Supplementary Material (ESI) for Analyst
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# Structural characterization by confocal laser scanning microscopy and electrochemical study of multi-walled carbon nanotube tyrosinase matrix for phenol detection

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**Supporting information**

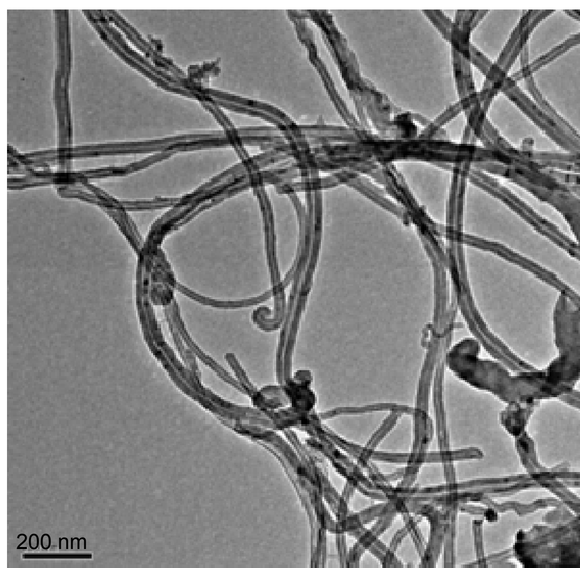


**Figure S1.** TEM image of the MWCNT purified and dispersed in THF (1mg of MWCNT/ 1ml THF).

- **Operational conditions in Confocal studies**

Roughness experiments were carried out using a Leica TCS SP2 AOBS confocal microscope (Leica Microsystems, Germany) equipped with a Plan Apo 20x (NA 0.4, dry). Stacks of 40 sections every 2µm were acquired along the z axis using the Leica Confocal Software. The profile roughness parameters were the average roughness (Pa) and the maximum profile peak height (Pp) and can be calculated according to DIN EN ISO 4287. The mean roughness, Pa, is the arithmetic average of the profile ordinates within the measured section (average height).

Leica TCS SP5 AOBS confocal laser scanning microscope (Leica Microsystems, Germany) was used to take 3D the images using a Plan Apo 63x (NA 1.4, oil HC x PL

APO lambda blue) objective. To determine the 3D structure, a series of horizontal (x-y) optical sections were collected at 0.5µ intervals along the material thickness. The projections of the series obtained were generated with Imaris v. 6.1.0. Software (Bitplane; Zürich, Switzerland). Profile analysis of fluorescence intensity was measured using Leica LAS AF software to determine the fluorescence intensity (FI) along the line segment.

To compare the fluorescence intensity, samples were observed under Olympus FluoView FV1000 microscope equited with a 60x/1.42 (oil PL APO) objective. The fluorescence intensity was measured at the same laser excitation and photomultiplier gain setting from SPE. Argon lasers were emitted at 488nm, at an intensity of 60%, a contrast of 1% and a sensibility of 800. To construct the fluorescent image, 20 sections separated by 0.5µm were taken. Images are presented as a *z*-projection of the 20 stacks acquired. Integrated fluorescene intensity of Anti-tyrosinase was quantified using the Metamorph software package (Universal Imaging Corporation Downington, PA). The data sets were exported into Microsoft Excel for analysis. The intensity of each pixel had a value ranging from 0 to 4095 level of gray.

- **Evaluation of possible autofluorescence of the materials**

Every layer that constitutes the electrodes studies (bare SPE and SPE modified with MWCNT) is studied in order to evaluate if they present auto fluorescence. Consequently, different materials were studied. First of all, support polystyrene sheet for both electrode configurations was evaluated for all the wavelengths, and specially for the range where our fluorophore (Alexa Fluor® 488) emits (the maximum adsorption is at 495nm and the maximum emission is on 519). In this case, polystyrene sheet only shows auto fluorescence in the blue spectral range. Therefore, polystyrene sheet doesn't represent any interference when the final experiments are held with Alexa Fluor® 488.

We did the control of the autofluorescence from the different components (Carbon, carbon nanotubes) including that of the tyrosinase without and with primary label alone and didn't found any fluorescence. Consequently the obtained signal, using the

secondary label (Alexa Fluor® 488), at the green spectra range, is only due to the presence of Tyrosinase marked with the primary and the secondary label.

