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1 Au-covered hollow urchin-like ZnO nanostructures for surface- 2 enhanced Raman scattering sensing

3 Octavio Graniel¹, Igor Iatsunskyi², Emerson Coy², Christophe Humbert³, Grégory Barbillon⁴, Michel
4 Thierry⁵, David Maurin⁵, Sébastien Balme¹, Philippe Miele^{1,6}, and Mikhael Bechelany^{*1}

5 ¹Institut Européen des Membranes (IEM), UMR-5635, Université de Montpellier, ENSCM, CNRS,
6 Place Eugène Bataillon, 34095 Montpellier, France

7 ²NanoBioMedical Centre, Adam Mickiewicz University, 3 Wszechnicy Piastowskiej str., 61-614,
8 Poznan, Poland

9 ³Univ. Paris Sud, Université Paris Saclay, Laboratoire de Chimie Physique, CNRS, Bâtiment 201 P2,
10 91405 Orsay, France

11 ⁴EPF-Ecole d'Ingénieurs, 3 bis rue Lakanal, 92330 Sceaux, France

12 ⁵Laboratoire Charles Coulomb (L2C), Université de Montpellier, CNRS, Montpellier, France

13 ⁶Institut Universitaire de France (IUF), MESRI, 1 rue Descartes, 75231 Paris, France

14 * Corresponding author: mikhael.bechelany@umontpellier.fr

15 Abstract

16 Au-covered hollow urchin-like ZnO nanostructures were prepared with controlled size by combining
17 nanosphere lithography (NSL), atomic layer deposition (ALD), electrodeposition, and electron beam
18 (e-beam) evaporation. The optimal Au film thickness was determined by measuring the surface-
19 enhanced Raman scattering (SERS) intensities of the substrates. Furthermore, the sensing
20 performances of these hybrid nanostructures have been investigated by using chemical and biological
21 molecules: thiophenol and adenine, respectively. Limits of detection (LOD) of 10^{-8} M and 10^{-6} M were
22 found for the detection of thiophenol and adenine, respectively. Additionally, the excellent uniformity
23 and batch-to-batch reproducibility of the substrates make them excellent candidates for reliable SERS
24 sensing and biosensing.

25 Highlights:

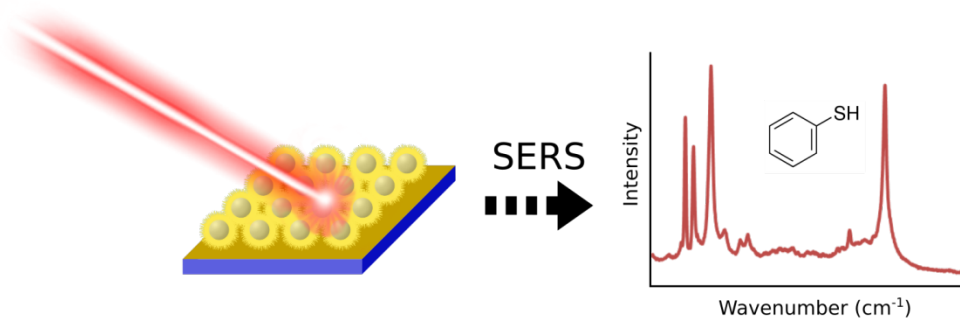
- 26 • Fabrication of Au-covered ZnO urchin-like structures for SERS sensing via high throughput
27 methods
- 28 • SERS signal is homogenous throughout the substrate and reproducible by testing different
29 batches
- 30 • The hybrid nanostructures offer promising prospects for the detection of adenine

31

32 **Keywords:** surface-enhanced Raman scattering, SERS substrates, atomic layer deposition, urchin-like
33 ZnO, ZnO/Au heterostructure

34

35 **Graphical abstract:**



36

37

38 **1. Introduction**

39 Surface-enhanced Raman scattering (SERS) has emerged as one of the most powerful analytical
40 techniques that offer single-molecule detection [1]. Its outstanding sensitivity and non-destructive
41 character have found many applications in many fields such as chemistry [2], medicine [3], biology [4],
42 and environmental sciences [5]. SERS substrates are mainly fabricated with noble metals (Ag, Au, Cu)
43 due to their strong interaction with electromagnetic waves and the excitation of the localized surface
44 plasmon resonance (LSPR) that can be tuned in the UV-Vis-NIR region [6,7]. These noble metals are
45 usually combined with nanostructures such as nanospheres [8], nanowires [9], nanogaps [10],
46 nanotrees [11], and nanorods [12] that provide roughened metal surfaces and plasmonically active
47 “hotspots” that enhance the Raman signal by several orders of magnitude [13]. Additionally, thanks to
48 their inherent SERS activity [14], semiconductors have been used to fabricate metal/semiconductor
49 hybrid nanostructures that exhibit both electromagnetic enhancement and charge-transfer effects [12].
50 Furthermore, these highly-efficient SERS hybrids present additional properties that make them
51 attractive for applications such as photocatalysis [15], water splitting [16], and solar energy conversion
52 [17]. As a semiconductor material with a wide direct bandgap (3.37 eV), biocompatibility [18], and
53 promising optoelectronic properties [19,20], ZnO has been employed for the construction of high-
54 surface-area SERS substrates with a wide range of tunable morphologies [21].

