Electronic Supplementary Information for:

Multiple Functionalization of Single-Walled Carbon Nanotubes by Dip Coating**

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Chemicals

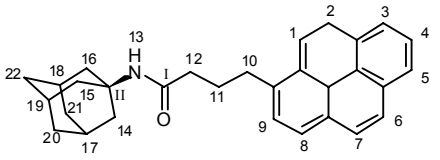
Avidin (A9275), FITC-labeled avidin, Na_2HPO_4 (S0878), NaH_2PO_4 (S0751) and glucose oxidase (GOX, G2133, 180.2 U mg^{-1}) were provided from Sigma. Glucose (24379) and $CuCl_2$ (23093) were purchased from Prolabo while EZ-link Sulfo-NHS-LC-biotin (21335) was acquired from Pierce. Single walled carbon nanotubes, produced by the HiPco® process (Purified, Unidym Grade/Lot # PO346), were purchased from Unidym Inc. and pretreated as described [1]. Such HiPco nanotubes have a diameter range between 0.8 and 1.2 nm and a length from 1 to 3 μm. The samples used for the experiments consist in large bundles of SWCNTs with diameters up to 100 nm and up to several tenth of micrometer length. Organic solutions were prepared with THF (Fischer scientific). Ultrapure water (18.2 Ω cm resistivity) was used for all aqueous solutions. All chemicals were of analytical grade and used as received. Solutions of glucose were allowed to mutarotate during one day and were kept refrigerated.

Synthesis of Pyrenebutyric acid adamantly amide

To a solution of 290 mg pyrene butyric acid (1 mmol) in 50 mL dry dichloromethane, 150 mg (1 mmol, 1 eq) of adamantylamine and 280 mg (1.2 mmol, 1.2 eq) of DCC was added. The reaction

mixture was stirred for 72 h at room temperature under reaction control by TLC (CH_2Cl_2, 100%). The produced solid was then filtered and the obtained yellow liquid was concentrated to obtain the pure product as slightly yellow powder after flash chromatography (SiO_2/CH_2Cl_2).

Yield: 320 mg (74%).



^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.31 (1H, d, **9**), 8.17 (1H, dd, **4**) 8.15 (1H, d, **1**) 8.11 (2H, 2d, **2, 7**) 8.02 (2H, 2d, **3, 8**) 8.00 (1H, d, **6**), 7.87 (1H,d, **5**), 5.02 (1H, s, **13**), 3.39 (2H, t, **10**), 2.05 (2H, q, **11**) 2.18 (2H, t, **12**), 2.16 (3H, m, **17-19**), 1.98 (6H, d, **14-16**), 1.66 (6H, t, **20-22**)

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 171 (1C, **I**), **Pyrene group (16C)**: 136.0 (1C), 131.4 (1C), 130.8 (1C), 129.8 (1C), 128.7 (1C), 127.4 (1C), 127.3 (1C), 126.6 (1C), 125.8 (1C), 125.1 (1C), 124.9 (1C), 124.9 (1C), 124.8 (1C), 124.7 (1C) 124.7 (1C), 123.4 (1C);

75.1 (1C, **II**), 51.8 (1C, **12**), 41.6 (3C, **14-16**) 36.3 (3C, **17-19**), 32.6 (1C, **10**), 29.4 (3C, 20-22), 27.5 (1C, **11**)

