Supplementary Information

Size-dependent direct electrochemical detection of gold nanoparticles: application in magnetoimmunoassays

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Screen-printed carbon electrodes (SPCEs) fabrication.

The full size of the sensor strip was 29mm x 6.7mm, and the working-electrode 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 95ºC, an Ag/AgCl layer was printed and cured for 15 minutes at 95ºC. Finally, the insulating ink was printed and cured at 95ºC for 20 minutes.

Images of the 45-sensor sheet obtained following the above experimental procedure and details of one of the SPCE are shown in figure S1.

AuNPs characterizations.

- *ICP-MS analysis:* The amount of total of gold in the AuNPs solutions was obtained by ICP-MS analysis. The samples were diluted in 1% HNO_3 and inserted in the ICP-MS spectrometer to obtain their total content of gold, expressed in mg L^{-1}.

- *Spectrophotometric analysis:* The spectrophotometric analysis of the different AuNPs solutions was performed by placing 275 µL of each solution on a plate of a 96-well plate, and measuring the OD in the range of 300-750 nm. The concentration of AuNPs in each sample was calculated as detailed in the following section.

- *TEM images:* The size and shape of the different AuNPs studied was observed in the transmission electron microscope. The AuNPs solutions were first sonicated in an ultrasonic bath and then a drop of 4 µL was placed in a disk of copper and let to dry during 2 hours before the measurements.

Estimation of the concentration of AuNPs from UV-Vis spectra

Knowing the value of ε_{450}, the particle concentration (c) in mol L^{-1} can be calculated from the absorption (A) at 450 nm for a standard path length (l) of 1 cm according to:

$$c = A_{450}/\varepsilon_{450}$$

The values of ε_{450} for each AuNP size has been calculated and experimentally verified in the range between 5-100 nm by Haiss's group (ref. 28 in the main text), being 7.20 x 10^6, 6.15 x10^7, 5.41 x

10^8, 4.92 x 10^9, 1.73x10^{10}, 3.89 x10^{10} and 6.44 x 10^{10} M^{-1} cm^{-1} for 5, 10, 20, 40, 60, 80 and 100-nm AuNPs respectively. Absorbance spectra for different AuNPs solutions and the estimation of the concentration of each solution are summarized in figure S2.

Estimation of settling velocity and deposition time for AuNPs of different sizes

The frictional force exerted on spherical objects with very small Reynolds numbers (e.g., nanoparticles) in a continuous viscous fluid can be calculated by the Stokes Law:

$$F_d = 6 \Pi \mu R V$$

where F_d is the frictional force (in N), μ is the fluid's dynamic viscosity (Pa s), R is the radius of the spherical object (m), and V is the particle's velocity (m s^{-1}).

If the particles are falling in a viscous fluid by their own weight due to gravity, then a terminal velocity, also known as the settling velocity, is reached when this frictional force combined with the buoyant force exactly balance the gravitational force. The resulting settling velocity (or terminal velocity) is given by the following equation, derived from the Stokes Law:

$$V_s = 2 (\rho_p - \rho_f) g R^2 / 9 \mu$$

where V_s is the particles' settling velocity (m s^{-1}) (vertically downwards if $\rho_p > \rho_f$, upwards if $\rho_p < \rho_f$), g is the gravitational acceleration (m s^{-2}), ρ_p is the mass density of the particles (kg m^{-3}) ρ_f is the mass density of the fluid (kg m^{-3}) and μ is the fluid's dynamic viscosity (Pa s).

Considering a gold density of 999.972 g L^{-1} and a fluid viscosity of 1 mPa s, the settling velocity of the AuNPs of different sizes from the suspension to the electrode surface and the necessary falling time to the electrode surface from an arbitrary distance of 50 nm can be estimated (Table S1).

Magnetosandwich immunoassay using as labels AuNPs of different sizes

Briefly, 150 µg (15 µL from the stock solution) of the magnetic beads solution (MBs) were transferred into 0.5 mL Eppendorf tube. The MBs were washed twice with 150 µL of B&W buffer.