55 However, the fabrication of reproducible and stable SERS substrates remains a major challenge [22].
56 Therefore, much attention has been paid to find facile, repeatable, and high-throughput fabrication
57 methods for large-area nanostructured substrates. Among these methods, nanosphere lithography
58 (NSL) has proven to be a reproducible and inexpensive technique that offers well-ordered arrays of
59 nanostructures with wafer-scale throughput [23,24]. Thus, NSL has been used in combination with
60 other techniques to fabricate different types of SERS substrates such as silver film over nanosphere
61 (AgFON) [25], gold semishells [26], nanoring cavities [27], nanopyramids [28], and optrodes [29].
62 Recently, high-surface-area urchin-like structures decorated with Ag have been successfully used as
63 SERS substrates [30–32]. Nevertheless, even though Ag is preferred over Au due to its higher SERS
64 activity, Ag is prone to oxidation in air or water environments [33]. Consequently, decreasing its SERS
65 enhancement factor (EF) as a result of changes in its chemical and plasmonic properties [34].

66 Herein, we report on a high-surface-area reproducible SERS substrate consisting of Au-covered ZnO
67 urchin-like hollow structures for the detection of thiophenol. This substrate was fabricated by using

68 scalable techniques that include NSL, atomic layer deposition (ALD) [35], electrodeposition, and
69 electron beam (e-beam) evaporation. NSL was used to obtain a template of well-organized spheres
70 that were later covered with a seed layer of ZnO deposited by ALD. Afterward, ZnO nanowires
71 (NWs) were grown via a hydrothermal method to obtain the urchin-like structures, which were finally
72 subjected to e-beam evaporation of Au. Furthermore, we investigated the SERS performances of these
73 hybrid nanostructures by detecting a small chemical molecule (thiophenol) and then a biomolecule
74 (adenine).

75

76 **2. Material and methods**

77 **2.1 Materials**

78 Polystyrene (PS) spheres (aqueous dispersion, 10 % w/w) with a diameter of ~500 nm and sodium
79 dodecyl sulfate (SDS, CAS: 151-21-3, purity > 99%) were purchased from Sigma-Aldrich and used as
80 received. Zinc chloride (CAS: 7646-85-7, purity > 98%) and potassium chloride (CAS: 7447-40-7, purity
81 > 99.0%) were purchased from Fluka. Thiophenol (CAS: 108-98-5, purity > 99%) was purchased from
82 Alfa Aesar. Diethylzinc (DEZ, CAS: 557-20-0, purity > 95%) was purchased from Strem Chemicals
83 Indium tin oxide (ITO) deposited on quartz was purchased from Präzisions Glas & Optik.

84

85 **2.2 Fabrication of urchin-like ZnO structures covered by Au**

86 First, ITO substrates (1 × 2 cm²) were cleaned by sonication for 15 min in acetone, ethanol, and
87 isopropanol sequentially and finally dried under air. After, the PS spheres were deposited on the ITO
88 substrates using a previously reported method with slight modifications [36]. Briefly, a 1:2 solution of
89 PS spheres and anhydrous ethanol was added dropwise with a micropipette onto the surface of a
90 tilted glass slide (previously treated with oxygen plasma) that was resting on the wall of a Petri dish
91 filled with deionized (DI) water. Afterwards, 5 μL of an SDS solution (10 wt. %) were added to the
92 water surface to change the surface tension and pack together the PS spheres. The self-assembled PS
93 spheres were then transferred to the ITO substrate (previously exposed to a 4 W, 254 nm UV lamp for
94 15 min to render it hydrophilic) by carefully introducing the substrate into the Petri dish and
95 removing it from the solution at an angle of 45°. Once dry, the substrates were heated in an oven at
96 100 °C for 30 min to promote the adhesion of the PS spheres to the substrate. Next, the size of the
97 spheres was reduced to produce a non-close-packed arrangement by reactive ion etching in O₂ plasma
98 (0.6 mbar, 50W). The size of the PS spheres was controlled by adjusting the oxygen plasma exposure
99 time.

100 Afterward, a 20-nm thick ZnO layer was deposited on the PS spheres-covered substrates by ALD in a
101 home-made system. The ZnO films were grown at 80°C using diethyl zinc (DEZ) and DI water as
102 precursors in a typical cycle described as follows: 0.2 s pulse of DEZ, 40 s exposure, and 60 s purge
103 with argon, followed by 2 s pulse of H₂O, 40 s exposure, and 60 s purge with argon.