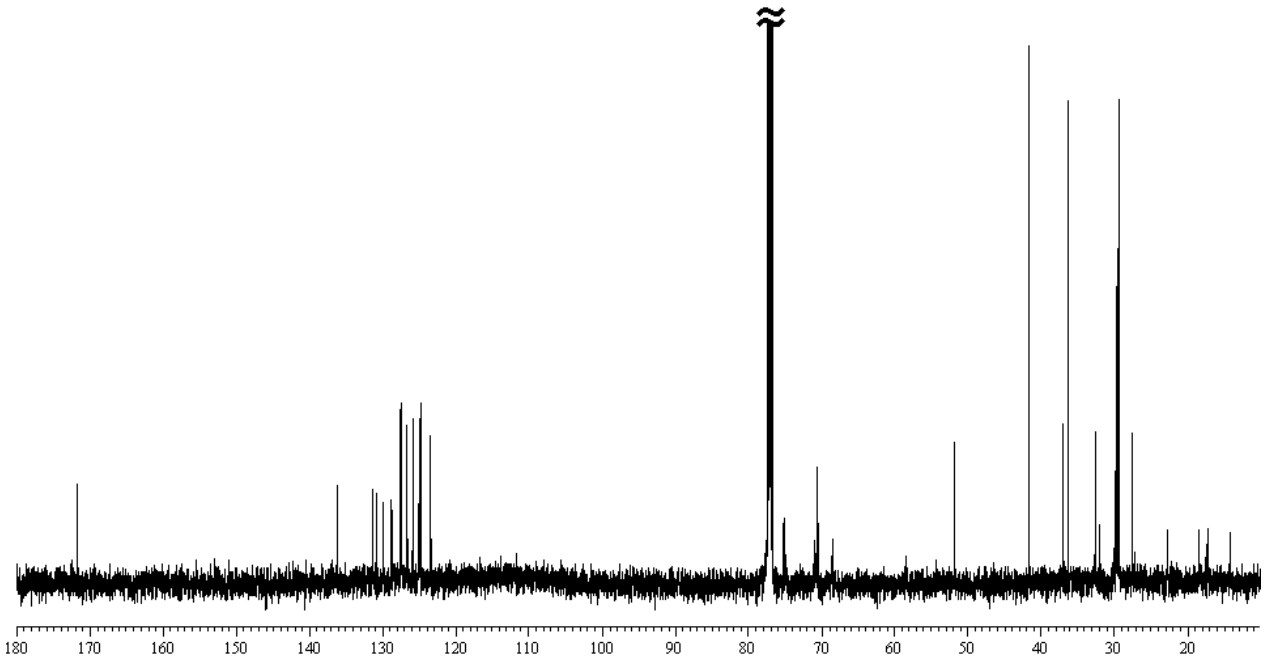


Figure 1 :^{13}C-NMR spectrum of pyrenebutyric acid adamantly amide

IR (KBr): ν (cm^{-1}) = 3411, 3291, 3035, 2904, 2848, 1641, 1542, 1453, 1359, 1181, 1095, 842, 752, 719, 679.

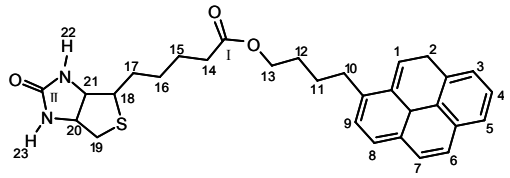
UV/Vis (THF): λ_{max} (nm) = 243, 256, 266, 276.5, 313.5, 327.5, 343.5.

MS (EI, 200 °C): m/z = 424 (MH^+, $C_{30}H_{33}NO$).

Synthesis of pyrene-butanol biotin ester

The synthesis of pyrene butanol biotin ester is already reported in literature[2] but will still be described here for completeness as some minor improvements have been made: pyrenebutanol (280 mg, 1.0 mmol) was dissolved in 20 mL DMF under argon atmosphere. The coupling reagents (DCC, 280 mg, 1.35 mmol; DMAP, 100 mg, 4.2 mmol) were added to this solution as well as biotin (0.250 g, 1.0 mmol). The reaction was observed by TLC (solvents: CH_2Cl_2 95% / EtOH 5%; retention 0.5). After one week reaction time, the solvent was evaporated and the yellow solid was dissolved in CH_2Cl_2. Biotin pyrene was purified by flash chromatography (mobile phase CH_2Cl_2 / EtOH 95:5) on silica gel 60 μm particle size as stationary phase.

Yield: 200 mg (0.4 mmol, 40%).



^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) = 8.23 (1H, d, **9**), 8.17 (1H, dd, **4**) 8.15 (1H, d, **1**) 8.11 (2H, 2d, **2, 7**) 8.02 (2H, 2d, **3, 8**) 8.00 (1H, d, **6**), 7.85 (1H,d, **5**), 5.52 (1H, s, **23**), 5.12 (1H, s, **22**), 4.25 (1H, t, **20**) 4.12 (2H, t, **13**), 4.05 (1H, d, **21**), 3.35 (2H, t, **10**), 2.92 (1H, d, **18**), 2.72 (2H, 2xdd, **19**), 2.27 (2H, t, **14**), 1.90-1.23 (10H, m, **11, 12, 15-17**).

