Robust, Reproducible, Recyclable SERS Substrates: monolayers of Gold NanoStars grafted on glass and coated with a thin silica layer.

B. Bassi,[1] B. Albini,[2] A. D'Agostino,[1] G. Dacarro,[1] P. Pallavicini,[1] P. Galinetto,[2*] and A. Taglietti[1*]

1) Dipartimento di Chimica, Sezione di Chimica Generale, Università di Pavia, viale Taramelli, 12 - 27100 Pavia – Italy;
2) Dipartimento di Fisica "A.Volta", Università di Pavia, Via Bassi 6, - 27100 Pavia – Italy;
Corresponding Author: * E-mail pietro.galinetto@unipv.it; angelo.taglietti@unipv.it;

Abstract. We prepared and characterized recyclable Surface Enhanced Raman Spectroscopy (SERS) active glass chips. Gold nanostars (GNS) were grafted on properly functionalized glasses by means of electrostatic interactions and then they were coated with a silica layer of controllable thickness in the nanometers range. The SERS activity of the obtained substrates were tested in terms of reproducibility and homogeneity intra-samples and inter-samples from different batches using as Raman reporter the model compound Rhodamine 6G. The uncoated substrates were used as reference to evaluate the effect of silica spacers on SERS enhancement factors. The chemical route to obtain silica coated SERS chips is described in details, and the morphology and the optical response of substrates have been characterized. We demonstrate that SERS substrates coated with 1 nm silica conserve a good Enhancement Factor, and that the coating confer to the SERS platform an extreme robustness leading to reusability of the substrates.

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Introduction

Surface Enhanced Raman Scattering (SERS) spectroscopy is nowadays a very diffuse analytical tool for surface chemical analysis. In SERS techniques, the intrinsic ability of Raman spectroscopy to recognize a molecule exploiting its vibrational fingerprint is coupled with the strong enhancement of signal intensity: the overall result is that SERS effects allow a rare combination of

sensitivity and specificity using a non-contact method, leading to several successful applications in a variety of fields, including electrochemistry, catalysis, biology, medicine, art conservation, materials science, [1] and in particular in biochemical optical sensing.[2,3]

The currently accepted theories state that the enhancement of Raman signal of a molecule observed close to noble metal nanostructured surfaces in SERS experiments arises by the combination of electromagnetic (EME) and chemical (CE) effects. [4-6] In SERS usually EME has the dominant role in boosting of Raman signal and it occurs as a consequence of the amplification of the incident and Raman scattered field close to the nano-objects surface due to Localized Surface Plasmon Resonance (LSPR) phenomena typical of noble metal nanoparticles (NP). Contribution from CE don't exceed usually a factor of 10^2-10^3 and it derives from charge transfer mechanisms activated by the proximity of molecule orbitals to metal nano-objects.

The specific frequency of LSPR absorption bands is a variable depending from the metal conductivity, the dielectric properties of the surrounding medium and from NP morphology (size and shape), with anisotropic particles usually showing red-shifted LSPR and even leading to near IR absorption. [7] NP architecture, thus, is a key element to efficiently confine the EM fields and then to control SERS enhancement. [8]

The whole pathway to optimal SERS substrate must be tuned to obtain the best compromise between different requirements [9-10]. Briefly, the substrate should i) have high SERS activity and therefore high sensitivity; ii) be uniform i.e. give the same EF on the whole surface of the sample; iii) have good chemical and mechanical stability with a stable Enhancement Factor (EF) even after a long shelf time; iv) have a good reproducibility of EF for different batches of substrates prepared by the same method. At this list two crucial constraints must be added to fulfill the roadmap to widespread commercial usage of SERS substrates: large scale applicability of a low cost synthetic pathway and reusability. Although the constantly increasing number of studies on SERS substrates, it's hard to find examples meeting all these criteria.

In the last decade, the efforts for increasing EF factors opened the way to the synthesis of asymmetric, branched, shaped metal nano objects. [11-12] SERS boosting decays rapidly with distance and, assuming a spherical model for the nano-object, few nanometers are enough to quench the enhancement [4] even if some authors [13] observed an unexpected persistence of SERS effect beyond tens of nanometers. Anyway it has been diffusely demonstrated [6,12] that EF increases markedly when SERS is probed in close proximity of sharp edges and tips of anisotropic NP (the so called "lightning rod effect"), or in small spaces comprised between two protruding edges of nano-objects. These confined spatial zones are called "hot spots": in general this definition includes highly localized regions formed within the interstitial spaces present in metallic nanostructures, where, as it is claimed, SERS response can reach up to 10^{12-13} compared to normal Raman signal, as a result of field confinement in narrow spatial regions.[14, 15] A possible drawback of "hot spots" strategy resides in its intrinsic morphology-dependent nature leading often to a non-homogeneous SERS response over a large surface area.[16]

Among anisotropic structures with high EF, gold nanostars (GNS) have emerged as excellent

SERS active NP, as their branches and terminal tips usually provide huge field enhancement and "hot spots" [17-19] showing higher SERS response when compared to spherical or other anisotropic structures such as nanorods and nanocubes.[20]

A homogeneous and dense organization of branched objects on flat, bulk surface suggests the presence of a conspicuous number of edges, tips and hot spots, a feature which is necessary to ensure high EF and consequent high SERS sensitivity, organized on a sensing chip of easy use. This field has been explored by several recent investigations, using different synthetic approaches. [14,21,22]

Surface assembly of noble metal nanoparticles driven by electrostatic interactions is a promising and economic approach used to realize SERS active substrates. A bulk, inexpensive surface such glass or ITO glass is coated whit a Self Assembled Monolayer (SAM) of an amine bearing alcoxysilane, for example aminopropiltrimethoxysilane (APTES), and subsequently, in the proper conditions, negatively charged nano-objects can interact with positively charged ammonium groups present on the bulk surfaces, allowing the preparation of uniform and reproducible substrates. [22,23]

Anyway, several critical issues for this type of materials may arise from the mechanical and chemical instability of the grafted GNS monolayer. The weakness of the electrostatic interactions leads to a situation in which even the simple handling can provoke the detachment of GNS from the glass. Moreover, competition with substances dissolved in analysed fluids (especially those of biological origin) which could lead to competitive interactions and eventually GNS detachment and release in analysed media must be considered. Last but not least, there is a problem which is independent from the approach used for the substrate assembly, and potentially affects all SERS substrates: chemical reactivity with contaminants and in general substances showing high affinity towards noble metal surfaces (i.e. thiols) can of course affect substrate stability, response and/or reusability.[9] All these issues represent limits of striking evidence from the point of view of synthetic reproducibility, robustness, reusability and, as a consequence, cheapness of routine applications.

Examples of substrates possessing the ability of regeneration of the SERS active surface for reuse are often based on photocatalytic degradation of molecules adsorbed on substrate, and usually exploit the presence of TiO_2 [24-26] or ZnO.[27] Other exploited methods to impart reusability include silver NP coated silicon nanospikes realized by femtosecond laser pulses [28], Ag nanowires in mesoporous silica [29] and Au sputtered silica nanorods.[30] In all cases, anyway, the substrate preparation is quite complex and/or time consuming. In addition specific treatments on the samples usually must be performed to gain reusability. For example, flower-like silver structures electrodeposited on ITO glass were found to partially regenerate after a very long (44 hours) immersion in ethanol solution [31] while in other lithographically constructed systems recyclability was obtained through a complex sequence of steps, including chemical etching and plasma cleaning.[32]

A more straightforward idea is to chemically stabilize and protect the GNS monolayer from the surrounding environment with a layer of silica, trying to conserve the SERS effects connected to

nanoscale surface features. In Ref. 33 Gold nanoparticles grafted on a capillary tube were coated with a layer of silica through APTES: in these case a reducing agent (sodium borohydride) must be present in a flowing washing solution to ensure regeneration of the surface for SERS based sensing purposes. [33] More recently, thin silica shell coated Ag NPs assembled on silica NPs were fabricated and used to simultaneously detect a mixture of aniline and 4-aminothiophenol by SERS.[34]

In this work we have prepared a monolayer of GNS grafted on bulk glass surfaces silanized with APTES, adding a further thin silica layer grown on the GNS monolayer. To do this, we followed a methodology which was widely employed, for example in order to protect gold island based transmission localized surface plasmon resonance (T-LSPR) sensing substrates, [35,36] and that we have recently used to obtain bulk surfaces with antibacterial action based on photothermal properties of gold nanostars.[37]

Using this approach we demonstrate that a complete stabilization can be obtained, as well protection and chemical isolation of SERS objects from the solution, inertness towards mechanical removal and chemical reactivity, conserving a good EF of SERS response. These surfaces, after easy and simple washing with water, can be reused, acting a prototype of a low cost, homogeneous, reproducible, robust and reusable SERS substrate.

