

## SUPPLEMENTARY INFORMATION

### **Immunoassay quantification using Surface-Enhanced Fluorescence (SEF) tags**

S. A. Camacho,<sup>a,b</sup> R. G. Sobral-Filho,<sup>b</sup> P. H. B. Aoki,<sup>b,c</sup> C. J. L. Constantino<sup>a</sup> and A. G. Brolo<sup>\*b</sup>

<sup>a</sup> São Paulo State University (UNESP), School of Technology and Applied Sciences, Campus Presidente Prudente, SP, Brazil, 19060-900.

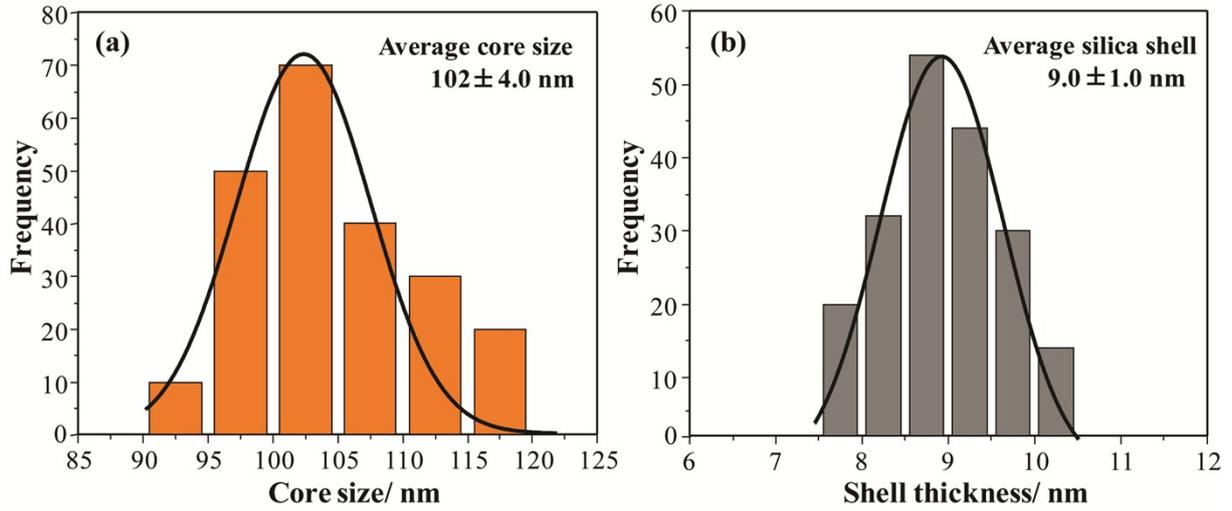
<sup>b</sup> University of Victoria, Department of Chemistry, Victoria, BC, Canada, V8P 5C2.

<sup>c</sup> São Paulo State University (UNESP), School of Sciences, Humanities and Languages, Campus Assis, SP, Brazil, 19806-900

\* E-mail: [agbrolo@uvic.ca](mailto:agbrolo@uvic.ca)

## Determination of the SHIN particle concentration

The determination of Au-SHINs concentration was performed by measuring the average diameter of Au-SHINs (core size + silica shell thickness dimensions) via TEM. Figures S11a and S11b display the histograms plotted for the Au-SHINs core size and silica shell thickness dimensions, respectively.



**Figure S11.** Histograms containing the average dimensions of Au-SHINs (a) core size and (b) silica shell thickness.

The average diameter of Au-SHINs ( $d_{Au-SHIN}$ ) is the sum of core size and silica shell thickness obtained from TEM histograms (Figure S11):

$$d_{Au-SHIN} = \text{core size} + \text{silica shell thickness} = 102 \text{ nm} + 9.0 \text{ nm}$$

$$\therefore d_{Au-SHIN} = 111 \text{ nm}$$

Considering that the Au-SHIN has spherical shape, the radius ( $R_{Au-SHIN}$ ) and the volume ( $V_{Au-SHIN}$ ) of one Au-SHIN were calculated:

$$d_{Au-SHIN} = 2R_{Au-SHIN}$$

$$\therefore R_{Au-SHIN} = 55.5 \text{ nm} = 55.5 \times 10^{-7} \text{ cm}$$

$$V_{Au-SHIN} = \frac{4}{3}\pi R^3$$

$$\therefore V_{Au-SHIN} = \frac{4}{3}\pi(55.5 \times 10^{-7} \text{ cm})^3 = 7.16 \times 10^{-16} \text{ cm}^3$$