^{13}C NMR (CDCl3, 300 MHz): δ = 173.7 (1C, **II**), 163.4 (1C, **I**), **Pyrene group (16C)**: 136.3 (1C), 131.4 (1C), 130.8 (1C), 129.8 (1C), 128.5 (1C), 127.4 (1C), 127.3 (1C), 127.2 (1C), 126.6 (1C), 125.8 (1C), 125.1 (1C), 125.0 (1C), 124.9 (1C), 124.8 (1C) 124.7 (1C), 123.2 (1C);

64.2 (1C, **21**), 61.7 (1C, **20**), 60.0 (1C, **18**), 55.2 (1C, **19**), 40.4 (1C, **13**), 33.9 (1C, **14**), 33.0 (1C, **15**), 28.6 (1C, **17**), 28.2 (1C, **10**), 28.1 (2C, **11**), 28.1 (2C, **12**), 24.7 (1C, **16**).

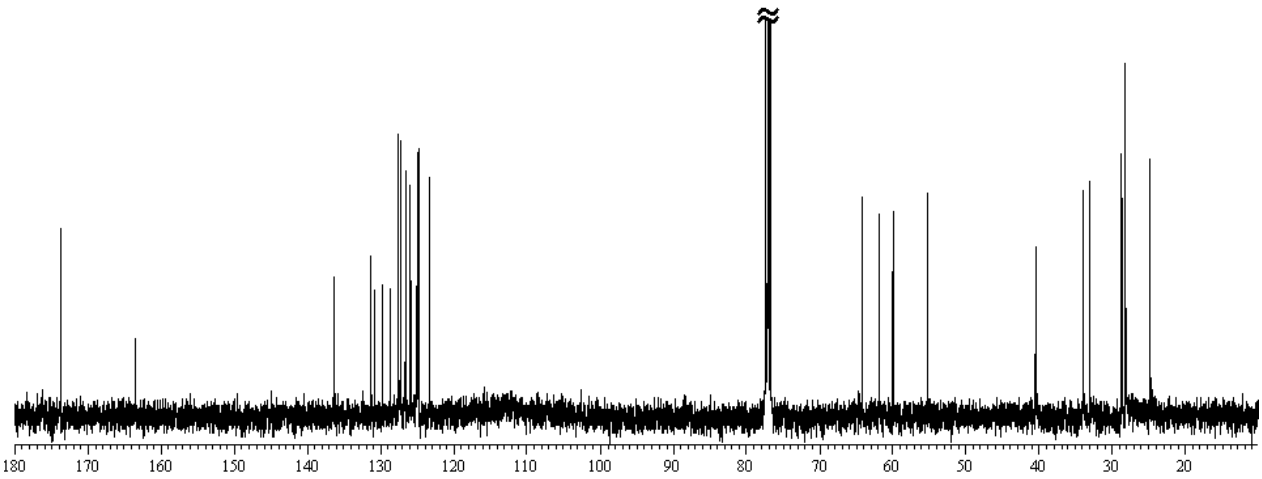


Figure 2: ^{13}C-NMR spectrum of pyrene-butanol biotin ester

IR (KBr): ν (cm^{-1}) = 3502, 3237, 3037, 2930, 2858, 1694, 1601, 1459, 1431, 1262, 1171, 842, 757, 707, 681.

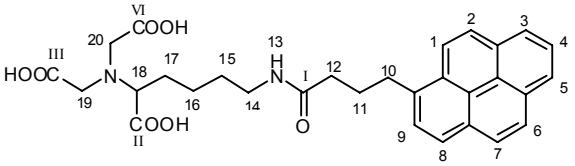
UV/Vis (THF): λ_{max} (nm) = 243, 256, 266, 276.5, 313.5, 327.0, 343.5.

MS (EI, 200 °C): m/z = 503 (MH^+, $C_{30}H_{34}N_2O_3S$).

