

Surface analysis using shell-isolated nanoparticle-enhanced Raman spectroscopy

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Surface-enhanced Raman scattering (SERS) is a powerful fingerprint vibrational spectroscopy with a single-molecule detection limit, but its applications are generally restricted to ‘free-electron-like’ metal substrates such as Au, Ag and Cu nanostructures. We have invented a shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) technique, using Au-core silica-shell nanoparticles (Au@SiO₂ NPs), which makes SERS universally applicable to surfaces with any composition and any morphology. This protocol describes how to prepare shell-isolated nanoparticles (SHINs) with different well-controlled core sizes (55 and 120 nm), shapes (nanospheres, nanorods and nanocubes) and shell thicknesses (1–20 nm). It then describes how to apply SHINs to Pt and Au single-crystal surfaces with different facets in an electrochemical environment, on Si wafer surfaces adsorbed with hydrogen, on ZnO nanorods, and on living bacteria and fruit. With this method, SHINs can be prepared for use in ~3 h, and each subsequent procedure for SHINERS measurement requires 1–2 h.

INTRODUCTION

Background

SERS was first observed in the 1970s on an electrochemically roughened Ag electrode^{1–3}, which showed an anomalous million-fold enhancement in Raman scattering intensity. The discovery of SERS had a considerable effect on spectroscopy and surface science because of the ultrahigh-sensitivity applications that it allowed. SERS is an intrinsically nanostructure-based phenomenon, as the intensity of the Raman scattered light depends crucially on size, shape and interparticle spacing at the nanoscale^{4–11}.

Since the mid-1990s, along with rapid developments in nanoscience and nanotechnology, SERS has been developing into a powerful tool for analytical chemistry, electrochemistry, catalysis and medical diagnostics (including trace detection of drugs and biomolecules in living cells)^{11–19}. SERS can provide ultrahigh sensitivity down to the single-molecule level, comparable to single-molecule fluorescence spectroscopy^{4,5,20}. However, two key stumbling blocks in the use of SERS are a lack of applicability to substrates of any composition and a lack of applicability to substrates of any surface morphology. Only a few ‘free-electron-like’ metals (mainly Au, Ag and Cu) provide a large SERS effect. Smooth surfaces are also excluded, especially the atomically flat surfaces of structurally well-defined single crystals that are commonly used in surface science.

Over the past 20 years, several groups have developed methods to overcome these two problems^{21–25}. For instance, Au or Ag NPs or nanostructures have been coated with ultrathin shells (1–10 atomic layers) of various other metals. Boosted by the long-range electromagnetic field enhancement effect associated with the highly SERS-active Au or Ag core, the inherently weak surface enhancement of the shell material can be substantially increased to obtain total enhancement factors of up to 10⁴–10⁵ for Pt, Pd, Ru, Rh, Ni and Co (refs. 21–23). However, for many other materials, such as oxides, insulators or biological membranes, it is very difficult if not impossible to coat them as uniform ultrathin shells on Au or Ag NPs.

Tip-enhanced Raman spectroscopy (TERS) was first reported in 2000, leading to a breakthrough in the substrate and surface generalities of SERS²⁶. The electric field enhancement generated at an atomic force microscopy (AFM) or scanning tunneling microscopy (STM) tip by excitation with a suitable laser can extend to any sample that is in close proximity to the tip apex. This new working mode separates the probed surface from the nanostructure that acts as the Raman signal amplifier. In principle, the Raman signal from any substrate, regardless of its material and surface morphology, can be enhanced by the tip apex. Another feature of TERS is its very high spatial resolution, in which the sampling area is reduced to 10–20 nm so that the total number of probed molecules is extremely small and even single molecules can be detected²⁴. However, the Raman signal from the tip area is quite weak. As a consequence, most TERS studies have been limited to molecules having very large Raman cross-sections (i.e., molecules that undergo efficient Raman scattering and easily yield large Raman signals). Carbon nanotubes and the dyes rhodamine 6G and crystal violet have sufficiently large Raman cross-sections when the right wavelengths are used. In contrast, adsorbed hydrogen cannot be studied by TERS. It is therefore highly desirable to explore an innovative approach based on the noncontact mode of TERS for substantially increasing the Raman signal and making Raman spectroscopy universally applicable in practice.

An alternative approach to solving this problem is the use of Au-core dielectric-shell NPs to enhance Raman scattering from surface species and is the subject of this protocol. The ultrathin yet continuous shell of silica (or alumina) isolates the Au core, which generates a large surface enhancement, and it ensures that there is no interference from processes on the Au itself. The distance between the Au core and the surface under investigation can easily be controlled by the shell thickness. The main virtue of such Au@SiO₂ NPs is that they can be easily prepared and then spread over surfaces with diverse compositions and morphologies. Thus, the optical properties of the Au core are borrowed to enhance the Raman vibrational spectra of nearby molecules²². About 1,000 SHINs with a 60-nm

diameter lie in a 2- μm laser spot and are excited simultaneously, and thus the total enhanced Raman signal is much greater than that obtained by TERS^{24,25}. We call this new technique, which has been applied very recently in surface science^{9,27–34}, shell-isolated nanoparticle-enhanced Raman spectroscopy or SHINERS⁹.

Advantages and limitations of SHINERS

The key advances made by SHINERS are a tremendous expansion of SERS versatility and a much higher detection sensitivity relative to TERS. SHINERS has largely broken the longstanding limitations of SERS by allowing the characterization of materials, surface morphologies and biological samples that were previously inaccessible. Shell-isolated enhancement may be applied to other spectroscopies as well, such as fluorescence³⁵, infrared absorption and sum frequency generation. SHINERS can also be developed into a simple, fast, cost-effective, nondestructive, flexible and portable characterization tool^{9,27}.

However, SHINERS does have its limitations and we are working to eliminate them. First, the silica and alumina shells described here are not completely inert. For example, a silica shell is not compatible with a high-pH environment, whereas an alumina shell is not compatible with a low-pH one. At present, we are learning how other materials can be deposited to form a shell that is chemically inert, ultrathin and pinhole free. Another limitation of SHINERS is the semiquantitative nature of the signal. If SHINs can be assembled on a surface in a uniform monolayer, Raman intensities will better correlate with the amount of the analyte present on the surface.

SHINERS applications

In this protocol, we include procedures that cover the application of SHINERS in surface science, electrochemistry, semiconductor materials, and the biological and food sciences. This is Step 3 of the PROCEDURE (an overview of the experimental procedure is provided in Fig. 1). Data using these methods have been published previously (ref. 9), and are also discussed in the ANTICIPATED RESULTS section.

First, we describe the use of SHINERS to investigate the atomically flat surfaces of different single-crystal metals (Box 1) in aqueous electrochemical environments. It is extremely difficult to study atomically flat single-crystal surfaces by SERS, because they cannot effectively support a strong surface plasmon resonance (SPR). Furthermore, signal is reduced when accumulating from an aqueous environment because of additional scattering and distortion of the optical path at whatever interface exists between the water and the optics.

