

1 **Universal Fabrication of Highly Efficient Plasmonic Thin-Films for Label-Free SERS**  
2 **Detection**

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45 **oxide, layer-by-layer assembly**

1 **Abstract**

2 The development of novel, highly efficient, reliable, and robust surface-enhanced Raman  
3 scattering (SERS) substrates containing a large number of hot spots with programmed size,  
4 geometry and density is extremely interesting since it allows the chemical sensing of numerous  
5 (bio-)chemical species. Herein, an extremely reliable, easy to fabricate and label-free SERS  
6 sensing platform based on metal nanoparticles (NPs) thin-film is developed by the layer-by-  
7 layer growth mediated by polyelectrolytes. A systematic study of the effect of NP composition  
8 and size as well as number of deposition steps on the substrate's performance is accomplished  
9 by monitoring the SERS enhancement of 1-naphtalenethiol, using a 532 nm excitation. Distinct  
10 evidence of the key role played by the interlayer (poly(diallyldimethylammonium chloride)  
11 (PDDA) or PDDA-functionalized graphene oxide (GO@PDDA)) on the overall SERS  
12 efficiency of the plasmonic platforms is provided, revealing in the latter the formation of more  
13 uniform hot spots by regulating the interparticle distances to  $5 \pm 1$  nm. Our SERS platform  
14 efficiency is demonstrated via its high analytical enhancement factor ( $\sim 10^6$ ) and the detection  
15 of a prototypical substance, i.e. drug tamoxifen (TAM), both in Milli-Q water and in a real  
16 matrix, viz. tap water, opening perspectives towards the use of our plasmonic platforms for  
17 future high-performance sensing applications.

## 1 1. Introduction

2 Surface-enhanced Raman scattering (SERS) spectroscopy is an extremely powerful analytical  
3 technique for the highly sensitive, and non-invasive chemical identification of multiple  
4 (bio-)chemical analytes.<sup>[1]</sup> Indeed, SERS (indirect or label-free) allows identification of the  
5 specific spectral fingerprint of probe analytes in close contact with a plasmonic substrate (i.e.  
6 noble metal nanostructures).<sup>[2]</sup> Importantly, SERS offers multiplexing capability, it requires  
7 little or no sample preparation, and it provides high spatial resolution in sample mapping.  
8 Within this context, SERS detection has been applied in several fields such as medical  
9 diagnostics,<sup>[3]</sup> environmental protection,<sup>[4]</sup> and food safety.<sup>[5]</sup> Because of the low Raman cross-  
10 section of many analytes, rational design and engineering of SERS substrates are crucial in  
11 order to collect SERS signals with high sensitivity, tunability, reproducibility and stability.<sup>[6]</sup>  
12 The presence of the so-called “hot spots” (such as junctions or gaps between metal NPs on the  
13 nm scale) in sufficient density on the substrate surface can markedly enhance the sensing  
14 capabilities of any SERS platform, allowing to reach lower limits of detection which is crucial  
15 for many applications.<sup>[7]</sup> In fact, it has been reported that a hot spot can produce enhancements  
16 of SERS intensity up to 15 orders of magnitude (proportional to  $|E|^4$ ) reaching single molecule  
17 detection.<sup>[8]</sup> Such colossal sensitivity enhancement has triggered a major research endeavour  
18 on the fabrication of plasmonic substrates containing a high density of hot spots. Nevertheless,  
19 the controlled fabrication of plasmonic substrates containing hot spots with designed size,  
20 geometry and density over an extensive area is still a challenge in terms of batch-to-batch  
21 reproducibility and large-scale homogeneity. Hot spots can be produced either via the assembly  
22 of nanoparticles in colloidal dispersion<sup>[9]</sup> or by preparing nanostructured surfaces using  
23 lithographic techniques,<sup>[10]</sup> as focused ion beam milling,<sup>[11]</sup> or by depositing nanoparticles onto  
24 solid supports.<sup>[4]</sup> Despite the several advantages of colloidal NPs in solution (i.e. high  
25 reproducibility, cost-effectiveness, or tailored optical properties),<sup>[12]</sup> it is well-known that hot  
26 spots can only be achieved through dynamic and uncontrolled aggregation processes, usually

1 taking place in the presence of the analyte. Aggregation heavily affects and limits the overall  
2 robustness of these SERS sensors in real life applications, since the reproducibility of the  
3 spectra is strongly dependent on the experimental conditions.<sup>[1]</sup> Although nanofabrication  
4 techniques (e.g., lithography-based like focused ion beam milling) allow for large-scale  
5 production, they also present unconvincing disadvantages, such as high cost and low resolution  
6 to reach lengthscale less than 10 nm. For all these reasons, a compelling alternative is  
7 represented by the direct self-assembly of colloidal NPs onto a solid support, towards the  
8 generation of operational devices suitable for commercialization.<sup>[13]</sup> In this framework,  
9 numerous strategies have been explored to achieve the controlled immobilization of NPs on  
10 solid supports, aiming to large surface sensing substrates with high enhancement and  
11 reproducibility.<sup>[14]</sup> Among them, the layer-by-layer (LbL) growth has emerged as one of the  
12 most powerful approaches for the fabrication of nanostructured thin-films<sup>[4]</sup> with tailored  
13 composition, structure, and thickness<sup>[15]</sup> on any surface, regardless of its shape and/or size. The  
14 growth process is ruled by either electrostatic or non-electrostatic interactions (i.e. hydrogen  
15 bonding, metal-ion coordination, host–guest interaction, covalent bonds, and so on).<sup>[16]</sup> In  
16 essence, this technology can be considered highly versatile, simple, and efficient, and as such,  
17 it holds great potential for the fabrication of SERS substrates. Although different components  
18 have been proposed as interlayer for the alternate LbL assembly with metal nanoparticles  
19 including polyelectrolytes,<sup>[17]</sup> macrocycles,<sup>[4]</sup> dendrimers<sup>[18]</sup> and nanosheets,<sup>[19]</sup> poor attention  
20 has been given to the key parameters to boost the SERS performance, such as the NP's  
21 composition, size and shape as well as control of the interparticle distance, being pivotal for the  
22 hot spot optimization. Crozier and co-workers have shown that the SERS enhancement  
23 increases when the interparticle distance in Au NP dimers decreases from 10 to 0.6 nm, while  
24 also a substantial drop is observed as the interparticle distance is reduced down to 0.2 nm, due  
25 to the strong electromagnetic (EM) field confinement.<sup>[20]</sup> Recent developments operating under  
26 strict electrodynamic conditions revealed that the EM field does not follow the predicted

1 behaviour of  $|E|^4 \sim 1/a^4$  (where  $a$  is the interparticle distance) but it satisfies universal behaviour,  
2 yielding an expression  $|E|^4 \sim 1/a^p$  (with  $p \approx 1.2-1.5$ ).<sup>[21]</sup> This discrepancy is associated with the  
3 emergence of electron tunnelling between the particles as the interparticle distance becomes  
4 less than 2 nm.<sup>[20]</sup> Once the interparticle distances reach sub-nm ( $< 1$  nm) size, the classical  
5 electromagnetic approach fails, and the non-local effects, such as the strong EM field  
6 confinement, come into play.<sup>[21]</sup> Hence, the ideal interparticle distance leading to the highest  
7 SERS enhancement should be comprised in the 1-10 nm range. However, while the majority of  
8 the studies on the hot spot engineering have been performed on Au NPs, the scenario is not well  
9 established yet in the case of Ag NPs and on more sophisticated metallic NPs compositions.