Synthesis of Pyrenebutyric acid N_α',N_α-Bis(carboxymethyl)-L-lysine (NTA) amide

To a solution of 400 mg pyrene butyric acid succineimid ester (1.0 mmol) in 50 mL of dry DMF, 350 mg (1.25 mmol, 1.25 eq) of N_α',N_α-Bis(carboxymethyl)-L-lysine hydrate (NTA+1xH_2O ; M = 280 g mol^{-1}) was added. To dissolve the NTA, 50 mg (one pellet) of NaOH in 2 mL of water was added and one drop of triethylamine assures the basic environment in the organic phase. The mixture was stirred for 3 days at 60 °C. After removal of the volatiles, the residue was dissolved in water and the product was precipitated after titration with HCl (1 mol L^{-1}). The obtained slight yellow solid was filtered and washed with water.

Yield: 450 mg (0.83 mmol; 83%).



^{1}H NMR (MeOD-d_4, 250 MHz): δ (ppm) = 8.38 (1H, d, **9**), 8.17 (1H, dd, **4)** 8.15 (1H, d, **1**) 8.11 (2H, 2d, **2, 7**) 8.02 (2H, 2d, **3, 8**) 8.00 (1H, d, **6**), 7.93 (1H,d, **5**), 3.87 (2H, t, **14**), 3.63 (4H, q, **19+20**), 3.45 (1H, t, **18**), 3.39 (2H, t, **10**), 3.17 (2H, t, **12**), 2.17 (2H, t, **17**), 1.95-1.23 (6H, m, **15, 16, 11**).

^{13}C NMR (DMSO-d_6, 300 MHz): δ = 174.0 (1C, **II**), 173.2 (2C, **III+IV**), 171.6 (1C, **I**), **Pyrene group (16C)**: 136.6 (1C), 130.8 (1C), 130.4 (1C), 129.3 (1C), 128.1 (1C), 127.5 (1C), 127.4 (1C), 127.2 (1C), 126.5 (1C), 126.1 (1C), 124.9 (1C), 124.8 (1C), 124.7 (1C), 124.2 (1C) 124.1 (1C), 123.5 (1C); 64.3 (1C, **18**), 53.5 (2C, **19**+**20**), 38.3 (1C, **14**), 35.0 (1C, **12**), 32.2 (1C, **10**), 29.3 (1C, **11**), 28.9 (1C, **15**), 27.5 (1C, **17**), 23.1 (1C, **16**).

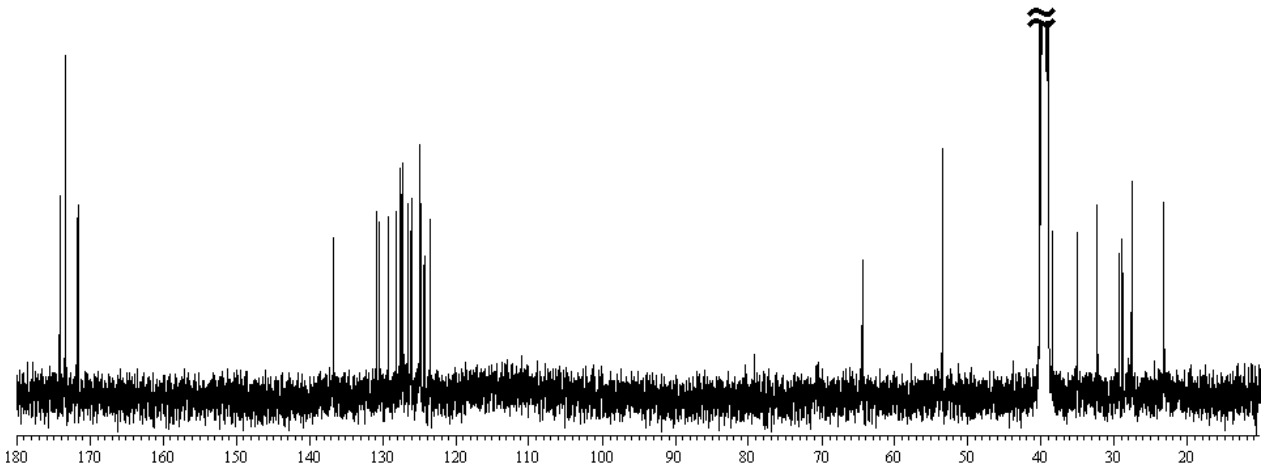


Figure 3: ^{13}C-NMR spectrum of pyrenebutyric acid N_α',N_α-Bis(carboxymethyl)-L-lysine (NTA) amide

IR (KBr): ν (cm^{-1}) = 3399, 3313, 3032, 2932, 2861, 1730, 1641, 1536, 1451, 1349, 1179, 841, 705.

