

Supporting Information for:

Laccase-catalyzed functionalization of phenol-modified carbon nanotubes: from grafting of metallopolyphenols to enzyme self-immobilization

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1. Materials and Methods

Chemicals and general procedures.

Multiwalled carbon nanotubes (MWCNT; 10 nm diameter, purity > 99%) were obtained from Sigma-Aldrich and were used as received without any purification. All the reagents were purchased from Sigma-Aldrich and were used without further purification. All solvents were of analytical grade. 4-hydroxybenzenediazonium tetrafluoroborate¹ and 4-ferrocenylphenol³⁸ were prepared as previously described. Distilled water was passed through a Milli-Q water purification system. The electrochemical experiments were carried out in a three-electrode electrochemical cell using a Biologic VMP3 Multi Potentiostat. The MWCNT electrodes were used as working electrodes. Pt wire was used as counter electrode and the reference electrode was based on the Ag/AgClO₄ 10⁻² M reference electrode in MeCN and Ag/AgCl in water. All potentials are given versus Fc/Fc⁺ in MeCN and the standard hydrogen electrode in water ($E^{\circ}_{\text{Ag/AgCl (KCl sat)}} = +0.197 \text{ V vs NHE at } 25^{\circ}\text{C}$). All current densities are normalized towards the geometrical surface of the glassy carbon electrode (0.071 cm²). Experiments were repeated on at least three electrodes.

The X-band EPR spectra were recorded on an EMX plus spectrometer equipped with a high sensitivity cavity and a Bruker nitrogen flow cryostat. Spectra were simulated using the Easyspin software package² operating in Matlab. First, MWCNTs were chemically-modified with 4-hydroxybenzenediazonium tetrafluoroborate according to a previously-described procedure.^{3,4} 200 μL of 1 mg mL⁻¹ dispersion of phenol-modified MWCNTs were prepared in 100 mM Acetate buffer (pH 5.5), 100 mM DMPO and 10 % DMSO. Laccase (2 μL of a 150 μM stock solution) was injected in the EPR tube and subsequently frozen at 100 K.

XPS analysis was performed using a Thermoelectron ESCALAB 250 device (ICGM, France). The X-ray excitation was provided by a monochromatic Al-K α ($h\nu=1486.6 \text{ eV}$) source. The analyzed area was $\sim 0.15 \text{ mm}^2$. The background signal was removed using the Shirley⁵ method. The surface atomic concentrations were determined from photoelectron peaks areas using the atomic sensitivity factors reported by Scofield⁶. Binding energies (BE) of all core levels were referred to the C=C of C1s carbon at 284.4 eV. *Caution: Perchlorate salts present a potential explosion risk; use with caution!*

UV-visible spectroscopy of reactivity of laccase with 4-ferrocenylphenol (FcPhOH). For all spectroscopical analysis was used spectrophotometer Lambda 650 (Perkin Elmer). The quartz glass cuvette (Hellma® Micro Cuvette, QS Quartz) was prepared with: 20 μL of 1 mM FcPhOH in 100% DMSO and 960 μL of 0.1 M Acetate buffer, pH 5.5. An FcPhOH spectrum was first recorded, after baseline correction (0.1 M Acetate buffer, pH 5.5, 2 % DMSO). Then 20 μL of Laccase 150 μM in 50 mM Acetate buffer, pH 5 was injected into the cuvette, mixed and then a spectrum was recorded after a total time of 2 minutes and then each 5 minutes thereafter.

A single wave time evolution, $\lambda = 350 \text{ nm}$, of the reactivity of the laccase with FcPhOH was recorded by injection of 20 μL of Laccase 150 μM in 50 mM Acetate buffer, pH 5 into the cuvette filled with 980 μL of 0.1M Acetate buffer, pH 5.5, 20 μM FcPhOH.

MALDI-TOF MS experiments

The MALDI-TOF experiments were performed with a Bruker Daltonics Autoflex Speed mass spectrometer in reflectron positive mode. Dithranol was used as matrix. The matrix solution was prepared by dissolving dithranol in tetrahydrofuran (THF) (2 mg ml^{-1}). Samples were diluted in THF. 2 μl of the sample solution were mixed with 4 μl of matrix solution then 1 μl of the mixture solution was deposited onto the MALDI target plate and dried at room temperature. Calibration was performed externally by using a peptide calibration mixture (Peptide Calibration Standard II -Bruker Daltonics).

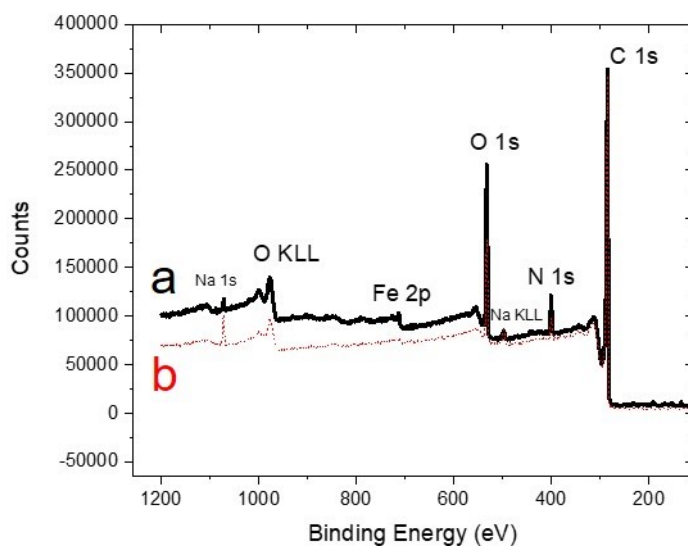
Preparation of MWCNT electrode modified with 4-hydroxybenzenediazonium tetrafluoroborate and laccase

MWCNT film were prepared by drop coating 20 μL of a 5 mg mL^{-1} dispersion of MWCNTs in 1-Methyl-2-pyrrolidinone (NMP) onto the glassy carbon (GC) electrode surface. The deposit was then allowed to dry under vacuum.

Electrografting of 4-hydroxybenzenediazonium on the surface of the MWCNT electrode was performed by cyclic voltammetry in MeCN with 0.1 M tetrabutylammonium hexafluorophosphate (TBAP) as the supporting electrolyte.

The modified MWCNT GC electrode were then incubated in 20 μL of Lac3 solution (70 μM in Acetate buffer 50 mM pH 5) for 6 h at 4°C.

Figure S1. XPS survey spectra the phenol-modified MWCNT electrodes modified with FcPhOH in the (a) presence and (b) absence of laccase.



References

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