Fabrication of a Au Nanoporous Film by Self-Organization of Networked Ultrathin Nanowires and Its Application as a Surface-Enhanced Raman Scattering Substrate for Single-Molecule Detection

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ABSTRACT: Due to its demonstrated usefulness in fields such as trace analysis, biodiagnosis, and in vivo study, surface-enhanced Raman scattering (SERS) has received renewed interest in recent years. Development of SERS substrates is of great importance as the SERS intensity and reproducibility depend strongly on the SERS substrates. In this paper we report the fabrication of Au nanoporous film (NPFs) by self-organization of networked ultrathin Au nanowires for use as SERS substrates. The acquired Au NPFs display controllable thickness, low relative density, and considerable specific surface area. Furthermore, this self-organization of nanowires not only provides abundant junctions between nanowires, 5–20 nm nanopores, and three-dimensional nanowells, but also makes nanopores/nanogaps down to 1–2 nm. These nanoscale characteristics result in a high spatial density of hotspots with Raman enhancement factors up to 10⁹. Combined with the uniformity and high purity, our Au NPF provides high-quality substrates for SERS sensing.

Surface-enhanced Raman scattering (SERS) is of great interest as a spectroscopic tool owing to its single-molecule-detection ability, capability of providing structure information for analytes of interest, minimal sample preparation, and ease of operation. SERS has great potential in the fields of Raman labeling, in situ monitoring of chemical reactions, cultural object authentication, homeland security, and chemical/biological analysis. Since SERS signals rely on the nanoscale characteristics of the metallic substrates, numerous studies have been conducted to design advanced nanostructures for generating SERS signals with a high-magnitude enhancement factor, good stability, and reproducibility. With the successful acquirement of an extremely high enhancement factor from the edges/corners of nanoparticles (NPs) and hotspots between NP dimers and trimers, as well as SiO₂ or Al₂O₃ shell-isolated Au nanoparticles, more and more efforts have been focused on constructing two-dimensional (2D) and three-dimensional (3D) nanoarchitectures to ameliorate the stability/reproducibility of signals and the practicality of operation. Various strategies, both bottom-up and top-down, have been proposed to fabricate such hierarchical nanostructures. To name a few, electrochemically roughened electrodes, self-assembled metal colloids, silver NP loaded exponentially grown layer-by-layer (e-LBL) films, isolated silver islands, metal films over nanospheres (MFONs), metallized organic or inorganic nanostructured films, macroporous microwell arrays, electron beam lithographed and ion beam etched microstructures, and nanoporous coin metals were reported to be effective SERS substrates. Among these nanostructures, nanoporous coin metal films are a group of promising SERS substrates, partly for their advantages of intense signals and relative ease of fabrication. Currently, the most successful approaches for generating nanoporous materials are based on chemical or electrochemical corrosion of alloys, galvanic displacement reactions, and self-assembly of NPs using an organic or even a biologic template. These processes are time- and labor-intensive and usually yield final products with large ligament size/low surface area. In addition, some of the existing processes cause impurities on the surfaces of the substrates, which have adverse effects on SERS analysis of trace or unknown samples. Therefore, it remains a great challenge to exploit green and straightforward tactics for fabricating high-quality nanoporous materials with low density, high purity, and large specific surface area.

Herein, we report the fabrication of a Au nanoporous film (NPF) for use as an SERS substrate by our very recently developed capping agent replacement induced self-organization of ultrathin nanowires (CARISUN) strategy. In comparison

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with other routes reported in the literature, our procedure for SERS substrate fabrication is of low cost, is easily operated, and is scalable. In addition, the Au NPF obtained with this novel procedure provides many advantages, including being relatively free of organic or inorganic impurities and having controllable thickness from tens of nanometers to several micrometers, low relative density (~7.5%), and large specific surface area (153 m²/g). Furthermore, this hierarchical nanostructure offers abundant potential Raman hotspots at three different levels: nanopores/nanogaps (down to 1–2 nm) that stem from incomplete fusion of nanowires, junctions between nanowires, and 3D nanowells. With these nanoscale characteristics, the as-fabricated Au NPF exhibits a satisfying SERS effect and is capable of detecting a frequently used Raman probe, \textit{trans}-1,2-bis-(4-pyridyl)ethylene (BPE) at the single-molecule level.

**EXPERIMENTAL SECTION**

**Chemicals and Materials.** BPE and Triton X-114 (TX-114) were purchased from Sigma-Aldrich. Auric acid (HAuCl₄) and anhydrous ethanol were supplied by the Sinopharm Chemical Reagent Co. (Beijing, China), while potassium borohydride (KBH₄) was purchased from the Jingke Chemical Institute (Tianjing, China). Silicon wafers were supplied by the Xin Xin Bai Ri Technology Co. (Beijing, China). All the chemicals were analytical grade or above, and Milli-Q water (Millipore) was used throughout the experiments.