104 Subsequently, electrodeposition of ZnO was performed using a previously reported procedure [37]. A
105 three-electrode configuration was used with the ITO/PS spheres/ZnO ensemble as a working
106 electrode, a Pt plate as the counter electrode, and Ag/AgCl as the reference electrode. An aqueous

107 solution of 0.05 mM ZnCl₂ (zinc precursor) and 0.1 M KCl (supporting electrolyte) was used as the
108 electrolyte and was continuously bubbled with O₂ during the whole electrodeposition process. The
109 electrodeposition was carried out at 80°C with a constant electric potential of -1 V during 15 min in a
110 VersaSTAT 3 potentiostat. Once the electrodeposition was completed, the PS spheres were burned off
111 in air at 600°C for 2 h. Finally, an Au layer (10, 30 and 50 nm) was deposited by a 350 UNIVEX e-beam
112 evaporation system (10 kV, 1×10⁻⁶ mbar) on top of the substrates. The deposition thickness of Au was
113 monitored using a quartz crystal monitor (deposition rate = 3 Å/s).

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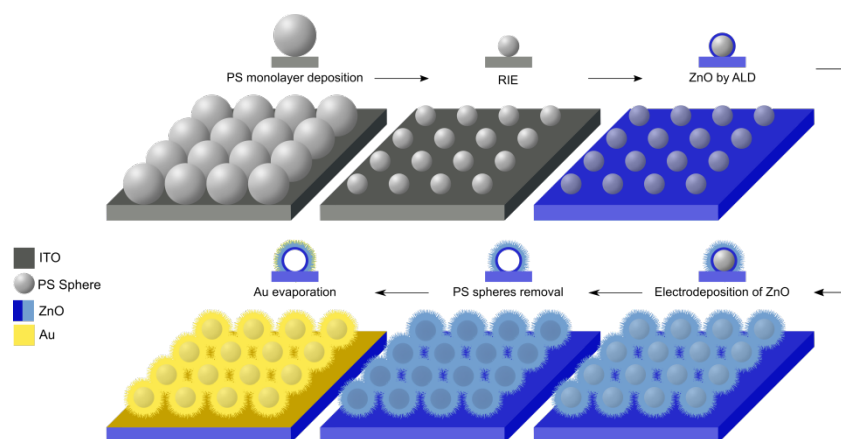
115 **2.3 Substrate preparation for SERS measurements**

116 To evaluate the enhancing capabilities of the substrates, thiophenol was selected as the test molecule.
117 The substrates were immersed in a freshly prepared ethanolic solution with different concentrations
118 (10⁻³ M – 10⁻⁸ M) of thiophenol for 18 h. After, the substrates were washed with copious amounts of
119 anhydrous ethanol and dried in air under ambient conditions. For adenine detection, the substrates
120 were immersed in different concentrations of adenine in DI water for 30 min. The substrates were then
121 removed from the adenine solution and rinsed thoroughly with DI water and dried in air under
122 ambient conditions.

123

124 **2.4 Structural, SERS and extinction characterizations**

125 The morphology of the as-prepared Au-covered urchin-like ZnO samples was characterized by a
126 Hitachi S4800 scanning electron microscope (SEM). In addition, samples were prepared for
127 Transmission electron (TEM) experiments by performing angular cuts using Focus Ion Beam (FIB)
128 JEOL JIB-4000 using a gallium source working at 10 kV, with 60°, 40° and 30° incident angle
129 geometries over a large area (50 μm). In this way, several particles were cut at different axis and
130 geometry. TEM experiments were performed in an HR-TEM JEOL ARM-200F, working at 200kV,
131 equipped with an energy-dispersive X-ray (EDX) detector. Samples were cut by FIB and then were
132 sonicated in ethanol after ion milling and drop cast on commercially available Cu grids and vacuum
133 dried overnight in a desiccator. X-Ray diffraction (XRD) was performed with a PANalytical X'pert-
134 PRO diffractometer equipped with an X'celerator detector using Ni-filtered Cu Kα radiation. The XRD
135 spectra were measured in the 2θ angular region between 20° and 70° with a scan speed of 2° min⁻¹ and
136 a step rate of roughly 0.02°/s. SERS measurements were performed with a Renishaw Invia Raman
137 microscope with laser beams at 633 nm and 785 nm. The beam was focused with a 100 × objective lens
138 with a N.A. = 0.9 in a backscattering geometry. The samples were irradiated with laser powers of 0.48
139 mW (633 nm) and 0.55 mW (785 nm) with acquisition times between 1 s and 10 s. For comparison, all
140 the collected spectra were normalized by laser power and acquisition time. The average SERS
141 intensities reported and relative standard deviations (RSD) were calculated with 25 SERS spectra.
142 Moreover, a LabRam spectrophotometer is employed for the Raman measurements in solution (non-
143 SERS reference) by using the same excitation wavelength and a macro-objective with a focal length of
144 40 mm (N.A. = 0.18). In addition, extinction spectra of samples were recorded in normal transmission
145 mode with a Cary-5000 spectrophotometer from Agilent.



