Electronic Supplementary Information

Cyclodextrin-based superparamagnetic host vesicles as ultra-sensitive nanobiocarriers for electrosensing

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Experimental Procedures

Chemicals and reagents

All commercially available chemicals were used as received and were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Synthesis of oleic acid capped iron oxide nanoparticles

Oleic acid coated magnetic nanoparticles were synthesized by co-precipitation of ferric and ferrous salts in alkaline medium as described in the literature.[1] To this end, 2 g of $FeSO_4 \cdot 7H_2O$ and 3.5 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 100 mL Milli-Q water. Next, 25 mL of an aqueous NH_3 solution (25%, w/w) was added to the mixture and the solution was kept stirring at RT. Once the solution turned black, 1 mL of oleic acid was added dropwise and aged at 80 ºC under Ar atmosphere. Finally, the product was well dispersed into water and transferred into a toluene phase.

DLS (diameter number mean): 37.0 ± 0.7 nm

mCDV preparation

mCDVs were synthesized following the method presented by Ravoo and co-workers.[2] 0.1 mg of oleic acid capped iron oxide nanoparticles and 6.5 mg of amphiphilic β–CD were dissolved in 100 µL of $CHCl_3$. The solvent was slowly evaporated from the solution in Ar stream while continually rotating the flask. The residual solvent was removed from the film in strong vacuum. Then, the film was hydrated by adding 2 mL of PBS (pH 7.2) and vigorous stirring overnight. Then, the solution was sonicated for 10 s and extruded through a 100 nm pore size polycarbonate membrane in a Liposofast manual extruder. mCDVs were stored in the fridge after use. A fresh batch of mCDVs was synthesized every week.

DLS (diameter number mean): 100 ± 3 nm

mNC–CPE fabrication

mNC–CPEs were fabricated by following the methodology employed by Muñoz and co-workers.[3] Graphite powder was hand-mixing dispersed within an insulating epoxy in a 1:4 (w/w) ratio. Then, a PVC tube (i.d.: 6 mm, length: 20 mm) containing a copper disk as electrical contact (body electrode) was tightly packed, where a small cylindrical neodymium magnet (3 mm diameter, N35) was placed into the center of this electrode. Finally, the electrode was cured at 80 ºC for 24 h and polished with different sandpapers of decreasing grain size. After each electrochemical measurement, the electrode surface was reset by a simple polishing step.

Apparatus and procedures

Dynamic light scattering (DLS): DLS measurements were recorded using a Nano-ZS Zetasizer (Malvern Instruments, Malvern, UK). While mCDVs were measured in PBS (pH 7.2), oleic acid coated magnetic nanoparticles were measured in ethanol. Samples were measured in disposable 1 mL semi-micro PMMA cuvettes (Brand GmbH & Co. KG, Wertheim, Germany). Data analysis was realized with Malvern Zetasizer software 7.12 (Malvern Instruments, Malvern, UK).

Transmission electron microscopy (TEM): Samples were deposited on a covered holey copper grid, by applying a drop of either mCDVs or oleic acid coated magnetic nanoparticles (blank). TEM images were acquired by using a Zeiss 200 FE electron microscope with schottky emmiter and energy Ω filter operating at 200 kV.

Electrochemical measurements: Electrochemical measurements were performed by Linear Sweep Voltammetry (LSV) and Electrochemical Impedance Spectroscopy (EIS) means employing an AutoLab Metrohm PGSTAT128N potentiostat/galvanostat equipped with NOVA 2.1.2 software, coupled with a conventional three-electrode configuration cell. The electrode configuration consists of: mNC–CPE as the working electrode, an Ag/AgCl wire as the reference electrode, and a Pt wire as the counter electrode. A 0.1 M KCl solution

containing 10 mM $[Fe(CN)_6]^{3-/4-}$ was utilized as the redox marker for EIS characterization. A phosphate-buffered saline (PBS) solution at pH 7.2 was the electrolyte employed for DLS electroanalysis.

Electroanalytical assay: The developed methodology is similar to a conventional antigen-antibody immunoassay, following the three sequential steps: *1)* 25 µL of the synthesized mCDVs were incorporated into a vial containing 1.0 mL of a desired concentration of T_4. The vial was aged for 5 min to promote the supramolecular β–CD-T_4 interactions. *2)* The loaded-mCDVs were captured by dipping the mNC–CPE into the vial and washed twice with a PBS solution (pH 7.2). *3)* The mNC–CPE containing the loaded-mCDVs was transferred into the three-electrode configuration cell filled with the electrolyte (PBS at pH 7.2) for LSV analysis. After each measurement, mCDVs were removed from the mNC–CPE by a simple polishing step, obtaining a reset electrode surface. For the control experiment, the same protocol was carried out using oleic acid coated magnetic nanoparticles.