**Synthesis of Networked Au Ultrathin Nanowires (NWs).** Ultrathin Au NWs were synthesized according to our previously reported procedure.

**Preparation of Au NPFs with Different Thicknesses and Ligament Sizes.** Silicon wafers (1 cm × 1 cm) were ultrasonically cleaned successively in 10% HCl, ethanol, and water for 30 min each and then dried in air. The cleaned silicon wafers were placed on the bottom of a 24-well plate or flat glass container, into which the freshly prepared Au NW solution/dispersion was added to reach the desired depth (0.25, 0.5, 1.0, 2.0, 3.0, or 4.0 cm). The container was stored undisturbed at 4 °C for 2 h, 50 °C for 2 h, and the resulting Au NPFs were denoted as NPF-RT, NPF-50 °C, NPF-100 °C, and NPF-150 °C, respectively. A Au NPF with ultrathin ligament size (about 5 nm in diameter) was fabricated according to our previously reported protocol.

The fabricated Au NPFs were well characterized by scanning electron microscopy (SEM; S-3000, Hitachi)/field emission scanning electron microscopy (FESEM; S-4800, Hitachi), or transmission electron microscopy (TEM; H-7500, Hitachi)/high-resolution transmission electron microscopy (HRTEM; JEM-2010F, JEOL). X-ray diffraction (XRD) analysis of the samples was performed with a Siemens D5000 X-ray diffractometer (Cu Kα radiation (λ = 0.15418 nm)). Electrochemical measurements were performed with a CHI 630C electrochemical analyzer (CHI Instruments, Shanghai, China). Pore size analysis was conducted on a Quadrasorb SI surface area analyzer and pore size analyzer (Quantachrome Instruments).

**RESULTS AND DISCUSSION**

**Characterization of Au NPFs.** The acquired nanostructure was at first characterized by TEM/HRTEM after being sliced from the silicon wafer. The TEM image (Figure 1a) showed that the Au NWs (Figure S1, Supporting Information) were further interconnected into large networks. It is noteworthy that during the deposition process the diameter of the Au NWs increased from ~3 to ~8 nm. This increase in diameter is a possible result of NW fusion in the sedimentation and drying process. The powder XRD pattern showed that the Au NPF maintained the face-centered cubic (fcc) structure (Figure S2, Supporting Information), and the diameter of the ligaments determined from the Scherrer line broadening analysis was ~10 nm. From the HRTEM image (Figure 1b), some small nanopores/nanogaps of less than 10 nm, or even down to 1 or 2 nm, become evident. These nanopores/nanogaps may stem from the incomplete fusion of Au NWs, which may function as Raman hotspots and are beneficial for the use of the Au NPF as a SERS substrate. Top-view FESEM images of the prepared Au nanostructure (Figure 1c) confirmed the successful construction of the Au NPF, which consists of uniform ligaments with a diameter of a few nanometers and channels in the range of 10−20 nm. This fits well with Chen’s description that the NPF from a dealloy is a quasiperiodic network comprised of randomly oriented Au NWs and hollow nanochannels. Pore size analysis (data not shown) showed that the diameter of the nanopores is in the range of 5−20 nm, which is similar to that of counterparts reported in the literature. Energy-dispersive X-ray spectroscopy (EDS) analysis (Figure 1d) demonstrated that the Au NPF is relatively free of impurities with a Au content of 93.4% (w/w).

**Au NPF as an SERS Substrate.** The absence of impurities is an important characteristic of high-quality SERS substrates, as the
Impurities interfere with the SERS analysis of trace or unknown samples. Besides elemental analysis, the surface cleanliness of the prepared Au NPF was further tested through recording the Raman spectrum of a Au NPF before probe molecule loading (Figure 2a). The absence of a distinguishable signal reveals its high purity and applicability as an SERS substrate. Another important requirement for SERS substrates is the existence of abundant Raman hotspots. The 3D nanoporous characteristics of our Au NPFs may generate Raman hotspots, which may stem from the considerable electromagnetic fields induced from nanopores/nanogaps inside the ligaments and the coupling of adjacent Au NWs and nanowells. Figure 2b displays the dark-field scattering spectrum of Au NPF-0.5, in which the much stronger scattering intensity in the near-infrared (NIR; ca. 785 nm) region than the visible region (ca. 532 or 633 nm) mainly results from a surface plasmon resonance (SPR) induced electromagnetic field. This also infers that our Au NPF may work as an NIR SERS substrate,27 which is very helpful in suppressing the fluorescence background.28 As BPE has a highly delocalized π-electron system and chemically active pyridyl nitrogen atoms for binding to the Au surface,28 it is employed as a probe molecule to evaluate Au NPFs as SERS substrates in this study. In accordance with the strong scattering at the NIR, the SERS activity of the Au NPF shows a strong excitation laser wavelength dependency (Figure 2c,d). Quantitatively, when excitation is done with a 785 nm laser, the Raman intensity at 1200 cm⁻¹ (the C=C stretching vibrational mode) is about 5 times and 6 times higher than those when excitation is done with a 633 or 532 nm laser,28 respectively.

