Universal One-Pot and Scalable Synthesis of SERS Encoded Nanoparticles

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ABSTRACT: Encoded particles are one of the most powerful approaches for multiplex high-throughput screening. Surface-enhanced Raman scattering (SERS) based codification can, in principle, avoid many of the intrinsic limitations due to conventional alternatives, as it decreases the reading time and particle size while allowing for almost unlimited codification. Unfortunately, methods for the synthetic preparation of these particles are tedious; often subjected to limited reproducibility (associated with large fluctuations in the size distributions of the polymers employed in the standard protocols); and to date, limited to a small amount of molecules. Herein, we report a universal, one-pot, inexpensive, and scalable synthetic protocol for the fabrication of SERS-encoded nanoparticles. This synthetic strategy is highly reproducible, independent of the chemical nature and size of the Raman code used (31 different codes were tested) and scalable in the liter range without affecting the final properties of the encoded structures. Furthermore, the SERS efficiency of the fabricated encoded nanoparticles is superior to that of the materials produced by conventional methods, while showing a remarkable reproducibility from batch to batch. This encoding strategy can easily be applied to nanoparticles of different materials and shapes.

INTRODUCTION

Encoded nanoparticles are among the most powerful alternatives for high-throughput multiplex screening in microarray technology, diagnosis, and bioimaging. These materials are simple and cost-effective platforms that allow for fast, sensitive, and reliable analyses. During the past decade, several encoded particles have been prepared using codification strategies based on changes in particle shape, composition, physical marks, or spectroscopic properties (e.g., luminescence or vibrational fingerprints). Among all of them, those based on surface-enhanced Raman scattering (SERS) are gaining importance because of (i) virtually unlimited multiplexing capability associated with the unique vibrational fingerprints of the different codes; (ii) short detection times (milliseconds) thanks to the intrinsic sensitivity of the SERS phenomenon; (iii) small size, allowing for biomimics and (iv) photostability and low toxicity (as compared to those of dyes or quantum dots).

In essence, a SERS-encoded nanoparticle (also called a SERS tag) comprises a plasmonic nucleus, responsible for the generation of the electric field necessary for the Raman amplification; a Raman probe (i.e., code), responsible for the unique vibrational fingerprint of the encoded particle; and a coating layer. This external coating is of key importance as it (i) prevents the code from leaching out into the medium, thus avoiding toxic effects or vibrational cross-contamination with the codes of other particles; (ii) protects the plasmonic particle from contaminations of the medium that could give rise to vibrational noise that would hinder the particle readout; (iii) increases the colloidal stability of the particle; (iv) provides a convenient surface for further chemical functionalization; and (v) protects the plasmonic core from interacting with other plasmonic particles, avoiding plasmon coupling and thus the uncontrolled generation of hotspots. Although polymers have been reported as particle coatings, the unique properties of silica (i.e., known surface chemistry, biocompatibility, optical transparency, and colloidal stability) make this material the most efficient protective layer for nanoparticles by far.

Silica coating of nanoparticles requires the colloidal stabilization of the particles in ethanolic solution prior to the hydrolysis/condensation of tetraethyl orthosilicate (TEOS). Although a range of polymers has been proposed for this task, the most common remains polyvinylpyrrolidone.
**Figure 1.** Schematic representation of the synthetic procedure for the production of SERS-encoded nanoparticles showing the different steps involved in the synthesis. First, citrate-capped Au nanoparticles are produced. Second, MUA is used to stabilize the particles in basic media. Third, an excess of SERS code is added to encode the particles. Fourth, a silica shell is grown on the particles to ensure stability for long periods of time and avoid undesired plasmon coupling.

(PVP). On the other hand, surfactants such as cetyltrimethylammonium bromide (CTAB) are also used commonly for this reaction. Nevertheless, with the exception of nanogapped core–shell nanoparticles, the most important factor for the generation of active SERS-encoded particles is intimate contact between the Raman code and the plasmonic structure.

This requirement introduces further complexity into the coating process associated with the surface chemistry properties. Both PVP and CTAB form solid layers of coating on the surface of the particles, limiting or even preventing the interaction of the encoding agent with the metallic core when added to the solution. Therefore, to increase the code adsorption efficiency on the plasmonic structure and, thus, the SERS signal, PVP and CTAB species need to be removed from the metallic surfaces.

On the other hand, this removal usually results in a dramatic reduction of the colloidal stability, which is further aggravated by the nonpolar nature of most codes, leading to uncontrolled particle agglomeration or even to irreversible precipitation. Aggregation of labeled nanoparticles into clusters of different sizes and geometries does generate very active SERS structures but with a highly inhomogeneous SERS response. Moreover, these fabrication methods normally work for a very limited number of encoding molecules as, in many cases, precipitation of the whole colloid occurs upon addition of the code (except for very low amounts of label). In fact, this explains why, in most of the literature, examples of SERS-encoded particles include small numbers of codes, usually just three or four.

As an alternative to conventional polymers or surfactants, thiolated poly(ethylene glycol) (PEG) has been successfully employed for the controlled silica coating of single metallic nanoparticles. The high polarity and porosity of this polymer efficiently stabilize particles in alcohol and water while allowing for the at least partial diffusion of the code to the metallic surface.

Importantly, polymers commonly suffer from large fluctuations in size distribution from batch to batch even for the same commercial brand. As a result, the synthetic protocol for encoding particles using these materials often needs to be retuned when a new polymer is purchased. Additionally, the high price of thiolated PEG hinders its use in the large-scale preparation of encoded particles as required for real-life applications. In contrast to most of the reported procedures, which typically involve complex steps, herein, we demonstrate an easy and fast one-pot approach for the production of SERS-encoded nanoparticles. As the initial plasmonic material, citrate-capped gold nanoparticles were selected, as most of the published research on SERS-encoded particles has been carried out on spherical gold nanoparticles. However, the encoding protocol can be readily applied to other metals, such as citrate-reduced silver colloids, and nanoparticle shapes, such as gold nanostars.

