# **Supporting Information for**

# Critical assessment of enhancement factors measurements in surface-enhanced Raman scattering on different substrates

Daniel C. Rodrigues, Michele L. Souza, Klester S. Souza, Diego P. dos Santos, Gustavo F. S. Andrade and Marcia L. A. Temperini<sup>,</sup>

### 1. Roughening Factor

As can be seen in the cyclic voltammogram (CV) in Figure S1A the Au electrode starts to oxidize at ca. +0.9 V and Au<sup>3+</sup> ions are released to the solution as  $[AuCl_x]^{3-x}$  ions; the potential sweeping is reversed at +1.2 V and the reduction of the gold ions in solution proceeds under potentials less positive than +1.0 V, presenting a well-defined peak at +0.6 V. The disorganized deposition of the Au atoms through the reduction of the ions at the electrode surface leads to a densely packed nanoscaled structures of a wide range of sizes which are associated to the SERS activity.



**Figure S1.** (A) CV of the Au electrode during the roughening process (KCl 0.1 mol L<sup>-1</sup> as electrolyte). (B) CV obtained to estimate the active area of the roughened Au electrode (H<sub>2</sub>SO<sub>4</sub>(aq) 0.5 mol L<sup>-1</sup> as electrolyte). Reference electrode  $Ag_{(s)}|AgCl_{(s)}|KCl_{(sat)}$ .

The electrochemical roughening causes an increase in the active surface area by the creation of nanostructures. One of the most used techniques to evaluate the increase on the

surface area on metallic electrodes consists on a potential scan in a  $H_2SO_4(aq) 0.5 \text{ mol } L^{-1}$ medium in the -0.3 V to +1.5 V range starting from 0 V at a scan rate of 100 mV s<sup>-1</sup>.

The cathodic peak at +0.9 V is assigned to the reduction of H<sup>+</sup>(aq) to H<sub>2</sub>(g) at the Au surface and resulted on a total charge of  $8.80 \times 10^{-5}$  C (hachured area in Figure S1B). In a similar experiment on an Au flat electrode Trasatti and Petrii determined a charge of  $390 \times 10^{-6}$  C cm<sup>-2</sup>.<sup>1</sup> Therefore, the active area ( $A_{active}$ ) of the electrochemically roughened electrode calculated from the CV was 0.22 cm<sup>2</sup>. The geometric area ( $A_{geometric}$ ) of this electrode was 0.12 cm<sup>2</sup>. A measure of the degree of roughening can be obtained as the ratio between active and geometric areas, or roughening factor (R):

$$R = \frac{A_{active}}{A_{geometric}}$$

In the present study R = 1.8, that is, the roughened Au electrode surface area was 180% larger than the estimated geometric area for the Au electrode.

#### 2. SEM images of the AuNT

Figure S2 presents two different regions of the AuNT substrate obtained from PCM  $\Phi$  400 nm. Most of the AuNT are tilted in respect to the gold film and they might present a distinct plasmon response.



Figure S2. SEM images from two different regions of the AuNT  $\Phi$  400 nm.

#### 3. SERS in AuNS in milimolar concentration range

Figure S3 A presents the SERS spectra for 4MPy at the AuNS suspension investigated and table S1 presents the enhancement factors (EF) for the studied AuNS systems. Figure S3 B present the extinction spectra of AuNS containing 4MPy in concentrations ranging from 10 to  $0.1 \times 10^{-3}$  mol L<sup>-1</sup> and KCl  $10 \times 10^{-3}$  mol L<sup>-1</sup>. The maximum extinction band related to disperse AuNS at 528 nm diminishes at the first 5 minutes after mixture the probe to all three concentrations and a broad band emerges in the near-infrared region. The suspensions remain stable after 60 minutes, necessary time to acquire the 350 SERS spectra.



**Figure S3.** (A) Representative SERS spectra of 4MPy in AuNS (bottom trace represents the normal Raman spectrum of 4MPy  $1.0 \times 10^{-2}$  mol L<sup>-1</sup>,  $\lambda_{exc.} = 633$  nm); (B) Extinction spectra of 4MPy in AuNS as a function of time after of mixture. Concentrations: (i)  $1.0 \times 10^{-2}$  mol L<sup>-1</sup>, (ii)  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> and (iii)  $1.0 \times 10^{-4}$  mol L<sup>-1</sup>.

Although the different number of scatterers according to the 4MPy concentration, the calculated EFs shown in table S1 present similar values when considering the standard deviations. This behavior is attributed to the achievement of the maximum surface coverage

even at the  $1.0 \times 10^{-4}$  mol L<sup>-1</sup> concentration evaluated in this study and to the exponential field decay which presents no enhancement on molecule excess in solution.

[4MPy] / 10 <sup>-3</sup> mol L <sup>-1</sup>	EF / 10 <sup>3</sup>	σ / 10 <sup>3</sup>
10	0.79	0.19
1.0	1.1	0.70
0.1	1.4	1.3

**Table S1.** EF values on the basis of the band at 1000 cm<sup>-1</sup> and respective standard-deviations to SERS spectra as a function of 4MPy concentration.

