

Supplementary information

Nanoparticle based enhancement of electrochemical DNA hybridization signal using nanoporous electrodes

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Materials

Apparatus and electrodes

Anodized aluminum oxide (AAO) nanoporous filter membranes (Whatman anodisc filters, 13 mm in diameter, 60 μm thick, containing 200 nm pores in a $1 \times 10^9 \text{ cm}^{-2}$ pore density) were purchased from Fisher-Bioblock (Spain).

Optical characterizations of the AAO filter membranes were performed using a Scanning Electron Microscope (SEM - Jeol JSM-6300, Jeol Ltd, Tokio, Japan) and the images of the gold nanoparticles shown in the *supplementary material* were obtained with a Transmission Electron Microscope (TEM) Jeol JEM-2011 (Jeol Ltd, Japan).

The electrochemical transducers used were homemade screen-printed carbon electrodes (SPCEs), consisting of three electrodes: working electrode, reference electrode and counter electrode in a single strip fabricated with a semi-automatic screen-printing machine DEK248 (DEK International, Switzerland). The reagents used for this process were: Autostat HT5 polyester sheet (McDermid Autotype, UK) and Electrodag 423SS carbon ink, Electrodag 6037SS silver/silver chloride ink and Minico 7000 Blue insulating ink (Acheson Industries, The Netherlands). The full size of the sensor strip was 29mm x 6.7mm, and the WE diameter was 3mm. The fabrication of the SPCEs was carried out in three steps. First, a graphite layer was printed onto the polyester sheet, using the screen-printing machine with the stencil (where it is the electron pattern). After curing for 15 minutes at 95°C, an Ag/AgCl layer was printed and cured for 15 minutes at 95°C. Finally, the insulating ink was printed and cured at 95°C for 20 minutes. Figure S2 shows images of the 45-sensor sheet obtained following the detailed experimental procedure (A) and a detail of a single sensor (B).

A home-made methacrylate electrochemical cell was used for the electrochemical measurements that were performed with an Autolab 20 (Eco-chemie, The Netherlands) connected to a PC.

Reagents and solutions

Synthetic 21-mer oligonucleotides : 5'-NH₂-GACTCTCAGCGAGCCTCAATG-3' (probe ssDNA), 5'-SH-CATTGAGGCTCGCTGAGAGTC-3 (target ssDNA) and 5'-SH-GACTCTCAGCGAGCCTCAATG-3' (non specific target ssDNA) were obtained from Alpha DNA (Canada). Oligonucleotide solutions were prepared in TE buffer, pH 8 (10 mM Tris-HCl buffer solution, 1 mM in EDTA). Aliquots were prepared and maintained at -20°C. Working solutions were made in Tris buffer (0.1 M Tris, pH 7.2). These solutions were conserved at 4 °C.

Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄•3H₂O, 99.9%), trisodium citrate, Tris(hydroxymethyl)aminomethane (Tris), EDTA, 3-amino-propyltrimethoxysilane (APS), glutaraldehyde, propylamine, acetone, potassium hexacyanoferrate (III), sodium chloride, sodium nitrate and phosphate salts were purchased from Sigma (Spain). All chemicals were used as received and all aqueous solutions were prepared in double-distilled water.

The 20-nm gold nanoparticles (AuNPs) were synthesized by reducing tetrachloroauric acid with trisodium citrate, a method pioneered by Turkevich et al.¹. A total of 200 mL of 0.01% HAuCl₄ solution was boiled with vigorous stirring. Some 5mL of a 1% trisodium citrate solution were added quickly to the boiling solution. When the solution turned deep red, indicating the formation of gold nanoparticles, it was left stirring and cooling down. In this way, a dispersed solution of 20-nm AuNPs was obtained. Figure

S3 shows TEM images and the Gaussian distribution of sizes of the used gold nanoparticles.