EXPERIMENTAL

Chemicals. Gold (III) chloride trihydrate (~30 wt % in HCl 99.99%), sodium borohydride (98%), L-ascorbic acid (≥99%), silver nitrate (99.8%), sodium citrate (≥99%), hydrochloric acid (≥37%), nitric acid (≥65%), sulfuric acid (95%), hydrogen peroxide (30 wt %), toluene (≥99.7%), ethanol (≥99.7%), N-Dodecyl-N,N-dimethyl-3-ammoniun-1-propansulfunate (≥99.7%), (3-aminopropyl)trimethoxysilane (APTES), sodium silicate solution ($Na_2O(SiO_2)_{3-5}$ 27% wt % SiO_2), (3-mercaptopropyl)trimetoxysilane (MPTS), were purchased from Aldrich. Amberlite® IR120 hydrogenated was purchased from Fluka Analytical. Microscopy cover glass slides 21x26 mm were purchased from DEL Chimica. All reagents were used as received. Water was deionized and bidistilled. Glassware was carefully cleaned with aqua regia, and then washed three times with bidistilled water for three minutes under sonication, before use.

Pretreatment of Glass Slides. Prior to APTES grafting, microscopy cover glass slides (21x26 mm) were treated with piranha solutions (3:1 sulfuric acid 95% and hydrogen peroxide 30 wt %) for 30 minutes. Then the slides were washed in water under sonication for three minutes, three times. Then the glasses were dried in an oven for 1 hour at 140°C.

Synthesis of Gold Nanostars (GNS)

Seed solution. The seeds were prepared in a vial by adding 5.0mL of LSB aqueous solution (0.2 M) and 5.0 mL of $HAuCl_4$ aqueous solution ($5*10^{-4}$ M). Subsequently, 600µL of an ice-cooled solution of $NaBH_4$ in water (0.01M) were added to the pale yellow solution of $AuCl_4^-$ obtained in the previous step. As prepared brown-orange solution was gently hand-shaken for a couple of second; this solution is efficient for the growth procedure of GNS for 180 minutes from preparation if kept cold.

Growth solution and GNS preparation. 50 mL of LSB solution in water at the same concentration of the seed solution (0.2 M), 1800 µL of $AgNO_3$ in water (0.004 M), 50 mL of aqueous $HAuCl_4$ (0.001 M) and 820 µL of an aqueous L-ascorbic acid solution (0.078 M) were mixed to obtain a colorless solution just after a few seconds of gentle mixing. Then 120 µL of seed solution were added to give a gray-blue color liquid, the intensity of which rapidly increased. The solution was allowed to react without agitation for 1h.

Preparation of "ZERO" glass samples. The pretreated slides (typically eight slides for each preparations) were fully immersed in a solution of APTES 10% (v/v) in ethanol contained in a Hellendhal type glass staining jar, for 5 minutes at 60°C. The obtained amino-modified glasses were washed three times with ethanol under sonication. After this step, the samples were gently dried under N_2 flux. The slides were then fully immersed in the GNS colloidal suspension for 14 hours. After immersion, the slides were washed three times in water without sonication and carefully dried in N_2 stream. Dried samples were stored in the dark in a box with dryers.

Preparation of "ONE" and "FOUR" samples. Tipically, seven dried "ZERO" samples were fully immersed in a MPTS ethanol solution 5% (v/v) for 10 minutes. After this step, the samples were washed three times with fresh ethanol and dried by N_2 flux. For silica deposition, the slides were then dipped for one or four hours in a 1.5 wt % sodium silicate solution (after dilution of a 27 wt % SiO_2 solution with water) kept at 90°C. The strongly acidic cation exchanger Amberlite IR-120 was used for the adjustment of the solution pH to 8.5-9. After the chosen immersion time, the silica-coated substrates were washed three times in ethanol under sonication and dried in N_2 stream. All steps are performed in Hellendhal type glass staining jars.

Preparation of samples for SERS measurements with MMC The chosen glass samples ("ZERO" samples and "FOUR" samples") were placed in a Hellendhal type glass staining jar containing a 10^{-5} - 10^{-3} M solution of MMC in ethanol. After fifteen minutes, glass samples were washed by 5 minutes immersion in fresh ethanol and dried under gentle flux of N_2. This washing procedure was repeated three times. A carefully dried in N_2 stream. Dried samples were stored in Hellendhal type glass staining jar kept in the dark in presence of dryers.

UV-Vis Spectroscopy. UV-Vis spectra on functionalized slides at normal incidence were measured in air using a Varian Cary 50 UV/Vis spectrophotometer. The wavelength scan range was 350-1000 nm. The samples were placed in a special holder enabling transmission measurement of the same spot on the slide during all experimental stages.

Contact Angle. Static contact angle determinations were made with a KSV CAM200 instrument, with the water sessile drop method.

Scanning Electron Microscopy (SEM) SEM images were taken from Tescan Mira XMU variable pressure Field Emission Scanning Electron Microscope – FEG SEM (Tescan USA Inc., USA) located at the Arvedi Laboratory, CISRiC, Pavia. Slides were mounted onto Aluminum stubs using double sided carbon adhesive tape and were then made electrically conductive by coating in vacuum with a thin layer of Pt/Pd (3‑5 nm). Observations were made in backscattered electrons mode (BSE) at 30 kV and with InBeam secondary electron detector for higher spatial resolution.

Preparation of samples for SERS measurements

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Preparation of samples for SERS measurements with R6G

The typical experimental setup for all samples investigated was performed placing on the 21x26 mm functionalized glass sample a 20 µL drop of a water solution of R6G at the desired concentration. After this, a blank and clean 21x26 mm glass slide was placed to cover functionalized glass in order to spread completely the drop and to obtain an almost homogeneous film of R6G solution between the two glass slides. The so-assembled sample was then used quickly for Raman analysis. For the re-usability tests, in the used sample the blank cover glass slide was removed after the first Raman measure, and the functionalized glass was washed with bi-distilled water, wiped with a lab tissue paper, and then used again in the same way as fresh samples.

Raman measurements SERS measurements were carried out at room temperature by using a Labram Dilor spectrometer equipped with an Olympus microscope HS BX40. The 632.8 nm light from He-Ne laser was employed as excitation radiation. The samples, mounted on a motorized xy stage, were tested with a 50x objective and with a laser spot of 2 µm of diameter. The spectral resolution was about 1 cm^{-1}. Neutral filters with different optical density were used to irradiate the samples at different light intensities leading to power density values from $5x10^3$ W/cm^2 to $5x10^5$ W/cm^2. The sample phase homogeneity was verified by mapping the Raman spectra from different regions of each sample. The parameters of the Raman spectra were extracted by using best fitting procedures based on Lorentzian functions. In this way the frequency, full width at half maximum, intensity and integrated intensity of the peaks were determined.

