

**Electrochemical biosensing of non-electroactive targets using
ferrocene-labeled magnetic particles and CNT wiring.**

Electronic Supplementary Information (ESI)

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1. MATERIAL AND METHODS

1.1. Chemical reagents and biocomponents

Phosphate-buffered saline dry powder (PBS; 0.01 M phosphate, 0.138 M NaCl, 0.0027 M KCl, pH 7.4), ferrocene carboxylic acid (Fc-COOH; ref: 46264-1G-F), bovine serum albumin (BSA), Tween 20, potassium ferricyanide ($\text{Fe}(\text{CN})_6^{4-}$), N-hydroxysuccinimide (NHS), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), TRIZMA hydrochloride and dimethylformamide (DMF) were from Sigma Aldrich (Barcelona, Spain).

Carboxylated single-walled carbon nanotubes (SWCNT-COOH) >90.0% (C), from the same provider, had a diameter of 4-5 nm and a length of 0.5-1.5 μm .

Biotin-XX Goat Antirabbit IgG (H+L) at a concentration of 2 mg mL⁻¹ and streptavidin-modified magnetic particles (MP; Dynabeads T1 and Dynabeads M280, 1 and 2.8 μm Φ , respectively, 10 mg mL⁻¹), were supplied by Invitrogen (Life Technologies, UK). According to the provider's description, MPs consist of polystyrene spheres evenly embedded with iron oxide and covered with a polymer shell that prevents iron leakage.

1.2. Electrode fabrication and characteristics

The Pt electrodes were fabricated by sputtering over pyrex wafers at the clean room facility of IK4-Ikerlan.

For that, first, OiR positive resist was spin coated over the pyrex wafer and baked for 5 min at 90 °C in a hot plate. It followed patterning by photolithography using an acetate mask, designed with the CleWin 4 program, and a Karl Süss MA6/B6 (Süss MicroTek) mask aligner. Then, metal sputtering was performed using a Pfeiffer Vacuum Classic 500 sputter (Germany). To ensure the adhesion of the 125 nm Pt layer, a 20 nm Ti layer was first deposited on top of the pyrex wafer. Finally, the resist was lifted off. Each

wafer contained 16 independent sensors and each device included a working, pseudo-reference and counter electrode. The diameter of the working electrode was of 2 mm.

Previous to their utilization, a pressure sensitive adhesive (PSA) layer was fixed onto each device to delimitate the active/exposing area of the sensor and protect/insulate the metal paths. The electrochemical measurements were performed in 40 μL solutions, deposited onto the device active area.

1.3. Preparation of SWCNT-COOH suspensions

For the preparation of SWCNT suspensions, 10 mg of SWCNT-COOH were diluted in 10 mL of deionized water and were sonicated in an S15H Elmasonic sonicator working at a frequency of 37 kHz for 1 h and 30 min, until a stable suspension was obtained. In order to prevent heating, water in the equipment tank was refreshed every 15 min. It was observed that the sonication time required for production of SWCNT suspensions of optimal electrochemical behavior depended on the sonicator operation frequency. Hence, after the first 60 min of treatment, the electrochemical behavior of the SWCNT was assessed every 5 min by producing CNT-electrodes as it will be described later in the text.

1.4. Magnetic particle modification with Fc-COOH

NHS, EDC and Fc-COOH were diluted in DMF to final concentrations of 200 mM, 100 mM and 100 mM respectively, and the mixture was incubated for 45 min at room temperature and protected from light. The EDC/NHS-activated Fc-COOH was then diluted to a final concentration of 16 mM by drop-wise addition to PBS while vortexing. In parallel, 150 μL of streptavidin coated MP (10 mg mL^{-1}) were washed three times with 300 μL of PBS using a magnetic rack (Bilatest magnetic separator, Sigma-Aldrich).

Then, the particles were resuspended in 150 μL of PBS containing the chosen concentration of EDC/NHS-activated Fc-COOH and were incubated for 2 hours and 30 min under continuous rotation protected from light. Next, the ferrocene functionalized MPs (MP-Fc) were washed twice and were blocked with 50 mM Tris for 15 min. Finally, the MP-Fc were washed three more times with PBS, were resuspended to a final concentration of 5 mg mL^{-1} in PBS containing BSA 0.05% and were stored at 4°C until used.