UV/Vis (THF): λ_{max} (nm) = 243, 255.5, 266, 276.5, 313.5, 327.5, 343.5.

MS (EI, 200 °C): m/z = 534 (MH^+, $C_{30}H_{32}N_2O_7$).

Modification of glucose oxidase with affinity partners

Glucose oxidase (5.3 mg, 33 nmol) was biotinylated with sulfo-NHS-LC-biotin (67 μL 10^{-2} mol L^{-1}, 670 nmol) in 5 mL phosphate buffer (pH 6, 0.1 mol L^{-1}) during 2 h at 4 °C. Histidine-glucose oxidase amides were synthesized as follows: to 5 mL of a 0.1 mol L^{-1} phosphate buffer (pH 6), 6.07 mg (*S*)-2-Amino-3-(4-imidazolyl)propionic acid (L-histidine, 39.1 μmol) and 5.08 mg 1-ethyl-3-(3-dimethylamino-propyl)carbodiimid hydrochloride (EDC, 26.5 μmol) were dispersed in an ultrasound bath. To this solution, 5.148 mg glucose oxidase was added. The reaction mixture was vigorously stirred for 24 h at 4 °C. The same procedure was used for the synthesis of β-cyclodextrin-glucose oxidase amides by taking 14.91 mg mono-6-deoxy-6-amino-β-cyclodextrin (13.1 μmol) instead of the histidine compound. Modified glucose oxidases (B-GOX, β-CD-GOX and His-GOX) were purified by centrifugation (30000 MWCO concentrator with PES membrane at 6000 G) at 4 °C. The obtained modified-glucose oxidases in phosphate buffer solution were quantified using UV spectroscopy by measuring the 280 nm-band intensity (yield: 40%). The activities of all modified enzymes, determined using amperometry are shown in Table 1 in the manuscript. Stock solutions of modified-GOX (0.5 mg mL^{-1}) were prepared with phosphate buffer (pH 7, 0.1 mol L^{-1}) and stored at -20 °C as aliquots (25 μL) until needed.

Preparation of the samples for SEM imaging

For the investigations of the morphology using SEM platinum microelectrodes were used instead of the platinum disc electrodes due to their reduced size. As for the amperometric measurement, the nanotubes deposits were formed by drop casting and the enzymes were immobilized as described in the manuscript in the same order.

Apparatus

Amperometric measurements were realized with PRG-DEL potentiostat (Tacussel, France) connected to a computer with E-corder interface and controlled by the E-chart software (eDAQ, Australia).

The electrode system consisted of a conventional three-electrode cell. Platinum disk electrodes (ø = 5 mm) were used as working electrodes and polished with 2 µm diamond paste (MECAPREX Press PM). A saturated calomel electrode (SCE) was used in aqueous solutions and a Pt wire placed in a separated compartment containing the supporting electrolyte was used as a counter electrode.

FEG-SEM images were recorded using ULTRA 55 FESEM based on the GEMENI FESEM column with beam booster (Nanotechnology Systems Division, Carl Zeiss NTS GmbH, Germany) and tungsten gun.

Biosensor performance of the SWCNT deposits modified by one affinity system and cross experiments for each affinity system

In order to illustrate the selectivity of our concept of SWCNT modification via incubation with pyrene substituted by an affinity system, electro-enzymatic control experiments were carried out. SWCNT coatings functionalized by one type of affinity system were incubated with an enzyme (glucose oxidase) bearing the complementary affinity partner or unmodified enzyme or enzymes modified by non-complementary affinity partners. The Figures 4-6 represent the amperometric response of electrodes modified by deposition of SWCNT functionalized by an affinity system and immobilized enzymes, as a function of glucose concentration. The resulting calibration curves for glucose detection present an increase with increasing glucose concentrations and then reached a plateau corresponding to the maximum current. The latter depends mainly from the amount of immobilized enzyme and its activity. For each affinity system, the comparison of the maximum current values clearly indicates the specific anchoring of GOX by affinity interactions instead of non-specific adsorption. The percentage of non-specific binding (5-10%) was determined from the comparison of maximum current values recorded with the different enzymes.