Sedimentation Temperature on the Surface Uniformity of Fabricated Au NPFs. Surface uniformity is an important issue of concern for SERS substrates; it determines the reproducibility of the SERS signal. As shown in Figure 3 and in Figure S3 (Supporting Information), Au NPFs fabricated at 4 and 15 °C were much more uniform than their counterpart constructed at 25 or 35 °C, though they almost have the identical microstructure. This difference in surface morphology may stem from the cloud point phenomenon. As the lower critical solution temperature of TX-114 is about 23–25 °C, above this temperature (ca. 25 and 35 °C), Au NWs tend to form Au NW/TX-114 assemblies with TX-114 micelles and then undergo deposition. This decreases the surface uniformity of the fabricated Au NPFs and excludes their use as substrates for SERS analysis.

Effect of the Thickness of the Au NPFs on Their SERS Performance. We further evaluated morphological parameters such as film thickness and ligament size on the SERS activities of Au NPFs. Figure S4 (Supporting Information) shows the cross-section view of Au NPFs prepared from a Au NW dispersion with different depths, except for that of Au NPF-0.25, which failed to be captured probably due to its relatively low thickness. The good linear relationship shown in Figure 4a (red line) indicates that the thickness of the Au NPFs can be easily and precisely designed by controlling the depth of the Au NW solution/dispersions. From the slope (1.337 μm/μm) of the plot of film thickness to Au NW solution depth and the Au concentration (1 mM) in the Au NW solution (see the details in the Supporting Information), it can be calculated that the relative density of these Au NPFs is 7.5%. This value is, to the best of our knowledge, the lowest one that has been reported in this group of nanostructures, indicating this method can reduce the cost of Au NPFs by a large margin and thus promote their application in optics/chemical sensing18,30 and related technical fields.31 Electrochemical measurement showed a surface area of 15.3 m²/g (blue line in Figure 4a and in Figure S5 in the Supporting Information), which is larger than that of nanoporous Au from dealloying (∼12 m²/g).26 Considering its smaller ligament diameter (∼10 nm) than that of the counterpart (10–40 nm), this increase in surface area is reasonable.

The SERS performance of Au NPFs with different thicknesses was evaluated at first by comparing the BPE SERS intensity at 1200 cm⁻¹. Parts b and c of Figure 4 show that the SERS intensity increased with the film thickness up to 0.5 μm and then decreased with a further increase of the film thickness, and empirically the thickest Au NPFs (NPF-4.0) give the highest reproducibility (relative standard deviation (RSD) 10%, average of 200 spectra). When the amount of probe molecule is insufficient to form a monolayer on Au NPF surface, which usually occurs in real sample analysis, the increase of the Au NPF thickness gives rise to higher surface area and lower surface

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Figure 1. (a, b) TEM and HRTEM images of a thin layer of a Au NPF. (c) Top-view FESEM image of a Au NPF. (d) Elemental composition of a Au NPF from EDXS.

Figure 2. (a) Raman spectrum recorded on a blank Au NPF. (b) Dark-field scattering spectrum of a Au NPF. (c) Raman intensity at 1200 cm⁻¹ with different laser wavelengths. (d) Surface-enhanced Raman spectra of BPE on a Au NPF with different laser wavelengths.
coverage, which results in the reduction of molecules being illuminated by laser and thus the signal intensity. In fact, on the assumption that $7 \times 10^{14}$ molecules/cm$^2$ exist in a monolayer, the estimated BPE molecular coverage on the nominal surface was only $\sim 0.01$ monolayer. The possible change of SERS activity is another explanation of this film thickness dependent SERS activity. On the other hand, thicker NPFs provide better surface uniformity and in turn generate a more reproducible SERS signal. Nevertheless, Au NPF-0.5 provided the most intense signal with acceptable reproducibility ($\sim 20\%$) and was adopted for further evaluation of its performance as an SERS substrate.

