Supporting Information

Carbon Nanotubes as Activating Tyrosinase Supports for the Selective Synthesis of Catechols

Fabiana Subrizi,[†] Marcello Crucianelli,[*,†] Valentina Grossi,[†] Maurizio Passacantando,[†] Lorenzo Pesci,[‡] and Raffaele Saladino[*,‡]

[†] Department of Physical and Chemical Sciences, University of L'Aquila, Via Vetoio, I-67100 Coppito (AQ), Italy

[‡] Department of Ecology and Biology, University of Tuscia, Largo dell'Università, 01100 Viterbo (VT), Italy

Corresponding Authors

Dr. M. Crucianelli, e-mail: marcello.crucianelli@univaq.it; fax: (+39) 0862-433753

Prof. R. Saladino, e-mail: saladino@unitus.it; fax: (+39) 0761-357242

A linear relationship between absorbance *versus* concentration of oxidized carbon nanotubes (ox-MWCNTs)

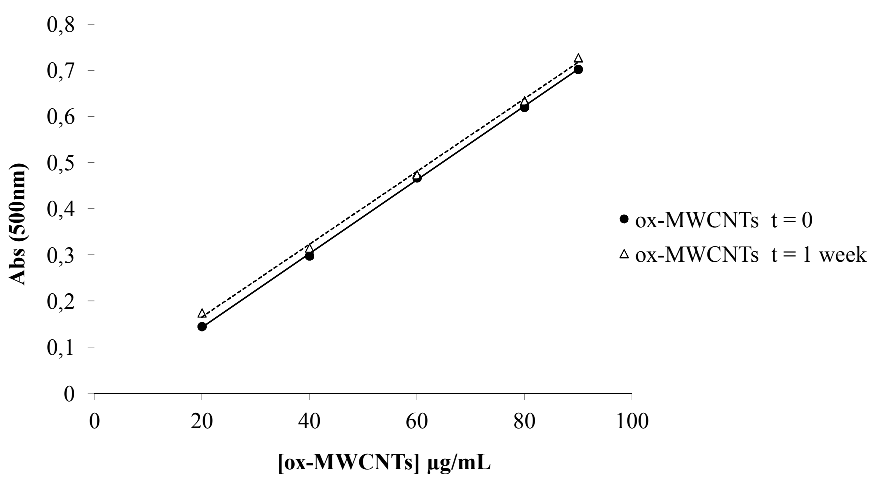

Figure S1. Absorbance of a solution of ox-MWCNTs as a function of its concentration: (•) freshly prepared (t = 0) and (∆) after a week of storage (t = 1 week).

Plot of activity (%) of catalysts I and IV *vs* run

The T_{50} value, defined as the run number at which the catalyst activity is reduced to 50% , was calculated by linear regressions of the percentage of activity *versus* run. Figures S2 and S3 report data for catalyst **I** and **IV** in buffer and organic medium. Tyro/E-LbL catalyst was included as reference.

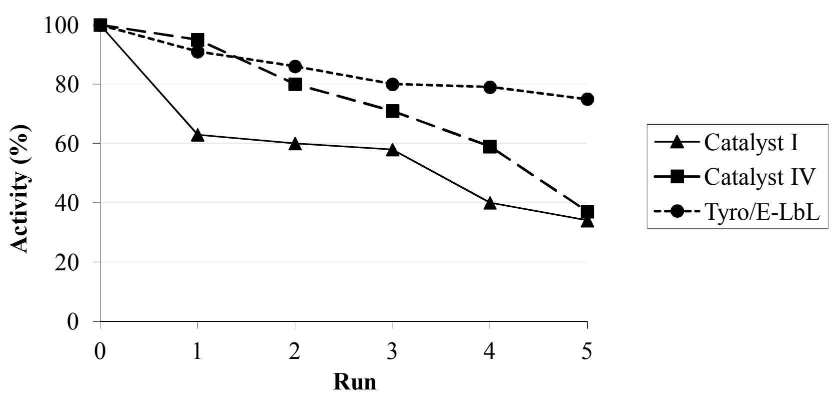

Figure S2. Activity (%) of catalyst **I** and **IV** *vs* run, in buffer.

