
Here, we report the immobilization of both the Fe<sup>III</sup>-MC-6 and Co<sup>III</sup>-MC-6 within 1  $\mu\text{m}$ -thick nanocrystalline mesoporous ITO films. These electrodes, prepared by GLAD (Glancing Angle Deposition), were recently successfully used for the immobilization and the spectroelectrochemical investigation of both heme-peptides and heme-proteins.<sup>9-11</sup> The modification of GLAD ITO electrodes was achieved by soaking them in 50  $\mu\text{M}$  MC-6 (10 mM MES buffer, pH 6.5). After equilibration (~14 hours), the electrodes were washed in a peptide-free MES buffer of high ionic strength (90 mM MES, 10 mM KPF<sub>6</sub>, pH 6.5) to remove the fraction of poorly adsorbed molecules.

The resulting electrodes were first characterized by UV-vis spectroscopy. Once corrected from their blank ITO film absorption contribution, a clear and well-defined Soret band was detected at 390 nm and 418 nm for Fe<sup>III</sup>-MC-6 and Co<sup>III</sup>-MC-6, respectively (Fig. 2 A-B). These wavelengths are similar to those obtained for the same complexes in solution, and indicate that the redox, spin and coordination states of the metal ions are not strongly affected by their physical adsorption onto the metal oxide surface (Tab. S1, S3 ESI).



**Figure 1** Surface representation of MC-6 model, with amino acid sequence. The two main polar regions are evidenced in yellow.<sup>3</sup>

In order to further study the coordination mode of the metal ions, the ferric and cobaltic complexes were investigated by resonance Raman, rR (Fig. 2 C). The high quality rR spectra obtained over a large range of frequencies confirm the molecular and structural identity of the immobilized complexes with those in solution. Accordingly, the molar extinction coefficients of the immobilized MC-6 compounds were assumed to be equal to that determined in solution, i.e.,  $\epsilon_{390} = 63 \text{ mM}^{-1} \text{ cm}^{-1}$  and  $\epsilon_{416} = 113 \text{ mM}^{-1} \text{ cm}^{-1}$  for  $\text{Fe}^{\text{III}}$ -MC-6 and  $\text{Co}^{\text{III}}$ -MC-6, respectively. Using these values, MC-6 surface concentration of  $\Gamma = 1\text{-}2 \times 10^{-9} \text{ mol cm}^{-2}$  was estimated. According to the  $\sim 66$ -fold surface enhancement, determined for a  $1 \mu\text{m}$ -thick GLAD ITO electrode, the  $\Gamma$  value is close to that expected for a monolayer covering the overall specific surface area of the porous metal oxide.  $\Gamma$  value is also in agreement with that previously obtained with a saturated monolayer of adsorbed  $\text{Fe}^{\text{III}}$ -MC-6 on decan-1-thiol-modified gold electrodes.<sup>2</sup>



**Figure 2** UV-vis spectra of (A)  $\text{Fe}^{\text{III}}$ -MC-6-ITO and (B)  $\text{Co}^{\text{III}}$ -MC-6-ITO electrodes (1- $\mu\text{m}$ -thick films): (A, B) (black) raw, (dotted) protein-free ITO, (red, orange) and blank-corrected spectra; (blue, A)  $58 \mu\text{M}$   $\text{Fe}^{\text{III}}$ -MC-6 and (green, B)  $54 \mu\text{M}$   $\text{Co}^{\text{III}}$ -MC-6 (1 mm path length cell). (C) rR ( $\lambda_{\text{exc}} = 405 \text{ nm}$ ), for respectively  $\text{Fe}^{\text{III}}$ -MC-6 and  $\text{Co}^{\text{III}}$ -MC-6, in solution (blue, green) or adsorbed in the ITO (red, orange), and (dotted) a protein-free ITO. All spectra were recorded in a 10 mM MES buffer at pH 6.5 ( $T = 20^\circ \text{C}$ ).

The adsorption kinetics of  $\text{Fe}^{\text{III}}$ -MC-6 within the mesoporous ITO film was explored by plotting the Soret band absorbance as a function of soaking time (Fig. S1, ESI). A first-order adsorption rate constant was determined ( $k_{\text{ads}} = 0.14 \text{ min}^{-1}$ ). An analogous value was reported for MP-11 adsorption on similar ITO electrodes.<sup>11</sup> It suggests a high affinity of  $\text{Fe}^{\text{III}}$ -MC-6 for the hydrophilic ITO film, thanks to the presence of several polar amino acid residues (Fig. 1), and a rapid saturation of the porous metal oxide surface after 30 min of immersion. Only  $\sim 10\%$  desorption was observed at high ionic strength, while the remaining 90% remained stable over hours.