In SHINERS, each SHIN (e.g., diameter = 55 nm) acts as an Au tip in a TERS experiment, and about one thousand ‘tips’ are simultaneously excited within a laser spot (e.g., diameter = 2 μm). This markedly increases the total Raman intensity available for detection. In addition, each Au core is protected by a silica or alumina shell that is relatively chemically and electrically inert so that the Raman signal comes only from the surface of interest.

Hydrogen adsorption is important in surface science, electrochemistry and various industrial processes, including fuel cell applications. To the best of our knowledge, no Raman spectra of hydrogen adsorbed on single-crystal surfaces were reported before our SHINERS spectra (published in ref. 9), because of hydrogen’s extremely low Raman scattering cross-section. The high surface sensitivity of SHINERS has allowed us to observe the Pt(111)-H stretching

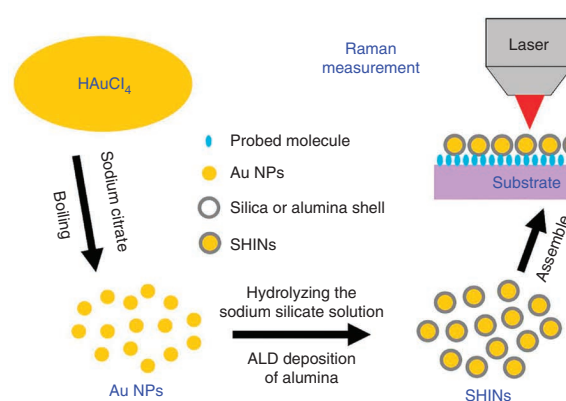


Figure 1 | Schematic illustration of SHINERS.

vibration (see Step 3A of the PROCEDURE) and distinguish two different single-crystal facets, Au(111) and Au(100), by using SCN⁻ as a probe molecule (see Step 3C of the PROCEDURE).

We also applied SHINERS to a number of nonmetal systems: a semiconductor, yeast cells and a fruit. We were able to see the Si(111)-H stretching vibration after a hydrogen fluoride (HF) surface treatment procedure that is widely used in the semiconductor industry (see Step 3B of the PROCEDURE). We have included a procedure that could be used to perform a SHINERS study of live yeast cell walls (Step 3F of the PROCEDURE). Finally, Step 3G of the PROCEDURE can be used to detect a pesticide residue on the surface of orange skin *in situ*. Inspection results can be obtained with a portable Raman spectrometer.

A comparison of the SHINERS shell-isolated mode with the bare Au/Ag NP SERS contact mode

In the 1980s, a ‘borrowing SERS’ strategy was proposed²². SERS-active metal nanostructures were deposited onto a surface that was not SERS active, or an SERS-inactive material was deposited onto an SERS-active one. These methods had three intrinsic problems. The first problem was contact with the chemical environment. The use of bare NPs is inapplicable to liquids (for biology and electrochemistry) and gases (for catalysis), which contain species capable of adsorbing on them. The SERS signals generated by these species may interfere with the SERS signal generated by the probed substrate (see Step 3E of the PROCEDURE). The second problem was contact with the probe molecule. As bare NPs lie in direct contact with one face of a self-assembled monolayer (SAM), the molecules located between the NPs and the substrate will most probably adopt a two-ended adsorption geometry instead of a one-ended adsorption geometry. For some systems, photocatalytic reactions can be initiated in SAM molecules by the NPs (see Step 3D of the PROCEDURE). The third problem was electrical contact between the surface of interest and the bare NPs. If this methodology is used to examine various metals and semiconductors that are not covered by SAMs, electrical contact can lead to unwanted charge-transfer effects. The difference in the Fermi levels of the NPs and the substrate may result in a contact potential that can significantly affect the electronic structure of any probe molecules in the area (see Step 3E of the PROCEDURE). All three of these problems are overcome in SHINERS, in which the Raman signal amplifier is isolated from species in the environment, probe molecules and the surface of interest.

TABLE 1 | Advantages and potential applications of various SHIN types.

SHIN types	55-nm-diameter spheres	120-nm-diameter spheres	Nanocubes and nanorods
Advantages	Simple preparation	Greater enhancement	Tunable SPR
Potential applications	Routine SHINERS measurements	SHINERS measurements on systems yielding very weak signals	Optimizing the enhancement for other excitation wavelengths

As an added practical benefit, SHINERS NPs are easier to clean and handle than bare metal NPs, because the shell prevents aggregation (Step 2C of the PROCEDURE). Moreover, they can be cleaned more extensively than bare metal NPs.

Experimental design

Various SHIN types. Different kinds of SHINs can be synthesized to meet different analysis requirements (Table 1). We use a 55-nm Au NP core in most cases, because the preparation of these SHINs is quick and easy⁹, and because they provide enough Raman-signal enhancement for most samples. If further enhancement is required, a 120-nm Au NP core is used³⁶. Numerical simulations have shown that this core size has the strongest SERS activity when a 633-nm excitation wavelength is used. Nanocube and nanorod SHINs^{37–39} were developed for their unique and tunable SPR properties. The SPR absorption band maximum can be forced into the near infrared by adjusting nanorod aspect ratio, for example, and this spectral region is essential for biosensor applications.

Shell thickness and shell uniformity. SHINERS intensity decreases in an exponential way with increasing shell thickness, as shown in Step 2A of the PROCEDURE. This means that the shell must be ultrathin for effective transfer of the strong electromagnetic field from the surface of the Au core to the surface of the silica shell.

However, pinholes in the shell can be problematic if it is too thin, as a comparatively strong SERS signal from molecules adsorbed directly on the Au core through them may interfere with the weaker SERS signal from analyte molecules adsorbed on the surface of interest. Therefore, the shell must be suitably thin yet pinhole free for SHINERS to be applicable in a wide variety of situations.

We use three methods to ensure that our silica shells are, in fact, pinhole free. First, the shell can be observed directly by high-resolution transmission electron microscopy (HR-TEM)⁹. Second, cyclic voltammetry may give a reduction peak for Au at ~0.9 V if the shell is not uniform⁹. Third, a SERS test using pyridine as a probe molecule (Step 3 in the PROCEDURE) is carried out on every new batch of SHINs, because it is the most reliable of the three tests⁹. First, some SHIN solution is dried to leave a film of SHINs; next, a drop of pyridine solution is added and a coverslip is placed on top to keep the pyridine solution from drying out. Pyridine is adsorbed on Au, but it is not strongly adsorbed on silica. If there are pinholes in the silica shell, pyridine molecules will absorb on the surface of the Au core and a SERS signal from pyridine will be observed at 1,009 cm⁻¹. If there are no pinholes, the pyridine will remain in solution and no Raman signal from pyridine will be obtained.

We note that pinholes in the shell are acceptable if the analyte in the SHINERS experiment is not adsorbed on the core material. As an example, hydrogen is not adsorbed on Au.