Results and discussion

GNS monolayer on glass

Formation of an APTES monolayer on glass slides was obtained using a reported procedure.[38] This step allows short functionalization times and ensures a good quality monolayer formation: efficiency of silanization is routinely controlled with contact angle (c.a.) measurements. The c.a. value moves from < 10° for cleaned glass samples (piranha solutions, see experimental), to a value of 52 (5)°, a data consistent with those found in the literature for the formation of a monolayer of APTES on bulk glass.[39]

GNS were prepared in water using an seed-growth method we already published and exploited for several purposes, [7,8,40,41] in the presence of ~10% (mol) silver ions and using a zwitterionic surfactant, laurylsulfobetaine (LSB), as the directing and coating agent. The colloidal suspensions obtained with this methodology consist of mixtures containing mainly asymmetric pentatwinned branched gold nanoparticles and a lower percentage of monocrystalline symmetric 6-branched

nanostars. Composition of these colloidal suspensions has been extensively characterized and described in our previous papers. Their extinction spectrum in solution is dominated by the LSPR of the main component, whose maximum can range in the 700-1150 nm interval as a function of synthetic parameters. It is important to notice that even when identical synthetic parameters (reactants concentrations, total volumes, temperature) are used, preparations can yield slightly different populations of objects, and thus colloidal suspensions are obtained with slight differences in position and intensities of LSPRs bands. We used reaction conditions to obtain colloidal suspensions with LSPR optical maxima always close to the same value (between 840 and 860 nm) in order to favour reproducibility in SERS tests. A representative spectrum of the preparations used is shown in Figure 1.

At the end of the growth process, GNS result coated on their surface by a double layer of LSB molecules, with an overall negative surface charge, which can be quantified (by DLS measurements) in a Z potential of ~ -15 mV.   Thus, immersion of APTES-terminated bulk glass samples in the colloidal GNS suspension for 15 hours yields the so called "zero" samples (i.e. in absence of silica coating), where a GNS layer formation is obtained, due to their electrostatic interaction with the positively charged amino-groups, which are in protonated form, as GNS colloidal solutions are slightly acidic (pH 5-6) because of the presence of excess ascorbic acid from the growth process. Glass samples were gently washed with ethanol and water, and then dried under nitrogen stream.

Success of GNS grafting on the APTES functionalized slides can be visually perceived directly, as slides turn to an evident blue colour. SEM images of the obtained samples show unchanged objects morphology with respect to the corresponding TEM image from the parent colloidal solution, as well a continuous and homogeneous coating of the slides with GNS as depicted in figure 1 and already reported.[37] A similar behaviour was also observed for monolayers of the same GNS grafted on a MPTS functionalized glass [42], for different GNS grafted on APTES-functionalised ITO glass samples [22] or tethered on smooth gold-coated Si surfaces by means of a SAM with 6-aminohexane-1-thiol.[14]
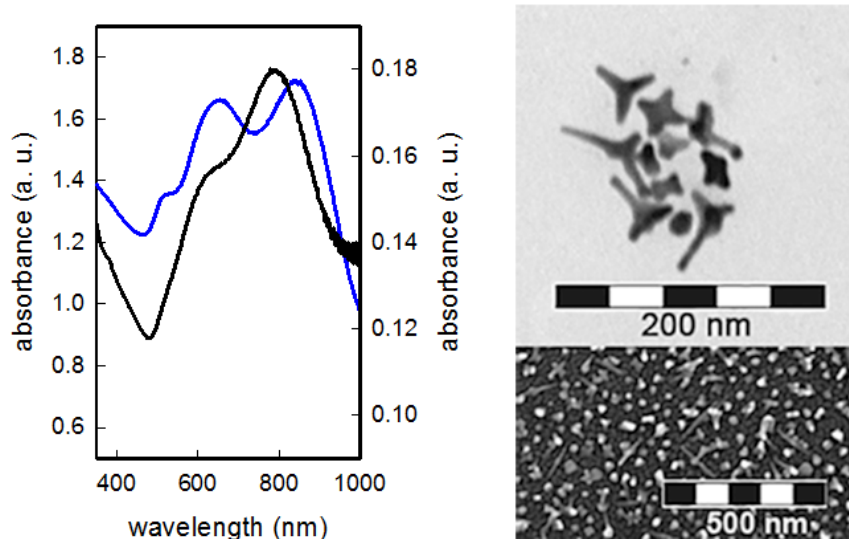
Figure 1 a) spectra of GNS in colloidal suspension (blue line, right scale) compared with the spectra of GNS grafted as a monolayer on amino terminate glass, the so called "ZERO" samples (black line, left scale); b) detail of a TEM image of the colloidal suspension giving the spectrum represente by the blu line of fig 1a; c) detail of SEM image of a "ZERO" sample giving the black line spectrum in fig 1a.

In figure 1a, the UV-Vis spectrum for a typical "ZERO" type glass (black line), is compared with the one of the GNS colloidal suspension (blue line), while in figure 1b a detail of a TEM image taken for a sample of the colloidal suspension is compared with a detail of SEM for one of the glass samples obtained. A LSPR blue shift of about 60 ± 10 nm (average of 32 glass samples, coming four preparations based on four slightly different starting colloidal suspensions: the spectrum for one representative preparation of eight samples is reported as an example in supporting information) is observed moving from the colloidal suspension in water to the monolayer on glass. This shift is due to the decrease of the local refractive index (n) on passing from water (n_{water} = 1.3339) to glass and air at the interface (n_{air} = 1.0003). Contact angle of type "ZERO" samples moves to 42 (5)°.

As a result of this blue shift, type "ZERO" samples coming from different preparations were found to have LSPR maxima ranging from 770 to 800 nm, due to the mentioned variability of the original colloidal solutions of GNS used for the coating, with an absorbance of the long LSPR band ranging between 0.17 and 0.19. Samples with spectral features outside of these ranges were discarded (less than 10% of prepared samples).

As already reported, [37] a gentle scratching, even with the soft surfaces of a tissue or a finger, leads to GNS monolayer removal, visually indicated by disappearing of the blue colour on glass surface. Detachment of GNS with subsequent decolouring is observed also after a three minutes
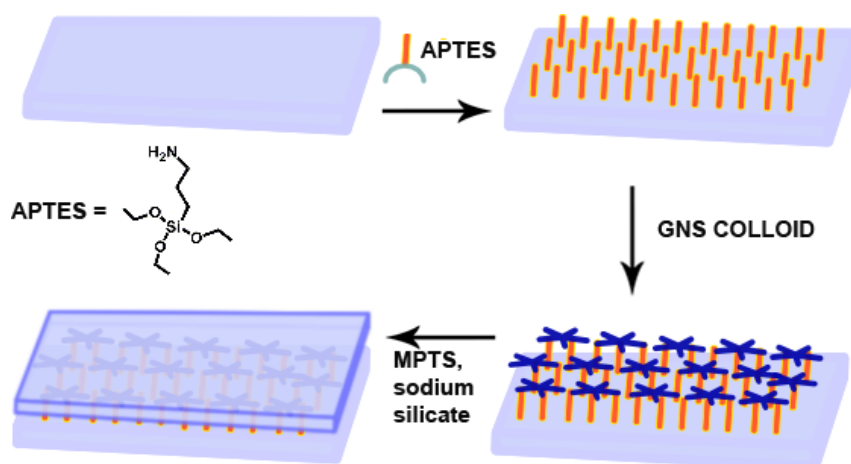
ultrasound treatment. In absence of ultrasound treatment and carefully manipulating them, the samples are anyway stable only for a few days, as evidenced by changes of their spectra: the complete loss of aniostropical features of the objects are observed after three months, as evidenced from changes in LSPR features (data not shown) and changes of colour from deep blue to a wine red colour which indicates the presence of spheroidal gold nano objects.

Coating with silica

Coating with silica gives to the described functionalized glass samples an extra stability.[37] In a first step, the uncoated ZERO samples were immersed in a MPTS solution: the thiol function of MPTS binds the gold surface, leaving the $Si(OCH_3)_3$ moieties pointing outside and exposed to the solution, and these groups guide the reaction with sodium metasilicate to form a thin layer of SiO_2 on the nanoparticles layer. [35,36] After the MPTS treatment, UV-Vis spectra on the glass slides undergoes a small red-shift (of about 10 nm) caused by thiol binding to gold surface. Contact angle increases to a value of 66 (4)°, demonstrating that binding with MPTS was successful, and the surface now is hydrophobic because of alcoxysilane moieties exposed on the surface.