Supplementary Information Figures

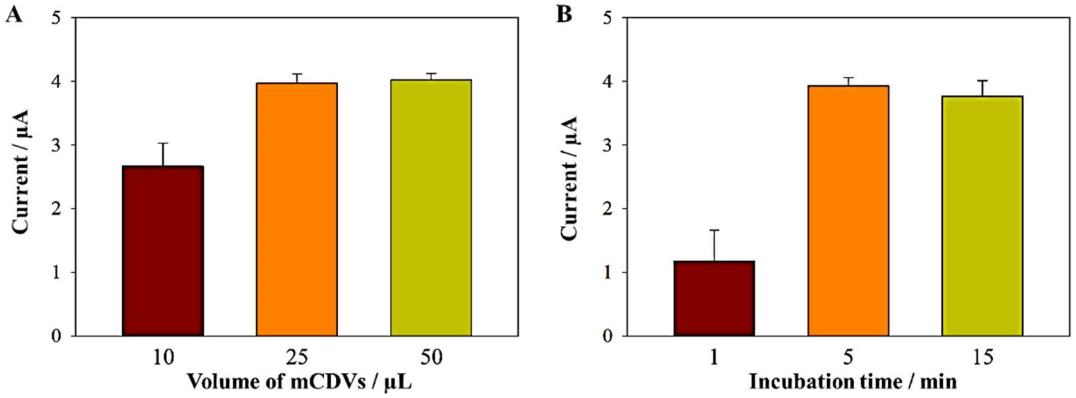

Figure S1. Optimization of some pivotal parameters for the electroanalytical assay. A) Study of the volume of mCDVs (10, 25 and 50 µL) for a fix T_4 concentration (60 pM); incubation time: 15 min. B) Determination of the optimum incubation time for the supramolecular CD-T_4 binding formation (1, 5 and 15 min) for a mix T_4 concentration (60 pM); volume of mCDVs: 25 µL. LSV analyses were run in a three-electrode configuration cell filled with 10 mL of electrolyte (PBS at pH 7.2), employing a scan rate of 25 $mV \cdot s^{-1}$.

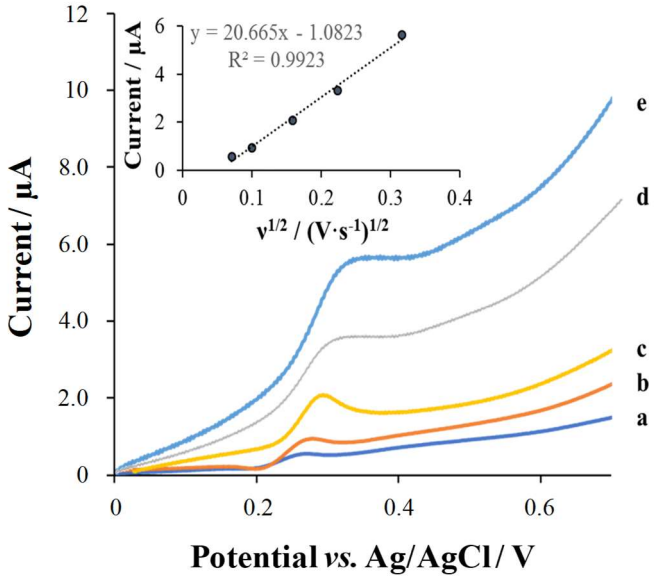

Figure S2. Influence of scan rate (υ) from 5 to 100 $mV \cdot s^{-1}$ (a to e) in the presence of 5.0 µM T_4 at the bio-recognition sensing system. Inset: lineal I_{pa} *vs.* $\upsilon^{1/2}$ dependence. Experiments were run in a PBS electrolyte at pH 7.2.