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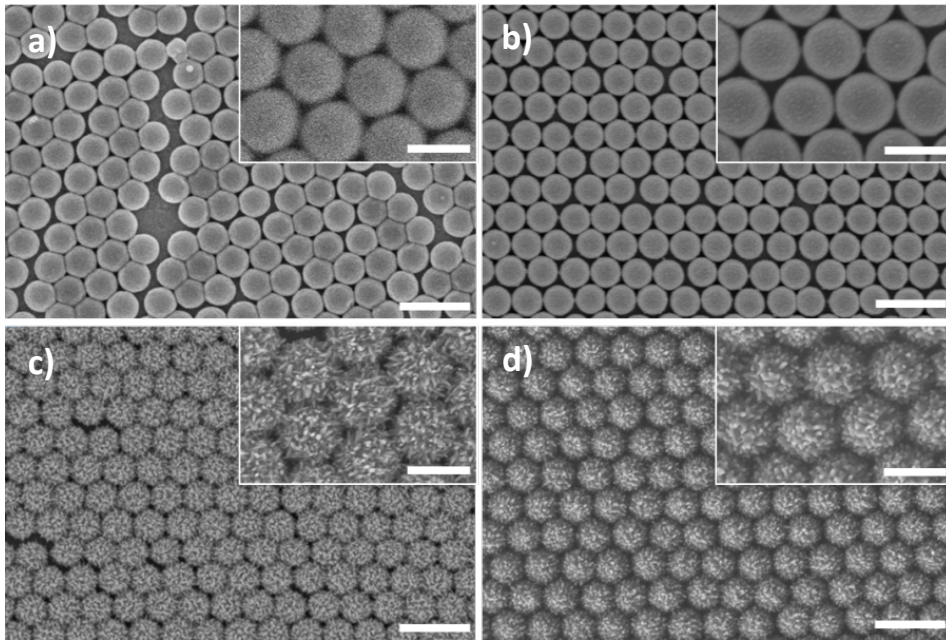
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Figure 1. Scheme of the overall fabrication process

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149 3. Results and discussion

150 The overall process for the fabrication of the Au-covered hollow urchin-like ZnO structures is
 151 depicted schematically in Fig. 1. Among the different approaches to fabricate ordered arrays of PS
 152 spheres, the direct assembly at the air-water interface has been demonstrated to be a simple and low-
 153 cost method that offers high-quality monolayers over large areas. Fig. 2a shows a SEM micrograph of
 154 a large area monolayer of PS spheres with a high degree of order. In order to produce a non-closed-
 155 packed monolayer, the PS spheres are exposed to oxygen plasma (Fig. 2b). This treatment allows
 156 controlling the size of the urchins and space between them. After reducing the size of the PS spheres,
 157 a thin 20-nm ZnO layer was deposited by ALD as a seed layer to promote the growth of ZnO NWs (Fig
 158 2b). Owing to its high conformality and homogeneity, the ALD method promotes the uniform
 159 generation of ZnO NWs on the surface of the PS spheres, unlike pure hydrothermal methods or
 160 sputtering where the thickness control of the seed layer becomes a challenge [38]. In addition, the ALD
 161 layer anchors the PS spheres to the ITO substrate and renders their surface electrically conductive for
 162 the electrodeposition process. Fig. 2c shows the morphology of the urchin-like ZnO structures. Thanks
 163 to the homogenous ALD seed layer, the electrodeposited ZnO NWs are evenly distributed on the
 164 surface of the PS spheres. The size and length of the ZnO NWs of the urchin-like structures deposited
 165 by electrodeposition can be tuned by changing the electrochemical conditions (i.e., zinc precursor
 166 concentration and charge density) [39]. Finally, the urchins are homogeneously covered by an Au film
 167 deposited via e-beam evaporation with thicknesses of 10, 30, and 50 nm, respectively. Fig. 2d shows
 168 the as-synthesized Au-covered urchin-like structures with a gold layer thickness of 30 nm (10 nm and
 169 50 nm are shown on Fig. S1).



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Figure 2. SEM micrographs of (a) an array of well-organized PS spheres, (b) PS spheres after been subjected to an oxygen plasma treatment, (c) ordered hollow urchin-like ZnO structures, and (d) urchin-like ZnO structures covered by a 30 nm-thick Au layer deposited by e-gun evaporation (scale bar = 1 μm). The insets show higher magnifications of the corresponding SEM images (scale bar = 500 nm).