The AuNPs loading with the thiolated target ssDNA was performed adapting the protocol established by Mirkin and co-workers². Briefly it consisted in mixing a 400 μL suspension of 20-nm AuNPs ($\sim 3 \text{ nM}$) with 100 μL of a 50 $\mu\text{g}/\mu\text{L}$ oligonucleotide solution. After standing for 24 h at room temperature, the solution was gently brought to 500 μL of 0.1 M NaCl, 10 mM phosphate buffer (pH 7) and allowed to stand for 40 h (final oligonucleotide concentration is 5 $\mu\text{g}/\mu\text{L}$). After that, it was centrifugated at 14,000 rpm for 20 min and reconstituted in 1 mL of Tris buffer. The same protocol was followed for the non-specific target ssDNA, used for testing the selectivity of the hybridization assay.

Methods

Porous membranes functionalization and immobilization of probe ssDNA inside the pores

A methodology previously reported by Smirnov's group³ was followed to functionalize the porous membranes. AAO nanoporous membranes, containing pores of 200 nm in diameter (see detailed characteristics in section 2.1) were boiled in ultra-pure water for 1 h and after drying in argon they were immersed into 5% acetone solution of APS for 1 h. After washing in acetone and baking at 120°C for 30 min, the filters were left overnight in 25% aqueous solution of glutaraldehyde. After that 30 μL of a 5 $\mu\text{g}/\mu\text{L}$ solution of the probe ssDNA were placed on the filtering side and left there for a determined time (usually from 30 min to overnight). Finally, in order to improve the

efficiency of the hybridization, the glutaraldehyde was neutralized overnight in a 10^{-5} M aqueous solution of propylamine.

DNA hybridization inside the pores

After washing in Tris buffer, 30 μ L of a solution of the target ssDNA (unlabeled or labeled with AuNPs) were placed on the filtering side of the AAO nanoporous membrane and left there for a determined time (usually from 30 min to 200 min). Finally, AAO nanoporous membranes were washed and stored in Tris buffer. Blank assays were performed following the same experimental procedure, but using the non specific ssDNA target described in section 2.2.

Nanoporous cell set-up and electrochemical detection

AAO nanoporous membranes were fixed onto the SPCE by a physical attachment, consisting in placing the SPCE onto a methacrylate block and putting the membrane with the filtering side up covering the three electrodes surface. Then, a second methacrylate block containing a hole of the same size of the working area of the SPCE is placed onto the AAO nanoporous membrane, using an insulating ring between them to avoid liquid leakage. Finally, the system is fixed using screws. In this way, a 300 μ L electrolytic cell of is defined, which is filled with a 100 μ L of 1mM $K_3[Fe(CN)_6]$ in 0.1M $NaNO_3$ solution. (see pictures of the cell-assembly in Figure S4). A pre-treatment process at -0.55 V during 30 s was carried out in order to have all the Fe ions in the form of Fe (II) and immediately after, differential pulse voltammetry (DPV) by scanning from -0.20 V to +0.50 V (resulting in an analytical signal due to oxidation of Fe (II) to Fe (III) at approximately +0.20V) was performed. A step potential of 10 mV, modulation amplitude of 50 mV and a scan rate of 33.5 mV/s were applied during the measurements in a nonstirred solution.