This versatile strategy allows for the SERS codification of particles with every molecule with affinity toward the metal surface, independently of its chemical nature, as exemplified here in the fabrication of 31 different encoded particles using exactly the same standard procedure. The method relies on the controlled coabsorption of mercaptoundecanoic acid (MUA) and the SERS code on the metallic surfaces. In addition to the ease of preparation, scalability to the liter range, and stability in aqueous solutions, our SERS-encoded particles show considerably higher optical efficiencies than those fabricated using PEG or PVP polymers.

### RESULTS AND DISCUSSION

A schematic outline of the universal protocol for the fabrication of encoded particles is presented in Figure 1. The process can be divided into four different steps: (1) synthesis of citrate-capped gold nanoparticles of ca. 50-nm diameter (see Figure S1 in the Supporting Information for transmission electron microscopy (TEM) images and size distribution histograms), (2) MUA functionalization, (3) SERS codification, and (4) silica coating. Because of the low stability of colloidal solutions upon functionalization with the SERS code, a stabilization step is required prior to the codification. MUA was chosen as the stabilizing agent because it binds covalently to the gold surface through the thiol group while providing particle stability with both a long aliphatic chain (steric repulsion) and a terminal carboxylic group (electrostatic repulsion). On the other hand, because of its aliphatic nature, its Raman cross-section is almost negligible as compared to those of aromatic compounds (Figure S2, Supporting Information). Nevertheless, the presence of a thiol group implies that MUA should be added in an adequate proportion to avoid the formation of a compact monolayer that could passivate the metallic surface, preventing the retention of the SERS codes, and with extreme care to avoid heterogeneous adsorption of the molecule by some of the colloids in the solution. Thus, in a second step, MUA was rapidly added under vigorous stirring at basic pH to yield MUA-functionalized gold nanoparticles (Au@MUA).

To maximize the final SERS efficiency of the encoded nanoparticles, the MUA surface coverage was decreased as much as possible to provide maximum accessibility to the metal surface while preserving the overall colloidal stability when exposed to an excess of the SERS code. The addition of the code is depicted as the third step in Figure 1. Among all of the investigated encoding molecules, 2-mercaptopyridine (MPy) was observed to induce the fastest colloidal aggregation upon
addition to the bare citrate-capped gold nanoparticles. For this reason, the optimization of the protocol was performed using this molecule (i.e., the worst colloidal stability scenario). MUA has been reported to occupy an area of 0.22 nm², corresponding to ca. 4.5 molecules nm⁻².⁴⁶ Therefore, experiments decreasing the amount of MUA were designed for concentrations of between 5.0 and 0.1 molecules nm⁻². After sufficient time had been allowed for MUA adsorption to reach its thermodynamic equilibrium (30 min), MPy was added in a large excess (15 molecules nm⁻²) to yield the corresponding Au@MUA/MPy nanoparticles. Aggregation events in the colloidal system were monitored by UV–vis–NIR spectroscopy (Figure 2) via comparison of the localized surface plasmon resonance (LSPR) at 535 nm, associated with isolated gold nanoparticles, and the absorption feature at 750 nm, attributed to plasmonic contributions of interacting particles and thus indicative of aggregation (Figure 2C). Concurrently, SERS was also monitored in the same samples to estimate the amount of adsorbed code (Figure 2B). The SERS measurements were performed by focusing a 785-nm laser onto the colloidal suspension ([Au] = 1 mM) with a long-working-distance objective. At concentrations between 5 and 0.8 MUA molecules nm⁻² (stable colloidal regime), the extinction spectra of Au@MUA/MPy exhibit the characteristic LSPR of monodisperse spherical gold nanoparticles in suspension. At 0.7 molecules nm⁻², the appearance of a shoulder at ca. 700 nm was observed, indicating the significant formation of nanoparticle aggregates. A further decrease of the MUA surface coverage led to a dramatic perturbation of the colloidal stability upon addition of MPy, as clearly revealed by the dominant plasmonic contribution at longer wavelengths. Therefore, the range of colloidal stability was identified as being between 5 and 0.8 MUA molecules nm⁻². Conversely, the monitoring of the SERS intensity of the MPy ring breathing mode at 1001 cm⁻¹ (Figure 2D) reveals the existence of three SERS regimes. The first corresponds to particle aggregation (below 0.8 MUA molecules nm⁻²) and, as expected, shows a marked increase of the intensity due to uncontrolled plasmon coupling (see also the very large standard deviation). The second, between 1.4 and 0.8 MUA molecules nm⁻², reveals a progressive decrease of the SERS intensity of MPy as the MUA content increases. In this regime, an increase in MUA surface coverage is directly reflected in the decrement of code adsorption onto the metal surface. In the third regime, 1.6–5 MUA molecules nm⁻², the SERS intensity remains constant as MUA forms a progressively fuller monolayer and only a fixed amount of MPy molecules can diffuse to the nanoparticle surfaces. Notably, for small molecules such as MPy, the SERS intensity never decays to zero. This is because, unlike for the crystalline arrangement of densely packed films of alkanethiols on gold surfaces,⁴⁷ the Coulomb repulsions between the negatively charged carboxylic groups of MUA limit the lateral interactions of the hydrophobic alkyl chains, preventing the formation of a thick molecular packing on the surface.⁴⁸,⁴⁹ Clearly, accessibility to the metal surface in this particular regime is highly dependent on the chemical and geometrical properties of the SERS code. For instance, for a MUA concentration of 4 molecules nm⁻², almost no SERS signals were observed for large molecular codes (Figure S3, Supporting Information). Therefore, 0.8 molecules nm⁻² was identified as the optimum MUA concentration for the production of our SERS-encoded gold nanoparticles, preventing colloidal aggregation and maximizing the final SERS signal.

