Electronic Supporting Information for the Article:

Amplified Electrochemical Detection of DNA through the Aggregation of Au Nanoparticles on Electrodes and the Incorporation of Methylene Blue into the DNA-Crosslinked Structure

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Experimental details of the preparation of the nucleic acid-functionalized Au NPs, assembly on the electrodes, and the experimental protocol for analyzing the DNA (3)

Preparation of DNA-modified Au nanoparticles

Gold nanoparticles (NPs) with an average diameter of 12 ± 1 nm were prepared using the citrate reduction method and the resulting NPs were modified with thiolated DNAs, (**1**) or (**2**) according to published protocols[1,2]. Briefly, Au nanoparticles were prepared by boiling an aqueous solution of 1 mM $HAuCl_4$ (100 ml) under rapid stirring and adding 10 ml of a 38 mM solution of sodium citrate. After 10 more minutes of boiling, the solution was allowed to cool to room temperature, and filtered through a 0.8 μm membrane.

The aqueous Au nanoparticle solution, 5ml, and thiolated DNAs **1** or **2**, respectively (final concentrations of 1.5 μM, about 1 OD, each), were incubated together for 16 h under constant stirring. The solution was slowly brought up to a final salt concentrations of 0.1 M NaCl and 10 mM phosphate (pH 7) and allowed to stand for 40 h. Centrifugation was performed for 40 min at 14,000 rpm in order to remove excess of reagents. The precipitate was washed with 0.1 M NaCl, 10 mM phosphate buffer (pH 7) solution, recentrifuged, and finally dispersed in 10 mM phosphate

buffer (pH 7) containing 0.3 M NaCl to yield stock solutions of 2.4×10^{-8} M DNA-modified Au NPs.

Modifying of Au electrode with Au NPs aggregates

Different concentrations of the analyte DNA (**3**) were interacted with a mixture of the (**1**)- and (**2**)-functionalized Au NPs, 6×10^{-9} M each, for 20 minutes. The resulting (**3**) crosslinked Au NP aggregates were centrifuged at 9000 rpm for 30 minutes, and the separated aggregates were redissolved in the buffer (10 mM phosphate, 0.3 M NaCl, pH=7.3). Then, the Au NP aggregates (600 μl) were allowed to react with the 1,9-Nonanedithiol modified Au surface for 2 hours, followed by rinse the surface with a buffer solution. Methylene blue was intercalated into the duplex-aggregated Au NPs by the interaction of the modified electrodes with a 0.1 mM of methylene blue for 30 minutes, following by rinsing the electrode with buffer (10 mM phosphate, 0.3 M NaCl, pH=7.3).

Electrochemical measurements

A conventional three-electrode cell, consisting of a modified Au wire working electrode (0.5 mm diameter, geometrical area ca. 0.16 cm^2, roughness factor ca. 1.4), a glassy carbon auxiliary electrode isolated by a glass frit, and a saturated calomel reference electrode (SCE) connected to the working volume with a Luggin capillary, was used for the electrochemical measurement. All potentials are reported with respect to the SCE. The cell was placed in a grounded Faraday cage. Differential pulse voltammetry and impedance spectra were performed using an Autolab electrochemical system (Eco Chemie, Netherlands). A phosphate buffer (0.1 M, pH=7.3) that included 0.3 M NaCl was used as electrolyte solution in all the experiments.

Figure S1 Differential pulse voltammagrams corresponding to: (a) The MB^+ interacted with dithiolated monolayer; (b) The MB^+ interacted with the surface subjected to the (1) and (2)-functionalized Au NPs (in the absence of (3)). (Curve (b) is identical to curve (a) in Figure 1(B) in the paper).