At this point, coating with a silica layer was obtained: as it was demonstrated by Rubinstein and others, [35, 36, 43] treatment of MPTS coated layer of gold nano-objects with a sodium silicate solution at a temperature of 90°, produces the growth of a silica layer with a rate of about 1 nm for hour of immersion. In order to obtain two different thickness, we used two immersion times: one hour, to obtain a ultrathin layer of an expected thickness of 1 nm (sample named "ONE"), and a more prolonged immersion (4 hours, named "FOUR") which, as we have already demonstrated, produces a thicker layer of about 4 nm.[37]

The general strategy used to produce silica-protected GNS layers is reported in scheme 1.

Scheme 1 General strategy used to produce silica-protected GNS layers

Silica coating can be immediately perceived: when silica coated samples ONE and FOUR undergo an ultrasound treatment of 5 minutes, no change in colour or appearance is observed. Even the scratching with a hard tip does not produce any degradation in the appearance of the coated glass, demonstrating that GNS layer is now protected from detachment and mechanical degradation. Contact angle shifts to not measurable values (< 10°) indicating the presence of an elevated

number of Si–OH groups which make the surface very hydrophilic. Moreover, LSPR features did not change in a 4-months period.
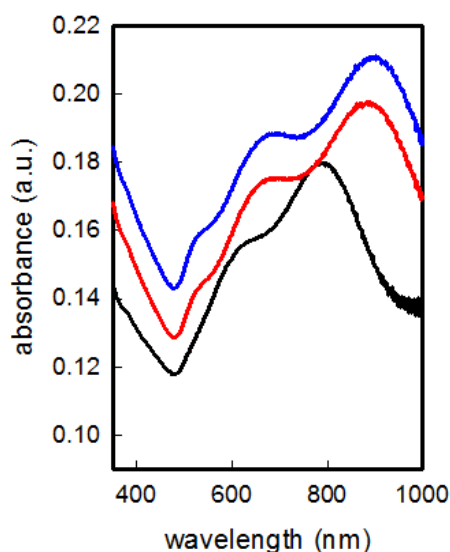

Figure 2: Uv-vis spectra of GNS functionalized glass samples: "ZERO" sample (black line), "ONE" sample (red line); "FOUR" sample (blue line)

Typically, the LSPR maxima for the long band experience a red shift of about 70-90 nm as a consequence of SiO_2 coating. [36, 44] The red shift is typical of Au nanoparticles when local refractive index (n) increase [45] as consequence of the formation of the silica layer (n = 1.4585 for SiO_2).

It is worth of note that, apart the consistent red shift, LSPR bands features do not change, indicating that no appreciable variations in the GNS morphology has happened upon silica coating, as we have already demonstrated on similar samples [37] using HRSEM imaging, which showed the homogeneous distribution of the unchanged GNS objects after the four-hours silica coating.

The coating process was found to be reproducible for both treatment times (one and four hours): only small differences in the measured spectra were found among ONE or FOUR samples, which are also due to the already observed differences among the parent ZERO samples (which may arise from the cited small differences between the starting colloidal solutions). Anyway, the few samples having the long LSPR band centred out of the 870-910 nm range were discarded. Uv-vis spectra of some representative preparation batches for ZERO (figure S1), ONE (figure S2) and FOUR (figure S3) samples are reported in the supplementary data. Reproducibility is observed not only between samples obtained from the same preparation vessel, but also between samples coming from different preparation batches: as an example, figure S3 reports the spectra obtained for two set of FOUR samples obtained from two preparation batches of ZERO samples.

SERS measures: the effect of silica coating on chemical inertness of substrates

As a first step we verified the ability of the silica layer to impart inertness to the GNS layer. To do this we chose 7-mercapto-4- metilcoumarine (MMC) as model interfering species, being a molecule with an intense Raman efficiency [46] and with a strong chemical affinity towards the gold nano-objects surfaces because of stable Au-S bonds. In other words MMC could be "chemisorbed" and not only "physisorbed" on the surface [14] and thus it has been chosen as a model of a passivating and interfering contaminant: hard to remove from the SERS active surface of the chip and with a strong SERS signal.

We immersed ZERO type samples and ONE type samples in an ethanol solution (10^{-5} M for ZERO samples, 10^{-3} M for ONE sample) of MMC. After 15 minutes of immersion in the MMC ethanol solution, the SERS substrates were washed three times with 5 minutes of immersions in fresh EtOH, in order to remove all the traces of the solution containing MMC and thus eliminate all the MMC molecules which were not bound to sample surfaces. After these three washing cycles, the samples were gently dried under nitrogen flux and then the SERS spectra were measured. The results are shown in figure 3, together with the typical SERS signal from MMC in GNS colloidal solution (black line).
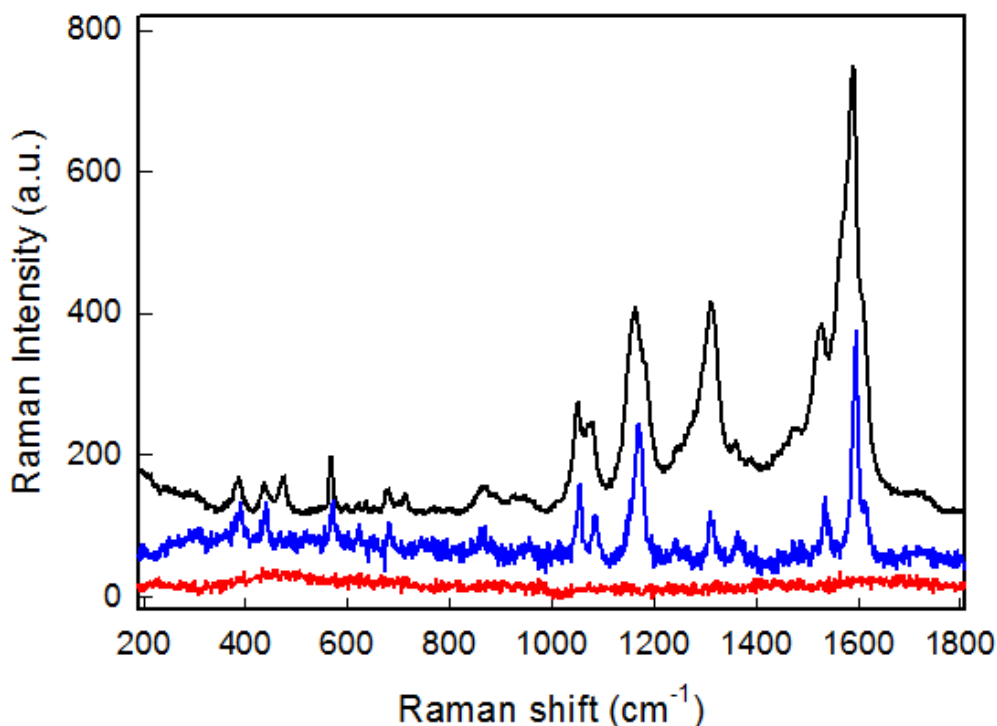

Figure 3 SERS spectrum of 10^{-3} M MMC in colloidal GNS solution (black line); SERS spectrum obtained from a "ZERO" sample after immersion in 10^{-3} M solution of MMC followed by three cycles of washing (blue line); SERS spectra obtained from a "ONE" sample after immersion in 10^{-3} M solution of MMC followed by three cycles of washing (red line)

The SERS signal of MMC is still observed in sample ZERO even after the three washing cycles (figure 3, blue line) while no traces of the typical MMC Raman lines can be recognized in sample

ONE after the same treatments (figure 3, red line). This fact demonstrated that the 1 nm thin silica coating layer prevents the formation of stable Au-S bond between MMC thiolic function and GNS surface. In turn, without this oxide spacer (in the ZERO sample) this bond cannot be removed even after three washing cycles with EtOH. Thus the silica coating protects the SERS active nanostructured substrates avoiding interferences and/or chemical degradation due to reaction of gold surfaces with thiols: in addition to mechanical inertness, a satisfying chemical stability with complete separation of gold surface from reactive species in solution is obtained with the silica coating process.

Effect of silica coating on SERS response

In Figure 4 SERS spectra obtained from 10^{-5} M Rhodamine 6G (R6G) dispersed on GNS substrates, with and without silica coating, are reported. For all the samples the SERS response has been sampled on four different zones of the glass samples.