**Effect of the Ligament Size on Au NPF SERS Activity.** It is well accepted that the sizes of both the ligament and nanopore influence the SERS activity of nanoporous SERS substrates. This is because the pore size determines the curvatures and interligament distance, which influences the intensity of localized electromagnetic fields, while the ligament size influences the degree of plasmon dumping and then the near-field SPR coupling. The morphology of Au NPF was modulated through changing the preparation conditions and aging temperature. Figure S1 shows the FESEM image of a Au NPF deposited at a basic condition, whose ligament size is about 6 nm. This change in solution pH, however, seems to have a negligible influence on its nanopore size, which is still about 10–20 nm. Besides changing the chemical condition in NPF fabrication, aging is another effective way to regulate the ligament/nanopore size of a Au NPF, as such material is prone to experiencing a coarsening process. Parts c–f of Figure S5 demonstrate that the morphology of the Au NPF was altered significantly by aging at different temperatures, even at room temperature for 24 h. As the aging temperature increases, the degree of coarsening increases accordingly. For example, after aging at room temperature, the ligament/nanopore size is about 20 nm/20–50 nm, while when the aging temperature is increased to 150 °C, those values markedly increase to about 200 nm/50–500 nm. It is noteworthy that if the Au NPF is placed in a refrigerator ($\sim 20$ °C) after preparation, no significant difference in its surface morphology/SERS performance can be detected, even after 1 week of storage.

As a consequence of this ligament/nanopore size change, their SERS activity also changed significantly. Parts g and h of Figure S5 show the distribution of Raman intensity at 1200 cm$^{-1}$ on Au NPFs with different morphologies. Au NPF-ultrathin, Au NPF-0.5, and Au NPF-RT display a narrow distribution, with an RSD of less than 20%, indicating good uniformity of these nanostructures. However, the results from Au NPFs aged at high temperature are rather different. They all show a very broad distribution, with an RSD approaching 100%, indicating their surface uniformity is also destroyed with the change of morphologies. In general, the Raman signal from Au NPF-0.5 shows the highest intensity, and we attribute this to the SERS hotspots generated by nanojunctions, nanowells between ligaments, and nanogaps/nanopores inside ligaments from incomplete fusion of nanowires. For NPF-ultrathin, although it shows an almost identical pore size compared with NPF-0.5, the absence of nanogaps/nanopores inside the ligaments devalues its performance as an SERS substrate, while for NPFs that experienced the aging process, besides the blocking of those nanogaps/nanopores, the increase of the ligament/nanopore size may also influence their SERS activities, and a Au NPF aged at 50 °C possesses the best SERS performance. It is noticeable that the average Raman intensity for a Au NPF aged at high temperature (50, 100, or 150 °C), especially for Au NPF-50 °C, is mainly contributed by a small portion, whose intensity is much higher than that of the rest. Figure S1 shows the Raman spectra whose intensity at 1200 cm$^{-1}$ is the most intense from different Au NPFs. Interestingly, the spectrum collected from Au NPF-50 °C is much more intense than its counterparts, even 2 times more intense than the spectrum collected from Au NPF-0.5. This phenomenon may be explained by the fact that, after the Au NPF was aged at 50 °C, although most of the nanoparticles showed an increase in their diameters, some nanoparticles with unchanged diameter remained. As was demonstrated previously by Chen et al, Au NPFs with small nanoparticles and large ligaments possess the best SERS activity.

**Au NPF as an SERS Substrate for Single-Molecule Detection.** To test the limit of detection (LOD) of the developed Au NPFs for SERS analysis, as well as to quantify the Raman enhancement effect, Raman spectra on a Au NPF loaded with different concentrations of BPE were recorded. For comparison,
the spectrum from normal Raman spectroscopy (NRS) on a 10 μL of 0.1 M BPE loaded Si wafer was also collected, and these results are presented in Figure 6. From Figure 6 a it is evident that the Raman spectrum is still discriminable even at probe molecule concentrations down to 10⁻¹⁰ M. As the probe molecule density is about 6 μm⁻² (10⁻⁵ L of 10⁻¹⁰ M BPE loaded on a 1 cm × 1 cm Au NPF), the laser spot size is about 2 μm and the number of BPE molecules being probed per laser shot is about 20, which is approaching single-molecule detection.