Finally, to ensure stability for long periods of time, protect both the SERS code and the plasmonic core, and generate a readily functionalizable external surface, the Au@MUA/MPy nanoparticles were encapsulated in a silica matrix. Silica coating was performed using a modification of the well-known Stöber method by exploiting the ability of ligands with terminal carboxylic acid groups, such as MUA, to induce silica growth.⁵¹⁻⁵⁵ To this end, appropriate amounts of ethanol and NH₄OH were added to the Au@MUA/MPy aqueous suspension to maintain an adequate pH of the solution and provide the correct EtOH/H₂O molar ratio (1.84) for the Stöber process. Then, TEOS was added to initiate silica growth. The solution was allowed to react for 14 h at room temperature before being subjected to several washing cycles (Figure 3B–D presents characteristic TEM images of Au@MUA/MPy@SiO₂ nanoparticles). A thick silica shell of ca. 30 nm was grown on top of the labeled nanoparticles to prevent the localization of intense local fields at the nanoparticle junctions of possible SERS-encoded nanoparticle agglomerates. Figure 3A displays the extinction spectra of the colloidal suspension after each fabrication step. As can be seen, the shift of the LSPRs of individual nanoparticles clearly reflects the changes in the
were signal. When PVP was used, citrate-stabilized Au nanoparticles followed and optimized to achieve the highest possible SERS on the metallic surface.

These washing cycles are critical to maximize the adsorption of the surface of the particles while maintaining the colloidal stability. Washed to remove as much as possible of the PVP from the code onto the metallic surface. Prior to code addition, the particles were extensively using PVP. Prior to code addition, the particles were extensively fabricated (when possible) with the most common polymer-based procedures used in the literature: PVP and thiolated PEG. Both polymer-based strategies rely on the universal applicability of this synthetic strategy. Figure 4 shows the SERS signatures and TEM images of 11 representative SERS-encoded nanoparticles, whereas another 20 codes are reported in the Supporting Information (Figure S5). Notably, the one-pot synthetic method was successfully employed in the fabrication of larger volumes of SERS-encoded nanoparticles (in the liter range; Figure S6, Supporting Information) without impacting the final characteristics of the substrate, which clearly demonstrates the scalability of the process.

To evaluate the optical efficiency of our protocol, we compared the SERS intensities provided by seven of our SERS-encoded nanoparticles with those yielded by their analogous counterparts fabricated (when possible) with the most common polymer-based procedures used in the literature: PVP and thiolated PEG. Both polymer-based strategies rely on the required stability to the Au particles during the whole process with no appreciable formation of aggregates. It is worth noting that, independently of the SERS encoding process and the related colloidal stability, homogeneous silica coatings could be achieved only for MUA concentration above 0.7 molecules nm$^{-2}$ (Figure S4, Supporting Information).

The described protocol was successfully extended to a large set of different codes, including thiolated and nonthiolated aromatic small molecules and dyes (phenothiazines, rhodamines, oxazines, triarylmethanes, tri- and tetrzaoles, etc.), confirming the universal applicability of this synthetic strategy. Figure 3A shows the extinction spectra of Au particles after each step of the MUA-based protocol. Inset: Detail of the plasmon absorption maxima. (B–D) Representative TEM images at different magnifications of the MPy SERS-encoded gold nanoparticles.

In the case of thiolated PEG, it was previously shown that the polymeric layer can significantly hinder the diffusion of molecules onto the metal surface. Consequently, because of the very limited number of Raman molecules capable of binding PEG-coated nanoparticles, the use of the corresponding Au@PEG@code particles is largely limited to surface-enhanced resonant Raman scattering (SERRS) conditions; i.e. when the electronic excitation of the dye is in resonance with the laser beam. An identical limitation applies if the codification step is performed prior to the PEG-coating step (Au@code@PEG) because, in this case, except for few fortunate cases of “nanoparticle-stabilizing” SERS code, the number of molecules per unit area that can be adsorbed onto the metal without perturbing the colloidal stability is very small. We therefore first tried to improve the accessibility of the Raman label to the metal surface (i.e., increasing the number of code molecules per nanoparticle) by progressively lowering the amount of added PEG-SH from 4 molecules nm$^{-2}$, as reported in the literature, to 0.2 molecules nm$^{-2}$. However, nanoparticle aggregation was already observed at a polymer concentration less than 3.5 molecules nm$^{-2}$, even prior to the addition of the excess of MPy (Figure S7, Supporting Information). Moreover, no distinguishable SERS signals were recorded upon functionalization of the PEG-coated gold nanoparticles.
nanoparticles with the excess of MPy unless in the presence of colloidal aggregation (Figure S7, Supporting Information). Discarding the possibility to increase the MPy surface coverage by decreasing the PEG-SH concentration, we pursued a different strategy.\(^{56,57}\) In this case, CTAB-stabilized Au nanoparticles of a similar size were prepared instead of citrate-capped colloids. The surfactant double layer offers an effective stabilizing shell, preventing nanoparticle aggregation when the colloids are exposed to lower PEG amounts (corresponding to 1.8 molecules nm\(^{-2}\) under our optimized experimental conditions). The resultant PEG/CTAB-stabilized nanoparticles were then subjected to, first, several washing cycles to remove excess surfactant and, second, exposure to the excess of MPy in the codification step without impacting the colloidal stability. This optimized protocol allowed for the synthesis of SERS-encoded nanoparticles with much higher SERS efficiencies, generally larger than that observed for the PVP approach, but still significantly lower than that provided by the MUA-based method (Figure 5A and Figure S8, Supporting Information). Furthermore, it is important to stress that, even when optimized, the combined CTAB/PEG approach retains intrinsic limitations and problems, such as the necessity for multiple cycles of centrifugation, separation, and resuspension (which are often critical for colloidal stability and also represent practical obstacles for large-scale production) and the use of large amounts of the highly cytotoxic CTAB.\(^{58}\)

Table 1 summarizes the SERS efficiency ratios calculated by comparing the SERS intensities of seven different SERS-encoded nanoparticles obtained by the MUA-based protocol with those yielded by the PEG-SH and PVP approaches (\(I_{\text{MUA}}/I_{\text{PVP}}\) and \(I_{\text{MUA}}/I_{\text{PEG-SH}}\), respectively). For all the investigated cases, the SERS intensity achieved with the MUA-based protocol lies between 140 to 10 times higher than in the case of PVP, and from 10 to 2 times higher than in the case of thiolated PEG. Such drastic differences from one Raman label to another can be ascribed to the different chemical natures and molecular sizes of the codes which clearly affect their ability to diffuse through the external polymeric layer and finally adsorb onto the metal surface.