MATERIALS

REAGENTS

- Milli-Q water (18.2 MΩ cm)
- Hydrogen tetrachloroaurate(III) trihydrate, 99.99% (Alfa Aesar, cat. no. 36400)
- Silver nitrate, 99.995% (AgNO₃, Alfa Aesar, cat. no. 43087)
▲ **CRITICAL** Silver nitrate should be stored in a foil-wrapped desiccator to avoid light-induced decomposition.
- Trisodium citrate dihydrate, 99.0% (Alfa Aesar, cat. no. 36439)
- (1-Hexadecyl)trimethylammonium bromide, 98% (Alfa Aesar, cat. no. A15235)
- Sodium borohydride, 98% (NaBH₄, Alfa Aesar, cat. no. 88983)
▲ **CRITICAL** Sodium borohydride should be stored under N₂, as it is moisture sensitive.
- (3-Aminopropyl)trimethoxysilane, 97% (APTMS; Alfa Aesar, cat. no. A11284)
- (3-Aminopropyl)triethoxysilane, 98% (APTES; Sigma-Aldrich, cat. no. A10668)
- (3-Mercaptopropyl)triethoxysilane, 94% (Alfa Aesar, cat. no. B21191)
- Sodium silicate solution, 27% SiO₂ (Sigma-Aldrich, cat. no. 338443)
- Sodium perchlorate, 98.0–102.0% (NaClO₄; Alfa Aesar, cat. no. 11623)
- Pyridine, 99.0% (Alfa Aesar, cat. no. 19378)
- Sodium thiocyanate, 98.0% (NaSCN; Alfa Aesar, cat. no. 33388)
- 4-Aminothiophenol, 97% (Alfa Aesar, cat. no. A14082)
- Perchloric acid, 99.9985% (HClO₄; Alfa Aesar, cat. no. 10983)
- Trimethylaluminum (Al(CH₃)₃; Sigma-Aldrich, cat. no. 663301)
- Ascorbic acid (G.R., Sinopharm Chemical Reagent)
- Hydroxylamine hydrochloride (A.R., Sinopharm Chemical Reagent)

- Hydrochloric acid (G.R., Sinopharm Chemical Reagent)
- Nitric acid (G.R., Sinopharm Chemical Reagent)
- Sulfuric acid (H₂SO₄; G.R., Sinopharm Chemical Reagent)
- Hydrofluoric acid, 30% (G.R., Sinopharm Chemical Reagent)
- Orthophosphoric acid (G.R., Sinopharm Chemical Reagent)
- Ethanol (A.R., Sinopharm Chemical Reagent)
- PBS
- Carbon monoxide, 99.9% (CO; Linde) **! CAUTION** Carbon monoxide is a poisonous, flammable, odorless high-pressure gas. It acts on blood, causing damage to the central nervous system (CNS). Ensure that appliances are installed and operated according to the manufacturer's instructions, and be sure to use a CO alarm, which can provide some added protection during the experiment.
- *Saccharomyces cerevisiae* strain (2.1882; China General Microbiological Culture Collection Center)
- YPD: 1% (wt/vol) yeast extract (Sigma-Aldrich, cat. no. Y1625-1KG); 2% (wt/vol) Bacto peptone (Sigma-Aldrich, cat. no. P6838-1KG); 2% (wt/vol) D-glucose (Sigma-Aldrich, cat. no. G7528-1KG)
- Methyl parathion (Standard Substance, Standard Number C 15890000, Standard Substance Net of The Agro-Environment Protection Institute, China) **! CAUTION** This material is a toxic liquid. Wear full chemically protective equipment during handling. Avoid all contact with eyes, skin and clothing. Do not inhale vapors or mists.

EQUIPMENT

- Savannah 100 atomic layer deposition (ALD) system (Cambridge NanoTech)
- Shimadzu UV-2100 spectrometer (Shimadzu Corporation)
- High-resolution TEM instrument (JEOL, cat. no. JEM 4000EX)



- High-resolution TEM instrument (Tecnai F30, FEI)
- Field emission microscope (Leo1530)
- CHI 631B electrochemical workstation (CH Instruments)
- Homemade spectroelectrochemical cell
- Plasma system (FEMTO timer, version 1; Diener Electronic)
- LabRam I confocal microprobe Raman system (Jobin-Yvon)
- DeltaNu Inspector Raman portable Raman spectrometer
- Stirring hot plate with temperature controller (IKA, C-MAG HP 7 IKATHERM hotplate, and IKA, ETS-D5 temperature controller)
- ▲ **CRITICAL** It is important to use a temperature controller to achieve a reliable rate of heating.
- Magnetic stirrer (IKA, color squid IKAMAG white, speed range from 0–2500 rpm, cat. no. 3672000)
- Shaker (MS 3 basic, speed range, 0–3,000 r.p.m., IKA, cat. no. 3617000)
- Microcentrifuge tubes, 1.5 ml (Axygen, cat. no. MCT-150)
- Cover glass (Fisher Scientific, Fisherfinest Premium Cover Glasses, cat. no. FIS12-544-14)
- Ultrasonic cleaner (Branson, 1510E-MT, cat. no. CPN-952-136)
- Round-bottom flasks
- Vacuum dryer
- Quartz coverslips

REAGENT SETUP

Chloroauric acid (0.01% (wt/vol) HAuCl₄) Dissolve 1 g of hydrogen tetrachloroaurate(III) trihydrate (solid) in 100 ml of Milli-Q water using a volumetric flask. Place 11.6 ml of this solution (0.86% (wt/vol) HAuCl₄) in a 1,000-ml volumetric flask and fill it to the mark with water. The final

concentration of HAuCl₄ is 0.01% (wt/vol). Store this solution at room temperature (25 °C) for at least 2 d before use.

APTMS solution (1 mM) Add 18.3 μl of APTMS (97%, wt/wt) to a 100-ml volumetric flask and fill it to the mark with Milli-Q water. The final concentration of APTMS is 1 mM. This solution is always freshly prepared before use.

Sodium silicate solution (0.54%, wt/wt) Add 2 ml of sodium silicate solution (27%, wt/wt) to a 100-ml volumetric flask and add ~20 ml of Milli-Q water. Add 60 ml of hydrochloric acid solution (0.01 M) to the flask with fast shaking to adjust the pH. Fill the flask to the mark with water. The concentration of sodium silicate is 0.54% (wt/wt), and the pH is ~10.2. This solution is also freshly prepared fresh before use.

EQUIPMENT SETUP

Raman instrument We recorded Raman spectra on a Jobin-Yvon LabRam I confocal microprobe Raman system. The excitation wavelength was 633 nm from a He-Ne laser, and power on the sample was about 1 mW. We used an ×50 magnification long-working-distance (8 mm) objective to focus the laser onto the sample and to collect the backscattered light. We used a DeltaNu Inspector Raman portable spectrometer, which offers an excitation wavelength of 785 nm, in addition to the LabRam I for detection of a pesticide residue on an orange skin (Step 3G).

Homemade spectroelectrochemical cell We used a homemade spectroelectrochemical cell, with a Pt wire and a saturated calomel electrode (SCE) serving as the counter and the reference electrodes, respectively, for the electrochemical SERS measurements.