1.5. SWCNT magnetic entrapment

MP-Fc, 5 μL , were washed three times in PBS and were resuspended in 10 μL of PBS. Then, 4 μL of a three-times concentrated PBS solution (3X PBS) and 5 μL of SWCNT-COOH (1 mg mL^{-1}) were added and the blend was mixed by pipetting for 20 s. Magnetic entrapment of MP/SWCNT complexes was promoted by placing a neodymium magnet (2 mm Φ ; Supermagnete, Gottmadingen, Germany) under the working electrode of the sensor.

1.6. Electrochemical measurements

Electrochemical measurements were carried out using an Autolab PGSTAT 128N (Eco-Chemie, The Netherlands). Before use, Pt electrodes were activated chronoamperometrically by applying three consecutive series of pulses, alternating between -1.4 V, 0 V and 1.4 V during 5 s each. It followed sensor characterization by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in ferricyanide. CV was performed from -0.2 V to 0.35 V, at a scan rate of 50 mV s^{-1} and a step potential of 5 mV. EIS measurements were performed at 10 mV input amplitude, between 0.1 Hz and 100 kHz.

After sensor modification with MP/SWCNT complexes, differential pulse voltammetry (DPV) was performed in PBS 0.01 M between -0.05 V and +0.5 V (modulation amplitude of 50 mV and step potential of 2 mV). To further characterize the modified electrode, EIS was performed in ferricyanide.

1.7. Data analysis

The assay lower limit of detection (LOD) was calculated as the average of at least three blank measurements (i.e.: the highest signal that could be registered) minus three times their standard deviation. The assay variability was calculated in terms of variation coefficient ($\%CV = (SD/mean) \times 100$). Each experiment was performed at least three times.

2. RESULTS

Figure S1. Scanning Electron Microscopy (SEM) images of streptavidin-coated M280 MP (a & c) and T1 MP (b & d) before (top) of after (bottom) SWCNT wiring. Upon binding onto the surface of MP, SWCNT form a web that physically wraps the MP, connecting the beads between them and with the electrode surface.