The MBs were then re-suspended in 108 μL of B&W buffer and 42 μL (from stock solution 0.36 mg mL^{-1}) of biotinylated anti-human IgG were added. The resulting MB and anti-human IgG (goat IgG for the blank assay) solution was incubated for 30 min at temperature 25 °C with gentle mixing in a TS-100 ThermoShaker. The formed MB/anti-human IgG were then separated from the incubation solution and washed 3 times with 150 μL of B&W buffer. The preparation process was followed by resuspending the MB/anti-human IgG in 150 μL of blocking buffer (PBS-BSA 5%) to block any remaining active surface of MBs and incubated at 25°C for 60 min. After the washing steps with B&W buffer, the MB/anti-human IgG were incubated at 25 °C for 30 min with 150 μL of 1 μg mL^{-1} of human IgG antigen, forming by this way the immunocomplex MB/anti-human IgG/Human IgG. Finally, after the washing steps, the MB/anti-human IgG/Human IgG immunocomplex was incubated at 25 ºC for 30 min with 150 μL of the previously synthesized AuNPs/anti-human IgG complex.

A blank (control) assay is performed using goat IgG instead of human IgG at the same concentration.

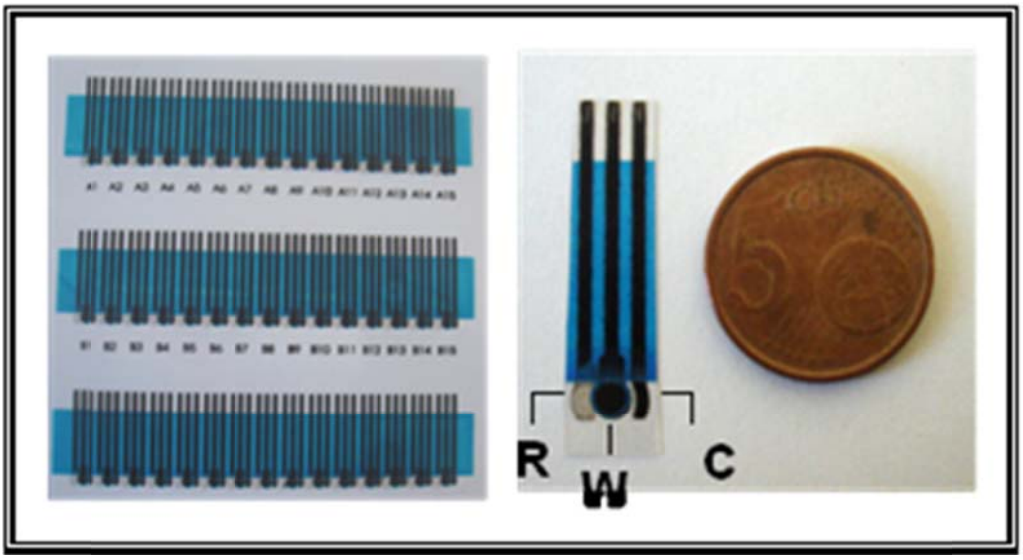
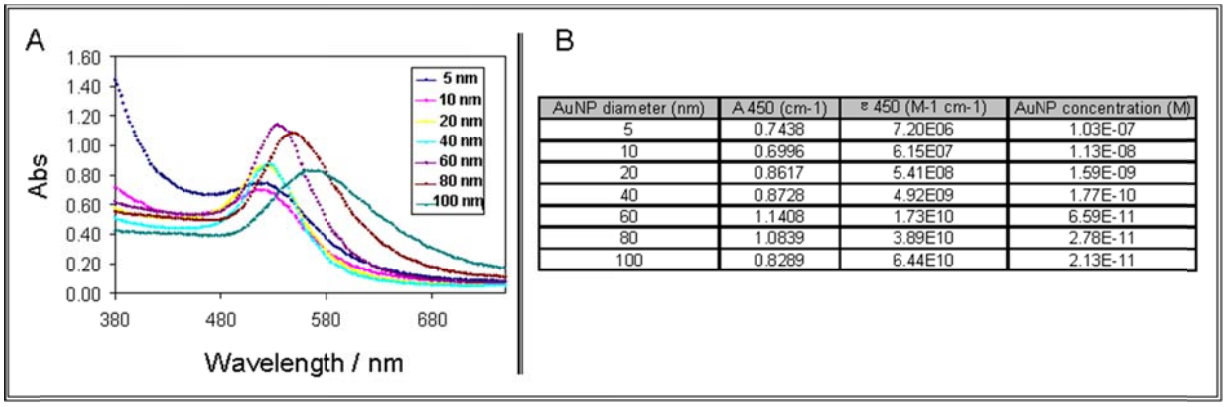


Figure S1. (Left) Images of the 45 SPCE sensors sheet obtained following the detailed experimental procedure. (Right) Detail of one SPCE, containing the three electrodes in the working area: R- Ag/AgCl reference electrode, W- carbon working electrode and C- carbon counter electrode.