PROCEDURE

Preparation of SHINs

1 | The first step is to prepare the SHINs appropriate for the experiments. Different kinds of SHINs are necessary to meet the different requirements. Therefore, there are a number of possible options that can be followed to prepare these NPs. These options are summarized in the in-text table below:

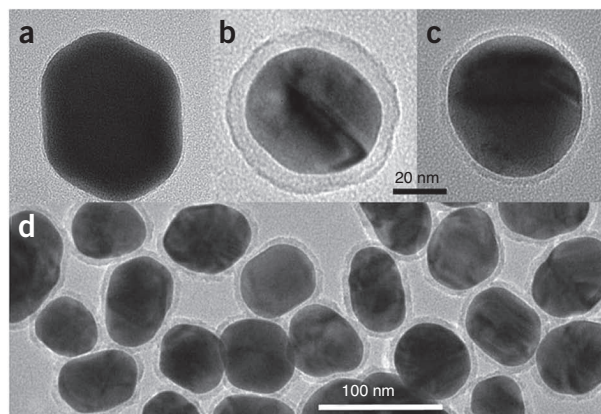
1A	55-nm-diameter Au nanosphere seeds and associated Au@SiO ₂ SHINs
1B	Au@Al ₂ O ₃ SHINs
1C	120-nm-diameter Au nanosphere seeds and associated Au@SiO ₂ SHINs
1D	Nanorod SHINs
1E	Nanocube SHINs

(A) Preparation of 55-nm-diameter Au nanosphere seeds and associated Au@SiO₂ SHINs ● TIMING 2 h

- Preparation of 55-nm-diameter Au nanosphere seeds.* Add 200 ml of chloroauric acid (0.01 wt%) into a round-bottom flask, and boil it with stirring and refluxing.
- Add 1.4 ml of sodium citrate (1 wt%) quickly into the boiling solution.
 - ▲ **CRITICAL STEP** Au NP size is determined by the quantity of sodium citrate added. Larger NPs may be produced by decreasing the quantity of sodium citrate and smaller NPs may be produced by increasing its quantity. The volume given here (1.4 ml) will produce NPs with a diameter of ~55 nm.
- Continue to boil the solution for 30 min.
- Cool it to room temperature.
 - **PAUSE POINT** Store the Au NPs in the dark at room temperature until they are used. The Au NPs can be stored for 1 month without major changes to their size and morphology if they are stored in the dark at room temperature.
- Preparation of the associated Au@SiO₂SHINs (Step 1A(v-x)).* Add 30 ml of the Au NP seed solution into a round-bottom flask.
- Add 0.4 ml of APTMS (1 mM) and stir for 15 min.
- Add 3.2 ml of sodium silicate solution (diluted to 0.54% (wt/wt) with Milli-Q water and adjusted to pH ~10.2 with hydrochloric acid) and stir for 3 min at room temperature.
 - ▲ **CRITICAL STEP** If the pH of the sodium silicate solution is too high (e.g., pH >11), dissolution of the shell by sodium hydroxide will interfere with its growth. Therefore, the shell remains very thin (**Fig. 2a**) and pinholes

PROTOCOL

Figure 2 | HR-TEM images of Au@SiO₂ NPs. (a–d) Their shells were prepared under different pH conditions. Sodium silicate solutions with high and low pH values (~11 and ~8) were used to prepare the SHINs in **a** and **b**, respectively, with a 60-min heating period. The Au@SiO₂ NPs shown in **c** and **d** were prepared at a pH of ~10.2 for 20 min and 60 min, respectively.



are detected. If the pH of the sodium silicate solution is too low (e.g., pH = 8), the silica shell grows quite rapidly and it can easily become too thick (**Fig. 2b**). Therefore, enhanced field strength from the surface of the core cannot extend beyond the surface of the shell, and no signal is acquired.

(viii) Place the flask in a 90 °C water bath and stir the contents for 10–60 min.

▲ **CRITICAL STEP** This elevated temperature is preferable to room temperature, because the time required for reaction is reduced from 2 or 3 d to about 1 h. The silica shell thickness is determined by the heating time, with longer heating time giving thicker silica shells. For example, a ~2-nm silica shell will result from 20 min of heating (**Fig. 2c**) and a ~4-nm silica shell will result from 60 min of heating (**Fig. 2d**).

(ix) Transfer 1.5 ml of the solution to each of four test tubes, and then place them in an ice bath to stop the reaction.

▲ **CRITICAL STEP** The reaction is slowed markedly and may even stop completely at this low temperature. Thus, one can obtain different silica shell thicknesses by controlling the time allowed for the reaction to occur.

(x) Centrifuge the four test tubes at 2,008g for 15 min at room temperature. Decant the supernatant, add 1.5 ml of Milli-Q water and disperse the SHINs again. Next, centrifuge the tubes for another 15 min and remove the supernatants to obtain clean SHINs.

(B) Preparation of Au@Al₂O₃ SHINs ● TIMING 2 h

(i) Drop some Au colloid solution (~20 μl) onto a clean glass microscope slide and dry it in a vacuum dryer for 15 min.

(ii) Perform the ALD⁴⁰. A deposition cycle includes the following: a 0.03-s pulse of Al(CH₃)₃ with a purge pulse of 15 s, and 0.07 s of pulse of H₂O with a pulse of 15 s.

▲ **CRITICAL STEP** The growth rate is about 0.1 nm per cycle, and the thickness of the alumina shell is controlled by adjusting the number of cycles. With such a high precision of control, the Al₂O₃ layer covers the entire sample uniformly and completely. However, we note that a pinhole will exist on each NP where it contacts the glass, and we suggest moving the NPs during the ALD process if pinhole-free SHINs are desired.

(iii) Place the glass microscope slide in water and use an ultrasonic treatment to detach the SHINs from it.

(iv) Centrifuge the NP solution at 2,008g for 15 min at room temperature to clean the Au@Al₂O₃ SHINs, and then continue to clean as in Step 1A(x).

(C) Preparation of 120-nm-diameter Au nanosphere seeds and associated Au@SiO₂ SHINs ● TIMING 4 h

(i) Add 100 ml of chloroauric acid (0.01 wt%) into a round-bottom flask, and boil it with stirring and refluxing.

(ii) Add 1.0 ml of sodium citrate (1 wt%) quickly into the boiling solution.

(iii) Continue to boil the solution for 30 min.

(iv) Cool the solution to room temperature.

(v) Place 4.0 ml of the solution containing the Au seeds (~40 nm in diameter at this point) into a round-bottom flask. Add another 53 ml of Milli-Q water.

(vi) Add 0.9 ml of sodium citrate (1 wt%) and stir for 3 min.

(vii) Add 0.9 ml of chloroauric acid (1 wt%) and stir for 8 min.

(viii) Add 1.4 ml of hydroxylamine hydrochloride (10 mM) to the round-bottom flask dropwise with continuous stirring. The reaction should be complete after 5 min, but we recommend stirring for 1 h at room temperature.