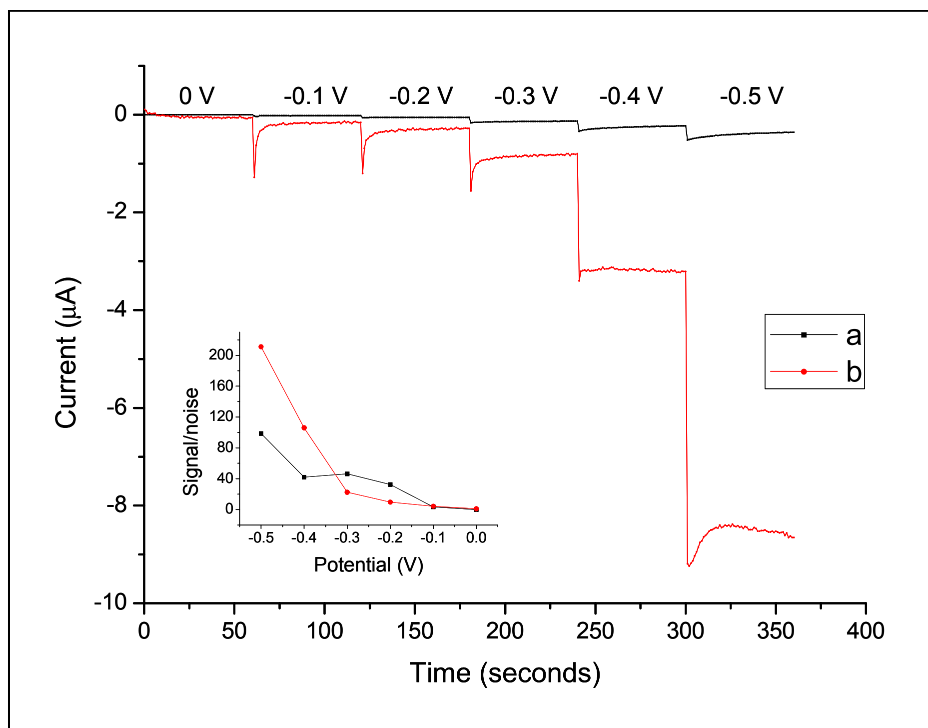


**Figure S2**. Hydrodynamic voltammograms for Tyr/SPE (a) and Tyr/MWCNT/SPE (b). Measurements are taken for a phenol concentration of 2.5 x 10-6 M into a 20 ml electrolytic cell containing 0.1M PBS (pH 6.5) under stirring conditions. **Inset**: corresponding graphic of signal/noise vs. potential for each formulation.