Biotin/avidin/biotin affinity system:

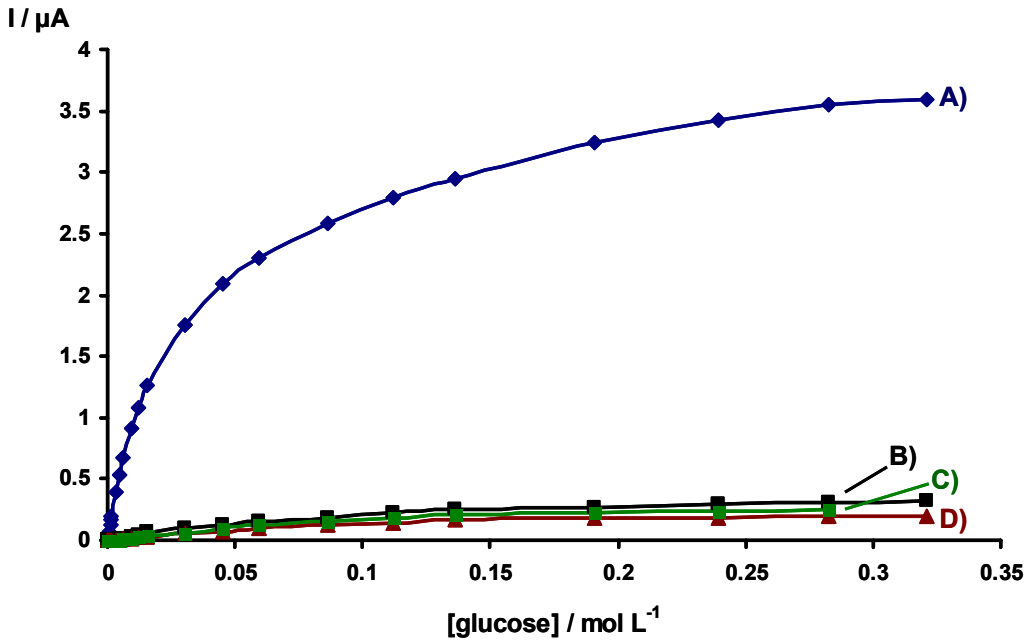


Figure 4: Calibration curves for the glucose responses after immobilization of A) B-GOX via avidin bridges on the pyrene-biotin functionalized nanotube deposit. The other curves represents cross experiments for nonspecific binding of B) β-CD-GOX and C) His-GOX on the pyrene-biotin functionalized nanotube deposit. D) cross experiments for nonspecific binding of unmodified GOX on a pyrenebutanol functionalized nanotube deposit.

NTA/Cu^{2+}/Histidine affinity system:

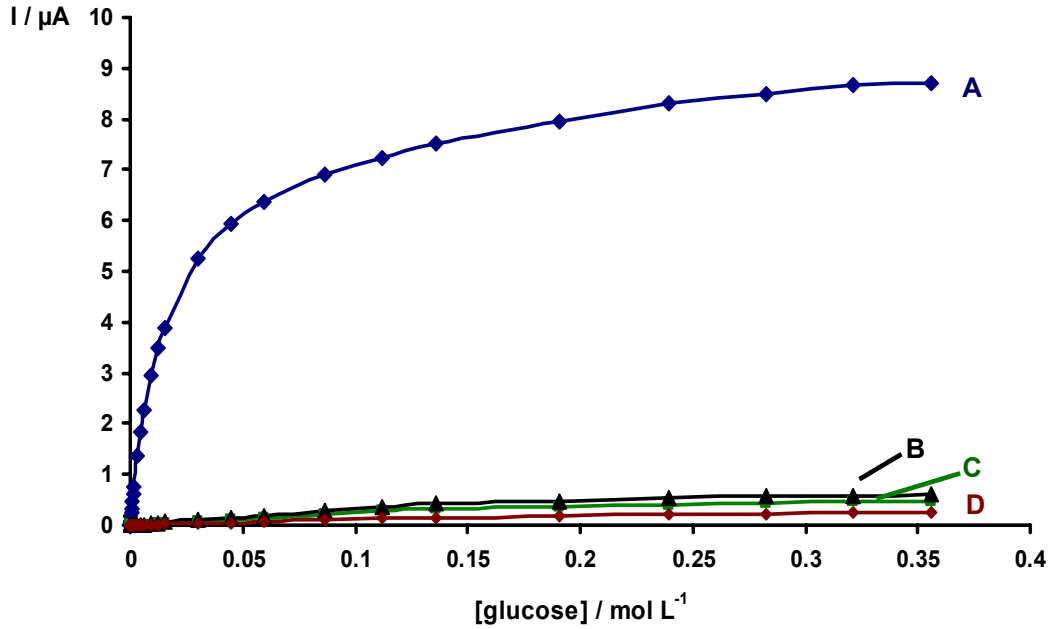


Figure 5: Calibration curves for the glucose responses after immobilization of A) His-GOX via coordination with the NTA- Cu2+ complex on the pyrene-NTA functionalized nanotube deposit. The other curves represents cross experiments for nonspecific binding of B) B-GOX and C) β-CD-GOX on the pyrene-NTA functionalized nanotube deposit (without coordinated Cu^{2+}). D) Cross experiments for nonspecific binding of unmodified GOX on a pyrene-butanol functionalized nanotube deposit.

Adamantane / β-cyclodextrin affinity system:

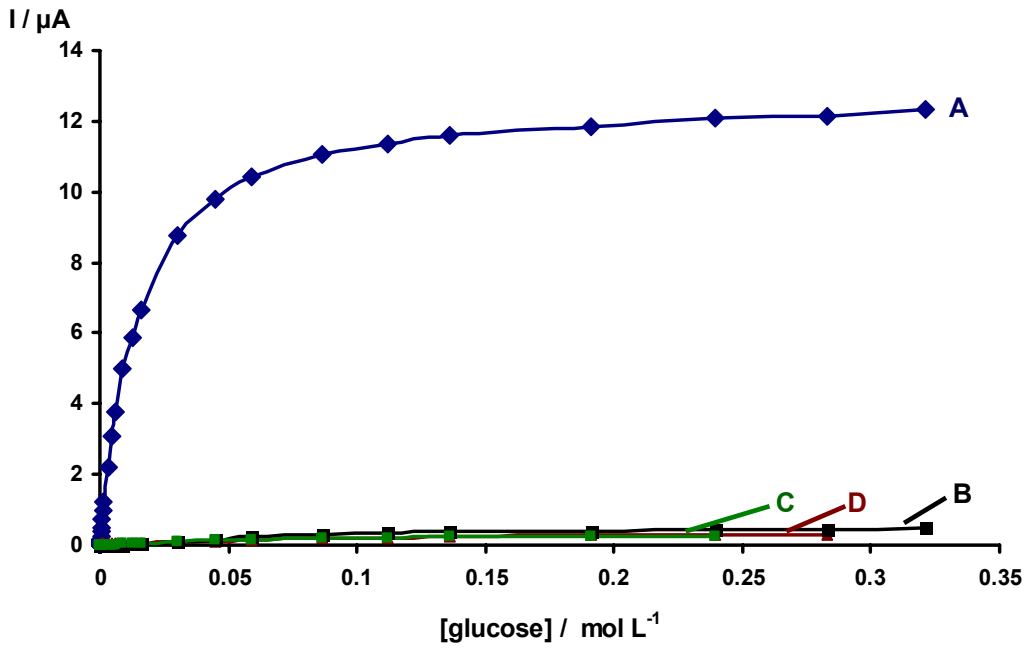


Figure 6: Calibration curves for the glucose responses after immobilization of A) β-CD-GOX via supramolecular assembly on the pyrene-adamantane functionalized nanotube deposit. The other curves represents cross experiments for nonspecific binding of B) B-GOX and C) His-GOX on the pyrene-adamantane functionalized nanotube deposit. D) cross experiments for nonspecific binding of unmodified GOX on a pyrene-butanol functionalized nanotube deposit.