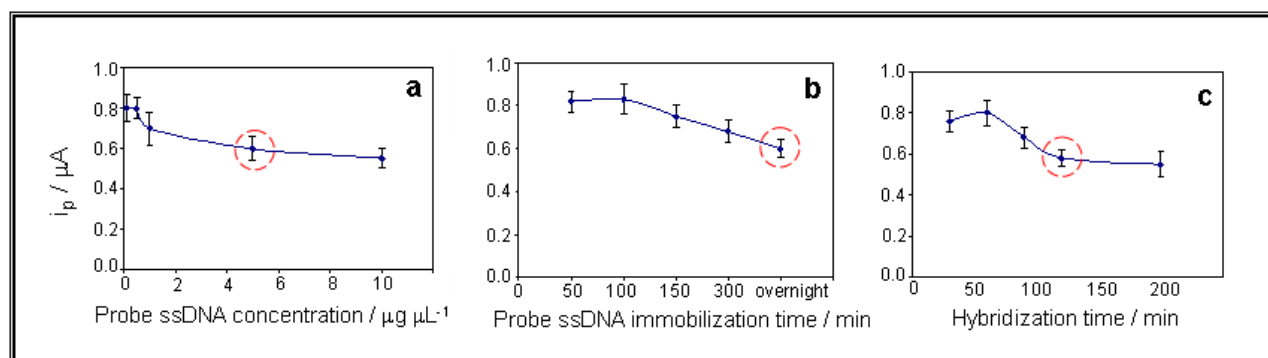


Figure S1. Optimization of the parameters affecting the analytical signal for a target ssDNA concentration of 5 $\mu g/\mu L$: (a) probe ssDNA concentration (probe immobilization time: overnight; hybridization time: 120 min) (b) probe ssDNA immobilization time (probe concentration: 5 $\mu g/\mu L$; hybridization time: 120 min); (c) Hybridization time (probe immobilization time: overnight; probe concentration: 5 $\mu g/\mu L$). Optimum values are circled in red.

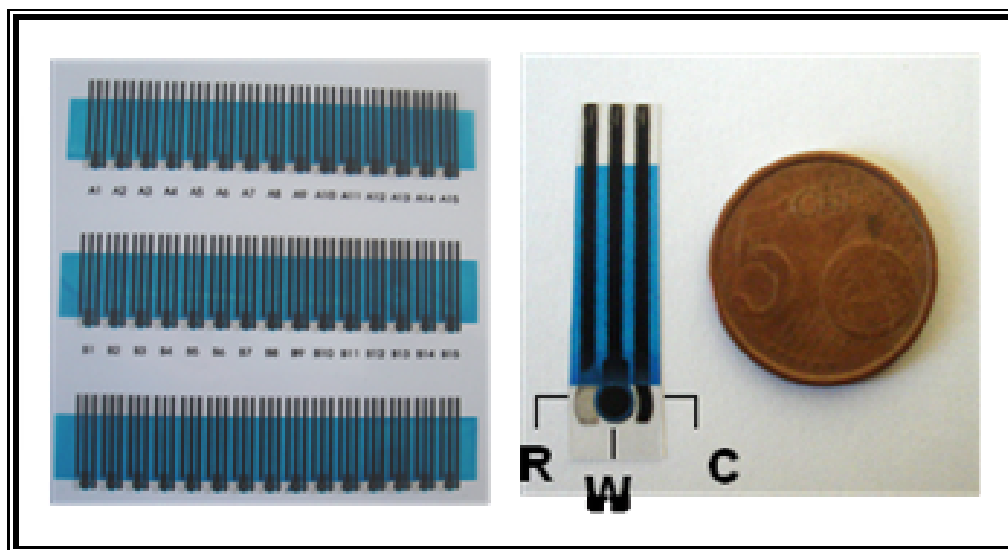


Figure S2. (left) A 45-sensor sheet obtained following the experimental procedure detailed, and (right) detail of a SPCE, showing the area with the three electrodes: reference silver electrode (R), carbon working electrode (W) and carbon counter electrode (C).