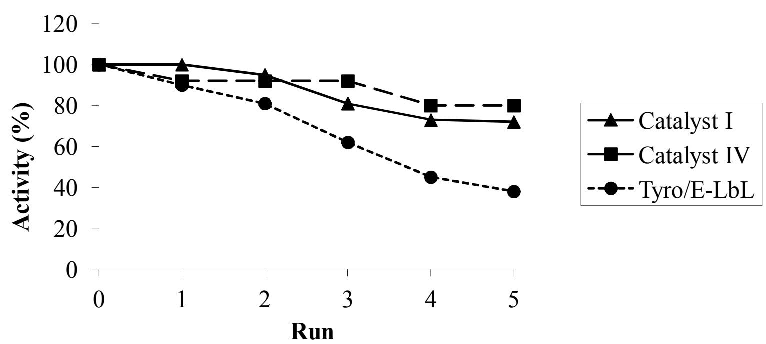

Figure S3. Activity (%) of catalyst **I** and **IV** *vs* run, in organic medium.

The T_{50} value for Tyro/E-LbL in buffer and in organic medium was calculated to be 10 and 4 respectively. These data suggest a better stability in water than in organic solvent. For the Catalyst **I** and **IV** an opposite behaviour was established.

Linear regression equations and plots of Lineweaver-Burk (Figure S4), Eadie-Hofstee (Figure S5) and Hanes (Figure S6)

$1/v = 1/V_{max} + (k_m/V_{max}) * 1/[\textit{para}\text{-cresol}]$

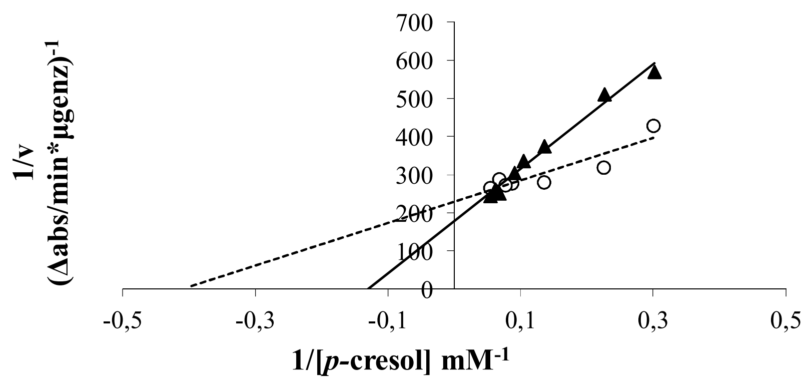

Figure S4. Lineweaver-Burk equation and plot for native tyrosinase (○) and catalyst **I** (▲)

$v = V_{max} - v\ (k_m/\ [\textit{para}\text{-cresol}])$

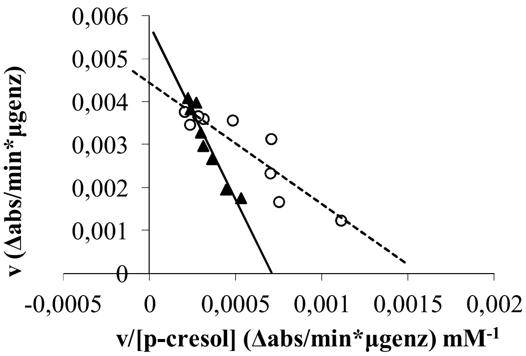

Figure S5. Eadie-Hofstee equation and plot for native tyrosinase (○) and catalyst **I** (▲)

$$[\textit{para}\text{-cresol}]/v = K_m/V_{max} + [\textit{para}\text{-cresol}]/V_{max}$$

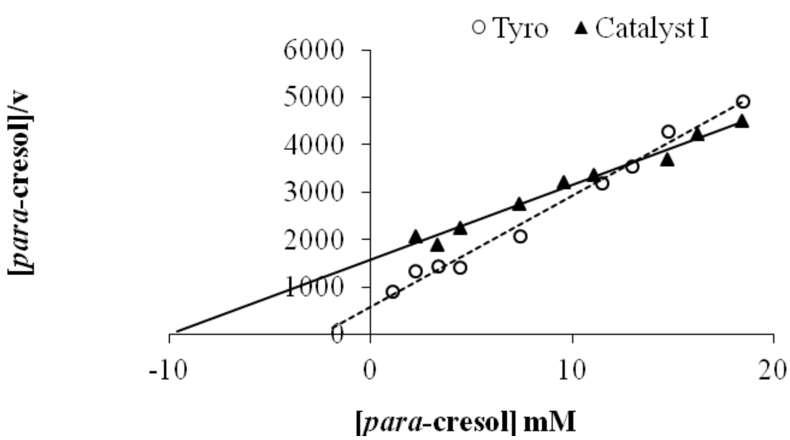

Figure S6. Hanes equation and plot for native tyrosinase (○) and catalyst **I** (▲). The initial velocity (v) was expressed as ΔAbs · min^{-1} · μg $enzyme^{-1}$