R6G molecule, containing two functional groups, a dibenzopyrene xanthene and a carboxyphenyl group tilted by about 90° with respect to the xanthene ring,[47] exhibits an intense Raman yield, so this dye molecule has been widely used as SERS marker and standard.[6] The main Raman modes of R6G are peaked at around 614, 766, 1178, 1306, 1361, 1509, and 1647 cm^{-1}, corresponding to the CH, COC, and CC vibrations of aromatic rings. The different vibrational units responsible for the above mentioned peaks are detailed in table 1, according to previous studies. [48,49]

Table 1

measured v_{SERS} (cm^{-1})	assignment
614	In plane deformation Xanthene ring
766	Out of plane vibration Xanthene ring; out of plane C-H bending
1178	C-H bending
1306	in plane breathing Xanthene ring; N-H bending
1361	in plane stretching Xanthene ring
1509	in plane stretching Xanthene ring, C-N str, C-H bend, N-H bend
1647	in plane stretching Xanthene ring; in plane C-H bend

GNS SERS substrates without silica coating ("ZERO" samples) exhibited high and reproducible SERS response: R6G signals are well defined, and intensities do not change when the measure is performed on different zones of the same sample or in samples coming from different preparation batches.
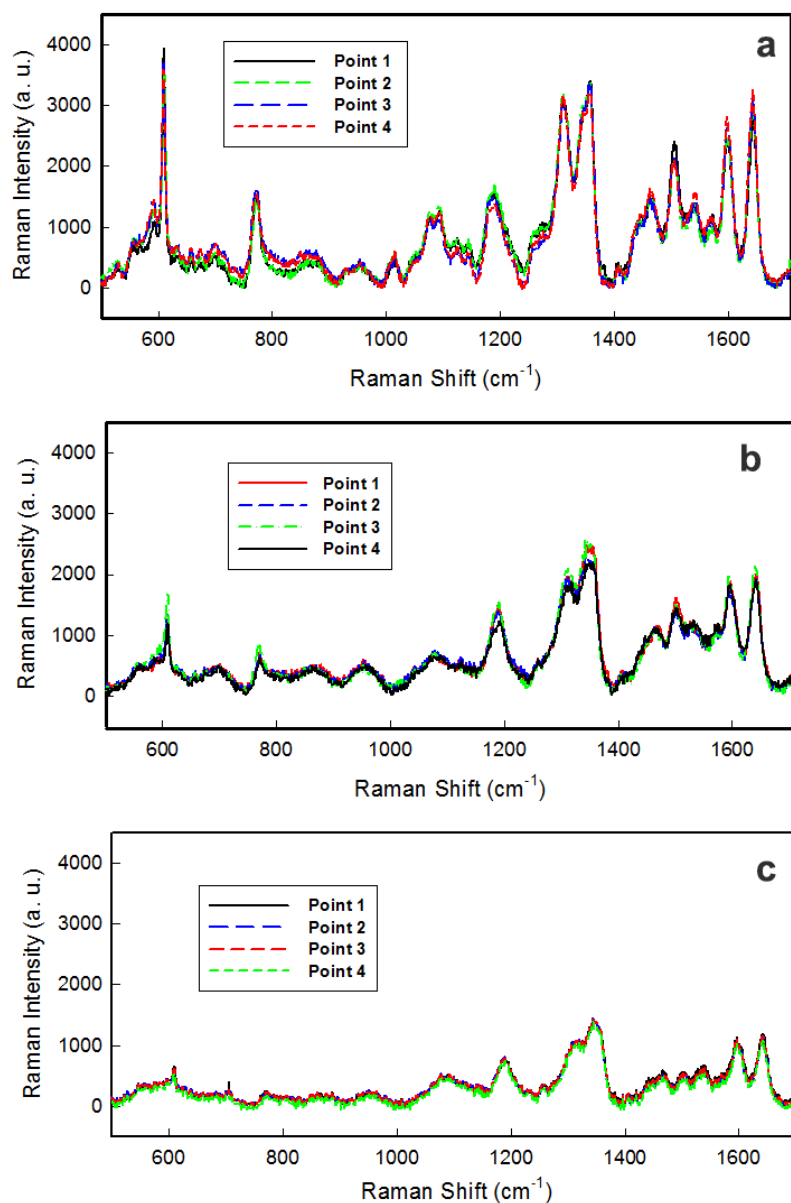
Figure 4 Reproducibility of SERS spectra of 10^{-5} M solutions of R6G using: a) ZERO , b) ONE, c) FOUR samples

Presence of the thin layer of silica reduces but does not depress very strongly the SERS response. Figure 4b shows a series of spectra obtained (in the same experimental conditions used for ZERO samples) for ONE samples in presence of 10^{-5} solution of R6G. Once again reproducible spectra are obtained, while an obvious decrease in SERS intensity is observed when comparing the measures obtained for R6G in absence of silica coating.

In addition, some changes in the lineshapes can be appreciated. This is particularly evident in the spectral regions where C-H bending modes are active, i.e. in the interval 1000-1200 cm^{-1} and 1400-1600 cm^{-1}. On the contrary the main features related to xanthene stretching modes seem to remain unchanged in shape and relative intensity ratio.

Reproducibility of measures demonstrates that not only we have an homogeneous GNS coating, but that also the further added silica coating has an homogeneous thickness and does not alter the GNS layer homogeneity.

A similar behaviour is obtained when using the FOUR samples, i.e. the samples obtained with a four hours immersion in silicate solution yielding a thicker silica coating (figure 4c). In this case the SERS response is less intense than for ONE samples, as can be expected from the increase of the silica layer [35,36,43], but the fingerprint of R6G is still clearly recognized. Once again sampling on different zones of a sample yielded reproducible intensities of the spectra, ensuring on the homogeneity of the silica coated SERS substrate.

A good reproducibility is observed also when using chips coming from different preparation batches: an example is given in figure S4 where SERS spectra of 10^{-5} M R6G measured using two ZERO glass chips coming from two different preparations, starting from two different (but almost identical in LSPR features) GNS colloids are reported. An identical test was repeated on two FOUR samples and is reported in figure S5. To further assess reproducibility, SERS measures on ZERO samples coming from different preparation batches were performed after immersion in 10^{-5} M MMC solutions and subsequent washing with fresh EtOH, and results are reported in figure S6.

The effect of silica thickness on SERS performances of the ONE and FOUR types of substrate can be evaluated in comparison with those of the uncoated ZERO samples, using the same solution and an identical scattering geometry. In figure 5a the SERS spectra for three samples representative of the three types of substrates are reported for a solution of R6G, choosing a concentration of 10^{-4} M for reasons which will be explained in the following. A similar plot for 10^{-5} concentration (which summarizes what already described in figure 3) is anyway reported in the supplementary information (figure S7).
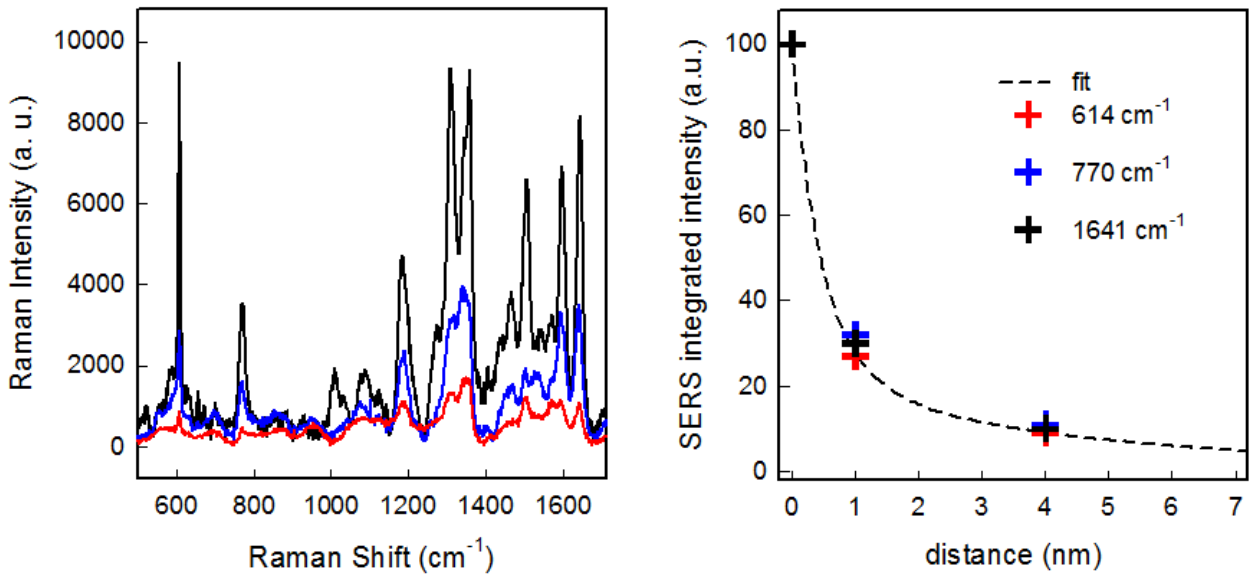
Figure 5 a) comparison of the SERS response, with R6G 10^{-4} M, of the different sample type: ZERO (black line), ONE (blue line) and FOUR (red line); b) dependence of the normalized SERS yield for the three samples i.e. for three silica thicknesses (0, 1 and 4 nm) for the Raman modes peaked at 614 (red symbol), 770 (blue symbol), 1641 cm^{-1} (black symbol) respectively. The line represents the output of best fitting procedure obtained for the mode at 614 cm^{-1}, according to equation (1), see text.