As previously indicated, modification of the nanoparticle surface chemistry by adsorption of Raman labels normally results in a decrease of the colloidal stability in suspension. However, in a very few cases, the specific chemical nature of the code molecule can still preserve such stability, with the code acting as a stabilizing agent. This is the case, for instance, for 4-mercaptobenzoic acid (MBA). As for MUA, the carboxylic groups of MBA bound to the metal surface are oriented toward the bulk solution, providing, at basic pH, the necessary electrostatic repulsion to avoid nanoparticle aggregation and, in the subsequent silica-coating step, promote silica growth. As a result, this SERS-encoded nanoparticle can be synthesized at full MBA coverage with no need for external stabilizing agents. We therefore assess the SERS performance of MBA-encoded nanoparticles produced by our MUA synthetic method (0.8 MUA molecules nm\(^{-2}\)) with respect to the same SERS tags obtained at full MBA coverage (i.e., 0 MUA molecules nm\(^{-2}\), maximum SERS efficiency). Figure 5C shows the corresponding SERS spectra as well as those of the analogous SERS tags synthesized by the PVP and thiolated-PEG methods. Notably, MUA functionalization results in a ca. 42% loss with respect to the maximum SERS efficiency, which is much less than those observed for the PVP (ca. 97% reduction) and thiolated-PEG (92% reduction) methods. The SERS enhancement factor for gold spherical nanoparticles fully coated with MBA was also calculated by direct comparison with the normal Raman spectrum of MBA in 0.1 M ethanol solution (Figure S9, Supporting Information). The estimated enhancement factor at

<table>
<thead>
<tr>
<th>code</th>
<th>(I_{\text{MUA}}/I_{\text{PVP}})</th>
<th>(I_{\text{MUA}}/I_{\text{PEG-SH}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBA</td>
<td>22.2</td>
<td>3.6</td>
</tr>
<tr>
<td>BT</td>
<td>14.9</td>
<td>3.8</td>
</tr>
<tr>
<td>MPy</td>
<td>26.5</td>
<td>2.7</td>
</tr>
<tr>
<td>INT</td>
<td>10</td>
<td>3.1</td>
</tr>
<tr>
<td>MBA</td>
<td>19.4</td>
<td>7</td>
</tr>
<tr>
<td>TFBT</td>
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<td>2.9</td>
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<td>4NBT</td>
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<td>2</td>
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Finally, in addition to spherical gold nanoparticles, we demonstrate the straightforward application of the MUA-based encoding protocol to other SERS-enhancing platforms such as gold nanostars prepared by the standard PVP protocol\(^\text{59}\) (i.e., similar shape but different material) or citrate-reduced silver colloids (i.e., similar shape but different material). Gold nanostars were selected as a representative example of highly SERS-active spiked nanostructures. Figure 6 presents representative SERS spectra and TEM images of differently shaped SERS-active spiked nanostructures. The enhancement factors associated with these structures were found to be 9.12 \(\times\) 10\(^7\) M for the silver nanostars and 6.73 \(\times\) 10\(^7\) M for the silver spherical particles. 

**CONCLUSIONS**

In summary, herein, we describe a universal, one-pot, inexpensive, and scalable synthetic protocol for the fabrication of SERS-encoded nanoparticles. This method relies on the functionalization of plasmonic nanoparticles with a submonolayer of mercaptoundecanoic acid, providing high colloidal stability during the codification process while allowing Raman labels to easily diffuse onto the metal. Furthermore, in a subsequent step, the carboxylic groups of MUA also act as functional sites promoting silica growth on the outer shell of the nanoparticles. This synthetic strategy was found to be successfully applicable to every Raman code we tested (31 codes) and scalable up to 2 L without affecting the final properties of the encoded structures. It is worth noticing that the MUA-based method avoids the use of high-molecular-weight polymers and their associated issues of reproducibility from batch-to-batch even for different manufacturers. The SERS efficiencies of the so-fabricated encoded nanoparticles were found to be 2–140 times higher than those of the corresponding SERS tags prepared by the common polymer-based methods (i.e., using PVP and thiolated PEG). The MUA-based protocol can be readily applied to metallic nanoparticles, such as citrate-reduced silver colloids or gold nanostars.

Figure 6. SERS spectra of encoded Ag nanoparticles (yellow curve; [Ag] = 1 mM; Raman probe = 3,4-dichlorobenzenethiol, DBT) and gold nanostars (red curve; [Au] = 1 mM; Raman probe = 3,4-dichlorobenzenethiol, DBT) and gold nanostars prepared by the standard PVP protocol\(^\text{59}\) (i.e., same material but different shape) or citrate-reduced silver colloids (i.e., similar shape but different material). Gold nanostars were selected as a representative example of highly SERS-active spiked nanostructures. Figure 6 presents representative SERS spectra and TEM images of the corresponding encoded nanoparticles are also included.