▲ **CRITICAL STEP** The key to preparing a stable solution of 120-nm Au NPs is to optimize the amount of HONH₃Cl used to reduce Au(+III). We have found that N(-I) in HONH₃Cl is oxidized to N(+V), instead of N(0), by a 1:2 mole ratio of HONH₃Cl/HAuCl₄. It is therefore not necessary to use large amounts of hydroxylamine hydrochloride, which will induce aggregation of the NPs. If prepared in this way, the 120-nm Au NP solution is very stable and the NPs are suitable for coating with silica. To synthesize monodisperse 120-nm Au NPs, the hydroxylamine hydrochloride solution should be added slowly, at a rate of about 1 drop every 10 s, while stirring at a speed of at least 2,000 r.p.m.

(ix) Dilute 15 ml of the 120-nm Au NP solution to 30 ml, and then place it in a round-bottom flask.

(x) Add 0.5 ml of APTES (0.5 mM) to the flask and stir for 20 min.

▲ **CRITICAL STEP** Here we use APTES rather than APTMS, because APTMS can induce aggregation of 120-nm NPs. The aqueous APTES solution should be allowed to hydrolyze for at least 10 min before it is added to the Au solution.

During this time we shake the volumetric flask in which it is prepared in order to improve the efficiency of the hydrolysis reaction.

- (xi) Add 2.8 ml of sodium silicate solution (diluted to 0.54% (wt/wt) with Milli-Q water and adjusted to pH ~10.2 with orthophosphoric acid) to the Au solution and stir for 3 min at room temperature.

▲ **CRITICAL STEP** Add the orthophosphoric acid to the sodium silicate solution with fast shaking. The pH of the resulting sodium silicate and Au solution mixture is ~9.4.

- (xii) Place the flask in a 90 °C water bath and stir for 1 h. The silica shell thickness will be ~4 nm (**Fig. 3a**).

▲ **CRITICAL STEP** The silica shell thickness is determined by the volume of sodium silicate solution added, the pH and the heating time. To prepare a 1-nm silica shell (**Fig. 3b,c**), add 1 ml of sodium silicate solution of pH ~11.6 (the pH of the resulting mixture is ~9.7) and heat for 20 min.

- (xiii) Transfer the resulting solution to a series of 1.5-ml centrifuge tubes, and place them in an ice bath to stop the reaction.
- (xiv) Centrifuge the test tubes at 598*g* for 10 min at room temperature. Decant the supernatant, and then add 1.5 ml of Milli-Q water and redisperse the SHINs. Centrifuge the test tubes at 598*g* for another 10 min. Decant the supernatant and obtain the clean, concentrated SHINs from the bottom of the test tube.

(D) Preparation of nanorod SHINs ● **TIMING 2–3 d**

- (i) Mix 250 μl of 10.0 mM chloroauric acid and 10 ml of 0.1 M (1-hexadecyl)trimethylammonium bromide (also known as cetyltrimethylammonium bromide or CTAB) to a round-bottom flask at 27 °C.
- (ii) Add 600 μl of ice-cold, freshly prepared 10 mM NaBH₄ to the flask all at once with vigorous stirring (1,200 r.p.m.).
 - ▲ **CRITICAL STEP** The Au(+III)-CTAB solution prepared in Step 1D(i) should be stirred gently (300–500 r.p.m.) to help prevent air from dissolving in it and causing a decrease in the reduction efficiency of the NaBH₄, which is added in Step 1D(ii). The stirring speed should be increased no more than a few seconds before the addition of NaBH₄. After this addition, a color change from yellow to brownish-yellow marks the successful formation of Au seeds.
- (iii) Place the Au seed solution from Step 1D(ii) into a 27 °C water bath without stirring for 2 h before use in Step 1D(vii).
- (iv) Add 40 μl of AgNO₃ (10 mM) into a test tube.
- (v) Add 4.75 ml of CTAB (0.1 M) and 200 μl of chloroauric acid (10.0 mM), one after the other in that order, to the test tube with gentle mixing.
- (vi) Add 32 μl of ascorbic acid (0.1 M) to the test tube and continue mixing gently.
 - ▲ **CRITICAL STEP** The solution will change from gold to colorless here, and this indicates that all of the Au(+III) has been converted to Au(+I).
- (vii) Add 20 μl of the Au seed solution prepared in Step 1D(iii) to the test tube and mix it gently for 30 s.
- (viii) Allow the test tube to stand for 6 h without stirring.
 - ▲ **CRITICAL STEP** The color will change to pale red after 30–60 min. The average length of the nanorods is ~75 nm and the average diameter is ~25 nm.
- (ix) Centrifuge the tube at 2,391*g* for 15 min at room temperature. Remove the supernatant and redisperse the nanorods in 5 ml of fresh Milli-Q water.
- (x) Add 200 μl of freshly prepared APTMS (1 mM) to 10 ml of the washed NP solution (concentration ~2.5 × 10⁻⁴ M) with vigorous stirring (1,200 r.p.m.).
- (xi) Continue to stir the solution vigorously for 15 min.
- (xii) Add 1.60 ml of sodium silicate solution (diluted to 0.54% (wt/wt) with Milli-Q water and acidified to pH ~10.2 with hydrochloric acid) and stir for 3 min at room temperature.
- (xiii) Allow the resulting solution to further react for 1–2 d with vigorous stirring.
 - ▲ **CRITICAL STEP** An elevated temperature is not preferable here because higher temperatures can lead to aggregation of the NPs in the presence of dilute CTAB.
- (xiv) Centrifuge the final products at 2,391*g* for 15 min at room temperature, remove the supernatant and redisperse the NPs in 1.5 ml of fresh Milli-Q water. Next, repeat this washing step a second time. Centrifuge, remove the supernatant and obtain the clean nanorod SHINs (**Fig. 4a**) as a concentrated solution.

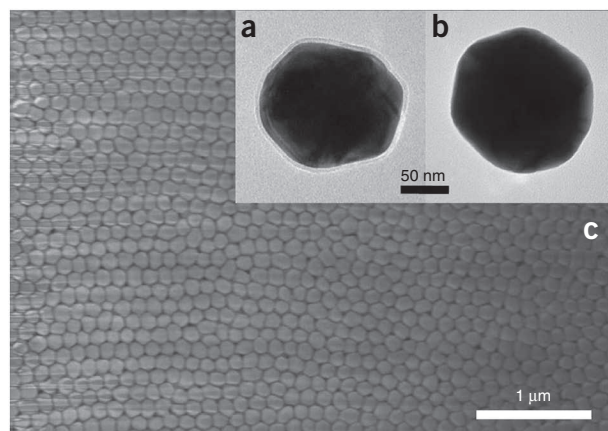


Figure 3 | Images of 120-nm Au@SiO₂ SHINs. (a–c) HR-TEM images of 120-nm Au@SiO₂ NPs with 4-nm (a) and 1-nm (b) shells, and a scanning electron microscopy image of 120-nm Au@1 nm SiO₂ NPs (c).