Concerning  $\text{Fe}^{\text{III}}$ -MC-6 rR spectra, the  $\nu_4$  and  $\nu_3$  oxidation marker bands are found at 1373 and  $1487 \text{ cm}^{-1}$ , respectively, as expected for 5-coordinated high spin (HS) ferric complexes.<sup>12</sup> On the contrary, the  $\nu_2$  spin state marker band is observed at  $1580 \text{ cm}^{-1}$ , a frequency characteristic of 6-coordinated low-spin complexes (LS) (Tab.2 ESI).<sup>13,14</sup> This could be due to the structural differences between MC-6 deuteroporphyrin and natural heme-protein protoporphyrin, but it may also be indicative of a spin-state admixture ( $S = 3/2, 5/2$  or QS state), in agreement to Maltempo and co-workers quantum mechanical theory.<sup>15</sup> Several class III peroxidases, cytochrome c', and heme-models were described by this model, on the basis of the of their rR, EPR, NMR, and UV-vis spectra.<sup>12,16-19</sup>

Regarding  $\text{Co}^{\text{III}}$ -MC-6,  $\nu_4$ ,  $\nu_3$  and  $\nu_2$  rR marker bands were found at 1379, 1508 and  $1591 \text{ cm}^{-1}$ , respectively. These wavenumbers are similar to those reported for 6-coordinated low spin bis-pyridine  $\text{Co}^{\text{III}}$ -porphyrins and for oxy-cobaltous-globins (Tab. S4 ESI).<sup>20,21</sup> Accordingly, they may be indicative of a 6-coordinated low spin complex. However, it is difficult to definitely assign spin and coordination states to  $\text{Co}^{\text{III}}$ -MC-6, due to the lack of reference rR data on 5-coordinated cobaltic porphyrin complexes. Hence, the use of multiple and combined analytical techniques, e.g. spectroscopic and spectroelectrochemical, is desirable to get deeper insight in  $\text{Co}^{\text{III}}$ -MC-6.

UV-vis spectroelectrochemistry allows for easy detection of the  $\text{M}^{\text{III}}/\text{M}^{\text{II}}$  interconversion during a potential-step redox titration or cyclic voltabsorptometric experiments (Fig. 3). When a constant cathodic potential was applied to the MC-6-modified ITO electrodes, the UV-vis spectra of the fully reduced species were obtained. The positions of  $\text{Co}^{\text{II}}$ - and  $\text{Fe}^{\text{II}}$ -MC-6 Soret bands were reasonably coincident with those obtained in solution. In particular, the  $\text{Fe}^{\text{II}}$ -MC-6 spectra were fully consistent with a high-spin five-coordinate  $\text{Fe}^{\text{II}}$  having an axial histidine at the fifth coordination site.<sup>3</sup> At the same time,  $\text{Co}^{\text{II}}$ -MC-6 spectra were in quite good agreement with a planar low spin  $\text{Co}^{\text{II}}$ -complex having a single axially coordinated nitrogen base (Tab. S1-S3 ESI).<sup>4,5</sup> Difference absorption spectra of both MC-6-ITO show well resolved  $\text{M}^{\text{III}}$  ( $\lambda_{\text{Fe}} = 392 \text{ nm}$ ,  $\lambda_{\text{Co}} = 418$ ) and  $\text{M}^{\text{II}}$  ( $\lambda_{\text{Fe}} = 420 \text{ nm}$ ,  $\lambda_{\text{Co}} = 390 \text{ nm}$ ) contributions (Fig. 3).

The potential-step redox titration of  $\text{Fe}^{\text{III}}$ -MC-6-ITO was fully reversible, with two well-defined isosbestic points at 403 and 450 nm (Fig. S2, ESI). The resulting titration curve was fitted to the Nernst equation from which an apparent standard potential of  $E^0 = -0.32 \text{ V}$  (vs. Ag/AgCl) and a value of  $n_{\text{app}} = 0.7$  electron transferred were inferred. The  $E^0$  value is similar to the one determined for the molecule in solution or immobilized on a gold electrode, thus confirming that the redox properties of  $\text{Fe}^{\text{III}}$ -MC-6 remain unmodified upon adsorption.<sup>2</sup> The low  $n_{\text{app}}$  value, which should theoretically be equal to 1 for the one-electron  $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$  couple, may derive from variable distribution of the artificial enzyme in (i) different orientations on the surface and/or (ii) diverse chemical microenvironments, as previously reported.<sup>11</sup>