The plot of the SERS yield of a specific Raman mode for the three samples allows to evaluate the effect of silica thickness on SERS EF's. To do this we interpolate the Raman features using one or more lorentzian curves as the fitting function.

In Figure 5b the integrated intensities for R6G 10^{-4} M Raman modes at 614 cm^{-1}, 770 cm^{-1} and 1641 cm^{-1} are plotted using as the independent variable the expected thickness, expressed in nm, of the silica layers in the three cases. These thickness values have been considered as the minimum distances from the metal nano-objects at which the vibrational units of the molecule experience the SERS enhancement effect. The line represent the output of the best fitting procedure where, according to Cotton et al.,[50] the following function has been used:

$$G_{em}(\nu_s) \sim [r/(r+d)]^{10} \quad (1)$$

In this formula $G_{em}(\nu_s)$ is the electromagnetic enhancement factor of a Raman mode at a given frequency ν_s, r is the radius of the spherical nanoparticle and d the distance where a specific monolayer experiences the confined EM field.[1]

The experimental behaviour is well reproduced by a linear combination of two terms with two values for r: the first one is close to 4 nm while the second is markedly larger ($\approx$ 43 nm). This result has been obtained using a weight for the smaller radius term 4 times greater than the other one.

The value for the smaller radius well agrees with the average curvature of the tips as derived by TEM images. [7] The higher value could be justified considering as the model spherical nano-object the mean radius of a sphere containing the whole GNS. The greater weight for the smaller radius contribution indicates a more efficient SERS enhancement mechanism for sharp tips, as expected, as well their number on the objects. [18] The second contribution is needed to account for a long-lasting component in SERS enhancement, not satisfactorily reproduced by a single function with a radius equal to the average curvature of the tips. An active role in sustaining SERS enhancement beyond the abrupt decrease imposed by equation (1) can be played by the presence of silica coating. Indeed some authors [13] observed a persistence of EF at longer distances, even over 30 nm, and tentatively they justified it in terms of the spatial spread or depth of penetration to the dielectric of collective surface plasmon polaritons just favoured by the presence of silica as dielectric medium. A similar treatment was repeated using the data obtained with 10^{-5} M R6G solutions and the integrated intensity for the same modes at 614 cm^{-1}, 770 cm^{-1} and 1641 cm^{-1}, yielding almost identical results as reported in figure S7.

Anyway the observed behaviour indicates that only a very thin part of the laser focus volume give sizeable SERS signal: molecular layers placed at distances greater than 10 nm from the nanoaggregates are practically expected to be SERS silent. In addition the good best fitting results obtained using a pure EM contribution indicate that chemical enhancement seems negligible even for ZERO samples.

These results allowed us to evaluate EF's. To estimate the SERS enhancement we used the following equation according to Le Ru et al. [6] :

$$EF = (I_{SERS} /I_{RS}) \times (N_{RS} /N_{SERS}) \qquad (2)$$

Where I_{RS} and I_{SERS} are the intensities of Raman and SERS peak of the same vibrational mode; N_{RS} and N_{SERS} are the numbers of R6G molecules respectively participating at the Raman and SERS phenomena in the two experiments.

For determination of N_{RS} and N_{SERS}, 10 µL of R6G solution (10^{-3} M) was spread onto a blank glass slide with the diameter of 1 cm, while a more diluted R6G solution (1×10^{-4} M, 10 µL) was dropped onto a ZERO substrates covering the same area. In both cases the drop was spread using a blank glass slide.

Some considerations are needed to explain the choice of R6G concentrations used to estimate EF factor. The comparison between Raman Spectroscopy and SERS signals are usually difficult and the first requirement is to be able to carefully control the experimental conditions. Just for this we used the same scattering geometry i.e. a sort of sandwich configuration. This allows to control the total volume of the drops. Then we choose 10^{-3} M concentration for Raman Spectroscopy experiments because this is the minimum value to obtain a sizeable signal in normal Raman experiments. We didn't use the same concentration in SERS to avoid contribution in SERS scattering from normal Raman process but on the other hand we selected 10^{-4} M to experience SERS in a similar concentration range thus avoiding SERS effects, sometimes unstable, from local hot spots, as we were interested in the average and stable SERS response.

From the analyses on the peak at 610 cm^{-1} comparing SERS response from ZERO sample for a 10^{-4} M solution and normal Raman response for a 10^{-3} M solution, we obtain a value for the term (I_{SERS}/I_{RS}) equal to ~50.

The term (N_{RS}/N_{SERS}) is strongly affected by the differences in scattering volumes, other than the factor 10 in concentration ratio. Due to the "sandwich" configuration the dimension that change in the two experiment is only the height of the beam contributing to the scattering. We have demonstrated that in the present case the SERS signal decreases dramatically with distance according to equation (1) (practically disappearing, see figure 5, at a distance longer than 10 nm) and thus we can consider that the effective volume, i.e. N_{SERS}, contributing to SERS signal is due to a 10^{-3} fraction of N_{RS} involved in normal Raman, being the whole thickness of the R6G dispersed drop calculated to be about 18 microns. More precisely, the ratio between the two heights should be 18000 nm/10 nm = 1.8x10^3. Considering the ratio in concentration, we obtain for (N_{RS}/N_{SERS}) a value of 1.8x10^4 and a total EF factor equal to ~9x10^5 for the ZERO samples. We underline that this value is in good agreement with that reported in Le Ru et al.[6]

Testing reusability

Recyclable SERS-active substrates which can regenerate their activity after every cycle of measurements are highly desired, and different approaches have been proposed for cleaning of these SERS-active substrates. In a recent example, reusability was obtained regenerating a silica coated AuNP surface by means of washing with sodium borohydride solutions, which were able to remove every absorbed molecule by simply flowing into the capillary which were used for continuous online analysis.[51] As reusability should be a key feature for all SERS-active substrates, we tested in a very simple way the reusability of our samples, which derives directly from the mechanical and chemical inertness we have described in the previous section.