AuNP diameter (nm)	A 450 (cm-1)	ε 450 (M-1 cm-1)	AuNP concentration (M)
5	0.7438	7.20E06	1.03E-07
10	0.6996	6.15E07	1.13E-08
20	0.8617	5.41E08	1.59E-09
40	0.8728	4.92E09	1.77E-10
60	1.1408	1.73E10	6.59E-11
80	1.0839	3.89E10	2.78E-11
100	0.8289	6.44E10	2.13E-11

Figure S2. (A) Absorbance spectra of different AuNPs solutions and (B) estimation of the concentration of each solution.

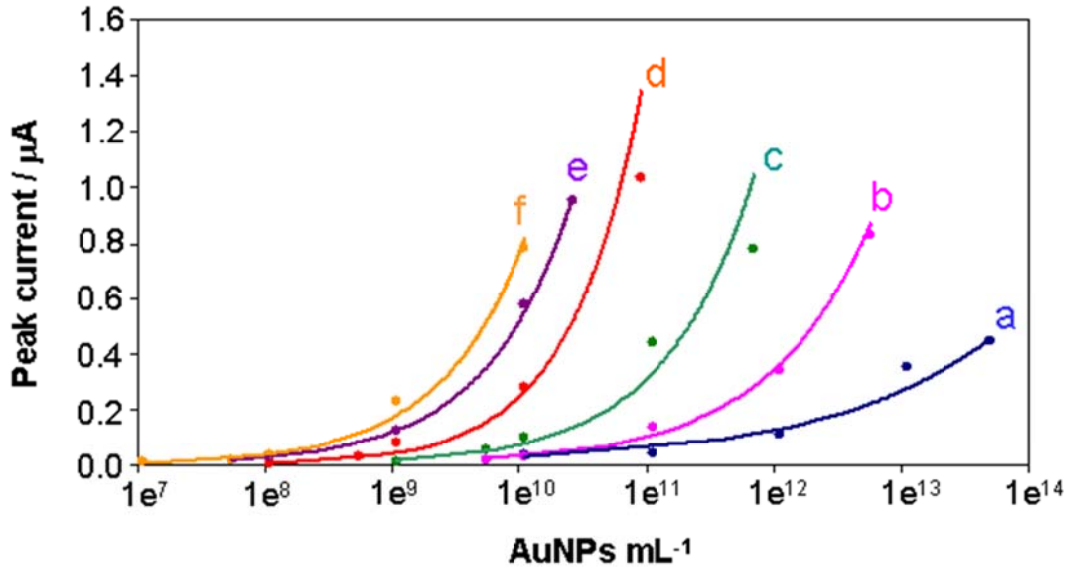


Figure S3. Full data of the electrochemical results obtained for different concentrations of AuNPs of different sizes: (a) 5 nm, (b) 10 nm, (c) 20 nm, (d) 40 nm, (e) 60 nm, (f) 80 nm.

AuNP diameter (nm)	**Velocity** (cm/min)	**Time*** (min)
5	1.57 e-6	31.97
10	6.30 e-6	7.99
20	2.52 e-5	2.00
40	1.01 e-4	0.50
60	2.27 e-4	0.22
80	4.04 e-4	0.12

* Considering an arbitrary distance of 50 nm that the AuNP has to travel from the solution to the electrode.

Table S1. Estimation of the falling velocity and time required for AuNPs of different sizes traveling from the solution to the electrode surface.

AuNPs diameter (nm)	**Nº atoms per nanoparticle**	**Nº NPs in 1 mol atoms**	**Nº NPs in 25μL of 50 μM gold solution**	**Nº surface atoms in 25 μL of 50 μM gold solution**
5	3859	1.50×10^{20}	1.95×10^{11}	1.99×10^{14}
20	246960	2.44×10^{18}	3.05×10^{9}	5.00×10^{13}
80	15806976	3.81×10^{16}	4.76×10^{7}	1.25×10^{13}

Table S2. Estimation of the number of surface atoms contained in 25 μL of a 50 μM gold solution of AuNPs of different sizes.