PROTOCOL

(E) Preparation of nanocube SHINs ● TIMING 2–3 d

- (i) Mix 250 μl of chloroauric acid (10.0 mM), 2.75 ml of Milli-Q water and 7.5 ml of CTAB (0.1 M) in a round-bottom flask and heat the mixture to 35 °C.
- (ii) Add 600 μl of ice-cold, freshly prepared NaBH_4 (10 mM) to the flask all at once with vigorous stirring (1,200 r.p.m.) and allow it to react for 1 min.
 - ▲ **CRITICAL STEP** The procedure and tips given here are similar to those in the preparation of Au nanorod seeds section. Extra Milli-Q water is added to dilute the seed solution, and the temperature is slightly higher in order to prevent CTAB from crystallizing.
- (iii) Keep the Au seed solution from Step 1E(ii) in a 35 °C water bath without stirring for 2 h, and then dilute it 100 times before use in Step 1E(vii).
 - ▲ **CRITICAL STEP** The seed solution is diluted 100 times after the 2-h period, not before.
- (iv) Add 8.0 ml of CTAB (0.1 M) and 1.0 ml of chloroauric acid (10.0 mM), one after the other in that order, to a test tube with gentle mixing.
- (v) Add 38 ml of Milli-Q water to the test tube.
- (vi) Add 3 ml of ascorbic acid (0.10 M) to the test tube with gentle mixing.
 - ▲ **CRITICAL STEP** The solution will change from gold to colorless here, and this indicates that all of the Au(+III) has been converted to Au(+I).
- (vii) Add 20 μl of the diluted Au seed solution prepared in Step 1E(iii) to the test tube and mix it gently for 30 s.
- (viii) Allow the solution to stand in the test tube overnight at 35 °C without stirring.
 - ▲ **CRITICAL STEP** A color change from transparent to pale red will be observed after 30 min. The average nanocube side will measure ~85 nm.
- (ix) Isolate the reaction products by centrifuging at 2,391g for 15 min at room temperature. Remove the supernatant and redisperse the nanocubes in 50 ml of fresh Milli-Q water.
- (x) Add 150 μl of freshly prepared APTMS (1 mM) to 10 ml of the washed NP solution with vigorous stirring (1,200 r.p.m.).
- (xi) Continue to stir the solution vigorously for 15 min.
- (xii) Add 1.22 ml of sodium silicate solution (diluted to 0.54% (wt/wt) with Milli-Q water and acidified to pH ~10.2 with hydrochloric acid) and stir for 3 min at room temperature.
- (xiii) Allow the resulting solution to further react for 1–2 d with vigorous stirring.
- (xiv) Centrifuge at 2,391g for 15 min at room temperature, remove the supernatant and redisperse the NPs in 1.5 ml of fresh Milli-Q water. Next, repeat this washing step a second time. Centrifuge the mixture, remove the supernatant and obtain the clean nanocube SHINs (Fig. 4b) as a concentrated solution.

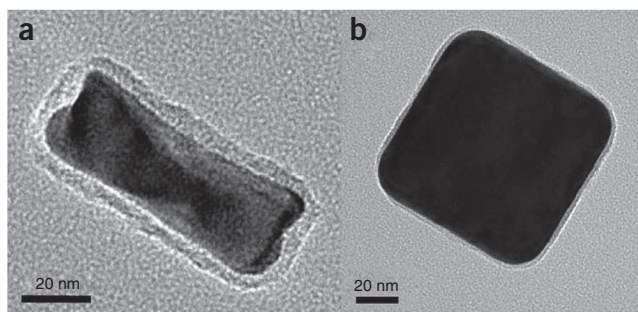


Figure 4 | HR-TEM images of nanorod and nanocube SHINs. (a) A nanorod SHIN with a 4-nm silica shell (prepared in 2 d). (b) A nanocube SHIN with a 2-nm silica shell (prepared in 2 d).

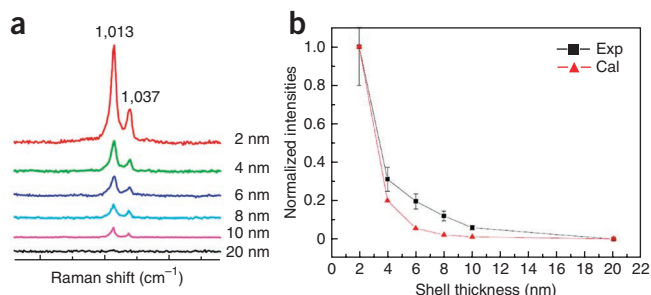
Quality control steps

2| Before using the SHINERS in experiments, it is important to ensure that they are of sufficient quality. Possible tests are described below:

2A	Test for the effect of shell thickness
2B	Test for pinholes in the silica shell
2C	Comparing the stability of SHINs and bare Au NPs

Figure 5 | Correlation of the SHINERS intensity and the shell thickness.

(a) SHINERS spectra of pyridine adsorbed on a smooth Au film coated with 55-nm Au@SiO₂ NPs having different silica shell thicknesses. (b) Integrated SHINERS intensity, obtained for pyridine's 1,013 cm⁻¹ band, plotted as a function of silica shell thickness after normalizing by the 2-nm shell-thickness intensity (black squares, from experiment (Exp)). Average electric field strengths calculated by 3D-FDTD (Box 2) were overlaid for comparison after normalizing by the 2-nm shell-thickness average field strength (red triangles, from calculation (Cal)).



(A) Test for the effect of shell thickness ● TIMING 1 h

- (i) Prepare SHINs with different silica shell thicknesses (2, 4, 6, 8 and 10 nm) by controlling the reaction time.
 - ▲ **CRITICAL STEP** Silica shell thickness is determined by the heating time: shells about 2, 4, 6, 8 and 10 nm thick are prepared by heating for 20 min, 1 h, 2 h, 4 h and 8 h, respectively.

? **TROUBLESHOOTING**

- (ii) Add 10 μ l of each concentrated SHIN solution to a different clean, smooth Au film. Dry all of the SHIN-coated Au films in a vacuum dryer for 15 min.
- (iii) Add a drop of 10 mM pyridine to each of the SHIN-coated Au films. Place a quartz coverslip on each sample so that a thin layer of solution is held under each coverslip. Allow \sim 3 min for pyridine to adsorb on the Au film.
- (iv) Carry out a SHINERS experiment on each of the samples in sequence to obtain a series of spectra similar to those shown in **Figure 5**.

(B) Test for pinholes in the silica shell ● TIMING 1 h

- (i) Drop 10 μ l of concentrated Au@1 nm SiO₂ sol (with pinholes) and 10 μ l of concentrated Au@4 nm SiO₂ sol (without pinholes) on two separate clean Si wafers. Dry them under vacuum.
- (ii) Add 20 μ l of pyridine (10 mM) onto each of the SHIN-coated Si wafers and place a quartz coverslip on top.
- (iii) Allow \sim 3 min for pyridine to adsorb on any exposed Au.
- (iv) Collect Raman signals from the Au@SiO₂ samples with (**Fig. 6a**) and without (**Fig. 6b**) pinholes. Pinholes in the silica shell allow pyridine to adsorb on the Au core, and they lead to a strong Raman signal. SHINs with a pinhole-free silica shell do not give a Raman signal.