A freshly prepared ONE silica coated sample was used to measure the SERS spectra on 20 μL of a 10^{-4} M solution of R6G using the already described methodology. After the measure, the blank glass used as a cover was removed, and the SERS active substrate was simply washed under a flow of bidistilled water, and then treated in bidistilled water with one minute of ultrasounds. At this point, a SERS spectra using 20 μL of water was taken. After this, water was removed with a clean tissue, and then the substrate was reused with another aliquot of 10^{-4} M solution of R6G to take another SERS spectra. The results are reported in figure 6: as can be clearly observed, an almost identical spectra was obtained. The described procedure can be repeated several times without any change in behaviour.
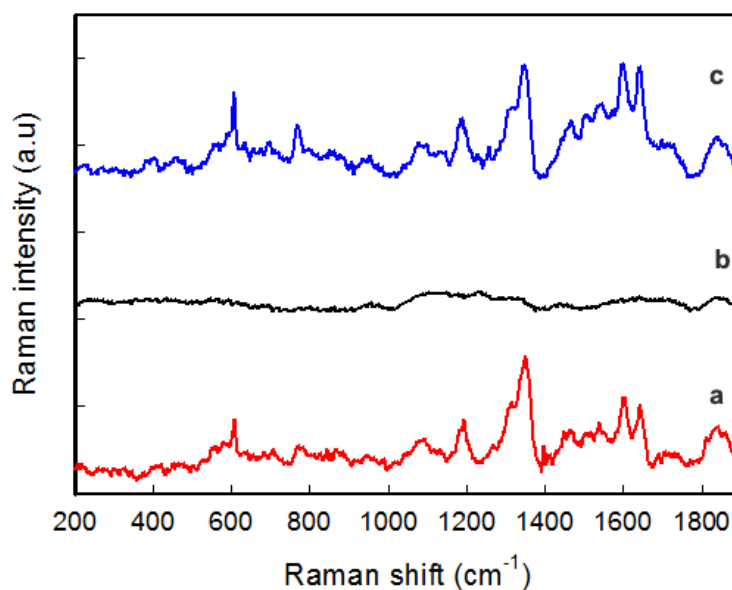
Figure 6 Reusability of silica coated SERS substrate: (a) SERS spectra of R6G 10^{-4} M on fresh new ONE substrate; (b) spectra of water on same substrate after washing and sonication; (c) SERS spectra of R6G 10^{-4} M on recycled substrate.

Conclusions

We have used a simple strategy to prepare GNS monolayers on glass and to coat them with a silica layer of controlled thickness, demonstrating that: i) silica coating avoids the contact of GNS with the solution, preventing chemical bonding of reactive molecules with the gold surface and subsequent passivation of nano-objects by reactive molecules like thiols, in the same time ensuring mechanical resistance to washing, effects of ultrasounds, scratching; ii) homogeneity obtained during GNS grafting and in silica coating processes ensure reproducibility of SERS measures; iii) the presence of a thin silica layer does not suppress the SERS response but reduces it as a function of distance from GNS layer, conserving a good EF and thus good sensitivity. Thus, we have realized GNS based chips which are robust, give reproducible results, and can be recycled by simply washing them under a water flow: we believe they will be precious prototypes to realize cost effective substrates for routine SERS applications.

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Supplementary data

Robust, Reproducible, Recyclable SERS Substrates: monolayers of Gold NanoStars grafted on glass and coated with a thin silica layer.

B. Bassi,[1] B. Albini,[2] A. D'Agostino,[1] G. Dacarro,[1] P. Pallavicini,[1] P. Galinetto,[2*] and A. Taglietti[1*]

1) Dipartimento di Chimica, Sezione di Chimica Generale, Università di Pavia, viale Taramelli, 12 - 27100 Pavia – Italy;
2) Dipartimento di Fisica "A.Volta", Università di Pavia, Via Bassi 6, - 27100 Pavia – Italy;
Corresponding Author: * E-mail pietro.galinetto@unipv.it; angelo.taglietti@unipv.it;

Figure S1 UV–vis–NIR spectra of eight ZERO type samples coming from the same preparation vessel
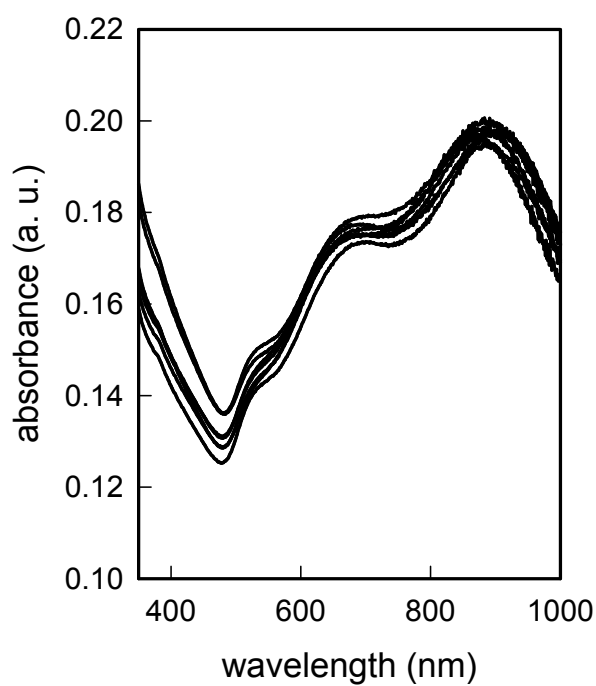
Figure S2 UV–vis–NIR spectra of eight ONE type samples coming from the same preparation vessel
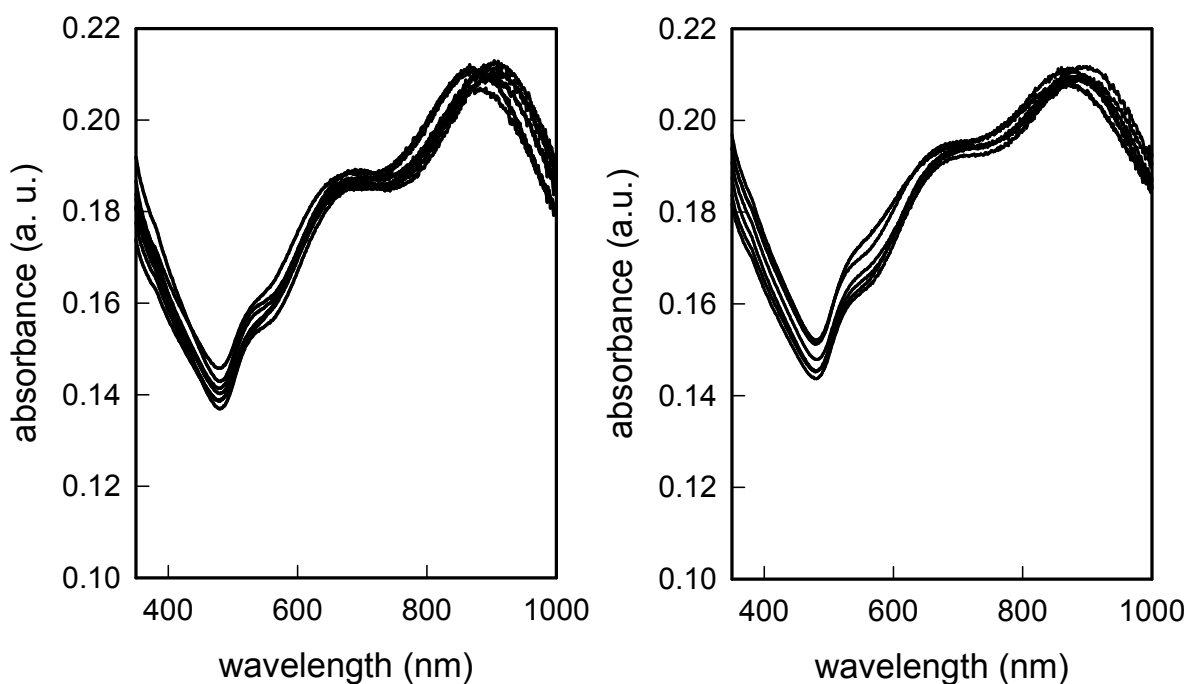
Figure S3 UV–vis–NIR spectra of two set of eight FOUR samples, each set coming from a different preparation vessel
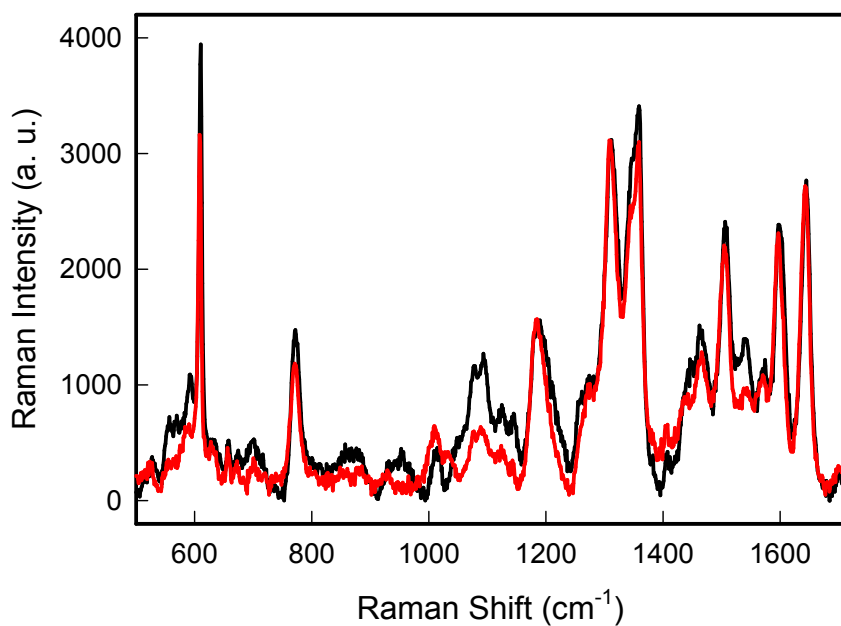