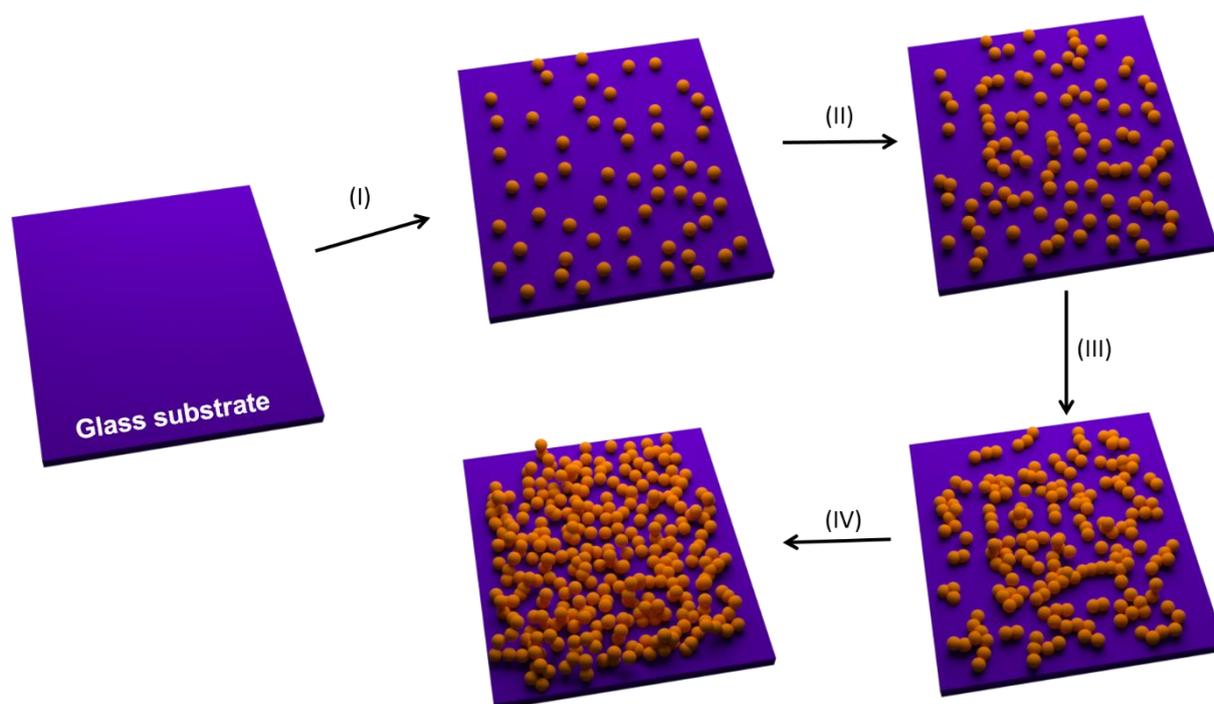
10 Herein, we report the fabrication of reliable, and label-free SERS sensing platforms, constituted  
11 by Ag and Au@Ag core@shell NPs thin-films assembled via the LbL technique, with an  
12 accurate design and engineering of the hot spot size and density. The thin-films supported on  
13 glass supports were manufactured by the alternate deposition of negatively charged (tannic  
14 acid)/citrate coated metal NPs and the positively charged poly(diallyldimethylammonium  
15 chloride) (PDDA) polyelectrolyte, held together by electrostatic interactions. We have studied  
16 the plasmonic response and the morphology (structure and hot spot density) of the films as a  
17 function of the NP composition (by exploring Ag, and Au@Ag core@shell NPs), the NP size  
18 (from 15 to 89 nm), the number of deposition steps (1 to 4) and the influence of an interlayer  
19 such as (poly(diallyldimethylammonium chloride) (PDDA) or PDDA-functionalized graphene  
20 oxide (GO@PDDA). Subsequently, the SERS performances for the different NPs platforms  
21 were investigated with a 532 nm laser line by employing 1-naphthalenethiol (1-NAT) as Raman  
22 reporter as well as other molecules such as methylene blue, methyl orange and dopamine.

23 Finally, the sensitivity of the best SERS platform was analysed for the label free detection of  
24 the antineoplastic drug tamoxifen (TAM) in tap water.

25

26 2. LbL assembly of metal NPs

1 Thin-films of (tannic acid) / citrate-stabilized metal nanospheres<sup>[22]</sup> have been controllably  
2 assembled by the LbL technique via the subsequent deposition of oppositely charged building  
3 blocks, i.e. negatively charged (tannic acid) / citrate -stabilized metal nanospheres and a cationic  
4 polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA).<sup>[4]</sup>  
5 In particular, the metal NPs assemblies have been grown onto glass supports following the  
6 mechanism portrayed in Scheme 1: first, to ensure an optimal distribution of positive charges  
7 on the surfaces, a triple layer of oppositely charged polyelectrolytes was deposited. This was  
8 obtained by immersing activated glass supports in an aqueous polycationic PDDA solution (0.1  
9 mg/mL, NaCl 0.5 M) followed by their immersion in an aqueous polyanionic poly(acrylic acid)  
10 (PAA) solution (0.1 mg/mL, NaCl 0.5 M) and then in PDDA, once again. Subsequently, such  
11 positively charged surfaces were exposed to an aqueous dispersion of negatively charged  
12 (tannic acid) /citrate-stabilized metal NPs (scheme 1, step I). PDDA and (tannic acid) / citrate-  
13 stabilized metal NPs, hereafter PDDA – metal NPs, deposition steps were alternatively repeated  
14 to produce controlled nanoparticle assemblies (scheme 1, step II, III and IV). Four PDDA -  
15 metal NPs deposition steps were performed in total.



1 **Scheme 1.** Schematic representation of assemblies of metal NP fabricated by electrostatic  
2 Layer-by-Layer deposition. Step I consists of a triple layer of oppositely charged  
3 polyelectrolytes (PDDA - PAA - PDDA). Steps II, III and IV correspond to the subsequent  
4 immersion of the substrate in PDDA solution (0.1 mg/mL, NaCl 0.5 M) for 15 min and in an  
5 aqueous solution of metal NPs for 3h.