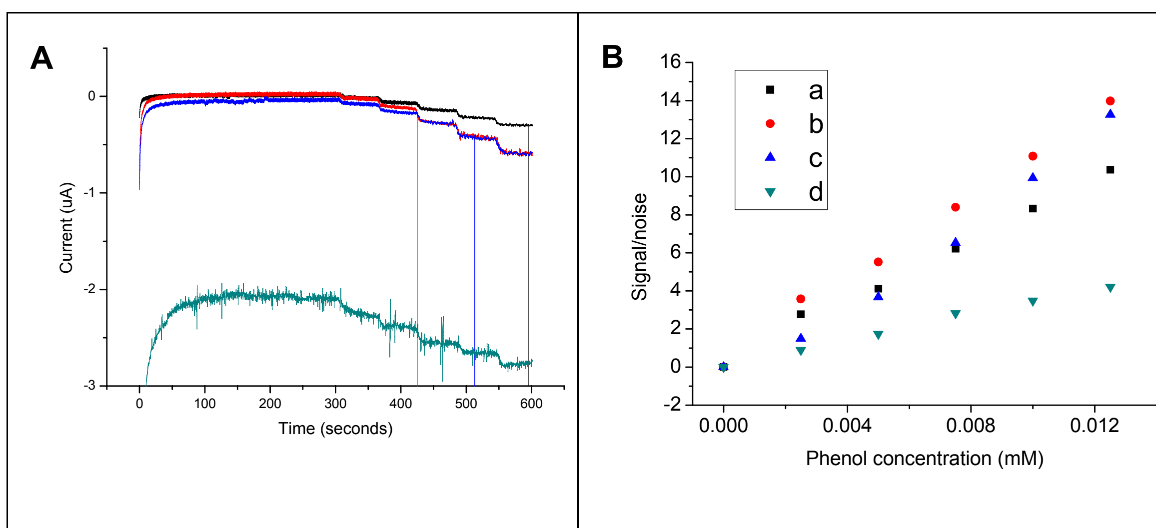


**Figure S3**. (**A**) Amperometric responses to a 2.5 x $10^{-6}$ M phenol solution successively added in 5µl (stock phenol solution: 0.01M) into a 20 ml electrolytic cell containing 0.1M PBS (pH 6.5) under stirring conditions. Tyr/MWCNT/SPE biosensors with tyrosinase concentration of (1mg tyrosinase/50µl buffer)

at different working potentials are evaluated: 0V (a), -0.1V (b), -0.2V (c) and -0.4V(d). (**B**) Corresponding calibration for the electrodes described in Figure 7**A**.

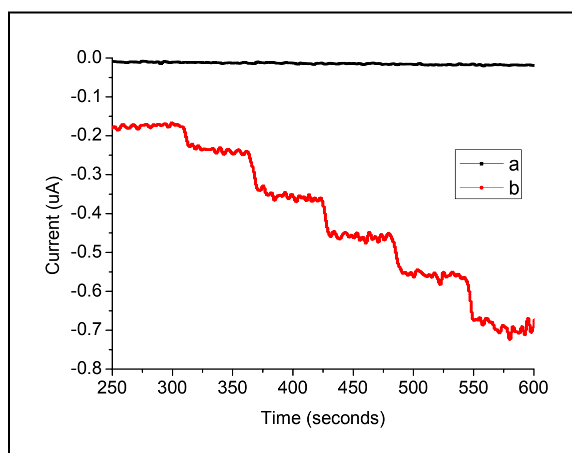


**Figure S4**. Amperometric responses for successive addition of 5µl of 2.5 x $10^{-6}$ M phenol solution into a 20 ml electrolytic cell containing 0.1M PBS (pH 6.5) under stirring conditions at a working potential of -0.2V for Tyr/SPE biosensor with tyrosinase concentration of 1mg tyrosinase/50µl buffer (a) and Tyr/MWCNT/SPE biosensor with tyrosinase concentration of 1mg tyrosinase/50µl buffer (b).

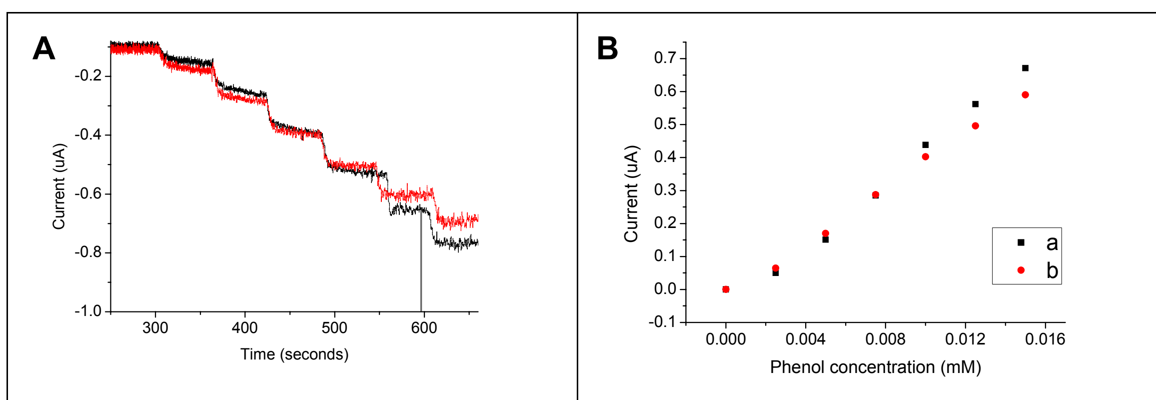


**Figure S5**. Shelf lifetime study. (**A**) Amperometric responses for successive additions of 25µl. of 5 x $10^{-6}$ M phenol solution successively added into a 20 ml 0.1M PBS (pH 6.5) under stirring conditions for a Tyr/MWCNT/SPE biosensor with tyrosinase concentration of 1mg tyrosinase/50µl buffer tested on the preparation day (24.11.08) (b) and after 68 days of being prepared (30.01.09). (**B**) Corresponding calibration plots