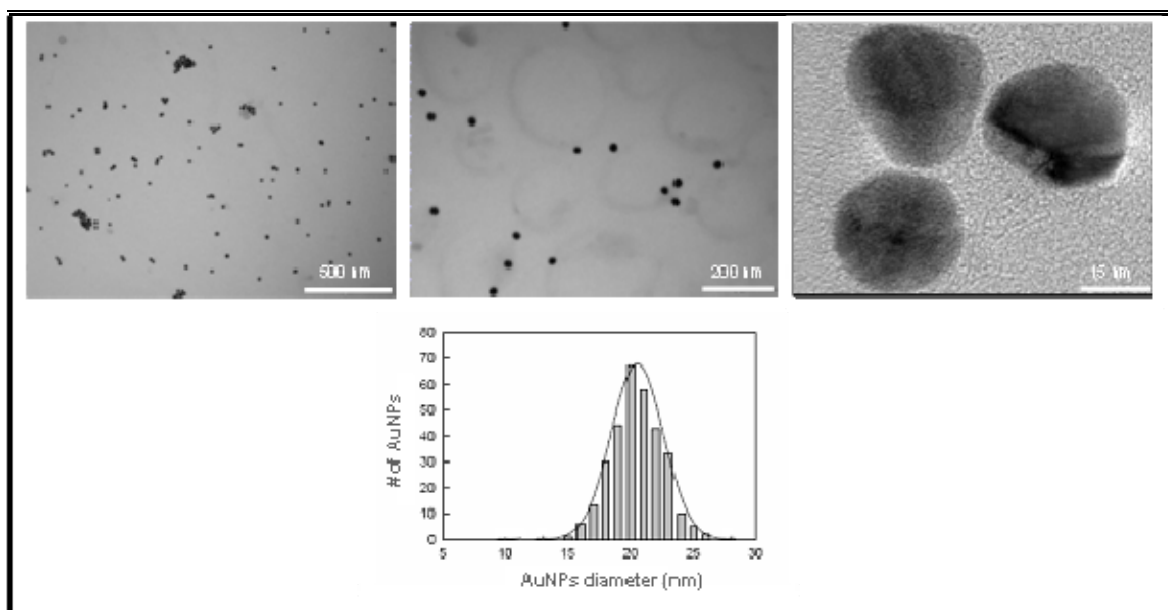


Figure S3. TEM images of the AuNPs suspension (above) and the Gaussian distribution of their sizes (below).

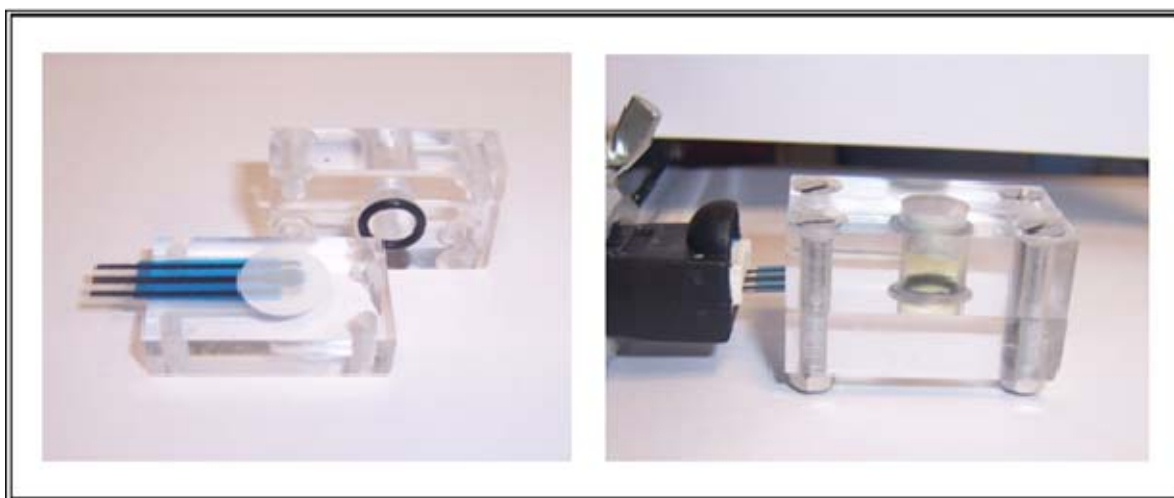


Figure S4. (Left) Picture of the SPCE placed into the two methacrylate blocks with the AAO nanoporous membrane covering the working area. **(Right)** Cell assembled, filled with 1mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ /0.1M NaNO_3 solution and connected to the potentiostat.

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