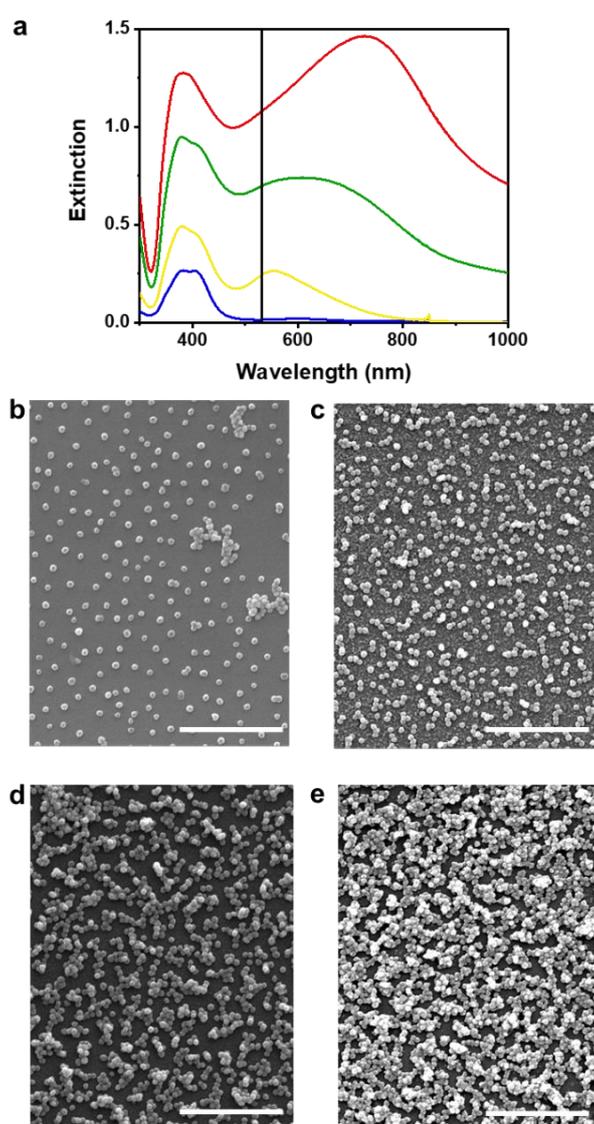
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### 7 **3. Optimization of SERS Substrates**

#### 8 **3.1. Mono-component metal NPs**

9 Despite the inherently higher SERS activity of Ag compared to Au,<sup>[23]</sup> sensing platforms based  
10 on Ag NPs are more unexplored because of their poor ambient stability resulting from their  
11 spontaneous oxidation. Visible–NIR spectroscopy and scanning electron microscopy (SEM)  
12 analyses made it possible to monitor the LbL growth of the PDDA - Ag NPs hybrid. **Figure 1a**  
13 shows the optical properties of PDDA - 37 nm Ag NPs assemblies in air obtained after one,  
14 two, three, and four PDDA - Ag NPs depositions steps on glass. In the case of one deposition  
15 step film, the optical response exhibits a main localized surface plasmon resonance (LSPR)  
16 band centered at 394 nm and a less intense and broader band at 607 nm (Figure 1a). The  
17 presence of isolated Ag NPs on the substrate surface is proved by the former band, which is  
18 blue-shifted with respect to the one typical for Ag colloids in water (407 nm, Figure S1,  
19 Supporting Information) due to the lower average refractive index of air compared to water.  
20 The band at lower energies indicates the presence of a small amount of Ag aggregates, as  
21 confirmed by SEM imaging. As displayed in Figure 1b, the film prepared via one deposition  
22 step mostly exhibits Ag NPs randomly distributed and well separated from each other of a few  
23 tens of nanometers, although a small percentage of dimers, trimers, and tetramers is also  
24 observed. When a second deposition step was accomplished, the optical response showed a  
25 broadening in the LSPR coupling band (> 730 nm), along with an increase in extinction (Figure  
26 1a). The optical response indicates that a larger number of particles are deposited on the surface,

1 resulting in the formation of Ag NPs assemblies causing an intense plasmon coupling among  
2 particles, and therefore hot spots. The greater interparticle interactions upon increased  
3 deposition steps, as observed by optical spectroscopy, is confirmed by SEM imaging (Figure  
4 1b-e). The same LbL protocol to create 37 nm Ag NP films has been successfully exploited to  
5 assemble Ag NPs with different diameters (i.e. 15, 23 and 56 nm) with the alternate deposition  
6 of PDDA and tannic acid /citrate-stabilized Ag NPs, leading to a similar variation of the optical  
7 properties with the increased number of deposition steps (Figure S4, Supporting Information).



8  
9 **Figure 1.** PDDA- 37 nm Ag NPs assemblies on activated glass: a) Visible–NIR extinction  
10 spectra recorded in air after different deposition steps: one (blue), two (yellow), three (green),  
11 and four (red). The black line highlights the position of the 532 nm laser line. b-e)

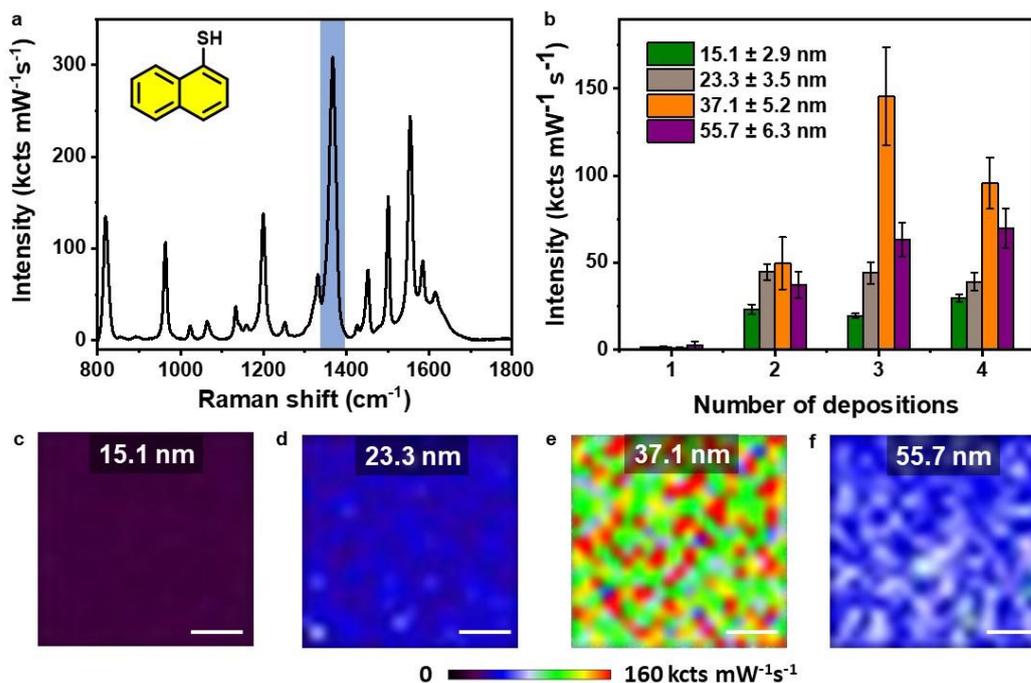
1 Representative SEM images obtained after b) one, c) two, d) three, and e) four deposition steps.  
2 Lateral scale bars = 1  $\mu\text{m}$ .