? **TROUBLESHOOTING**

(C) Comparing the stability of SHINs and bare Au NPs ● TIMING 10 d

- (i) Place 0.5 ml of pyridine solution (0.01 M) into each of two cuvettes.
- (ii) Add 10 μ l of concentrated SHINs (\sim 1.07 nM) to one of the cuvettes, and add 10 μ l of concentrated bare Au NPs (\sim 1.07 nM) to the other.
- (iii) Take photographs of the mixtures at different times (**Fig. 7a,b**).
- (iv) Collect Raman signals from the solutions in the cuvettes and compare the stability of these Raman signals (**Fig. 7c,d**).

SHINERS experimental procedures

3| Procedures for examples of experiments using SHINs are included in options A–G.

- 3A SHINERS experiment on Pt(111) single-crystal electrodes adsorbed with hydrogen
- 3B SHINERS experiment on Si(111) wafers adsorbed with hydrogen
- 3C SHINERS experiment on Au(111) and Au(100) single-crystal electrodes adsorbed with SCN⁻
- 3D SHINERS experiment on PATP adsorption using SHINs and bare Au NPs
- 3E Experiment on Pt(111) single-crystal electrodes adsorbed with CO using SHINs and bare Au NPs
- 3F SHINERS experiment on yeast cell walls
- 3G SHINERS detection of a pesticide residue on a fruit

(A) SHINERS experiment on Pt(111) single-crystal electrodes adsorbed with hydrogen ● TIMING 2 h

- (i) Drop 2 μ l of concentrated SHIN solution onto a freshly prepared Pt(111) single-crystal surface (**Box 1**) and dry it in a vacuum dryer for 15 min.
 - ▲ **CRITICAL STEP** If the single-crystal surface has very small facets, a micropipette should be used to add the SHIN solution, and a charge-coupled device (CCD) should be used to test the resulting sample. Neither too many nor too few SHINs should be spread on the sample surface. A monolayer of SHINs will generate the strongest Raman signal.

? **TROUBLESHOOTING**

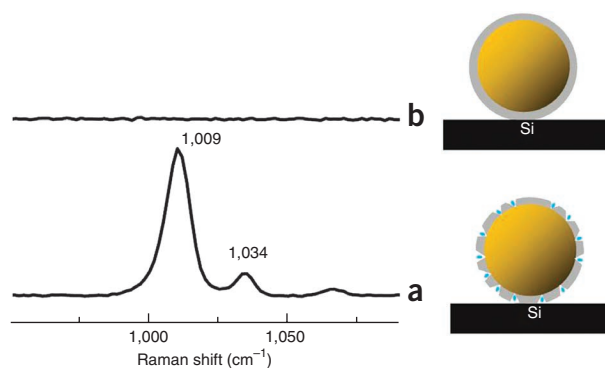


Figure 6 | Pinhole test for SHINs. (a,b) SHINERS spectra obtained with 55-nm Au@1 nm SiO₂ (with pinholes, a) and 55-nm Au@4 nm SiO₂ (without pinholes, b) on Si wafers in 10 mM pyridine.

PROTOCOL

- (ii) Place the Pt(111) single crystal in a spectroelectrochemical cell similar to the one shown in **Figure 8**. Add 0.1 M NaClO₄ solution.
- (iii) Hold the potential in the region of the hydrogen evolution reaction. This occurs between about -1.9 V and about -1.2 V versus a SCE. Collect the Raman signal from the Pt(111) electrode.

▲ **CRITICAL STEP** The electrode should be pushed very close to the quartz window (but not into contact with it), so that a very thin layer of solution (50–100 μm) remains between the electrode and the window. This will minimize the scattering of light by hydrogen bubbles in the electrochemical cell.

(B) SHINERS experiment on Si(111) wafers adsorbed with hydrogen

● TIMING 2 h

- (i) Clean a Si(111) wafer by submerging it in H₂SO₄ for 30 min, rinsing it thoroughly with water and drying it. Drop 10 μl of concentrated SHIN solution onto the surface and dry it in a vacuum dryer for 15 min.
- (ii) Clean a second Si(111) wafer as in Step 5B(i), and then treat it with a 30% (wt/wt) HF solution for 30 s. Drop 10 μl of concentrated SHIN solution onto the surface and dry it in a vacuum dryer for 15 min.
- (iii) Clean a third Si(111) wafer as in Step 5B(i); next, treat it with a 30% (wt/wt) HF solution for 30 s and an oxygen plasma for 15 min. Drop 10 μl of concentrated SHIN solution onto the surface and dry it in a vacuum dryer for 15 min.
- (iv) Collect Raman signals from the three Si wafers.

(C) SHINERS experiment on Au(111) and Au(100) single-crystal electrodes adsorbed with SCN⁻

● TIMING 2 h

- (i) Drop 2 μl of concentrated SHIN solution onto freshly prepared Au(111) and Au(100) single-crystal surfaces (**Box 1**), and then dry them in a vacuum dryer for 15 min.

? TROUBLESHOOTING

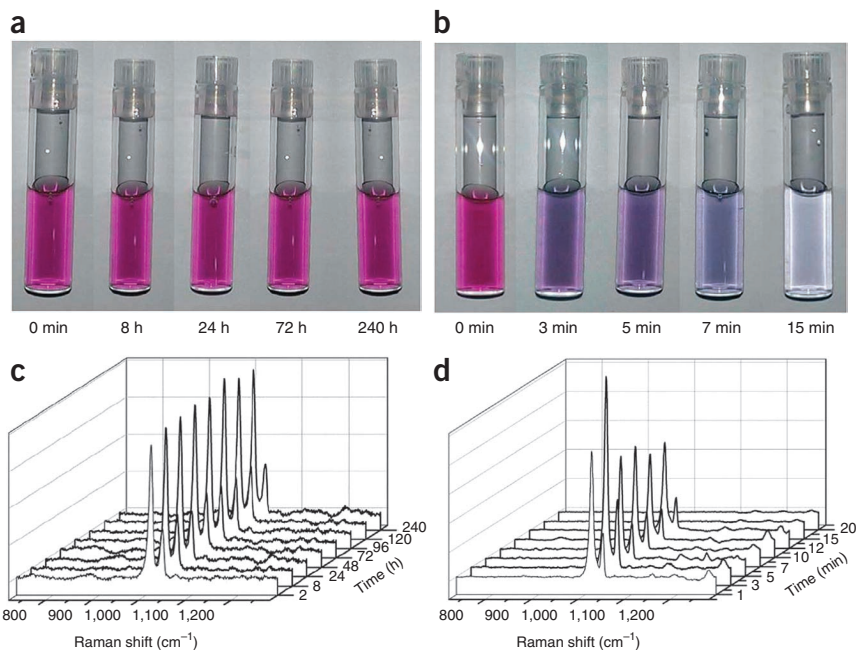


Figure 7 | Stability comparison of SHINs and bare Au NPs. (a,b) Photos of SHINs (a) and bare Au NPs (b) in 0.01 M pyridine solution at different times. (c,d) Corresponding SERS signals from pyridine obtained with SHINs (c) and bare Au NPs (d).