**EXPERIMENTAL SECTION**

**Materials and Methods.** Gold(III) chloride trihydrate (99.9%, HAuCl₄·3H₂O), dehydrated trisodium citrate (299.5%, C₆H₅Na₃O₇·2H₂O), ammonia solution (29%, NH₄OH), t-succinic acid (299.0%, AA), tetraethoxysilane (99.999%, TEOS), ethanol (99.5%, EtOH), polyvinylpyrrolidone (average MW = 58000, PVPk58), polyvinylpyrrolidone (average MW = 8000, PVPk38), cetyltrimethylammonium bromide (99.72%, CTAB), 11-mercaptoundecanoic acid (95%, MUA), 2-mercaptopyridine (97%, MPy), 4-nitrobenzenethiol (80%, 4NBT), mercaptophenol (97%, 4MP), mercaptobenzoic acid (99%, MBA), 3,5-bis(trifluoromethyl)benzenethiol (97%, 35BTFMB), 4-fluorophenol (98%, 4FPT), 2,3,5,6-tetrafluorobenzothiophenol (97%, 2356TFBT), 2-(trifluoromethyl)benzenethiol (96%, 2TFMBT), 3-fluorophenol (95%, 3FPT), Nile blue A (95%, NBA), 2-fluorophenol (97%, 2FPT), toluidine blue O (≥84%, TB), benzenethiol (97%, BT), 4-((3-mercaptopropyl)-2-methoxybenzyl-4H-1,2,4-triazole-4-yl)imino)methyl)phenol (97%, MMPHTYIMP), 4-((3-mercaptopropyl)-2-pyridinyl)-4H-1,2,4-triazole-4-yl)imino)methyl)benzoic acid (MPHTYIMBA), 4-((3-mercaptopropyl-2-pyridinyl)-4H-1,2,4-triazole-4-yl)imino)methyl)-1,2-benzenediol acid (MPHTYIMBD), 1-(4-hydroxyphenyl)-1H-tetrazole-5-thiol (97%, HPHTT), 1,1′,4′-terphenyl-4-thiol (97%, TPT), 1-naphtalenethiol (99%, 1NT), 2-naphtalenethiol (99%, 2NT), 5-(4-methoxyphenyl)-1,3,4-oxidazoline-2-thiol (97%, MPOT), 2-methylene blue (≥92%, MB), 3,4-dichlorobenzenethiol (97%, DCBT), diphenyl-4-thiol (97%, BPT), 7-mercaptop-4-methylcoumarin (≥97%, MMC), biphenyl-4-4′-dithiol (95%, BDPT), thiosalicylic acid (97%, TSA), 5-amino-1,3,4-thiazidazole-2-thiol (≥87%, ATT), 4-aminothiophenol (97%, 4ATP), 2-phenylethanethiol (98%, 2PET), and crystal violet (≥90%, CV) were purchased from Sigma-Aldrich GmbH (Munich, Germany). All reagents were used without further purification. Milli-Q water (18 MΩ cm\(^{-2}\)) was used in all aqueous solutions, and all glassware was cleaned with aqua regia before the experiments.

**Synthesis of Citrate-Stabilized Spherical Gold Nanoparticles.** Spherical gold nanoparticles of approximately 50-nm diameter were produced by codification with a modification of the well-known Turkevich method.\(^\text{60}\) Briefly, 308 µL of an aqueous solution of HAuCl₄\((0.081 \text{ M})\) was added to a boiling aqueous solution of sodium citrate (100 mL, 0.27 mM) under vigorous stirring. Heating and stirring were continued for 30 min. A condenser was utilized to prevent the evaporation of the solvent. During this time, the color of the solution gradually changed from colorless to purple to finally become deep red. After this time, heating was continued, and the condenser was removed to allow evaporation of the solvent to half its initial volume, to achieve a final gold concentration of 5 \(\times\) 10\(^{-4}\) M.

**Mercaptoundecanoic Acid Functionalization of Gold Nanoparticles.** To provide colloidal stability to the Au nanoparticles during the encoding process and later promote the growth of silica, 50 mL of the as-produced spherical gold nanoparticles was functionalized with a small amount of MUA (0.8 molecules nm\(^{-2}\)). To this end, a solution containing NH₄OH (879 µL, 29% aqueous solution) and MUA (1 mL, 3.99 \(\times\) 10\(^{-3}\) M in EtOH) was prepared. This solution was then rapidly added under vigorous stirring to the gold nanoparticle sol (50 mL). Agitation was continued for 30 min to ensure MUA functionalization of the Au surface.

**Gold Nanoparticle Codification.** With the aim of demonstrating the versatility of the presented method, 31 different SERS-active molecules were used, namely, MPy, 4NBT, 4MP, MBA, 35BTFMB, 4FPT, 2356TFBT, 2TFMBT, 3FPT, NBA, 2FPT, TB, BT, MMPHTYIMP, HPHTT, TPT, 1NT, 2NT, MPOT, MB, DCBT, BPT, MMC, BPDT, CV, 2FET, 4ATP, ATT, TSA, MMPHTYIMBD, and MPHTYIMBA. The exact same procedure was used for each molecule. Briefly, a solution containing EtOH (324.89 mL) and NH₄OH (5.73 mL, 29% aqueous solution) was prepared. This solution was then rapidly added under vigorous stirring to the MUA-stabilized gold nanoparticles (51.88 mL). Next, a stock solution of the SERS-active molecule was prepared (10⁻² M, in EtOH), and 74.8 µL of this solution was added to the MUA-functionalized Au particles (382 mL) under strong magnetic stirring for 30 min (to ensure proper
Au functionalization). The amount of SERS-active molecules added was calculated to be 15 molecules nm\(^{-2}\).