3

4 To gain insight into the SERS performance of this new type of plasmonic substrates, we have  
5 studied the overall efficiency in terms of the number of PDDA - Ag NPs deposition steps and  
6 Ag nanoparticle size. For the SERS analysis, 1-naphthalenethiol (1-NAT) was chosen as a  
7 standard Raman reporter molecule (**Figure 2a**)<sup>[24]</sup> Prior to use, Ag NPs films were immersed  
8 for 30 min in a 50 mM NaBH<sub>4</sub> aqueous solution to remove tannic acid and citrate stabilizing  
9 agents. Figure S5 displays the SERS spectrum of the pristine 37 nm Ag NPs substrate after four  
10 deposition steps and exposure to NaBH<sub>4</sub> (black spectra) and the Raman spectra of 1-NAT (red  
11 spectra). As clearly shown, the SERS fingerprint of the plasmonic substrate does not overlap  
12 with the main characteristic peaks of 1-NAT, making it an ideal Raman reporter molecule for  
13 our SERS platform. To record the SERS spectra of 1-NAT, the plasmonic thin-films were  
14 immersed in a 10<sup>-5</sup> M 1-NAT aqueous solution for 3 h and then dried under a gentle stream of  
15 N<sub>2</sub>. Figure 2a shows a representative SERS spectrum. It revealed the characteristic signals of  
16 chemisorbed 1-NAT molecules (Table S2), including the C–H bending (1195 cm<sup>-1</sup>), ring  
17 stretching (1367, 1500, and 1553 cm<sup>-1</sup>), and ring breathing (818 and 964 cm<sup>-1</sup>).<sup>[24-25]</sup> We focused  
18 our attention on the ring stretching of 1-NAT at 1367 cm<sup>-1</sup>, which has been mapped over  
19 extended areas (typically, 40 x 40  $\mu\text{m}^2$  with 2  $\mu\text{m}$  step size). Firstly, we plotted the average  
20 SERS intensity (441 points in total) at 1367 cm<sup>-1</sup> (Figure 2b) as a function of deposition steps  
21 and Ag NPs size in order to evaluate the efficiency of the different plasmonic substrates. To  
22 acquire a meaningful statistic, our mappings were recorded on three different PDDA – Ag NPs  
23 plasmonic substrates, which exhibited similar intensities. Interestingly, a marked increase of  
24 the signal intensity has been observed by moving from the first to the second deposition step,  
25 independently of the Ag NPs size. This was predicted from the optical properties (Figure 1a-b  
26 and S4, Supporting Information), revealing that the one deposition substrate shows a lack of

1 plasmonic coupling deriving from an interparticle distance of few tens of nanometers. The third  
2 deposition step shows a further improvement of SERS signal in the case of 37 and 56 nm Ag  
3 NPs assemblies, whereas it remains nearly constant when smaller Ag NPs (viz. 15 and 23 nm)  
4 are used, presumably due to the mismatch between the excitation wavelength (532 nm) and the  
5 LSPR coupling band in the latter cases. This result is in agreement with the electromagnetic  
6 mechanism, which predicts the highest SERS intensity when the  $LSPR_{max}$  is located between  
7 excitation and vibrational wavelengths,  $\lambda_{ex}$  and  $\lambda_{vib}$ , respectively.<sup>[26]</sup> Nevertheless, a plateau or  
8 a clear worsening of the signal is recorded for the fourth deposition step, independently of the  
9 NPs size. This trend is the direct effect of the stepwise increase of NPs density onto our substrate,  
10 featuring a poor control of the NPs positioning. As a result, the SERS efficiency reaches a  
11 maximum and then, an overall worsening which stems from the hot spot deactivation due to  
12 symmetry effects and differences in plasmonic coupling strength.<sup>[4, 27]</sup> A similar hot spot  
13 deactivation has been previously reported for the LbL assembly of Au nanospheres mediated  
14 by pillar[5]arene<sup>[4, 28]</sup> and for self-assembled Au nanostars on polystyrene beads,<sup>[29]</sup> where the  
15 random distribution of NPs leads to an initial increase of the SERS intensity proportional to the  
16 amount of NPs and then to an overall decrease due to the excessive and non-controllable  
17 plasmon coupling. 37 nm Ag NPs exhibited enhanced SERS efficiency compared to 56 nm  
18 ones, since, as the nanoparticles size increases, the convex shape of the surface becomes flatter,  
19 so that less inelastic scattering can occur on it, leading to a weaker electromagnetic field on the  
20 surface and a lower overall SERS intensity.<sup>[30]</sup> Finally, figure 2d-f and Figures S6-7 reveal that  
21 the intensity maps related to 1-4 deposition steps are highly uniform and homogeneous  
22 throughout the sample area, independently of the NPs size (Figure 2d-f) and number of  
23 depositions (Figures S6-7, Supporting Information). Overall, 3 deposition steps of 37 nm Ag  
24 NPs turned out being the best performing SERS platform.

25



1  
 2 **Figure 2.** a) Representative SERS spectrum of 1-NAT, whose molecular structure is shown in  
 3 the inset. b) Average intensities of the ring stretching band ( $1367\text{ cm}^{-1}$ ) as a function of PDDA-  
 4 Ag NPs deposition steps for four Ag NPs sizes. All SERS measurements were carried out with  
 5 a  $100\times$  objective and a maximum power of  $0.02\text{ mW}$  for the  $532\text{ nm}$  laser line. The acquisition  
 6 time was  $1\text{ s}$ . c-f) SERS mappings obtained at  $1367\text{ cm}^{-1}$  after three deposition steps of PDDA-  
 7 Ag NPs as a function of Ag NPs size. Lateral scale bars =  $10\text{ }\mu\text{m}$ .

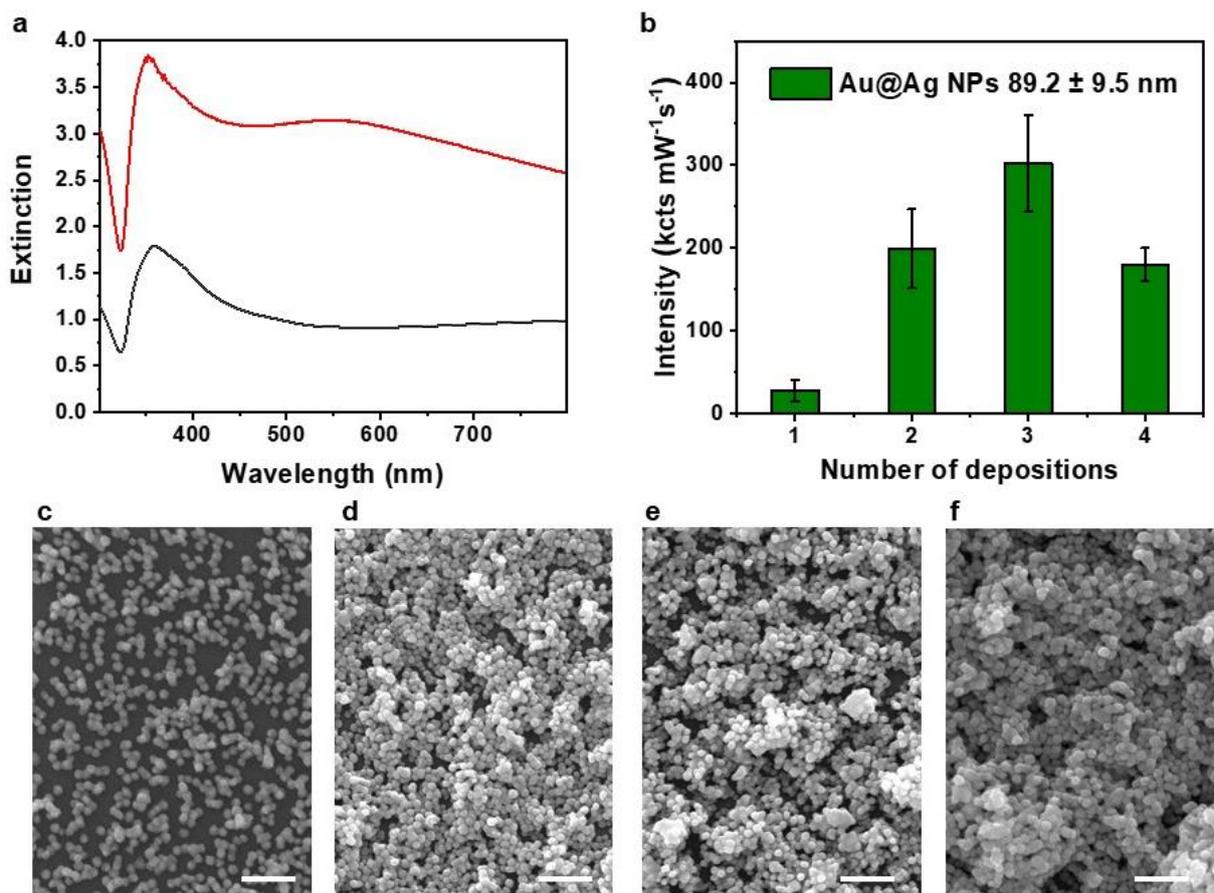
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### 9 3.2. Core@shell metal NPs