Figure S4 SERS spectra of a 10^{-5} M R6G solution spread on two ZERO samples coming from two different preparation batches
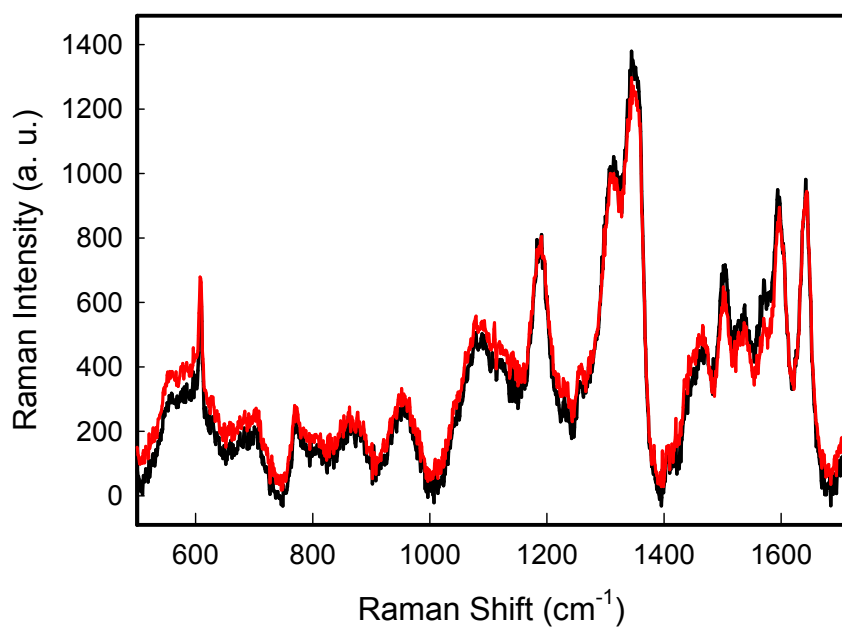
Figure S5 SERS spectra of a 10⁻⁵ M R6G solution spread on two FOUR samples coming from two different preparation batches
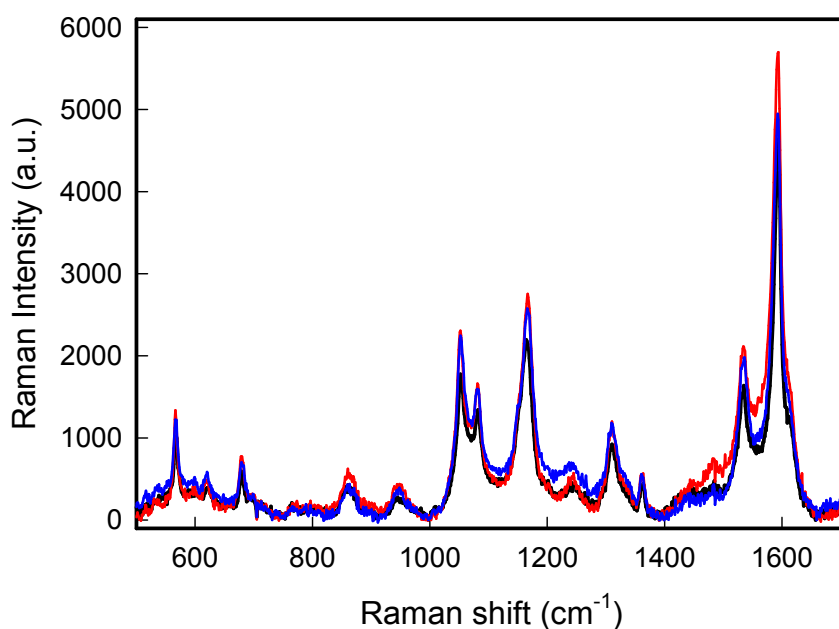

Figure S6 SERS spectra of three ZERO samples, coming from three different preparation batches, after immersion for fifteen minutes in a 10⁻⁵ M MMC EtOH solution and subsequent washing with fresh EtOH.
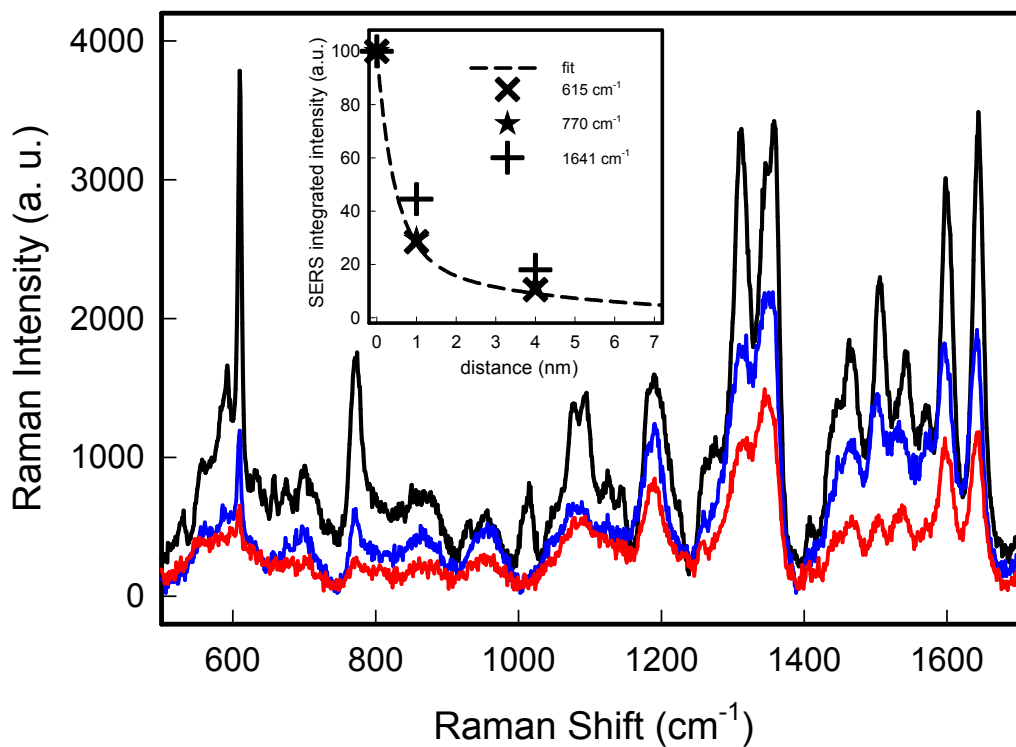
Figure S7 Comparison of the SERS response, with a 10^{-5} M solution of R6G, of the different sample types: ZERO (black line), ONE (blue line) and FOUR (red line). Inset: dependence of the normalized SERS yield for the three samples i.e. for three silica thicknesses (0, 1 and 4 nm) for the Raman modes peaked at 614, 770, 1641 cm^{-1} respectively. The line represents the output of best fitting procedure obtained for the mode at 614 cm^{-1} using a 10^{-4} M R6G solution, according to equation (1), see text