The Raman enhancement factor (REF) was estimated by comparing the measured SERS intensities of BPE at 1200 cm⁻¹ on the Au NPFs and the normal Raman intensity at 1197 cm⁻¹ using the following equation:

\[
\text{REF} = \frac{I_{\text{SERS}}}{I_{\text{NRS}}} \frac{N_{\text{bulk}}}{N_{\text{SERS}}} = \frac{P_{\text{bulk}}}{P_{\text{SERS}}} \frac{T_{\text{bulk}}}{T_{\text{SERS}}}
\]

where \( I \), \( N \), \( P \), and \( T \) represent the Raman intensity, number of probe molecules, laser power, and acquisition time, respectively. The molecule density of normal Raman analysis is 6 × 10⁹ μm⁻² (10⁻⁵ L of 0.1 M BPE loaded on a 1 cm × 1 cm Si wafer), and that for SERS analysis ranges from 6 to 6 × 10⁵ μm⁻² (10⁻⁵–10⁻¹⁰ M BPE loaded on a 1 cm × 1 cm Au NPF). As the SERS intensity does not increase linearly with the probe molecule concentration (Figure 6 b), using different probe molecule concentrations for REF calculation will result in different values.

The calculated REF is in the range of 6 × 10⁵ to 1.6 × 10⁹, and this result is comparable with or higher than the reported value (10⁵–10⁶) in the literature. We hypothesize that this enhancement stems from abundant Raman hotspots such as nanopores/nanogaps, 3D nanowells, and the junctions between NWs. The much larger enhancement factor at lower probe molecule concentration may be attributed to the fact that in such a case most of the analyte molecules are expected to migrate and adsorb at SERS-active sites.

Large-Area Raman Mapping. Besides sheer SERS enhancement, the homogeneity of the SERS signal across a large area is another important parameter in evaluating the usefulness of an SERS substrate. To demonstrate the applicability of Au NPFs as viable SERS sensing materials, point-by-point Raman mapping was recorded on a random selected 22 × 20 μm area with a step size of 1 μm on Au NPF-0.5. Parts a–d of Figure 7 show the white light Raman intensity mapping at 1016, 1200, and 1608 cm⁻¹. The homogeneous distribution of the Raman intensity of all three main peaks strongly supports the notion that the optimal SERS substrate is a Au NPF.
that the Au NPF is uniform in a large area and capable of generating an SERS signal with good reproducibility. Figure 7 e shows the Raman spectra collected from the strongest point (point A) and the weakest point (point B). Although the former is about 2 times more intense than the latter, from the intensity distribution graph (Figure 7 f), both the points with high Raman intensity (for the peak at 1200 cm\(^{-1}\), \(\geq 2000\) au S\(^{-1}\) mW\(^{-1}\)) and low intensity (\(\leq 1300\) au S\(^{-1}\) mW\(^{-1}\)) are less than 10% of the total point, and the RSDs of all three peaks are all less than 15%, again confirming the good uniformity of the Au NPF. The exceptional uniformity and relatively large SERS enhancements make these Au NPF substrates ideal detection platforms for eventual single-molecule vibrational spectroscopy using low-cost SERS sensors. Besides the good uniformity/reproducibility in SERS intensity on a single Au NPF, the repeatability of the Au nanostructures produced at any given condition is also very high, and this can be demonstrated by electron microscopy and SERS experiments (data not shown).

**CONCLUSIONS**

In summary, we report a new route for fabricating Au NPFs as highly effective SERS substrates by self-organization of ultrathin Au NWs. The proposed procedure is of low cost, is easily operated, and is environmentally friendly, while the as-prepared Au NPFs are featured with controllable thickness, high purity and porosity, large surface area, and small ligament diameter. We anticipate they may find applications in areas such as environmental pollutant screening\(^7\),\(^8\) and hope this novel strategy is effective SERS substrates by self-organization of ultrathin hybrid nanoporous films which may possess unique properties and finds applications in a variety of areas such as bioanalysis. Furthermore, considering the good biocompatibility of Au nanostructures and the bottom-up nature of the proposed Au NPF fabrication strategy, this method may provide a possible way to trap living objects such as cells or bacteria inside Au NPFs to sense the biochemical process of these biospecies,\(^7\) and research is ongoing in our laboratory.

**ASSOCIATED CONTENT**

Supporting Information. Chemicals, experimental procedures, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure 7. (a) White light image of a Au NPF (22 \(\times\) 20 \(\mu\)m). (b–d) SERS intensity maps at 1016, 1200, and 1608 cm\(^{-1}\) corresponding to the area shown in (a). (e) Raman spectra obtained at the strongest signal point (point A in (b)) and the weakest signal point (point B in (b)). (f) Distributions of the frequencies of occurrence of the Raman intensity at 1200 cm\(^{-1}\) in the 440 spectra measured.