10 The metal composition of the NPs is a key parameter affecting the SERS efficiency.<sup>[31]</sup> A  
 11 representative example is the one of bimetallic NPs with a core@shell structure which provides  
 12 richer plasmonic modes, thanks to the combined material- and size/shape-dependent  
 13 plasmonics.<sup>[32]</sup> Bimetallic core@shell NPs have been the subject of SERS studies in solution as  
 14 a function of the size of both core and shell and also excitation wavelength.<sup>[22c]</sup> Samal and co-  
 15 workers have shown that the SERS efficiency of the Au@Ag NPs in solution increased with  
 16 size.<sup>[22c]</sup> To the best of our knowledge, a similar study on the SERS efficiency as a function of  
 17 the core and shell size has never been performed in thin-film of self-assembled Au@Ag NPs.

1 Here we investigated the SERS effect as a function of the NPs composition using 57 nm  
2 Au@Ag NPs (Au core of 30 nm and 13 nm Ag shell) (Figure S8, Supporting Information) and  
3 89 nm Au@Ag NPs (Au core of 50 nm and 20 nm Ag shell) (Figure 3 and S9), being the same  
4 size of the hybrid NPs reported by Samal et al,<sup>[22c]</sup> allowing us to compare results in aqueous  
5 solution with those obtained in thin solid films. Interestingly, our study of the SERS efficiency  
6 on the two different NP sizes as a function of deposition steps revealed the same behavior of  
7 the Ag NPs, i.e. the best signal was obtained at the third deposition step (Figure 3b and Figure  
8 S8b, Supporting Information) and hot spot deactivation occurs when a fourth deposition step is  
9 performed. Concerning the effect of the core@shell NPs size, we observed the same trend  
10 previously reported for colloidal systems. In particular, PDDA - 57 nm Au@Ag NPs (Au core  
11 of 30 nm and 13 nm Ag shell) assembly exhibits a LSPR maximum (780 nm) highly misaligned  
12 with the excitation wavelength, i.e. 532 nm (Figure S8), leading to a worse SERS efficiency.  
13 On the contrary, the PDDA – 89 nm Au@Ag NPs assembly displays a broad and blue-shifted  
14 extinction (LSPR maximum at 550 nm), which yields a stronger electromagnetic coupling with  
15 the excitation laser (Figure 3a and S8).

16



1  
2 **Figure 3.** a) Visible–NIR extinction spectra recorded in air after different 89 nm Au@Ag NPs  
3 deposition steps: one (black spectrum), and two (red). The extinction spectra of the three and  
4 four depositions substrates were not included due to saturation. b) Average intensities of the 1-  
5 NAT ring stretching band ( $1367\text{ cm}^{-1}$ ) as a function of PDDA-Au@Ag NPs depositions steps.  
6 All SERS measurements were carried out with a  $100\times$  objective and a maximum power of 0.02  
7 mW for the 532 nm laser line. The acquisition time was 1 s. c-f) Representative SEM images  
8 of 89 nm PDDA-Au@Ag NPs assemblies on activated glass obtained after: c) one, d) two, e)  
9 three, and f) four PDDA-Au@Ag NPs deposition steps. Scale bars = 500 nm.

10

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### 12 3.3. Interlayer effect

13 As aforementioned, the ideal interparticle distance leading to the highest SERS enhancement  
14 should be comprised in the range 1 - 10 nm, which can be hardly achieved when using a mono-

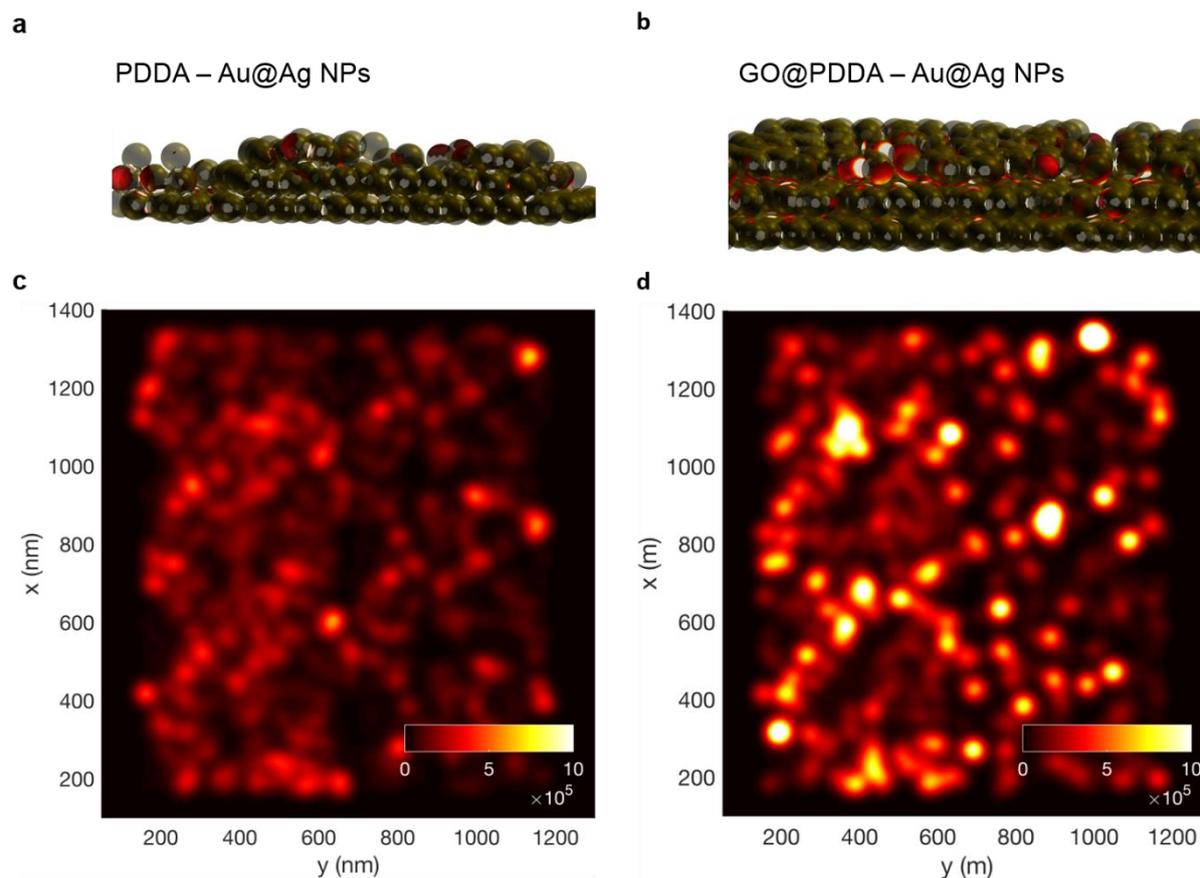
1 component PDDA film as interlayer, being the van der Waals cross-section of the  
2 macromolecule  $\sim 0.5$  nm, when it adopts a stretched conformation. We propose a  
3 straightforward protocol to modulate the interparticle distance by varying the interlayer nature.  
4 In the present case, graphene oxide (GO) (thickness of  $\sim 0.8$  nm) was chosen as interlayer, not  
5 only to act as a more controlled spacer but also because of its inherent SERS effect. However,  
6 the anionic nature of GO makes it unsuitable to be used as a building block for the negatively  
7 charged citrate Au@Ag NPs assembly. For this reason, GO was previously functionalized with  
8 a PDDA layer (GO@PDDA) (See experimental details in the Supporting Information), leading  
9 to an overall thickness of  $5 \pm 1$  nm which falls in the ideal interparticle distance range. The  
10 previously used LbL protocol was employed for the fabrication of 89 nm Au@Ag NPs  
11 assemblies by using GO@PDDA as interlayer (Figure S10, Supporting Information) but  
12 exposing the plasmonic substrates to the GO@PDDA for 3 hours instead of 15 min as in the  
13 case of PDDA due to the slower kinetics of attachment of GO@PDDA (data not shown). Firstly,  
14 Figure S11 reveals that the intensity maps related to 1-4 deposition steps are highly uniform  
15 and homogeneous throughout the sample area, independently of the NPs size, number of  
16 depositions and nature of the interlayer. Interestingly, in the case of GO@PDDA, when a fourth  
17 deposition is performed, a further SERS enhancement is clearly observed for GO@PDDA – 89  
18 nm Au@Ag NPs while not for PDDA – 89 nm Au@Ag NPs, despite these two systems show  
19 identical extinction spectra (Figure S12). The different behavior when GO is used may have  
20 two different explanations. It is well-known that GO, and more generally 2D materials, exhibit  
21 SERS effect.<sup>[33]</sup> As they support plasmons only in the terahertz region, the enhancement  
22 mechanism relies on chemical factors such as charge transfer or dipole-dipole formation.  
23 Within this context, the proper combination of metal nanoparticles and 2D materials had already  
24 led to excellent systems featuring unprecedented SERS sensitivity.<sup>[34]</sup> In addition to the  
25 contribution of GO to the overall SERS enhancement, the different trend of the SERS efficiency  
26 with the number of deposition steps can also be ascribed to GO@PDDA ensuring a better

