Supplementary Material (ESI) for Chemical Communications

Highly ordered transparent mesoporous TiO_2 thin films: an attractive matrix for efficient immobilization and spectroelectrochemical characterization of cytochrome c

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Experimental section

Semi-transparent (40% Transmittance) gold-coated microscope glass slides were purchased from Ssens (The Netherlands). All chemicals were purchased from Sigma-Aldrich, including equine heart cytochrome c, and used without further purification and all aqueous solutions were prepared with milli-Q water obtained from a Millipore purification system. Highly ordered mesoporous TiO_2 thin films were prepared as previously described.[1] Briefly, the films were deposited at room temperature by dip-coating glass, silicon wafer, or semi-transparent gold-coated glass substrates in a solution with a molar composition of 1 $TiCl_4$, 10 H_2O, 40 EtOH and 0.005 $HO(CH_2CH_2O)_{106}$-$(CH_2$-$CH(CH_3)O)_{70}(CH_2CH_2O)_{106}H$ (Pluronic F127). The relative humidity inside the dip-coater chamber was carefully maintained at 15 % until the interference fringes disappeared and then increased up to 70% for 5 min. Films were next aged for 48 hrs under a relative humidity of 75 % and then gradually heated in air at 350°C for 3 hours. The films were finally calcinated in air at 500°C (ramp 5°C min^{-1}) for 10 minutes. The mesoporous film thickness and pore size distribution were measured by ellipsometry and ellipsoporosimetry, respectively using a Woolam VASE M-2000U apparatus.[1, 2]

The substrates were cut in rectangular pieces of ~1 × 1 cm^2 or ~0.8 × 2.5 cm^2 for UV-visible experiments or UV-visible spectroelectrochemical measurements, respectively.

Cytochrome c solution were prepared in Hepes Buffer (10 mM, pH 7.0) and its concentration determined by UV-visible spectroscopy using ε_{409} = 106 $mmol^{-1}.L.cm^{-1}$. The hepes buffer was prefered to the classical phosphate buffer because of the high affinity of the phosphate ions for the TiO_2 surface. Ferrocytochrome c (10 μM solution) was prepared from ferricytochrome c (10 μM solution in 10 mM Hepes buffer, pH 7.0) by addition of an excess of sodium ascorbate (0.1 mM) under air.

UV-visible absorption spectra were monitored on a SPECORD S-600 diode-array spectrophotometer (AnalyticJena). A GG395 filter, *ie.* a high-pass glass color filter with a cut-off wavelength at 395 nm (Ocean Optics), was installed between the light sources and the sample to avoid

photoexcitation of the TiO_2 network during measurements. The optical transmittances of the modified substrates were analyzed at 409 nm and values of 90 % and 60 % were respectively obtained for the TiO_2-modified microscope glass slides and the TiO_2-modified semi-transparent gold-coated glass slides. Spectroelectrochemistry was performed in a home-made one-compartment three-electrode cell (Fig. 1). A FLEXREF Ag/AgCl/KCl 3M (World Precision Instruments) was used as the reference electrode and a platinum wire (1-mm diameter) was used as an auxiliary electrode. The three electrodes were inserted into a 2-mm 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 0.35 mL buffer, continuously purged with argon during the entire experiment and thermostated to 20°C. Electrolysis at controlled potential was carried out with a home-made potentiostat.

Figure

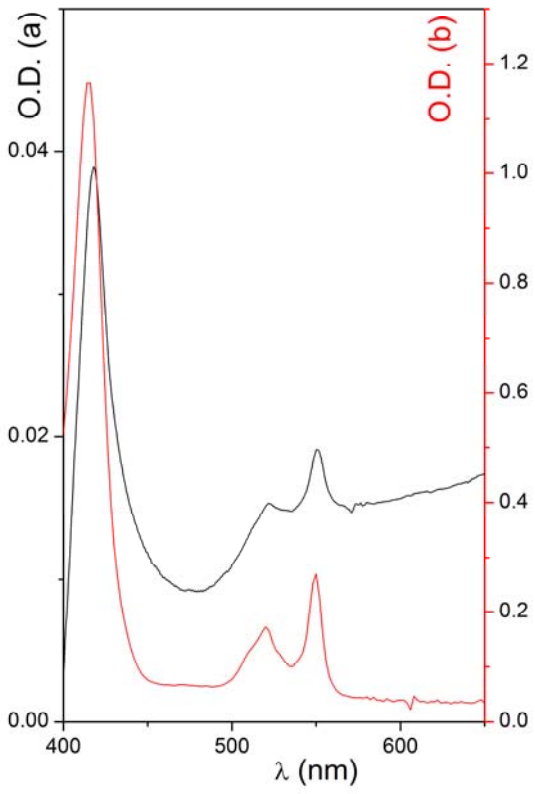

Fig. S1 (a) UV-visible spectrum of adsorbed Fe^{II}-cyt c within a mesoporous TiO_2 thin film (200-nm thick) on a gold-conductive electrode obtained under application of a -0.8 V redox potential (the blank spectra was subtracted). (b) UV-visible spectrum of ferrocytochrome c in homogeneous solution (9 μM in a quartz cell of 1-cm path length) prepared under air by addition of an excess of sodium ascorbate.

1. Y. Sakatani, D. Grosso, L. Nicole, C. Boissiere, G. J. d. A. A. Soler-Illia and C. Sanchez, *J. Mater. Chem.*, 2006, **16**, 77-82.
2. C. Boissiere, D. Grosso, S. Lepoutre, L. Nicole, A. B. Bruneau and C. Sanchez, *Langmuir*, 2005, **21**, 12362-12371.