Using this volume ( $V_{Au-SHIN}$ ) and the density of the gold ( $\rho_{gold}$ ) the mass of one Au-SHIN ( $m_{Au-SHIN}$ ) was also calculated:

$$\rho_{gold} = \frac{m_{Au-SHIN}}{V_{Au-SHIN}} \rightarrow m_{Au-SHIN} = \rho_{gold} \times V_{Au-SHIN}$$

$$m_{Au-SHIN} = 19.32 \frac{\text{g}}{\text{cm}^3} \times 7.16 \times 10^{-16} \text{ cm}^3$$

$$\therefore m_{Au-SHIN} = 1.38 \times 10^{-14} \text{ g}$$

For the experimental procedure, a known volume of Au-SHINs colloidal suspension ( $V = 33 \mu\text{L}$ ) was brought to dry and the resulting mass was measured ( $m = 5 \times 10^{-4} \text{ g}$ ). Then, considering the relation described below, the concentration of Au-SHINs in the colloidal suspension was determined:

$$\frac{1 \text{ Au-SHIN}}{x} \rightarrow \frac{1.38 \times 10^{-14} \text{ g}}{5 \times 10^{-4} \text{ g}}$$

$$\therefore x = 3.6 \times 10^{10} \text{ Au-SHINs}$$

This is the number of Au-SHINs in 33  $\mu\text{L}$  of colloidal suspension. Then, to estimate the concentration of Au-SHINs, the mathematical relation described below was applied:

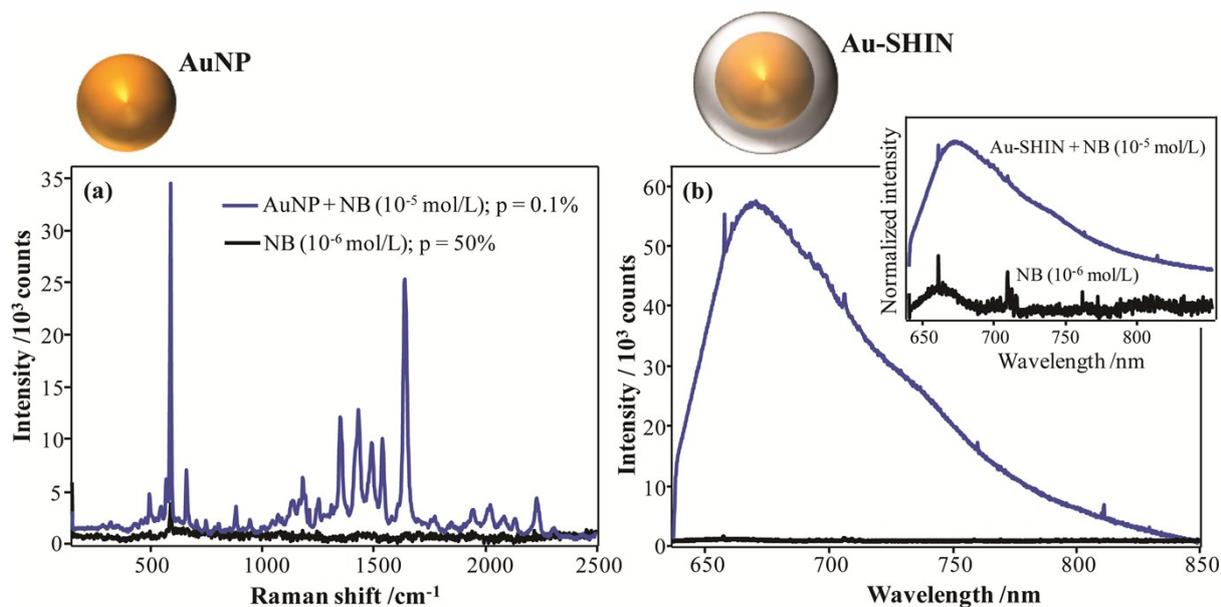
$$\frac{3.6 \times 10^{10} \text{ Au-SHINs}}{x'} \rightarrow \frac{33 \mu\text{L}}{1000 \mu\text{L}}$$

$$\therefore x' = 1.1 \times 10^{12} \text{ Au-SHINs/mL}$$

## Determination of the Fluorescence Enhancement Factor

The efficiency of the fluorescence enhancement were obtained by analyzing the emission from NB solution + uncoated AuNPs-100 (NB final concentration:  $10^{-6}$  mol/L) and NB solution + Au-SHINs (NB final concentration:  $10^{-6}$  mol/L). The results are summarized in Figures SI2a and SI2b, respectively.