Silica Encapsulation. The silica encapsulation of the encoded nanoparticles was achieved through a modified Stöber method using the MUA carboxylic group to promote the growth of silica as follows: The proper concentrations of H\(_2\)O, NH\(_4\)OH, and EtOH for silica growth on the MUA-SERS-encoded particle solution was previously adjusted, during the codification step, to yield final concentrations of 7.94, 0.128, and 14.60 M, respectively (EtOH/H\(_2\)O molar ratio of 1.84). Then, TEOS (13.2 \(\mu\)L) was added, and the solution was energetically shaken and left undisturbed at room temperature for 14 h. Finally, the resulting core−shell nanoparticles were cleaned to remove excess reactants by centrifugation (3 \(\times\) 6000 rpm, 20 min) and redispersed in ethanol. To concentrate the solution (10\(^{-3}\) M) to perform the SERS characterization, 15.3 M of this solution was centrifuged again (2 \(\times\) 6000 rpm, 20 min) and redispersed in water. After the final centrifugation step, everything was resuspended in a final volume of 1 mL.

Synthesis of SERS-Active Silica-Encapsulated Gold Nanoparticles. Different SERS-active molecules were used, namely, iNT, MPy, 4NBt, MBA, NBA, TB, and 2356TFBT. Spherical gold nanoparticles of approximately 50-nm diameter were produced as previously described. To 75 mL of a PVP\(_{98}\) solution (0.69 mM) was added 50 mL of the citrate Au particles dropwise, and the mixture was left to react overnight under stirring. Next, the solution was centrifuged (5400 rpm, 20 min) and redispersed in 50 mL of EtOH ([Au] = 0.5 M). To remove as much as possible of the excess PVP\(_{98}\), this process was repeated four times. Then, the code molecule (74.8 \(\mu\)M, 10\(^{-2}\) M) was added under stirring for 2 h. Finally, silica coating was carried through adjustment of the final concentrations as follows (in a 50 mL solution): [Au] = 0.5 mM, [H\(_2\)O] = 10.55 M, [NH\(_4\)] = 0.2 M, [EtOH] = 13.4 M, and [TEOS] = 1.12 mM. The reaction mixture was allowed to react for 24 h. When the reaction time was completed, the particles were centrifuged and washed with ethanol. To concentrate the solution (10\(^{-3}\) M) to perform the SERS measurements, 2 mL of this solution was centrifuged again (2 \(\times\) 6000 rpm, 20 min) and redispersed in water. After the final centrifugation step, everything was resuspended in a final volume of 1 mL.

Synthesis of PEG-Based Spherical Encoded Gold Nanoparticles. Different SERS-active molecules were used, namely, iNT, MPy, 4NBt, MBA, NBA, TB, and 2356TFBT. First, CTAB-stabilized gold nanospheres of 50-nm diameter were produced as previously described. To 75 mL of a PVP\(_{98}\) solution (0.69 mM) was added 50 mL of the as-synthesized Au spheres ([Au] = 0.25 M) and vigorously shaken. Next, 187 \(\mu\)L of the particles. The second step involved o-[2-(5-mercapto-2-propionylamino)ethyl]-o-n-methyloxyethylene glycol (PEG-SH, MW = 5000) capping, ethanol transfer, and silica coating. To do this, 100 mL of the as-synthesized Au spheres ([Au] = 0.25 M) was centrifuged for 20 min (6000 rpm), and the precipitate was redispersed in a CTAB solution ([CTAB] = 0.5 mM) to clean the CTAB as much as possible without compromising the colloidal stability of the particles. This process was repeated three times to finally redisperse in a final volume of 50 mL to obtain CTAB and Au concentrations of ~0.5 M each. Next, a stock solution of PEG-SH was prepared and sonicated for 15 min (10\(^{-4}\) M, in H\(_2\)O), and 89.8 \(\mu\)L of this solution was added to the CTAB-stabilized Au particles (50 mL) under strong magnetic stirring for 30 min (to ensure proper Au functionalization). The amount of PEG-SH added was calculated to be 1.8 molecules nm\(^{-2}\). The PEG-modified particles were centrifuged twice to remove excess PEG-SH and redispersed in ethanol (50 mL); in the second centrifugation, the particles were redispersed in a solution (50 mL) adjusted to the following final concentrations: [Au] = 0.5 mM, [H\(_2\)O] = 10.55 M, [NH\(_4\)] = 0.2 M, and [EtOH] = 13.39 M. The third step was the encoding of the nanoparticles. For this step, a stock solution of the SERS-active molecule was prepared (10\(^{-2}\) M, in EtOH), and 74.8 \(\mu\)L of this solution was added to the PEG-SH-functionalized Au particles (50 mL) under strong magnetic stirring for 2 h. The amount of SERS-active molecules added was calculated to be 15 molecules nm\(^{-2}\).

Finally, TEOS (13.20 \(\mu\)L) was added, and the solution was energetically shaken and left undisturbed at room temperature 14 h. To concentrate the solution ([Au] = 1 mM) to perform the SERS measurements, 2 mL of this solution was centrifuged again (2 \(\times\) 6000 rpm, 20 min) and redispersed in water. After the final centrifugation step, everything was resuspended in a final volume of 1 mL.

Synthesis of PVP-Based Spherical Encoded Gold Nanoparticles. Spherical silver nanoparticles (Ag NPs) of approximately 50-nm diameter were produced by adding AA (50 \(\mu\)L, 0.10 M) to 47.5 mL of boiling water. One minute after the addition, a solution previously incubated for 5 min containing AgNO\(_3\) (0.25 M, 1 wt %), sodium citrate (1 mL, 1 wt %), and 1.25 mL of Milli Q water was injected into the boiling aqueous solution. The color of the reaction solution quickly changed from colorless to yellow. The solution was further boiled for 1 h under stirring to guarantee the formation of uniform quasispherical silver nanoparticles.