1 control over the NPs positioning (more control over the interparticle distance) during the  
2 deposition steps with respect to the simple PDDA, directly influencing the plasmonic coupling.  
3 We have therefore performed two set of experiments to assess the interlayer distance in the  
4 presence of GO@PDDA: the former relies on Raman spectroscopy in order to determine the  
5 number of GO layers enveloped by PDDA, and the latter consists of using electron tomography  
6 for imaging the particles and their inter-distance. Figure S13a shows a representative Raman  
7 spectrum of GO, obtained after the immersion of a glass substrate in a diluted GO@PDDA  
8 solution overnight. A 10-fold dilution of the 0.04 mg/mL GO@PDDA solution was performed  
9 in order to isolate GO@PDDA flakes for Raman analysis. The detailed experimental procedure  
10 is reported in the Supporting Information. The Raman spectrum of GO exhibits the 2D and the  
11 overtone of the G+D peaks.<sup>[35]</sup> A distribution histogram indicates the position of the 2D peak  
12 for 5 isolated GO flakes, extrapolated from Raman mappings over the whole area of the flakes  
13 (Figure S13b). The peak position of the 2D band can be correlated with the number of GO  
14 layers: the spectral feature at 2698 and 2680  $\text{cm}^{-1}$  can be ascribed to 3 and 5 layers in thickness,  
15 respectively.<sup>[36]</sup>

16 Electron tomography was performed on a hybrid system consisting of GO@PDDA  
17 functionalized with Au@Ag NPs in a colloidal solution. The detailed experimental procedure  
18 is reported in the Supporting Information. The thickness of GO@PDDA interlayer was  
19 measured from the 3D reconstruction shown in Figure S14a-e, in the area highlighted in Figure  
20 S14f. The thickness was found to range between 3 and 7 nm. These values indicate a film  
21 thickness ranging between 3.4 and 5 nm, taking into account a thickness of GO and PDDA  
22 amounting to 0.8 nm and 0.5 nm, respectively. Overall, both electron tomography and Raman  
23 spectroscopy showed that the average interparticle distance amounts to  $5 \pm 1$  nm.

24 To provide strong support to our experimental evidence, plasmon-driven SERS intensities of  
25 the proposed platforms have been simulated as the product of near electric-field intensity  
26 enhancement produced upon normal irradiation with the excitation laser light and the

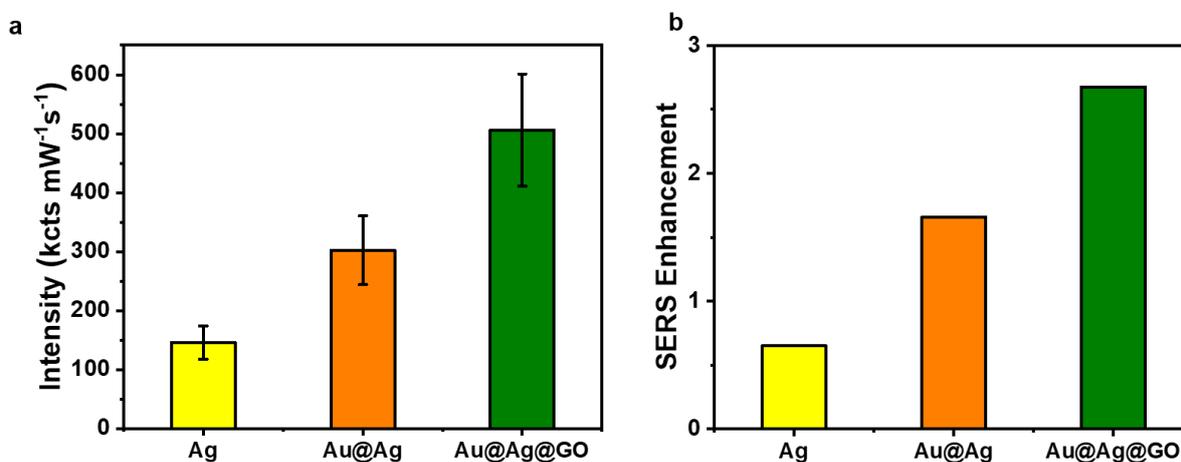
1 inelastically emitted Raman signal.<sup>[7c]</sup> This method allows the simulation of the SERS  
 2 enhancement as a function of the interparticle distance, nanoparticles composition (the different  
 3 metals are described through their frequency-dependent complex permittivity, taken from the  
 4 optical measurements) and density on the substrate surface (Figure 4 and S15-17).<sup>[37]</sup>  
 5



6  
 7 **Figure 4.** a-b) SERS enhancement distribution, and c-d) filtered SERS of Au@Ag NPs  
 8 assemblies as a function of the adhesive layer being a,c) PDDA, and b,d) GO@PDDA.