Non-linear regression plot

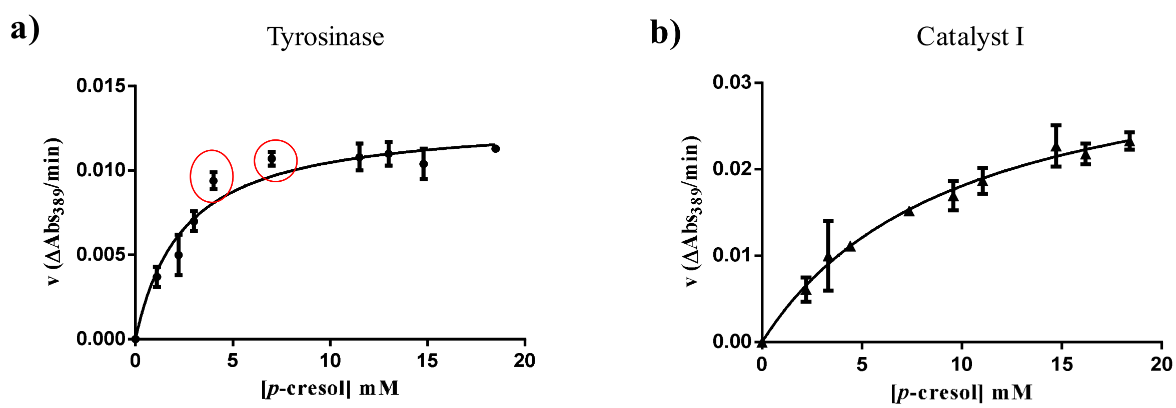

Figure S7. Non-linear regression for native tyrosinase (a) and catalyst **I** (b). Evidenced points were discarded from the regression curve.

Identification and characterization of oxidation products

All products were identified by ^{1}H NMR, ^{13}C NMR and GC-MS. ^{1}H NMR and ^{13}C NMR were recorded on a Varian Crioprobe 400 MHz spectrometer using $CDCl_3$ as solvent. All chemical shift are expressed in parts per million (δ scale).[a] GC-MS analysis were performed on a GCMS-QP5050 Shimadzu apparatus using a SPB column (25 m × 0.25 mm and 0.25 mm film thickness) and an isothermal temperature profile of 100°C for 2 min, followed by a 10 °C/min temperature gradient to 280°C for 25 min. The injector temperature was 280°C. Chromatography-grade helium was used as the carrier gas with a flow of 2.7 mL min^{-1}. Mass spectra were recorded with an electron beam of 70 eV. Quantitative analyses were performed using dodecane as internal standard.

4-Methylcatechol (4-Methyl-1,2-benzenediol) (1a): Oil. ^{1}H NMR[b] (400 MHz, $CDCl_3$) δ_H (ppm) 2.24 (3H, s, CH_3), 5.04 (1H, br s, OH), 5.18 (1H, br s, OH), 6.61- 6.76 (3H, m, Ph-H); ^{13}C NMR[b] (100 MHz, $CDCl_3$) δ_C (ppm) 20.8 (CH_3), 115.3 (CH), 116.2 (CH), 121.5 (CH), 131.1 (C), 141.0 (C), 143.3 (C); *MS, (m/z):* 268 (M^+), 253 (M-CH_3), 238 [M-$(CH_3)_2$], 223 [M-$(CH_3)_3$], 195 [M-$Si(CH_3)_3$], 179 [M-$OSi(CH_3)_3$], 164 [M-$OSi(CH_3)_4$], 149 [M-$OSi(CH_3)_5$], 134 [M-$OSi(CH_3)_6$], 106 [M-$OSi_2(CH_3)_6$], 90 [M-$O_2Si_2(CH_3)_6$].

Dimer (1b): Oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 2.19 (3H, s, CH_3), 2.42 (3H, s, CH_3), 6.51 (3H, br s, OH), 6.61-7.12 (5H, m, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 16.0 (CH_3), 22.1 (CH_3), 116.6 (CH), 118.2 (CH), 122.0 (C), 125.6 (C), 127.0 (CH), 127.2 (C), 131.2 (CH), 133.1 (CH), 136.7 (C), 141.3 (C), 150.1 (C), 158.2 (C); *MS, m/z:* 446 (M^+), 431 (M-CH_3), 329 [M-Si(CH_3)$_3$], 313 [M-OSi(CH_3)$_3$], 298 [M-OSi(CH_3)$_4$], 268 [M-OSi(CH_3)$_6$], 180 [M-O_2Si_2(CH_3)$_6$].