Then, the synthesized Ag NPs were cleaned by centrifugation (5400 rpm, 30 min) and redispersed in a volume of Milli Q water to achieve a silver concentration of 2.5 \(\times\) 10\(^{-4}\) M. The Ag NPs were then functionalized with MUA (1.8 molecules nm\(^{-2}\)). Specifically, 5 mL of Ag NPs ([Ag] = 2.5 \(\times\) 10\(^{-4}\) M) was rapidly added to a solution containing NH\(_4\)OH (662 \(\mu\)L, 29% aqueous solution), MUA (41.6 \(\mu\)L, 1.0 \(\times\) 10\(^{-3}\) M in EtOH), and EtOH (32.50 mL) under vigorous stirring. Agitation was continued for 1 h to ensure the complete MUA functionalization of the Ag surface. Then, 3.8 \(\mu\)L of a 10\(^{-2}\) M stock solution of different SERS probes was added under strong magnetic stirring. Once more, stirring was continued for another 1 h. The amount of SERS-active molecules added was calculated to be 15 molecules nm\(^{-2}\). Finally, TEOS (12 \(\mu\)L, 10% v/v) was added, and the solution was energetically shaken and left undisturbed at room temperature for 14 h. The solution was centrifuged and redispersed in water following the same procedure as for spherical gold nanoparticles.

The nanoparticle concentration was calculated to be 0.26 nM by the Lambert−Beer law using the extinction coefficient for silver nanoparticles of 5.37 \(\times\) 10\(^{10}\) M\(^{-1}\) cm\(^{-1}\), derived from the literature.44 The synthesis, Codification, and Silica Coating of Silver Nanoparticles. Spherical silver nanoparticles (Ag NPs) of approximately 50-nm diameter were produced by adding AA (50 \(\mu\)L, 0.10 M) to 47.5 mL of boiling water. One minute after the addition, a solution previously incubated for 5 min containing AgNO\(_3\) (0.25 M, 1 wt %), sodium citrate (1 mL, 1 wt %), and 1.25 mL of Milli Q water was injected into the boiling aqueous solution. The color of the reaction solution quickly changed from colorless to yellow. The solution was further boiled for 1 h under stirring to guarantee the formation of uniform quasispherical silver nanoparticles.

Then, the synthesized Ag NPs were cleaned by centrifugation (5400 rpm, 30 min) and redispersed in a volume of Milli Q water to achieve a silver concentration of 2.5 \(\times\) 10\(^{-4}\) M. The Ag NPs were then functionalized with MUA (1.8 molecules nm\(^{-2}\)). Specifically, 5 mL of Ag NPs ([Ag] = 2.5 \(\times\) 10\(^{-4}\) M) was rapidly added to a solution containing NH\(_4\)OH (662 \(\mu\)L, 29% aqueous solution), MUA (41.6 \(\mu\)L, 1.0 \(\times\) 10\(^{-3}\) M in EtOH), and EtOH (32.50 mL) under vigorous stirring. Agitation was continued for 1 h to ensure the complete MUA functionalization of the Ag surface. Then, 3.8 \(\mu\)L of a 10\(^{-2}\) M stock solution of different SERS probes was added under strong magnetic stirring. Once more, stirring was continued for another 1 h. The amount of SERS-active molecules added was calculated to be 15 molecules nm\(^{-2}\). Finally, TEOS (12 \(\mu\)L, 10% v/v) was added, and the solution was energetically shaken and left undisturbed at room temperature for 14 h. The solution was centrifuged and redispersed in water following the same procedure as for spherical gold nanoparticles.

The nanoparticle concentration was calculated to be 0.26 nM by the Lambert−Beer law using the extinction coefficient for silver nanoparticles of 5.37 \(\times\) 10\(^{10}\) M\(^{-1}\) cm\(^{-1}\), derived from the literature.44
M with a constant flow of N_2 for 120 min. In the second step, a solution of PVP_k (15 mM) in dimethylformamide (35 mL) was sonicated for 15 min. Then, 216 μL of H_{2}AuCl_4 (0.081 M) was added to the mixture under rapid stirring at room temperature, followed by the concentrated seeds (1.108 mL, [Au] = 9.45 × 10^-4 M). Within 5 min, the color of the solution changed from pink to blue, indicating the formation of gold nanoparticles. The solution was left stirring overnight. Then, the excess PVP_k was removed by a 6-fold centrifugation (7000 rpm, 15 min) and redispersed in ethanol (particle solution was adjusted to yield a final concentration of [Au] = 4.5 × 10^-4 M).

The as-synthesized GNS were then functionalized with MUA (0.8 molecules nm^-²) following the previously described protocol. Specifically, a solution containing NH_2OH (87.9 μL, 29% aqueous solution) and MUA (31.6 μL, 1.0 × 10^-4 M in EtOH) was rapidly added under vigorous stirring to 5 mL of GNS suspension ([Au] = 4.5 × 10^-4 M). Agitation was continued for 3 h to ensure the complete MUA adhesion on the Au surface. Next, 5.9 μL of a 10^-2 M stock solution of different SERS probes was added under strong magnetic stirring. Once more, stirring was continued for another 3 h. The amount of SERS-active molecules added was calculated to be 15 molecules nm^-².

Finally, the same modified Stöber method as applied to spherical gold nanoparticles was followed to obtain a homogeneous silica shell around the Raman-labeled GNSs. The colloidal suspension was concentrated to a Au concentration of 1 mM prior to the SERS measurements.

**Characterization.** UV–vis spectroscopy (Perkin-Elmer, Lambda 19) and transmission electron microscopy (TEM, LEO 922 EFTEM operating at 80 kV) were applied to characterize the optical response, structure, and size of the nanoparticles during the encoding process. SERS spectra were collected in backscattering geometry with a Leica confocal microscope. The spectrograph used a high-resolution Renishaw Invia ReSi and a laser operating at 80 kV were applied to characterize the optical response, indicating the formation of gold nanostars. The solution was left stirring overnight. Then, the excess PVP_k was removed by a 6-fold centrifugation (7000 rpm, 15 min) and redispersed in ethanol (particle solution was adjusted to yield a final concentration of [Au] = 4.5 × 10^-4 M).