9  
 10 **Figure 4** clearly show the interlayer dependence NPs positioning, where controlled interparticle  
 11 distances of 5 nm has led to a more efficient system, containing a higher number of hot spots  
 12 which are pivotal for ultrasensitive SERS detection. The results obtained in this work varying  
 13 the nature of the interlayer (PDDA vs GO@PDDA) showed that the SERS enhancement  
 14 follows the same trend previously reported for NPs dimers by increasing the interparticle  
 15 distance from 0.5 to 5 nm. All these findings highlight the importance of a systematic study of

1 the SERS performance in terms of NPs size, composition, number of deposition steps and  
 2 interparticle distance to maximize the size, geometry and density of the hot spots in a reliable  
 3 manner.



4  
 5 **Figure 5.** a) Average intensities of the 1-NAT ring stretching (1367cm<sup>-1</sup>) as a function of metal  
 6 NPs composition; PDDA - 37 Ag NPs (yellow), PDDA – 89 nm Au@Ag NPs (orange) and  
 7 GO@PDDA – 89 nm Au@Ag NPs (green). Note that for PDDA - 37 Ag NPs and PDDA – 89  
 8 nm Au@Ag NPs the average intensity corresponds to three deposition steps, while, in the case  
 9 of GO@PDDA – 89 nm Au@Ag NPs, average intensity corresponds to four deposition steps.  
 10 All SERS measurements were carried out with a 100× objective and a maximum power of 0.02  
 11 mW for the 532 nm laser line. The acquisition time was 1 s. b) Simulated average SERS  
 12 enhancement as a function of metal NPs composition (see the Supporting Information for  
 13 details).

14  
 15 **Figure 5a-b** compares the experimental data acquired (441 points in total) at 1367 cm<sup>-1</sup> with  
 16 the theoretical ones stemming from the average SERS enhancement corresponding to the third  
 17 deposition step for PDDA - 37 Ag NPs and PDDA – 89 nm Au@Ag NPs or fourth deposition  
 18 step for the GO@PDDA – 89 nm Au@Ag NPs. Alongside, calculated SERS mappings  
 19 depicting the weighted SERS for the different nanoparticle composition systems is displayed in

1 Figure S17. The use of GO@PDDA to modulate the assembly of 89 nm Au@Ag NPs has  
2 resulted in an enhancement in the intensity of a factor of 1.7 or 3.5 for PDDA – 89 nm Au@Ag  
3 NPs or PDDA – 37 nm Ag NPs, respectively. The SERS performance was finally quantified  
4 for GO@PDDA – 89 nm Au@Ag NPs thin-films estimating the analytical enhancement factor  
5 (AEF) which can be defined as:

$$6 \quad AEF = \frac{I_{SERS}/C_{SERS}}{I_{Raman}/C_{Raman}} \quad (1)$$

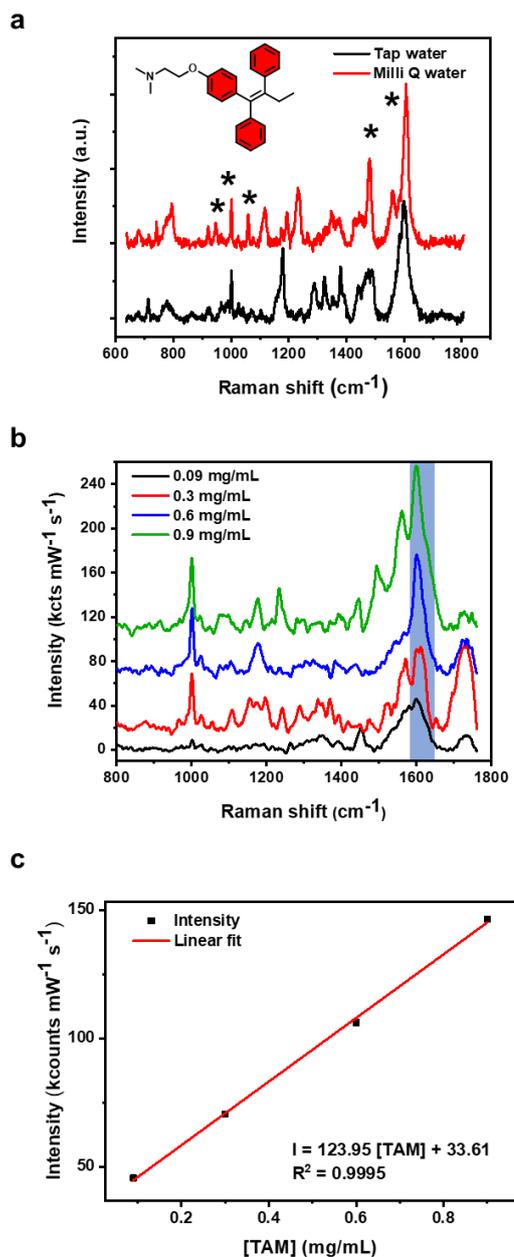
7  
8 with  $I_{SERS}$  being the SERS intensity at  $1367 \text{ cm}^{-1}$  of 1-NAT with a concentration  $C_{SERS}$  and  
9  $I_{Raman}$  Raman intensity of 1-NAT with a concentration of  $C_{Raman}$  both recorded under identical  
10 experimental conditions (laser wavelength, laser power, microscope objective, spectrometer,  
11 etc.). In this particular case, the AEF obtained was  $\sim 10^6$ , in good agreement with AEFs reported  
12 for other metal NPs platforms.<sup>[38]</sup> Finally, 2D materials may play an additional role on the  
13 fabrication of hybrid SERS plasmonic substrates. Graphene and other 2D materials have been  
14 recently reported as a protective barrier against oxidation and corrosion processes.<sup>[34, 39]</sup>  
15 Oxidation processes, especially for Ag NPs, inevitably affect the long-term stability of SERS  
16 substrates, which is a commonly neglected but critical problem for practical applications. We  
17 have evaluated the stability of 89 nm -Au@Ag NPs assemblies when combined with both type  
18 of interlayers, i.e. PDDA or GO@PDDA (Figure S18) for 60 days. As can be seen in Figure  
19 S12, the NPs oxidation is drastically reduced for the substrates fabricated when using  
20 GO@PDDA, as the optical properties remain practically constant. On the contrary, in the case  
21 of PDDA the extinction is drastically reduced, as a consequence of the oxidation of Au@Ag  
22 NPs. Hence, the combination of plasmonic substrates with 2D materials not only boosted its  
23 SERS performance but also reinforced its long-term stability, being both characteristics of high  
24 interest for real life practical applications.

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#### 4. Sensing capabilities

Our stepwise approach to the fabrication of an efficient label-free SERS platform allows the identification of several analytes through their own Raman spectrum. Such label-free sensors can find also application in the identification and differentiation of analytes in complex matrices (i.e. tap water or biological fluids), using complex statistical models for unknown compositions.<sup>[1]</sup> In this work, we show the sensing capabilities of our best performing label-free SERS platform (*viz.* GO@PDDA- 89 nm Au@Ag NPs) for the detection of different molecules. In particular, in Figure S14 we display the comparison between the SERS and Raman spectra of methylene blue, methyl orange and dopamine. The most significant and typical bands for these target molecules are clearly visible and enhanced in the SERS spectra, while most of them are not clearly discernible in the Raman spectra. Furthermore, the N-Ag stretching band is also visible at  $\sim 237\text{ cm}^{-1}$ , suggesting that the adsorption of those molecules occurs via N.<sup>[40]</sup> The Tables S3-5 report a detailed vibrational band assignment for methylene blue, methyl orange and dopamine. Furthermore, we challenge our label-free SERS platform practical applicability to track tamoxifen (TAM), a well-known anticancer drug that can be used against estrogen receptor-positive breast cancer cells.<sup>[41]</sup> It has been demonstrated that many cytotoxic drugs are present in hospital effluents as well as in aquifer.<sup>[42]</sup> Furthermore, TAM can lead to corneal toxicity<sup>[43]</sup> and hence its accurate and fast detection is of high importance in terms of diagnosis.