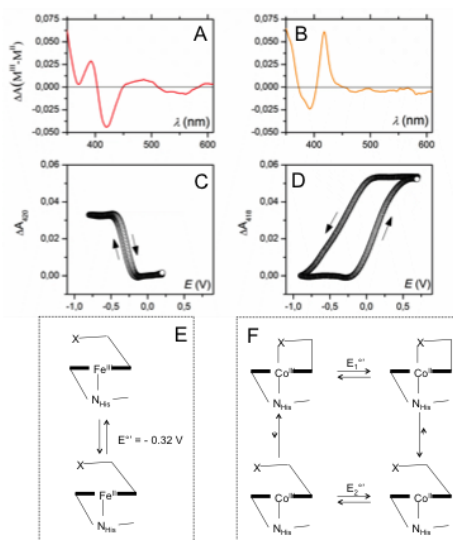
$\text{Fe}^{\text{III}}$ -MC-6-ITO cyclic voltabsorptometry evidences a typical thin-layer redox behaviour process since, at low scan rates ( $\nu$ ), (i) the sigmoidal plots of reduction and oxidation on the cyclic voltabsorptogram (CVA) are almost superimposed (Fig. 3), (ii) the anodic and cathodic waves in cyclic voltammetry (CV) are highly symmetric, showing a peak potential difference relatively close to 0 (Fig. S3, ESI), and (iii) up to  $0.1 \text{ V s}^{-1}$ , the cathodic and anodic peak intensities are a linear function of  $\nu$  (Fig. S4, ESI). As the scan rate is further increased, the hysteresis between the reduction and oxidation curves is enlarged, indicating an increasing kinetic control of the redox process by the heterogeneous electron transfer rate ( $\sim 4 \text{ s}^{-1}$ ).

In contrast to the relatively well-defined reversible one-electron transfer process observed with  $\text{Fe}^{\text{III}}$ -MC-6, the CVA of  $\text{Co}^{\text{III}}$ -MC-6-ITO show a large hysteresis between the reduction and oxidation processes (e.g.  $\Delta E_p = 0.5 \text{ V}$  at  $\nu = 0.01 \text{ V s}^{-1}$ ).

In CV, such a sluggish redox processes was very difficult to identify from the large capacitive background current (Fig. S3, ESI), thus explaining the pertinence and advantage of using a spectroelectrochemical method, which is totally free of these capacitive background current responses.

Attempts to diminish or suppress the oxidation/reduction hysteresis by decreasing the scan rate were revealed unsuccessful. The large hysteresis reported here is thus indicative of a

square scheme mechanism involving fast and irreversible chemical processes coupled to the preceding electron transfer (E.T.) reactions.



**Figure 3** Difference spectra obtained for (A) Fe<sup>III</sup>-MC-6-ITO and (B) Co<sup>III</sup>-MC-6-ITO, when the applied reducing potentials are -0.5 V, and -0.9 V, respectively. C) CVAs recorded at 0.01 V s<sup>-1</sup> corresponds to (C) Fe<sup>III</sup>-MC-6-ITO, and (D) Co<sup>III</sup>-MC-6-ITO. ( $\lambda = 420$  nm, indicative of Fe<sup>II</sup>-MC-6 increase;  $\lambda = 418$  nm, revealing Co<sup>III</sup>-MC-6 decrease). Integration time: 10 ms; experimental conditions: 90 mM MES buffer, 10 mM KPF<sub>6</sub> pH 6.5; T = 20 °C. Bottom schemes: (E) Simple E.T. mechanism observed for Fe<sup>III</sup> MC-6 and (F) square-scheme mechanism suggested for Co<sup>III</sup> MC-6.

Similar phenomena were reported for the base-off/base-on equilibrium in vitamin B12, and the E.T. associated to a large conformational change found in cytochrome cd1.<sup>22,23</sup> In vitamin B12, breaking of intramolecular ligand coordination occurred upon Co<sup>II</sup>/Co<sup>I</sup> reduction, resulting into a large potential difference between the reduction and the oxidation waves in CV, under appropriate scan rate.<sup>22</sup>

In analogy, binding/unbinding of an intramolecular sixth ligand could be associated with a large structural reorganization of the peptide conformation in Co<sup>III</sup>-MC-6 complex (Fig. 3). This hypothesis is supported by the spectroscopic data recorded for the oxidized and reduced species, which both are consistent with a low spin 6-coordinated Co<sup>III</sup> ion and a 5-coordinated Co<sup>II</sup> ion. Difference in coordination mode of the Co<sup>III</sup>- and Co<sup>II</sup>-MC-6 is further supported by the low reduction potential reported for the Co<sup>III</sup>/Co<sup>II</sup> redox transition.<sup>24</sup> Since no exogenous ligand is present in solution, we assume that the sixth ligand comes from the distal peptide chain (e.g. D<sup>1</sup> or E<sup>2</sup>).

