Supporting materials

Efficient direct oxygen reduction by laccases attached and oriented on pyrene-functionalized polypyrrole/carbon nanotube electrodes

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Experimental

Pyrrole-NHS and 11-pyrrol-1-yl-undecyl ester (pyrrole-pyrene) were prepared as previously described [1]. 4-Pyren-1-yl-butyric acid, Tetrabutylammonium perchlorate (TBAP), sodium phosphate monobasic, sodium phosphate dibasic, 1-Methyl-2-pyrrolidinone (NMP), and Laccase (120 Umg^{-1}) from *Trametes versicolor* were purchased from Sigma-Aldrich. When not used the enzymes were stored at 4°C. Multi-Walled Carbon Nanotubes (MWCNT) (9.5 nm diameter, purity >95%), were obtained from Nanocyl. All the reagents were used without further purification.

NMP dispersions of carbon nanotubes were prepared by 30 min sonication of 5 mg nanotubes in 1 ml NMP until a homogenous black suspension was obtained. Then, 20 µL of the MWCNT solution was drop-casted on a GC electrode and NMP was removed under vacuum, leaving a thick MWCNT film on the GC electrode. Poly (pyrrole–pyrene) films were generated on GC electrodes by repeated potential scanning of pyrrole–pyrene (1 mM) from 0V to 0.7V in 0.1 M TBAP + CH_3CN for various scan number. After transfer of the resulting electrode into a monomer free solution of 0.1 M + CH_3CN TBAP for its characterization, all the modified electrodes were thoroughly washed with CH_3CN and deionized water. The functionalized electrodes were then incubated with 20 µL of a (5 mg/ml) laccase PB overnight at 4°C. The electrodes were finally dried and washed with PB.Electrochemical measurements of each electrode were carried out in a three-electrode electrochemical cell using an Autolab potentiostat 100. The working electrodes were glassy carbon electrodes (3 mm diameter). A Pt wire, placed in a separate compartment containing the supporting electrolyte, was used as a counter electrode. Potentials are reported to the Ag/10 mM $AgNO_3$ in CH_3CN reference electrode containing 0.1 M TBAP for organic solutions and to saturated calomel electrode (SCE) for aqueous solutions. Phosphate buffer (PB, pH 5, 25°C) was used as supporting electrolytes in aqueous media at room temperature.

Figures

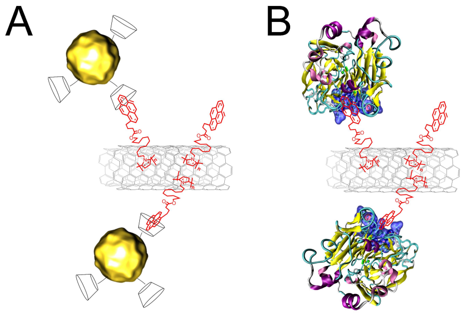

Figure S1. Sketch of the supramolecular interaction between pyrene and (A) the hydrophobic pocket of laccase and (B) a β-cyclodextrin modified gold nanoparticle (β-CD AuNP) used for SEM investigations.

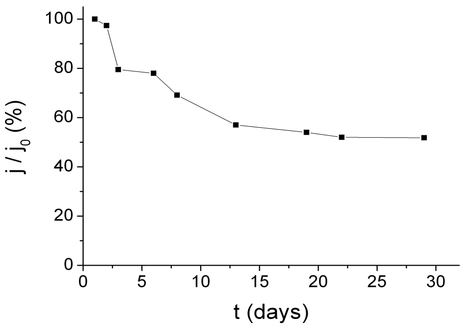

Figure S2. Stability of the MWCNT/Polypyrrole-pyrene/Lac catalytic response over one month. Catalytic current densities were measured by cyclodextrin-modified gold nanoparticles.

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[1] S. Cosnier and M. Holzinger, *Electrochim. Acta*, 2008, **53**, 3948–3954.

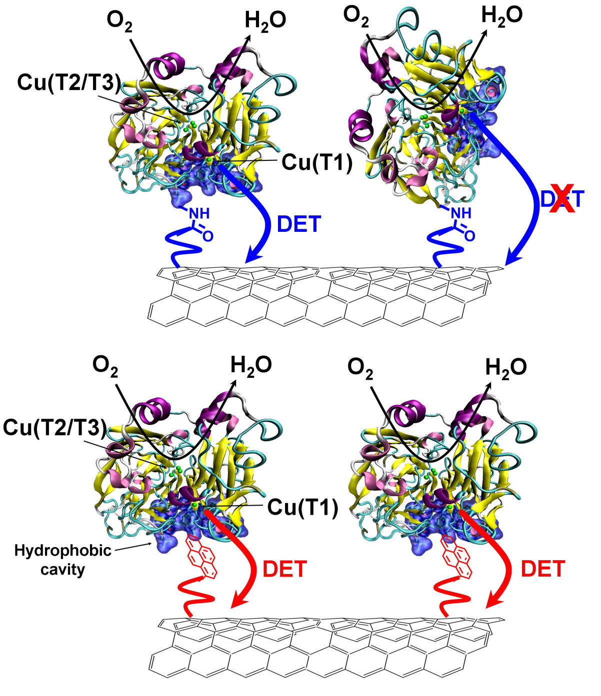

Figure S3. Schematic representation of the favourable orientation of laccases on MWCNT coatings functionalized by poly(pyrrole-pyrene) film compared to the chemical grafting onto poly(pyrrole-NHS) film.