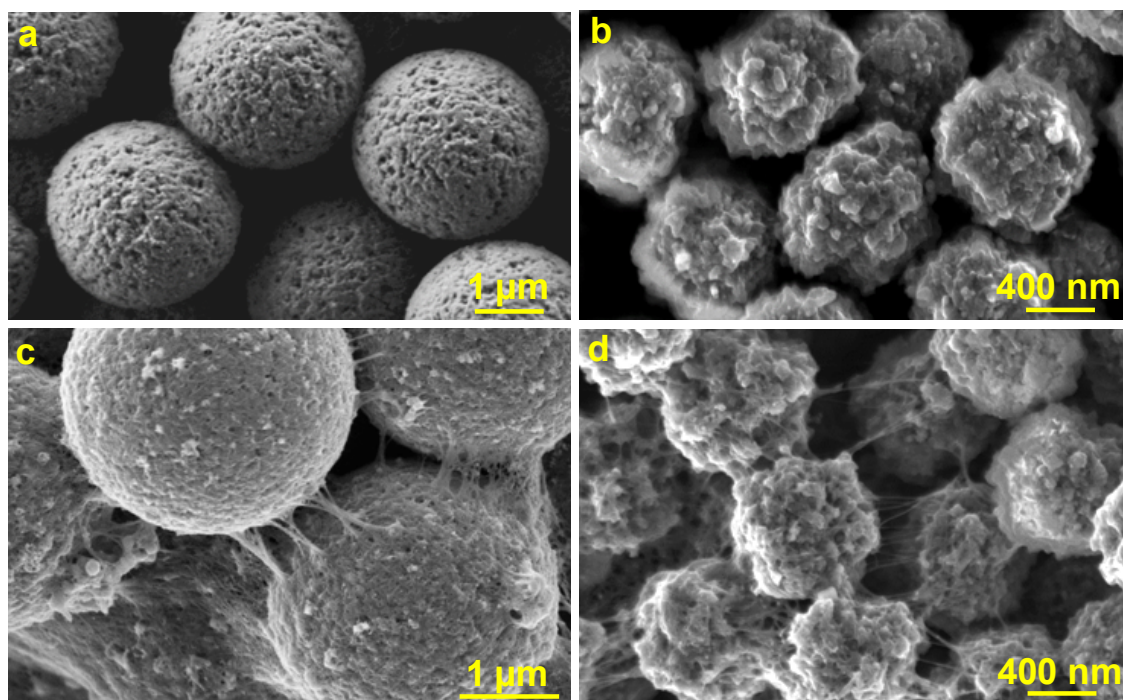


Figure S2. Example of a DPV measurement performed using 10 μL of T1 MP-Fc₁₆ either directly deposited onto the electrode surface (grey line) or detected via SWCNT wiring (black line).

While the DPV registered for MP-Fc₁₆ showed a peak of 0.83 μA , the DPV registered for MP-Fc₁₆/SWCNT showed a peak of 21.68 μA . Hence, the peak registered via SWCNT wiring was 26 times higher than that provided by straightforward DPV detection of the MP-Fc, where only those segments of the MP-Fc placed in close proximity of the electrode surface are efficiently detected.

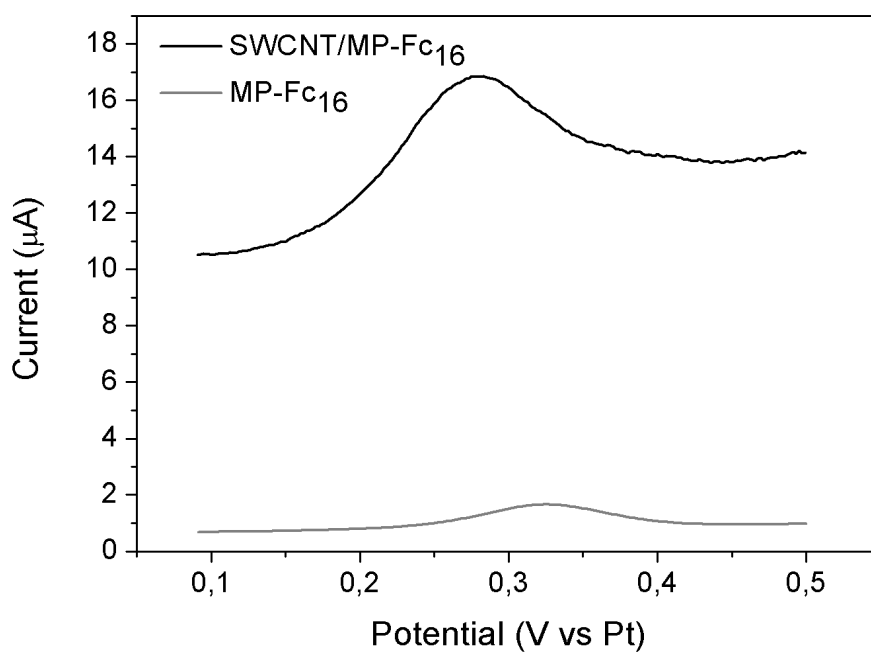


Figure S3. Characterization of PM/SWCNT electrodes by electrochemical impedance spectroscopy (EIS).

The value of the solution resistance (R_s) was extrapolated from the intersection of the leftmost end of the semicircle with the X axis in the Nyquist plot and the charge transfer resistance (R_{ct}) was calculated by fitting the semicircle diameter. Since R_s depends basically on the ionic concentration and the type of ions present in solution, which were kept constant during the measurements, R_s had a value around $100\ \Omega$ in all the cases.

The R_{ct} parameter is inversely proportional to the heterogeneous charge transfer rate and affects the sensitivity and response time of the electrode. Therefore, the lower the R_{ct} value the faster the electron transfer.