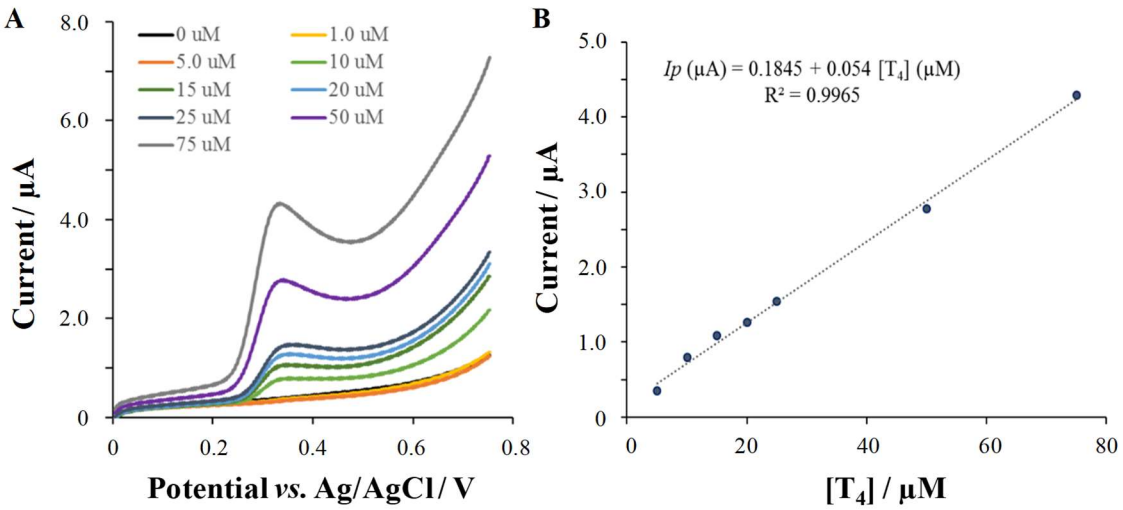

Figure S3. A) LSV response at the naked NC–CPE (blank experiment) with increasing the [T_4] from 1.0 to 75 µM. B) Linear response with a LOD of 5.0 µM. Experiments were carried out in a PBS electrolyte at pH 7.2 (scan rate: 25 $mV \cdot s^{-1}$).

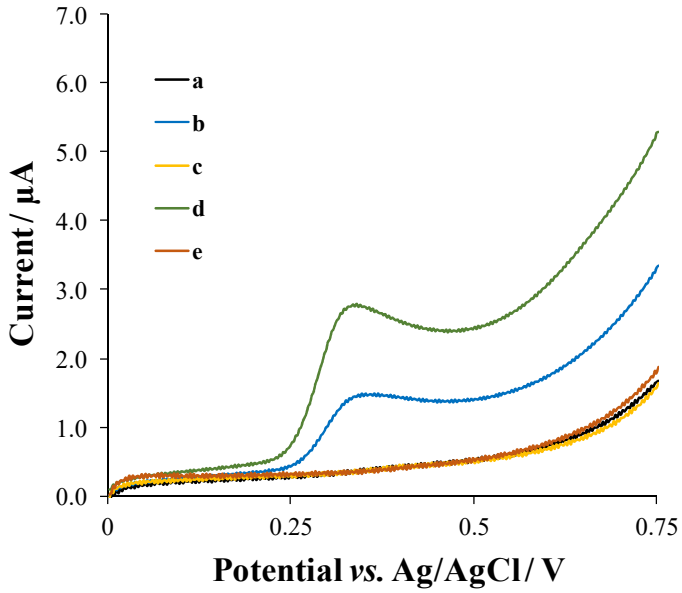

Figure S4. Regeneration capability of the mNC–CPE surface before (*a, c)* and after sensing different [T_4] (*i.e.,* (*b*) 10 pM and (*d*) 25 pM T_4). After the polishing procedure (*c*, *e*), the electrode recovers its initial electrochemical performance. Experiments were carried out in a PBS electrolyte at pH 7.2 (scan rate: 25 $mV \cdot s^{-1}$).

Supplementary Information Tables

Table S1. Study of four potentially interfering biomolecules on the R_{CT} signal of 5.0 μM T_4. The selectivity EIS test was carried out by incubating the mCDVs with a T_4 solution doped with tyrosine (Tyr), ascorbic acid (AA), uric acid (UA) and dopamine (DA) in a 1:1 ratio.

Compounds	Concentration (μM)	R_{CT} change (%)
T_4	5.0	100
Tyr	5.0	+2.57
AA	5.0	+1.28
UA	5.0	+0.97
DA	5.0	+0.99

References

[1] Sun, Y.; Ding, X.; Zheng, Z.; Cheng, X.; Hu, X.; Peng, Y., *European Polymer Journal* 2007, **43** (3), 762-772.
[2] Schenkel, J. H.; Samanta, A.; Ravoo, B. J., *Advanced Materials* 2014, **26** (7), 1076-1080.
[3] Muñoz, J.; González-Campo, A.; Riba-Moliner, M.; Baeza, M.; Mas-Torrent, M., *Biosensors and Bioelectronics* 2018, **105**, 95-102.