Supporting informations

The Laccase mediator system at carbon nanotubes for anthracene oxidation and femtomolar electrochemical biosensing

Ilaria Sorrentino,[a] Marie Carrière, [a] Hélène Jamet,[a] Ilaria Stanzione,[b] Alessandra Piscitelli[b], Paola Giardina,[b]* Alan Le Goff [a]*

alan.le-goff@univ-grenoble-alpes.fr

giardina@unina.it

[a] Univ. Grenoble Alpes, CNRS, DCM, 38000 Grenoble, France

[b] Department of Chemical Sciences, University of Naples Federico II, 80126 Naples, Italy

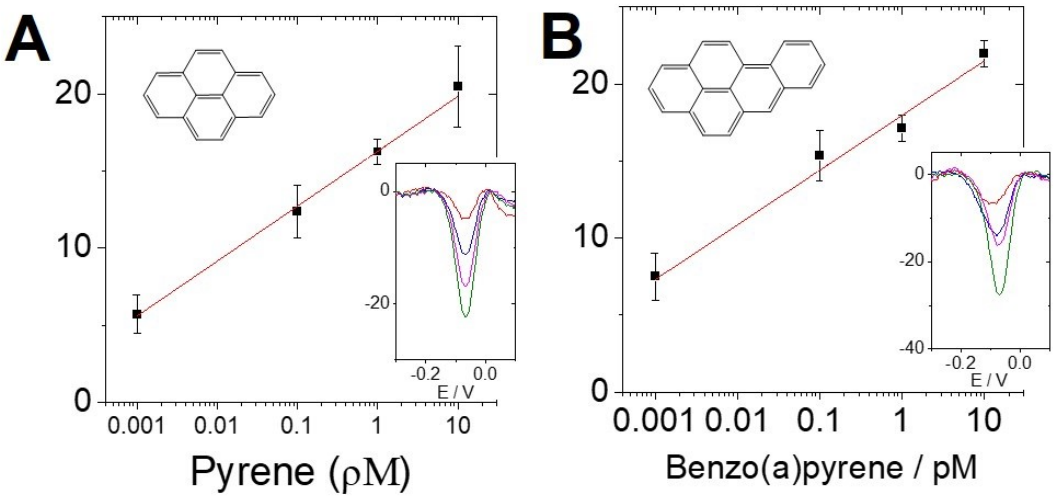

Figure S1. Linear part of the logarithmic plot of the SWV peak current towards (A) pyrene and (B) benzo(a)pyrene concentration at a MWCNT electrode soaked (10 min) in a solution of 1 U mL^{-1} of laccase and 20 mM ABTS left to react for 1 h at 25 °C min with the corresponding PAH. (0.1 M McIlvaine buffer pH 5, 25°C, pulse height = 25 mV, pulse width = 0.5 s, step height = -5 mV)

Methods

Reagents

All reagents were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA) and were used without further purification. All chemicals employed were of analytical grade. Distilled water was passed through a Milli-Q water purification system to obtain 18.2 MΩ cm−1 ultrapure water. Phosphate/citrate (McIlvaine) and Tris-HCl buffer solutions were prepared from Milli-Q water. The

PAH stock solutions (10mM) and their serial dilutions are prepared in acetone. The addition of PAHs, during calibration curves, is carried out in order to maintain the final concentration of acetone in the reaction solution constant.

Electrochemical Measurements

The electrochemical experiments were carried out in a three-electrode electrochemical cell using a Biologic VMP3 Multi Potentiostat. The saturated calomel electrode (SCE) served as the reference electrode, a Pt wire was used as the counter electrode and MWCNT bioelectrodes were used as working electrodes. All experiments were conducted at room temperature. All simulated curves were obtained via Origin Pro 9.0. Error bars were estimated from three measurements recorded per sample.

Preparation of the MWCNT-modified Glassy Carbon (GC) Electrode

The working electrodes were glassy carbon (GC) electrodes (3 mm diameter). 5 mg mL^{-1} 1-Methyl-2-pyrrolidinone (NMP) dispersions of MWCNTs (Multi-Walled Carbon Nanotube, purity > 99% Sigma-Aldrich) were prepared by 30 min in ultrasonic bath (Fisher scientific FB 15050) until homogeneous black suspension was obtained. Then 20 µL of the MWCNTs solution were drop-casted on a GCE and NMP was removed under vacuum obtaining a 5-µm-thick film.

Laccase Enzymes

POXA1b laccase was produced in *Pichia pastoris* as described in Pezzella et al., 2017[1]. Hydrophobin chimera-laccase was produced in *Pichia pastoris* as described in Sorrentino et al., 2019[2].

The laccase activity was assayed at room temperature, monitoring the oxidation of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) at 420 nm (ε_{420} nm = 3.6×10^4 M^{-1} cm^{-1}). The assay mixture was composed of 2 mM ABTS and 50 mM phosphate/citrate buffer, pH 3.0.

100 µL of pristine magnetic beads (MBs) (Absolute Mag™ Magnetic Particles, 3.0-3.9 µm, Creative Diagnostics) were washed once with 500 µL of water and three times with 500 µL of 50 mM Tris HCl pH 8. Then, MBs were incubated with 500 µL of laccase chimera (0.2 U mL^{-1}) on a rotary tube mixer overnight at 4°C. The functionalized MBs were precipitated by using a magnet and, after three washes with buffer, the immobilization yield was calculated as a difference between the incubated laccase units and those measured in the supernatant and in the washes.

PAH biosensing

Two different strategies have been set up:

- 40°C/ no ABTS, immobilized enzyme

The MWCNT-modified GC electrodes modified MWCNT electrodes were incubated in 20 µL of laccase (100 U mL^{-1}) for 2 h at room temperature. Electrodes were then rinsed with 50mM Tris-HCl buffer solution at pH = 8 and stored at 4°C. The modified bioelectrodes were immersed in a phosphate/citrate buffer solution (2 mL at pH= 5) at increasing concentrations of Anthracene (from 0 to 1 mM) and incubated at 40°C for 2h. Then, the detection of as-produced and adsorbed oxidized

products were measured by SWV in a phosphate/citrate buffer solution pH 5. SWV parameters were as follow: pulse height = 25 mV, pulse width = 0.5 s, step height = -5 mV.

- Laccase in solution

In the second developed assay to detect PAHs, a reaction solution was prepared with 2 U of laccase, 0.01 mM ABTS as a redox mediator in phosphate/citrate (2 mL at pH = 5) at increasing anthracene concentrations (from 0 to 1mM) and incubated at room temperature for 1 hour. MWCNT electrodes were immersed in the reaction solution for 10 minutes, and the detection of as-produced and adsorbed oxidized products were measured by SWV in a phosphate/citrate buffer solution pH 5. SWV parameters were as follow: pulse height = 25 mV, pulse width = 0.5 s, step height = -5 mV.

DFT calculations:

All calculations were performed using the Gaussian16 package[3] at the WB97XD/6-311G(d,p) level in gas phase. The MWCNT electrodes were modelized using a graphene ribbon of 18*12 $Å^2$. Benchmarks were done at the B3LYP/6-311G(d,p) level, but a perpendicular orientation of the anthracene was obtained on the graphene. Vibrational frequency calculations were performed to ensure that each geometry optimization converged to a real minimum. Orbitals were computed using the cubegen of Gaussian16 and their representations done using the software VMD.[4]

References

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