1  
2 **Figure 6.** a) Representative SERS spectrum of 0.9 mg/mL tamoxifen in Milli Q and tap water.  
3 The acquisition time was 10 s. The main peaks are highlighted with a star. b) SERS TAM  
4 spectra at different concentrations. All the spectra were acquired after drop casting of 5  $\mu\text{L}$  of  
5 the TAM solution in Milli Q water. All the SERS measurements were carried out with a 100 $\times$   
6 objective and a maximum power of 0.02 mW for the 532 nm laser line. The acquisition time  
7 was 1 s and 20 accumulations were collected. c) SERS intensity at 1611  $\text{cm}^{-1}$  as a function of  
8 tamoxifen concentration.

9

1 Thus, as a proof-of-concept, we investigated its detection both in Milli Q and tap water by drop  
2 casting 20  $\mu$ l of TAM aqueous samples (0.9 mg/mL) on our best performing SERS platform  
3 (viz. GO@PDDA- 89 nm Au@Ag NPs). The SERS recording has been performed after air-  
4 drying (**Figure 6a**). Table S6 shows a detailed vibrational band assignment for TAM. Figure  
5 6a displays a representative vibrational spectrum acquired for TAM in Milli Q (red spectrum)  
6 and tap water (black spectrum). Interestingly, the characteristic TAM peaks were clearly  
7 distinguished and compared in the two samples. It was found that the SERS intensities were  
8 similar, suggesting no matrix interferences. In other words, our platform can be used as  
9 disposable and single shot selective sensor. In fact, our device is capable to detect the  
10 spectroscopic TAM fingerprints in a real matrix like tap water with a sensitivity in the ppm  
11 range, thus complying with the state of the art of plasmonic sensors and ultimately matching  
12 the nowadays commercial requirements. To demonstrate the sensing capabilities of such  
13 plasmonic substrate, we investigated the limit of detection (LoD) and the sensitivity towards  
14 TAM. It was performed by drop-casting (TAM volume droplet corresponding to 5  $\mu$ L) on the  
15 plasmonic substrate at different concentrations (0.09 – 0.9 mg/mL) and recording their SERS  
16 spectra. A linear variation of the SERS signal is observed as a function of the analyte  
17 concentration (Figure 6b-c). The SERS measurements showed the feasibility to achieve a  
18 quantitative TAM detection within the above-mentioned range of concentrations. The final  
19 sensitivity corresponds to 123.95 mL/mg and a linear response has been defined by the  
20 following formula:  $I = 123.95 [\text{TAM}] + 33.61$  ( $R^2 = 0.9995$ ), where I is the SERS intensity (in  
21 kcounts/mW s) and [TAM] is the TAM concentration (in mg/mL). The LoD of such sensing  
22 platform has been calculated as  $3.3 \times 10^{-2}$  mg/mL as:

$$23 \quad LoD = \frac{(3 SD)}{\sigma} \quad (2)$$

24 where SD is the standard error of the regression and  $\sigma$  is the sensitivity. To the best of our  
25 knowledge, there is only one published paper showing the SERS detection of TAM, where the

1 lowest detected concentration is  $3.7 \times 10^{-1}$  mg/mL.<sup>[38]</sup> The detection of cytostatic drugs such as  
2 TAM in water or sewage effluent and surface waters is usually based on chromatographic  
3 methods associated to mass spectrometry, as shown in Table S1. Despite the extremely low  
4 LoD up to  $\sim 10^{-10}$  mg/mL in the case of TAM,<sup>[44]</sup> these sophisticated methods are standard bench  
5 analysis, which cannot be exploited for in-field analysis. Furthermore, these techniques show  
6 low response time, they are based on expensive equipments, and they require specialized  
7 personnel. Conversely, SERS spectroscopy can be adapted for the unequivocal, rapid and on-  
8 site analyte detection

## 10 **5. Conclusion**

11 In summary, we have proposed a straightforward fabrication protocol for reliable and label-free  
12 SERS platforms based on the electrostatic LbL assembly of metal nanoparticles and  
13 polyelectrolytes, with an accurate design and engineering of the hot spot size and density. The  
14 plasmonic performance was optimized based on the composition of metal NPs (single vs  
15 core@shell NPs), size of NPs (15-89 nm), number of deposition steps (1-4) and interlayer  
16 (PDDA vs GO@PDDA). The analysis of the SERS performance of the different platforms  
17 revealed that core@shell nanostructures (89 nm Au@Ag NPs), which have not been studied  
18 before as thin-films, exhibited a better performance compared to mono-component metal NPs  
19 (vs 37 nm Ag NPs) due to their inherent unique plasmonic modes. In addition, the use of  
20 GO@PDDA as building block ensured the formation of more uniform hot spots by regulating  
21 the interparticle distances to  $5 \pm 1$  nm, leading to an increase in the overall SERS efficiency and  
22 a robust shelf-life (viz. at least 60 days). Importantly, all the experimental findings are in  
23 accordance with the simulated SERS enhancement. Interestingly, the AEF obtained for  
24 GO@PDDA- 89 nm Au@Ag NPs thin-films was  $\sim 10^6$ , in good accordance with AEF reported  
25 for other metal NPs platforms. The high performance of this plasmonic platform, as a label-free  
26 SERS substrate, has been successfully tested by comparing the SERS and Raman spectra of

1 methylene blue, methyl orange and dopamine in Milli-Q water. A linear variation of the TAM  
2 signal is observed as a function of its concentration in the range 0.09 – 0.9 mg/mL and its LoD  
3 was calculated as  $3 \times 10^{-2}$  mg/mL in Milli Q water. Furthermore, our SERS platform succeeded  
4 to detect TAM even in tap water, as a result of the excellent capability of this sensor. Finally,  
5 such results meet the stringent requirements of in-field sensing applications, making this  
6 technology a promising route to be explored towards the development of novel SERS sensors.

7  
8

### 9 **Supporting Information**

10 Supporting Information is available from the Wiley Online Library or from the author.

11

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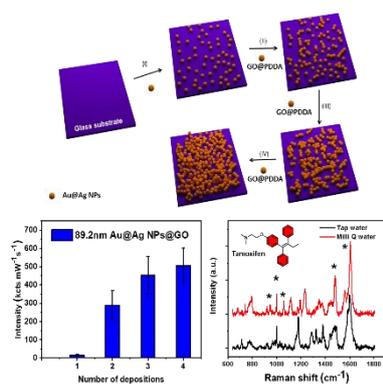
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1  
 2 A reliable, easy to fabricate and label-free surface-enhanced Raman scattering sensing  
 3 platforms is realized, constituted by Au@Ag core@shell NPs thin-films assembled via the  
 4 layer-by-layer technique, with an accurate design and engineering of the hot spot size and  
 5 density. The use of graphene oxide functionalized with polyelectrolytes allows to regulate the  
 6 interparticle distances to  $5 \pm 1$  nm, leading to an increased overall SERS efficiency and a robust  
 7 shelf-life.

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