5,5'-Dimethyl-[1,1'-Biphenyl]-2,2',3,3'-tetrol (1c): Oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 2.41 (6H, s, 2xCH_3), 6.62-6.91 (4H, m, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 22.1 (2xCH_3), 117.2 (2xCH), 122.1 (2xC), 132.0 (2xCH), 138.1 (2xC), 141.3 (2xC), 150.3 (2xC); *MS, m/z:* 534 (M^+), 519 (M-CH_3), 417 [M-Si(CH_3)$_3$], 401 [M-OSi(CH_3)$_3$], 386 [M-OSi(CH_3)$_4$], 371 [M-OSi(CH_3)$_5$], 268 [M-O_2Si_2(CH_3)$_6$].

4-Ethylcatechol (4-ethyl-1,2-Benzenediol) (2a): Oil. ^{1}H NMR^{c} (400 MHz, $CDCl_3$) δ_H (ppm) 1.04 (3H, m, CH_3), 2.36 (2H, m, CH_2), 6.00-7.25 (3H, m, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 15.2 (CH_3), 28.1 (CH_2), 116.5 (CH), 117.4 (CH), 124.2 (CH), 139.3 (C), 145.7 (C), 148.4 (C); *MS, m/z:* 282 (M^+), 267 (M-CH_3), 252 [M-(CH_3)$_2$], 237 [M-(CH_3)$_3$], 209 [M-Si(CH_3)$_3$], 193 [M-OSi(CH_3)$_3$], 179 [M-OSi(CH_3)$_4$], 164 [M-OSi(CH_3)$_5$], 148 [M-OSi(CH_3)$_6$], 120 [M-OSi_2(CH_3)$_6$].

Dimer (2b): Oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.21 (3H, m, CH_3), 1.32 (3H, m, CH_3), 2.71 (2H, m, CH_2), 2.82 (2H, m, CH_2), 6.4-7.2 (5H, m, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 15.2 (CH_3), 15.7 (CH_3), 27.2 (CH_2), 28.3 (CH_2), 106.4 (C), 107.2 (C), 112.3 (CH), 114.1 (CH), 128.3 (C), 130.1 (CH), 134.1 (CH), 134.6 (C), 135.1 (C), 136.2 (CH), 143.1 (C), 155.2 (C); *MS, m/z:* 474 (M^+), 459 (M-CH_3), 429 (M-CH_3)$_3$, 341 [M-OSi(CH_3)$_3$], 326 [M-OSi(CH_3)$_4$], 311 [M-OSi(CH_3)$_5$].

4-*sec*-Butylcatechol (4-(1-methylpropyl)-1,2-Benzenediol) (3a): Oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 1.33 (9H, s, CH_3), 6.63-7.11 (3H, m, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 31.2 (3xCH_3), 34.5 (C), 116.5 (CH), 116.9 (CH), 122 (CH), 144.3 (C), 146.2 (C), 147.1 (C); *MS, m/z:* 310 (M^+), 295 (M-CH_3), 280 [M-(CH_3)$_2$], 237 [M-Si(CH_3)$_3$], 222 [M-OSi(CH_3)$_3$], 207 [M-OSi(CH_3)$_4$], 192 [M-OSi(CH_3)$_5$], 149 [M-OSi_2(CH_3)$_6$], 133 [M-O_2Si_2(CH_3)$_6$].

4-*tert*-Butylcatechol (4-tert-Butylbenzene-1,2-diol) (4a): Oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ_H (ppm) 1.10 (3H, m, CH_3), 1.22 (3H, m, CH_3), 1.53 (2H, m, CH_2), 3.23 (1H, m, CH), 6.52-6.84 (3H, m, Ph-H); ^{13}CNMR (100 MHz, $CDCl_3$) δ_C (ppm) 11.2 (CH_3), 22.3 (CH_3), 31.2 (CH_2), 43.1 (CH), 113.3 (CH), 114.1 (CH), 124.4 (CH), 136.2 (C), 145.1 (C), 147.0 (C); *MS, m/z:* 310 (M^+), 295 (M-

CH_3), 280 [M-$(CH_3)_2$], 265 [M-$(CH_3)_3$], 237 [M-$Si(CH_3)_3$], 222 [M-$OSi(CH_3)_3$], 207 [M-$OSi(CH_3)_4$], 192 [M-$OSi(CH_3)_5$], 176 [M-$OSi(CH_3)_6$], 148 [M-$OSi_2(CH_3)_6$].

References:

a) Guazzaroni, M.; Pasqualini, M.; Botta, G.; Saladino, R. *ChemCatChem* **2012**, *4*, 89.

b) Chernyak, N.; Dudnik, A. S.; Huang, C.; Gevorgyan, V. *J. Am. Chem. Soc.* **2010**, *132*, 8270.

c) Nakayama, S.; Ikeda, F. *US Patent* 5102906, 4985458, **1988**.