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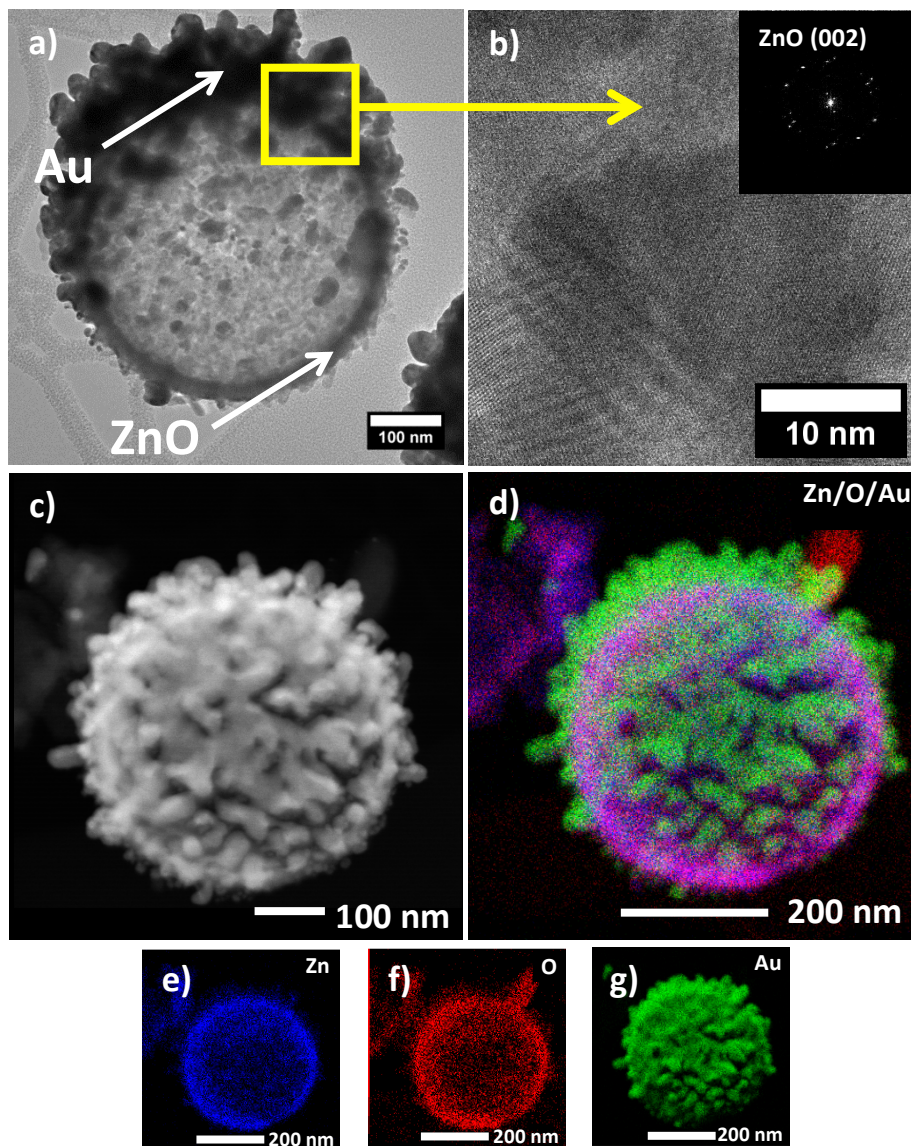
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The morphology and crystallinity of the urchin-like structures were studied by TEM. Fig. 3a clearly shows the Au film that covers the top of the urchin-like structures and the 20 nm ZnO seed film deposited by ALD. Notably, the TEM images display a cavity with a reduced contrast and a shell with a uniform dark gray color (Fig. S2), clearly showing the hollow character of the structures. The high-resolution (HR) TEM image from Fig. 3b shows the lattice fringes that correspond to ZnO and Au. Likewise, the FFT of the HRTEM image shows the (002) plane of ZnO. Additionally, to further confirm that the PS core of the urchin structures is removed after annealing, an EDX mapping was performed. Fig. 3c shows a scanning TEM (STEM) image of a single urchin structure and Fig. 3d shows the corresponding EDX elemental mapping of Fig. 3c. Fig. 3d shows that Zn and O are located in the core of the urchin, while Au is covering the outer shell of the structure.



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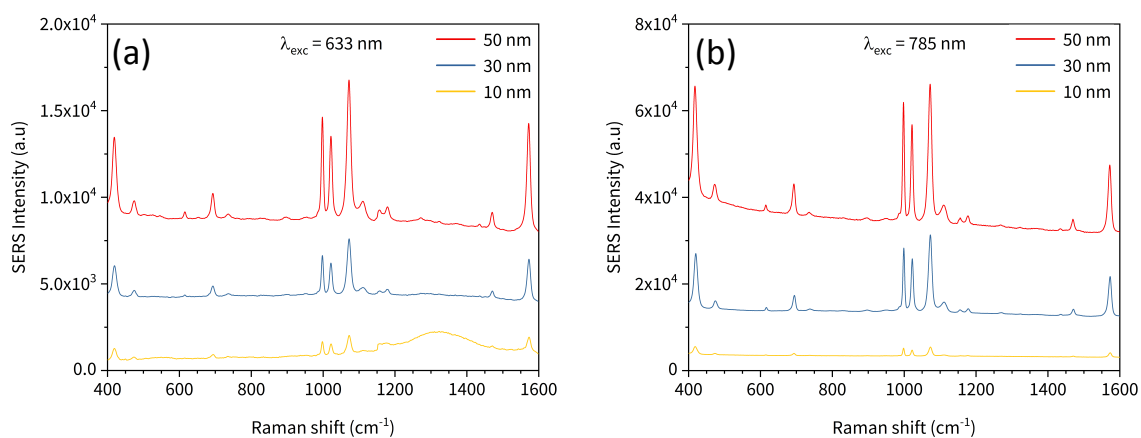
189 **Figure 3.** a) TEM image of an Au-covered ZnO urchin-like structure and b) high-resolution
190 image of the selected area in image a). The inset in b) shows the FFT operation of the image.
191 c) STEM and (d-g) EDX elemental mapping images of an Au-covered hollow ZnO urchin-
192 like structure. Blue, red, and green illustrate Zn, O, and Au, respectively.