In conclusion, the comparative spectroelectrochemical study of Fe<sup>III</sup>- and Co<sup>III</sup>-MC-6 adsorbed in mesoporous ITO electrodes reveals contrasting redox behaviours that seem related to different coordination modes in their oxidized states.

MC-6 was conceived to stabilize 5-coordinated complexes, since no ligand is supplied by the peptide in deuteroporphyrin distal side. The present results indicate that conformational preferences of the peptide chains in Co<sup>III</sup>- and Fe<sup>III</sup>-MC-6 complexes might be different. This information could be exploited for the redesigning of MC-6 aimed at stabilizing a 5-coordinated Co<sup>III</sup>.

## Notes and references

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1. A. Lombardi, F. Nistri, and V. Pavone, *Chem. Rev.*, 2001, **101**, 3165–3189.
2. A. Ranieri, S. Monari, M. Sola, M. Borsari, G. Battistuzzi, P. Ringhieri, F. Nistri, V. Pavone, and A. Lombardi, *Langmuir*, 2010, **26**, 17831–17835.
3. F. Nistri, L. Lista, P. Ringhieri, R. Vitale, M. Faiella, C. Andreozzi, P. Travascio, O. Maglio, A. Lombardi, and V. Pavone, *Chem. a Eur. J.*, 2011, **17**, 4444–4453.
4. L. C. Dickinson and C. W. Chien, *J. Biol. Chem.*, 1973, **248**, 5005–5011.
5. T. Yonetani, Haruiko, and G. V. Woodrow, *J. Biol. Chem.*, 1974, **10**, 682–690.
6. L. C. Dickinson and J. C. Chien, *Biochemistry*, 1975, **14**, 3526–3534.
7. H. M. Marques, *Dalton Trans.*, 2007, 4371–4385.
8. K. M. Krause, M. T. Taschuk, K. D. Harris, D. A. Rider, N. G. Wakefield, J. C. Sit, J. M. Buriak, M. Thommes, and M. J. Brett, *Langmuir*, 2010, **26**, 4368–4376.
9. C. Renault, K. D. Harris, M. J. Brett, V. Balland, and B. Limoges, *Chem. Commun.*, 2011, **47**, 1863.
10. D. Schaming, C. Renault, R. T. Tucker, S. Lau-Truong, J. Aubard, M. J. Brett, V. Balland, and B. Limoges, *Langmuir*, 2012, **28**, 14065–14072.
11. C. Renault, C. P. Andrieux, R. T. Tucker, M. J. Brett, V. Balland, and B. Limoges, *J. Am. Chem. Soc.*, 2012, **134**, 6834–6845.
12. R. Weiss, A. Gold, and J. Turner, *Chem. Rev.*, 2006, **106**, 2550–2579.
13. T. G. Spiro and T. C. Streckas, *J. Am. Chem. Soc.*, 1974, **96**, 338–345.
14. F. Nistri, A. Lombardi, G. Morelli, C. Pedone, V. Pavone, G. Chottard, P. Battioni, and D. Mansuy, *J. Biol. Inorg. Chem.*, 1998, **3**, 671–681.
15. M. M. Maltempo, *Q. Rev. Biophys.*, 1976, **9**, 181–215.
16. A. Feis, B. D. Howes, C. Indiani, and G. Smulevich, *J. Raman Spectrosc.*, 1998, **29**, 933–938.
17. B. D. Howes, C. B. Schiodt, K. G. Welinder, M. P. Marzocchi, J. G. Ma, J. Zhang, J. A. Shelnutt, and G. Smulevich, *Biophys. J.*, 1999, **77**, 478–492.
18. B. D. Howes, N. C. Veitch, A. T. Smith, C. G. White, and G. Smulevich, *Biochem. J.*, 2001, **353**, 181–191.
19. K. M. Kadish, K. M. Smith, and R. Guillard, *The porphyrin handbook*, Academic Press, San Diego, 2000, vol. 6.
20. W. H. Woodruff, T. G. Spiro, and T. Yonetani, *Proc. Natl. Acad. Sci. U. S. A.*, 1974, **71**, 1065–1069.
21. W. H. Woodruff, D. H. Adams, T. G. Spiro, and T. Yonetani, *J. Am. Chem. Soc.*, 1975, **97**, 1695–1698.
22. D. Lexa and J. M. Saveant, *Accounts Chem. Res.*, 1983, **16**, 235–243.
23. A. Koppenhöfer, K. L. Turner, J. W. A. Allen, S. K. Chapman, and S. J. Ferguson, *Biochemistry*, 2000, **39**, 4243–4249.
24. M. T. de Groot and M. T. M. Koper, *Phys. Chem. Chem. Phys.*, 2008, **10**, 1023.