## **Colorimetric immunosensor by aggregation of photo-chemically functionalized gold nanoparticles**

Marzia Iarossi,<sup>1,†</sup> Chiara Schiattarella,<sup>1,2</sup> Ilaria Rea,<sup>2</sup> Luca De Stefano,<sup>2</sup> Rosalba Fittipaldi,<sup>3</sup> Antonio Vecchione,<sup>3</sup> Raffaele Velotta<sup>1,\*</sup> and Bartolomeo Della Ventura<sup>1</sup>

<sup>1</sup>Department of Physics "E. Pancini" – Università di Napoli "Federico II" – Via Cintia, 26 Ed. 6 – 80126 Napoli (Italy).

<sup>2</sup>National Research Council - Institute for Microelectronics and Microsystems, Unit of Naples, Via P. Castellino 111, 80131, Napoli (Italy).

<sup>3</sup>National Research Council - SPIN Institute, Unit of Salerno and Department of Physics "E. R. Caianiello", Università di Salerno, Via Ponte don Mellillo, 84084 Fisciano, Salerno (Italy).

*KEYWORDS:* gold nanoparticles, localized surface plasmon resonance, antibody functionalization, photochemical immobilization technique (PIT), colorimetric immunosensor, nanoparticle aggregation, IgG detection.

\*Present Addresses: Italian Institute of Technology, Via Morego 30, 16163 Genova, Italy.\*Corresponding author: Raffaele Velotta, email: rvelotta@unina.it

Table of contents

Figure S1. Absorption spectrum of synthetized AuNPs.

Figure S2. DLS measurements.

Figure S3. Zeta potential measurements.

Figure S4. UV-Vis absorption spectra of functionalized AuNPs after different incubation times.

Figure S5. UV-Vis absorption spectra of functionalized AuNPs after centrifugation and blocking.

Figure S6. SEM images of functionalized AuNPs and aggregates at lower magnification.

Figure S7. Interaction kinetics between human IgG and the functionalized AuNPs.

Figure S8. Specificity test and stability.



**Figure S1**. Absorption spectrum of synthetized AuNPs. The absorbance at 450 nm is approximately 0.6 and considering the molar extinction coefficient of  $5 \times 10^9$  Mol<sup>-1</sup>·cm<sup>-1</sup> for 40 nm AuNPs,<sup>1</sup> we obtain a nanoparticle concentration of  $6 \times 10^{10}$  particles/mL.



Figure S2. Intensity-size distribution of the synthetized AuNPs obtained from DLS measurements.

(1) Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV–Vis Spectra. *Anal. Chem.* **2007**, *79*, 4215–4221.



Figure S3. Zeta potential distribution of the synthetized AuNPs.



**Figure S4.** UV-Vis absorption spectra of the functionalized AuNPs collected after various incubation times, which show that less than five minutes are required to reach the equilibrium.



**Figure S5**. UV-Vis absorption spectra of the functionalized AuNPs before and after the purification treatment and the addition of BSA.



**Figure S6.** SEM images at lower magnification of functionalized AuNPs interacting with human-IgG at different concentrations: (a) no IgG (0 ng/mL), (b) 50 ng/mL and (c) 200 ng/mL.



Figure S7. Change of the wavelength of the maximum absorption measured at human IgG of 200 ng/mL.



**Figure S8.** UV-Vis absorption spectrum of functionalized AuNPs (black solid line). The same spectrum after 1 h (red dashed line) and 24 h (blue dotted line) incubation time with 200 ng/mL mouse IgG. The inset shows the cuvette with functionalized AuNPs (left) and after the addition of 4  $\mu$ L of a solution containing mouse IgG 50  $\mu$ g/mL (right).