193 XRD measurements were carried out to study the crystallinity of the samples. The XRD patterns of
194 ITO, urchin-like ZnO, and Au-covered urchin-like ZnO structures are displayed in Fig. S3. The
195 diffraction peaks at 31.8°, 34.4°, and 36.4° can be assigned to the hexagonal würtzite planes of (100),
196 (002), and (101) of ZnO [40,41], while the peaks at 38.2°, 44.5°, and 64.6° can be assigned to the face-
197 centered-cubic (fcc) planes of (111), (200), and (220) of Au [42,43]. These results show the crystalline
198 nature of the samples and the successful fabrication of a ZnO/Au composite. The remaining peaks
199 correspond to the ITO film deposited on quartz that is used as a substrate [44].

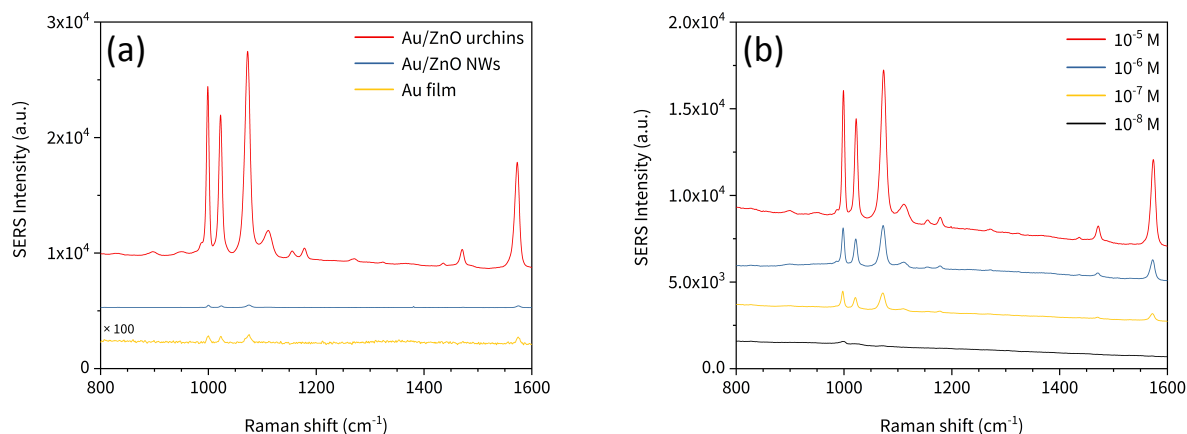
200 3.1 SERS performances for chemical sensing

201 The SERS activity of the substrates was optimized by depositing different Au thicknesses. Fig. 4 shows
202 the SERS spectra of thiophenol molecules grafted on the ZnO urchin-like structures covered with 10,

203 30, and 50 nm of gold for the two excitation wavelengths used here. Thiophenol was selected due to
 204 its ability to form self-assembled monolayers (SAMs) via S-Au bonds [45] and the absence of
 205 molecular vibrational activity near the selected excitation wavelengths (633 nm and 785 nm). The
 206 characteristic vibrational modes of thiophenol molecules (10^{-3} M) are well-observed (see refs [46-48])
 207 like those at 999 cm^{-1} corresponding to the ring out-of-plane deformation and the C-H out-of-plane
 208 bending (noted, respectively: r-o-d and $\gamma(\text{CH})$), 1022 cm^{-1} corresponding to the C-C symmetric
 209 stretching and the ring in-plane deformation (noted, respectively: $\nu(\text{CC})$ and r-i-d), 1072 cm^{-1}
 210 corresponding to the C-C symmetric stretching and C-S stretching (noted, respectively: $\nu(\text{CC})$ and
 211 $\nu(\text{CS})$), and at 1572 cm^{-1} the C-C symmetric stretching (noted: $\nu(\text{CC})$). For comparison, we studied the
 212 peak intensity at 999 cm^{-1} . It is noticeable that the Raman peaks intensities become larger as we
 213 increase the Au layer thickness for the two excitation wavelengths. This is in accordance with
 214 literature reports where it is shown that the thickness of the metallic layer plays an important role on
 215 the overall SERS enhancement [49–51]. For our complete study, we chose the 30-nm thickness with the
 216 excitation wavelength of 785 nm in order to obtain a good compromise between the SERS signal
 217 enhancement and a low overall cost of production of these SERS substrates. Fig. 5a shows the
 218 thiophenol spectra obtained from different structures for the purpose of comparison. When ZnO
 219 nanowires covered with Au (30 nm) of a size comparable from those on the ZnO urchin-like structures
 220 are used, the characteristic Raman peaks of thiophenol can barely be distinguished. Similarly, for the
 221 bare Au film (30 nm), the thiophenol peaks can be hardly identified. This result shows that the urchin-
 222 like structures present the highest SERS activity from these three types of substrate configurations.