**ASSOCIATED CONTENT**

**Supporting Information**
Additional UV–vis–NIR, TEM, and SERS characterization data. SERS spectra of another 20 encoded particles. Comparison of SERS intensities of different encoded particles prepared with MUA, PVP, and PEG for different codes. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Author Contributions**
The manuscript was written through contributions of all authors.

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
This work was funded by Medcom Advance SA.

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Supporting Information

Universal One-pot and Scalable Synthesis of SERS

Encoded Nanoparticles

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Figure S1. Representative TEM image (A) and size distribution histograms (B) of the synthesized citrate-capped gold nanoparticles.
Figure S2. SERS spectra of Au particles coated with a full monolayer of MUA showing its small Raman cross section.
Figure S3. Comparison of the SERS spectra of CV at two different MUA molecules nm$^{-2}$ (0.8 black and 4 red). Showing that almost no signal is obtained when 4 molecules nm$^{-2}$ were used.
Figure S4. (A, B, and C) TEM images showing the results of encoding nanoparticles at MUA concentrations below 0.8 molecules nm$^{-2}$ (0.7, 0.6 and 0.5 respectively). (D) TEM image showing the uncontrolled aggregation of the particles below 0.7 molecules nm$^{-2}$. 
Figure S5. SERS spectra of 20 representative SERS-encoded nanoparticles. Left column: Toluidine Blue O, 2-Phenylethanethiol, 4-Mercaptophenol, Biphenyl-4-thiol, 7-Mercapto-4-methylcoumarin, 4-Hydroxyphenyl)-1H-tetrazole-5-thiol, 2-Fluorothiophenol, Crystal Violet, 2-Naphthalenethiol, 4-((3-Mercapto-5-(2-methoxyphenyl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol. Right column: (2-Trifluoromethyl)benzenethiol, 4-Aminothiophenol, 1-Naphthalenethiol, 1,1',4,1''-Terphenyl-4-Thiol, Biphenyl-4,4'-dithiol, Thiosalicylic acid, 4-((3-Mercapto-5-(2-pyridinyl)-4H-1,2,4-triazol-4-yl)imino)methyl)-1,2-benzenediol, 4-((3-Mercapto-5-(2-pyridinyl)-4H-1,2,4-triazol-4-yl)imino)methyl)benzoic, 2,3,5,6-Tetrafluorobenzenethiol, 5-(4-Methoxyphenyl)-1,3,4-oxidazole-2-thiol).
Figure S6. (A) Photography of the one-pot synthesis of a liter batch of MPy encoded nanoparticles. (B) UV-Vis, (C) SERS spectra, and (D) TEM image of the encoded nanoparticles.
Figure S7. (A) Extinction spectra of the Au@PEG-SH nanoparticles with different amount of PEG-SH (molecules nm$^{-2}$). Below 3.5 PEG-SH molecules nm$^{-2}$, it is clearly seen that nanoparticles start to aggregate. (B) SERS spectra of Au@PEG-SH/MPy nanoparticles with different amount of PEG-SH, 4, 3.5, and 3 molecules nm$^{-2}$ (red, blue, and magenta, respectively). The MPy SERS spectra is only obtained below 3.5 molecules nm$^{-2}$. (C) Comparison of the extinction spectra of Au@PEG-SH nanoparticles with different amounts of PEG-SH (molecules nm$^{-2}$) before and after the addition of MPy. Showing that after addition of MPy, the particles aggregate more especially below 3.5 molecules nm$^{-2}$ of PEG-SH.
Figure S8. SERS spectra comparison of encoded gold nanoparticles prepared using the PVP, PEG-SH and MUA approaches (blue, red, and green respectively) of MBA, 4NBT, NBA, TB, TFBT, and 1NT.
Figure S9. Normal Raman spectrum of EtOH and MBA (0.1 M in EtOH). SERS spectrum of Au@MBA@SiO₂ suspension in EtOH ([Au] = 1 mM), [NP] = 0.244 nM). All the spectra were acquired under the same experimental conditions (785 nm laser line, 100% laser power, 1 s exposition time, 5 accumulations).

The SERS enhancement factor was calculated using the following definition:

\[ EF = \frac{I_{\text{SERS}} / N_{\text{Surf}}}{I_{\text{Raman}} / N_{\text{Vol}}} \]

where \( N_{\text{Vol}} \) is the average number of molecules in the scattering volume, \( V \), for the normal Raman measurement, and \( N_{\text{Surf}} \) is the average number of adsorbed molecules in the same scattering volume for the SERS measurement.¹ As normal Raman and SERS intensities (\( I_{\text{Raman}} \) and \( I_{\text{SERS}} \)) we selected the height of the \( \nu(\text{C=C}) \) band at ca. 1600 cm\(^{-1}\). Both Raman and SERS measurements were performed under the same experimental set-up (i.e. identical scattering volume). Nanoparticle concentration of 0.244 nM was calculated by Lambert-Beer’s law using the extinction coefficient for gold nanoparticles of \( 4.2 \times 10^{10} \text{ M}^{-1} \text{ cm}^{-1} \), derived from literature.² The molecular footprint of MBA on gold, for a full monolayer, was reported to be 0.44 nm\(^2\).³ Thus, for gold spheres of ca. 50 nm diameter functionalized with a monolayer of MBA molecules, we estimate to have ca. 18570 molecules per particle, which corresponds to \( 4.53 \times 10^{-6} \text{ M} \) in the colloidal suspension. The estimated EF at 785 nm is therefore ca. \( 5.3 \times 10^{-4} \text{ M} \).
Figure S10. SERS spectra and TEM images of different SERS encoded gold nanostars prepared using the MUA approach. Normalized extinction spectra of GNS@MPy@SiO$_2$ encoded nanoparticles.
Figure S11. SERS spectra and TEM images of different SERS encoded silver nanoparticles prepared using the MUA approach. Normalized extinction spectra of Ag@DBT@SiO$_2$ encoded nanoparticles.

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