Fluorescence imaging of the SWCNT deposits modified by a pyrene-biotin or a mixture of the three pyrene derivatives

With the aim to demonstrate qualitatively the specific immobilization of avidin by affinity interactions, fluorescence experiments were performed with a fluorescent avidin (FITC-labeled avidin). Nanotube deposits were incubated in different solutions containing only one affinity partner or a 1:1:1 mixture of all three affinity partners. After rinsing, a drop of FITC-labeled avidin (0.5 mg mL^{-1}) in phosphate buffer (pH 7, 0.1 mol L^{-1}) was put on these modified nanotube deposit, respectively. The fluorescence microscopy images were recorded after 30 min adsorption time followed by rinsing with phosphate buffer and distilled water.

Figure 7 A,B,D shows the fluorescence images of SWCNT coatings functionalized by one type of affinity system. A fluorescence phenomenon only appears onto the biotinylated SWCNT. This indicates that the fluorescent avidin was bound by affinity interactions to the biotinylated SWCNT deposit while no avidin adsorption occurred onto SWCNT coatings modified by incubation with pyrene-adamantane or NTA-pyrene.

In addition, the higher fluorescence intensity for a deposit SWCNT modified only by the pyrene-biotin (Figure 7D) compared to a mixture of three derivatives of pyrene (Figure 7C) seems to indicate the possibility to modulate the amount of immobilized B-GOX (Figure X A,D). Unfortunately, no quantification of fluorescence is possible with this setup.

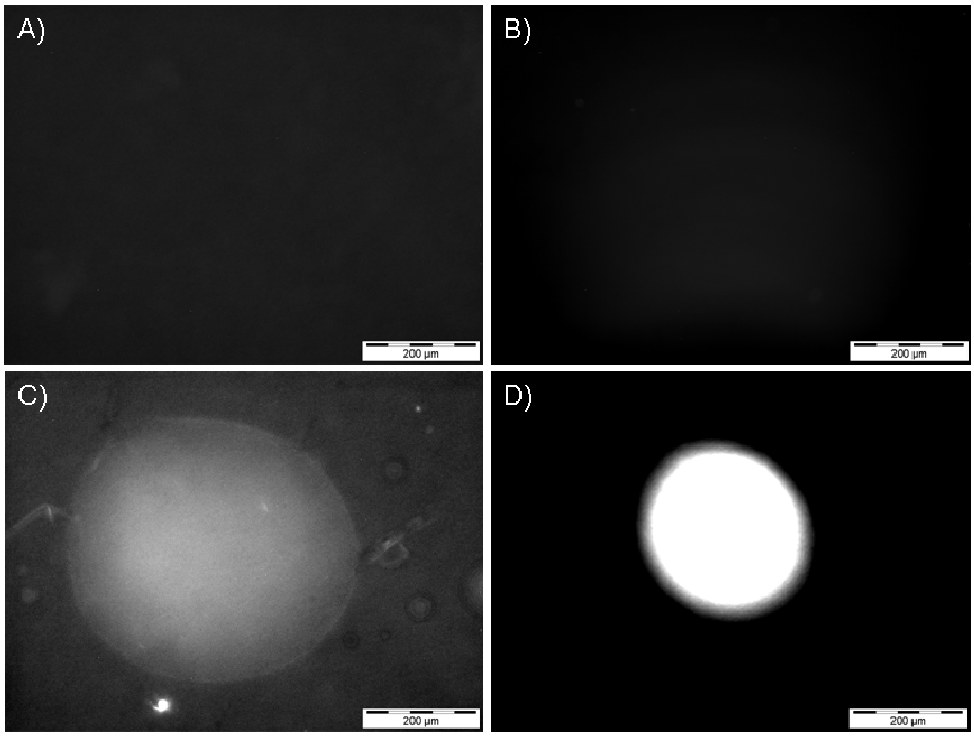


Figure 7: Fluorescence microscopy images of FITC-labeled avidin, deposited on SWCNT layers functionzalized with A) pyrene-adamantane, B) pyrene-NTA, C) pyrene-adamantane/pyrene-NTA/pyrene-biotin (ratio 1:1:1), and D) pyrene-biotin.

Reference

1. R. Haddad, S. Cosnier, A. Maaref and M. Holzinger, *Analyst*, 2009, **134**, 2412–2418.
2. M. Holzinger, R. Haddad, A. Maaref and S. Cosnier, *J. Nanosci. Nanotechnol.*, 2009, **9**, 6042-6046.