223
 224 **Figure 4.** SERS spectra of thiophenol (10^{-3} M) from different Au film thicknesses (10, 30, and 50 nm) for the two
 225 excitation wavelengths used in our experiments: (a) 633 nm and (b) 785 nm.
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227

228 **Figure 5.** (a) SERS spectra of thiophenol (10^{-3} M) from a 30-nm Au film, a 30-nm Au ZnO NWs, and a 30-
 229 nm Au covered urchin-like ZnO, which present an offset to improve the data visualization. (b) SERS
 230 spectra of the 30 nm Au-covered ZnO urchins with different thiophenol concentrations. The spectra are
 231 not background-corrected, and recorded at the excitation wavelength of 785 nm.

232 Given the complex 3D morphology of the urchins, it is difficult to know the exact number of
 233 molecules that are excited in the SERS measurements [52,53]. For this reason, the EF for the 30 nm Au-
 234 covered ZnO urchins SERS substrates was calculated by using the formula: $EF \approx I_{SERS}/I_{ref}$ [10] where I_{ref}
 235 represents the Raman intensity of a thiophenol solution in ethanol (1 M; see fig. S4 in SI) and I_{SERS}
 236 represents the Raman intensity of the SERS measurements. Thus, the highest EF value for the Raman
 237 peak at 1572 cm^{-1} was 5.3×10^2 (Table 1). From the extinction spectra (see figure 6), we observed that
 238 the excitation wavelengths of 633 nm and 785 nm are, respectively, more and more close to maximum
 239 of one resonance when the gold thickness increases that can significantly improve the enhancement
 240 factor. Moreover, it is known that EF is proportional to the extinction intensities (Q_e) [50,54], i.e.
 241 $EF \sim Q_e(\lambda_{exc}) \times Q_e(\lambda_{Raman})$. We observed that the EF values (see Table 1) follow qualitatively the same
 242 trend than those observed from the extinction spectra. These EF values can be attributed to the
 243 combination of different effects such as efficient light trapping [31] and the generation of hotspots
 244 from almost-touching NWs from adjacent urchins [55]. Additionally, the size of the gaps between the
 245 Au-covered urchin-like structures is in the same range as the excitation wavelength, which could
 246 contribute to the enhancement of localized electromagnetic fields and effective absorption of light by
 247 the LSPR [10,32]. SERS measurements with a range of molecular concentrations from 10^{-5} M – 10^{-8} M
 248 were performed in order to assess the limit of detection (LOD) of the substrates. Fig. 5b shows the
 249 Raman spectra of the substrates grafted with different thiophenol concentrations. The intensities of the
 250 Raman peaks remain strong up to a concentration of 10^{-7} M and then fall considerably for the
 251 concentration of 10^{-8} M where only the peak at 999 cm^{-1} remains visible. This LOD is comparable with
 252 values reported in the literature for thiophenol and similar Au-based structures [47,50].

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Raman shift (cm ⁻¹)	999	1022	1072	1572
I _{SERS}	15066	12199	16926	9539
I _{Raman}	79	32	55	18
EF	1.9×10^2	3.8×10^2	3.1×10^2	5.3×10^2

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Table 1. Calculated enhancement factors (EF) for four Raman peaks (1 mM of thiophenol) at the excitation wavelength of 785 nm for ZnO urchin-like structures covered with a 30-nm Au film.

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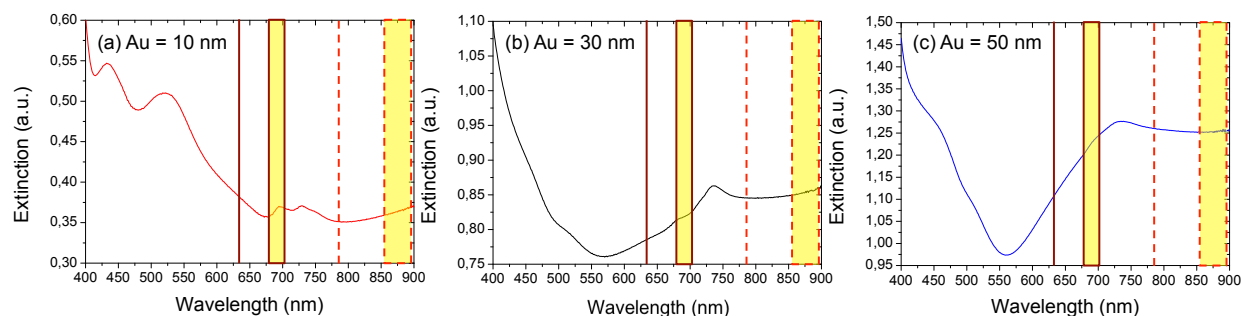
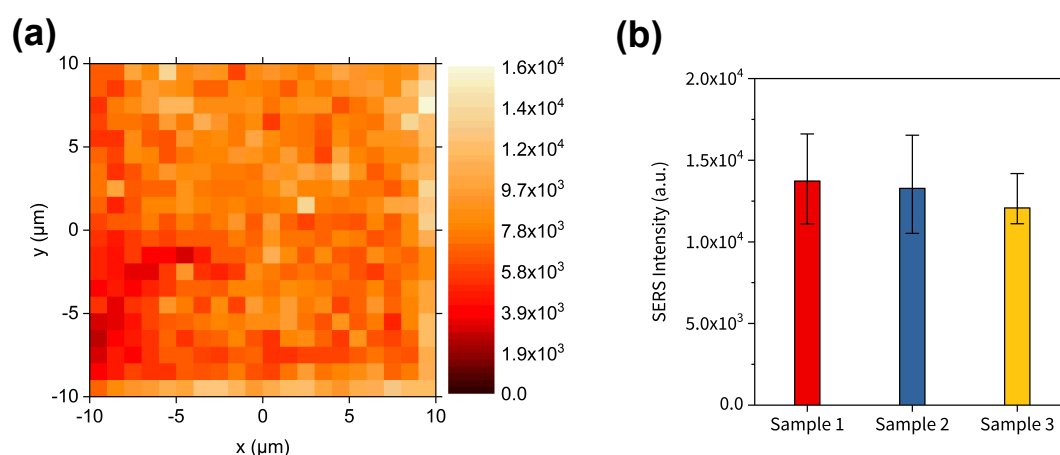