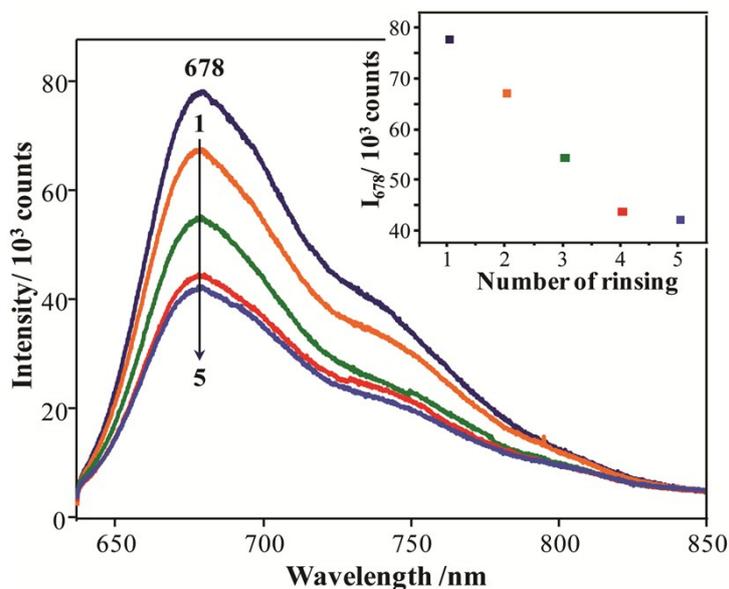
In Figure SI2a the fluorescence emission is totally quenched and NB SERS spectra are acquired. The main enhanced band at  $592\text{ cm}^{-1}$  is assigned to phenoxazine ring stretching.<sup>1,2</sup> Considering the SERS/Raman intensity ratio at  $592\text{ cm}^{-1}$ , with the Raman spectrum recorded for NB solution ( $10^{-6}$  mol/L), an enhancement factor (EF) of ca.  $10^3$  was achieved, which is in agreement with the EFs found in the literature (the average EF lies between  $10^3$ - $10^9$ ).<sup>3-5</sup> In Figure SI2b the enhanced fluorescence is observed at 669 nm from the NB monomer emission.<sup>6</sup> Surface-enhanced fluorescence (SEF)<sup>7,8</sup> is observed from molecules located at a certain distance from plasmonic nanostructures that can strongly interact with adjacent fluorophores and is proportional to  $E^2$ <sup>5,9</sup> leading to EF commonly between 1 and 100.<sup>10</sup> Considering the intensity ratio between SEF (recorded for NB solution + Au-SHINs) and fluorescence (recorded for NB solution ( $10^{-6}$  mol/L)), the EF found was ca. 70, which is in good agreement with the EF predict for SEF using the electromagnetic mechanism.<sup>8,10</sup>



**Figure SI2.** Validation of silica coating efficiency for fluorescence enhancement. NB solutions containing (a) AuNPs-100 (NB final concentration:  $10^{-6}$  mol/L) and (b) Au-SHINs (NB final concentration:  $10^{-6}$  mol/L).

### Removal of residual NB from SHINs

The variation of the SEF tags fluorescence intensity along the rinsing process is shown in Figure SI3. The fluorescence intensity from the NB monomer emission (peak at 678 nm) decreases according to the rinsing process of the SEF tags, with further decrease of the NB dimmer emission (at ca. 740 nm),<sup>6,11</sup> suggesting that the excess of NB molecules aggregated around the SEF tags are being removed. After the fifth rising, the fluorescence intensity reaches a plateau (inset – Figure SI3) indicating that the SEF signal solely arises from the NB molecules chemically bounded to the Au-SHINs.



**Figure S13.** Variation of SEF tags fluorescence intensity along the rinsing process. The inset shows the fluorescence band intensity at 678 nm versus the number of rinsing.

## References

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