## 4. SEM images of the AuNS aggregation under 4MPy low concentration

Figures S4, S5 and S6 show scanning electron microscopy (SEM) images for the AuNS substrate after aggregation promoted by the addition of 4MPy to a final concentration in nanomolar range (20, 40 and 60 nmol L<sup>-1</sup>, respectively). Several of the observed aggregates in the figures present an linear-like configuration, as it will be discussed in Monte Carlo simulations in section 4 of this SI file.



Figure S4. SEM images for AuNS after addition of 4MPy to a final concentration of 20 nmol L<sup>-1</sup>.



Figure S5. SEM images for AuNS after addition of 4MPy to a final concentration of 40 nmol L<sup>-1</sup>.



Figure S6. SEM images for AuNS after addition of 4MPy to a final concentration of 60 nmol L<sup>-1</sup>.

### 5. Monte Carlo simulations

The aggregation dynamics of the AuNS colloid upon KCl and 4MPy addition was simulated by a simple Monte Carlo simulation. The aggregation occurs due to the decrease in the electrostatic repulsions among the nanospheres in solution, which occurs due to (i) nanosphere surface charge decrease (exchange between stabilizing citrate layer and 4MPy molecule) and (ii) a screened electrostatic potential (addition of the salt to the solution). Such a potential can be qualitatively well described by the DLVO (Derjagun, Landau, Verwey and Overbeek) theory, which can described by the pairwise interaction potential energy (in units of  $k_BT$ ,  $k_B$  = Boltzmann constant and T = Temperature):

$$\frac{V(r;R_1;R_2)}{k_BT} = \frac{e^2 Z^2}{4\pi\varepsilon} \frac{e^{\kappa R_1}}{1+\kappa R_1} \frac{e^{\kappa R_2}}{1+\kappa R_2} \frac{e^{-\kappa r}}{r} - \frac{A_{eff}}{6k_BT} \left[ \frac{2R_1R_2}{r^2 - (R_1 + R_2)^2} + \frac{2R_1R_2}{r^2 - (R_1 - R_2)^2} + \ln\left(\frac{r^2 - (R_1 + R_2)^2}{r^2 - (R_1 - R_2)^2}\right) \right] \quad (Eq.1),$$

where

 $\kappa^2 = \frac{e^2}{\varepsilon k_B T} \sum_{j=1}^N n_j z_j^2$  and  $\sum_{j=1}^N n_j z_j^2$  describe the ionic strength of the medium. The first term in

Eq.1 corresponds to the screened electrostatic potential energy between two spheres of radius  $R_1$  and  $R_2$ , and surface charge Z (taken to be on the order of  $Q_0 = -700$  C, for a sphere of radius 20 nm<sup>2</sup>). Such electrostatic potential energy is dependent upon the salt concentration (ionic strength). The second term in Eq. 1 describes the dispersive attraction potential energy between the two spheres of a material characterized by  $A_{eff}$ , the Hamacker constant, which is of the order of 1.9 eV for Au colloid in water.<sup>3</sup> Figure S7 shows the resultant potential energy between two spheres separated by a distance r, for different values of initial nanosphere surface charge:



Figure S7. Potential energy for varying nanosphere surface charge.

The important qualitative result from Figure S6 is that, as the surface concentration of 4MPy increases the nanosphere surface charge decreases, which promotes a decrease in the potential energy barrier increasing the probability of aggregation. The Monte Carlo simulation was performed by considering such potential energy and that the movement of each of the particles in the simulation domain is constrained by such potential. In another words, the

probability (p) for a particle in a given initial point to move in a given direction to a final point depends on the potential energy difference between the two points, *i.e.*:

$$p = \begin{cases} 1, & \text{if } \Delta V < 0\\ \exp\left(\frac{-\Delta V}{k_B T}\right), & \text{if } \Delta V \ge 0 \end{cases}$$
(Eq. 2)

The results (snapshot) of such a probabilistic simulation is presented in Figure S8 for different initial surface concentrations.



Figure S8. Monte Carlo simulations for the AuNS aggregation dynamics based on the DLVO theory. Each panel in the figure corresponds to a simulation for the process of decreasing the AuNS initial surface charge ( $Q_0$ ) by exchange of the citrate layer by 4MPy molecules. The region marked by a red ellipse was used in the main paper for Generalized Mie Theory simulations.

From Figure S8 it is possible to observe the aggregation dynamics upon the decrease of surface charge, promoted by 4MPy addition to the system. It is important noticing that aggregates assume an almost linear geometry to decrease the potential repulsion; such result was used as a first approximation to study the surface plasmon in the AuNS in the main paper. The marked region (red ellipse) in Figure S8 was used in the main paper as a model to describe the local field enhancement factors for different laser excitations.

- 1. S. Trasatti and O. A. Petrii, *Pure and Applied Chemistry*, 1991, **63**, 711-734.
- 2. M. Meyer, E. C. Le Ru and P. G. Etchegoin, *The Journal of Physical Chemistry B*, 2006, **110**, 6040-6047.

3. E. C. Le Ru and P. G. Etchegoin, *Principles of Surface-Enhanced Raman Spectroscopy: and related plasmonic effects*, Elsevier, Amsterdam, 2009.