Figure 6. Extinction spectra of Au/ZnO-urchins with different thicknesses of gold: (a) Au = 10 nm, (b) Au = 30 nm, and (c) Au = 50 nm. On each extinction spectrum, the excitation wavelengths of 633 nm (dark red line) and 785 nm (red dashed line) are displayed, and also the region of the corresponding Raman scattering wavelengths (999 to 1572 cm⁻¹; dark red/yellow rectangle associated to the excitation wavelength of 633 nm, and dashed red/yellow rectangle those associated to the excitation wavelength of 785 nm).

271 3.2 Uniformity and substrate-to-substrate reproducibility of SERS signal

272 To demonstrate the large-area uniformity of the Au-covered hollow urchin-like structures, we
 273 constructed a Raman intensity map of the 999 cm⁻¹ peak over a 20 × 20 μm² area with a step size of 1
 274 μm (Fig. 7a). The 400-point map shows an almost continuous bright-colored area with a few darker
 275 spots where the intensity is lower, which demonstrates the reasonable uniformity of the substrate.



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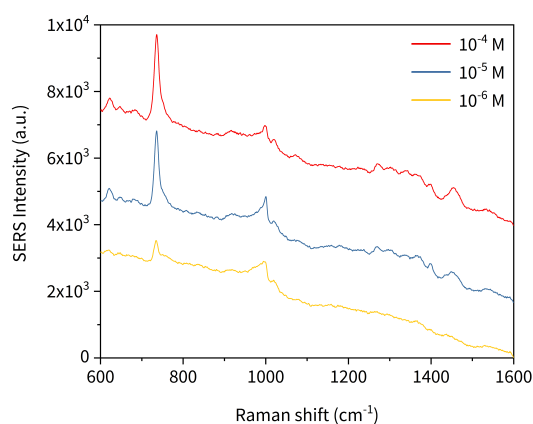
Figure 7. (a) Raman map image of the 999 cm⁻¹ peak from a randomly selected area (20 × 20 μm²) on the Au-covered hollow urchin-like structures at the 785-nm excitation wavelength. (b) Intensity distribution of the 999 cm⁻¹ peak of three different samples with an average RSD value < 10%.

279

280 Additionally, the substrate-to-substrate reproducibility was investigated by measuring the intensity of
281 the 999 cm^{-1} peak from three different batches. Fig. 7b shows the intensity distribution for three
282 different samples. The average RSD value was found to be $< 10\%$, which indicates a good
283 reproducibility of the SERS substrates and shows their excellent performance.

284 3.2 SERS performances for biological sensing

285 Finally, to demonstrate the biosensing capability of these substrates, we chose the biomolecule
286 adenine which can be used in the detection of DNA or RNA analytes [10,27]. Fig. 8 shows SERS
287 spectra of Adenine at three different concentrations using the urchin-like ZnO structures covered with
288 30 nm of Au, and recorded at the excitation wavelength of 785 nm. The characteristic purine stretch at
289 736 cm^{-1} is noticeable up to a concentration of 1 μM and the spectra show a low signal-to-noise ratio,
290 which is beneficial for future biosensing applications and is comparable to results published in the
291 literature [10,27].



292

293 **Figure 8.** SERS spectra for different concentrations of adenine with a LOD of
294 1 μM .

293

294

295 4. Conclusion

296 In summary, high-surface-area Au-covered hollow ZnO urchin-like structures were prepared by
297 combining NSL, ALD, electrodeposition, and e-beam evaporation. These high-throughput methods
298 are readily scalable and allow the precise control of the size of the urchins and the interparticle
299 distance. The amplification of the SERS signal for thiophenol was investigated by depositing different
300 thicknesses of Au, and the 30-nm thickness was selected for its good compromise between the
301 enhancement of the SERS intensity and the low cost of production. The LOD of the selected substrates
302 was tested by detecting thiophenol down to 10^{-8} M. Additionally, the batch-to-batch repeatability was
303 demonstrated and an average RSD $< 10\%$ was obtained. Finally, adenine was detected up to a
304 concentration of 10^{-6} M in order to demonstrate the biosensing capability of our substrate. These
305 results suggest that the Au-covered hollow urchin-like ZnO structures are viable candidates for
306 ultrasensitive and reproducible SERS sensing and biosensing.

307

308

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