Box 1 | Preparation of Au or Pt single crystals ● TIMING 1 h

MATERIALS

Au wire, 99.999% (Sigma-Aldrich, cat. no. 349305)
Pt wire, 99.95% (Alfa Aesar, cat. no. 43288)
Hydrochloric acid (G.R., Sinopharm Chemical Reagent)
Nitric acid (G.R., Sinopharm Chemical Reagent)
Hydrogen gas (Linde)
Oxygen gas (Linde)

PROCEDURE

1. Use a hydrogen/oxygen flame to melt one end of a piece of Au or Pt wire (at least 0.5 mm in diameter and at least 5 cm long) into a bead. The bead should remain attached to the wire.
2. Use aqua regia (nitrohydrochloric acid, HCl/HNO₃ = 3:1 by volume) to remove impurities from the surface of the Au or Pt bead.
3. Melt the Au or Pt bead again.
4. Repeat steps 2 and 3 a total of four or five times.

▲ **CRITICAL STEP** Steps 3 and 4 must be repeated until the Au or Pt bead is free of impurities.

5. During the final repetition of steps 3 and 4, maintain the liquid-solid transition temperature for more than 60 s and allow the bead to cool very slowly from its face to the end that remains attached to the wire.

▲ **CRITICAL STEP** If carried out correctly, step 5 will form single-crystal facets of good quality.

6. Choose the large (111) and microscopic (100) facets by eye and by using CCD imaging, respectively.

- (ii) Place the Au(111) and Au(100) single crystals in a spectroelectrochemical cell similar to the one shown in **Figure 8**. Add 0.01 M NaSCN/0.1 M NaClO₄ solution.
- (iii) Hold the potential at 0.0 V versus SCE and collect the Raman signals from the Au(111) and Au(100) electrode surfaces.
 - ▲ **CRITICAL STEP** The potential should be held between -0.6 V and 0.4 V. A higher potential may cause oxidation of the SCN⁻ and a lower potential may cause desorption. A solution thickness of 0.15–0.30 mm between the electrode and the window appears to be optimal for this system. More solution will decrease the Raman signal intensity, and less solution will increase the resistance and thereby affect the applied potential.

(D) SHINERS experiment on PATP adsorption using SHINs and bare Au NPs ● TIMING 2 h

- (i) Immerse a smooth Au film and a piece of glass coated with ZnO nanorods in a 1 mM solution of PATP in ethanol for 30 min.
- (ii) Rinse them with pure ethanol to remove excess (physically adsorbed) PATP.
- (iii) Drop 10 μl of concentrated SHIN solution and 10 μl of concentrated bare Au NP solution onto the smooth Au film and the piece of glass coated with ZnO nanorods. Dry them in a vacuum dryer for 15 min.
- (iv) Collect the Raman signals.
 - ▲ **CRITICAL STEP** The laser power must be >1 mW for this demonstration to work. If it is too weak, photocatalytic dimerization of PATP will not occur.

(E) Experiment on Pt(111) single-crystal electrodes adsorbed with CO using SHINs and bare Au NPs ● TIMING 3 h

- (i) Drop 2 μl of concentrated SHIN solution and 2 μl of concentrated bare Au NP solution on two separate, freshly prepared Pt(111) single crystals. Dry them in a vacuum dryer for 15 min.
- (ii) Sequentially, place each of the two Pt(111) single crystals in a spectroelectrochemical cell similar to the one shown in **Figure 8** and add 0.01 M HClO₄ saturated with CO gas.
- (iii) Hold the potential at 0.0 V versus SCE and collect the Raman signals.
 - ▲ **CRITICAL STEP** The potential should be held between -0.25 V and 0.50 V. A higher potential may cause oxidation of the CO and a lower potential may cause hydrogen evolution.

(F) SHINERS experiment on yeast cell walls ● TIMING 2 d

- (i) Clean a quartz slide sequentially with acetone, ethanol and distilled water in an ultrasonic cleaner for 5 min each. Immerse it in piranha solution (H₂SO₄/30% H₂O₂ = 3:1) for 1 h. Wash it with distilled water, blow dry with nitrogen and then dry it in an oven at 70 °C for 1 h.
 - ▲ **CRITICAL STEP** The quartz slide must be completely clean in order to avoid contaminant spectral peaks.
- (ii) Incubate yeast in YPD medium (1%) at 30 °C for 24 h while centrifuging at 2.7g.
- (iii) Wash the yeast cells three times with 0.1 M PBS to remove the YPD. Use a centrifuge-based procedure similar to the ones described previously, applying 598g for 1 min at room temperature during each cleaning step.
- (iv) Add 2 μl of the resulting solution to the quartz slide. This will be used to collect normal (unenhanced) Raman spectra from the yeast cells.
- (v) Suspend the washed yeast cells again in fresh YPD at a concentration of ~3.2 × 10⁷ cells per ml. Mix 100 μl of this with 100 μl of concentrated SHIN solution, and then incubate at 30 °C for 3 h.
- (vi) Add 10 μl of the solution containing yeast cells and SHINs to the quartz slide.
- (vii) Collect Raman signals from the samples.

(G) SHINERS detection of a pesticide residue on a fruit ● TIMING 1 h

- (i) Wash an orange peel with distilled water and then with Milli-Q water.
 - ▲ **CRITICAL STEP** The purpose of Step 5G(i) is to remove most of the contaminants present on the orange peel (some could interfere with the desired Raman information) so that this demonstration can be carried out under controlled conditions.
- (ii) Spray a methyl parathion solution (0.1–1.0 mM in ethanol) onto the orange peel and allow it to dry.

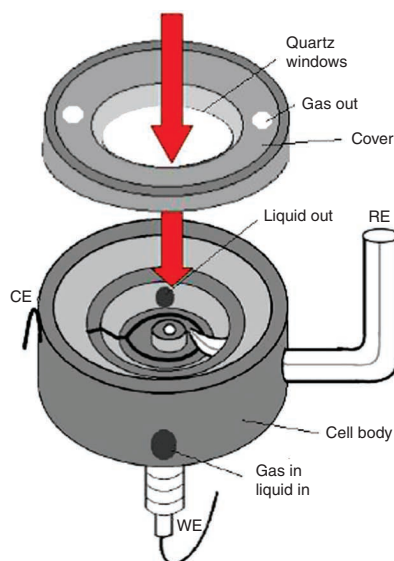


Figure 8 | Schematic illustration of our homemade spectroelectrochemical cell. The reference, working and counter electrodes are indicated by RE, WE and CE.

Box 2 | 3D-FDTD calculations ● TIMING 2–3 d

Run 3D-FDTD calculations using the XFDTD commercially available software package (Remcom XFDTD 6.3).

PROCEDURE

(A) Choose the calculation model

- (i) Model a 2×2 array of spherical Au@SiO₂ NPs on a smooth Au substrate. Set the Au cores to have a 55-nm diameter and the silica shells to have 0- to 50-nm thicknesses. Set both the length and the width of the smooth Au substrate at 260 nm.
- (ii) Set uniform 1 nm × 1 nm × 1 nm or 2 nm × 2 nm × 2 nm Yee cells in the software's automatic mesh mode. The calculation model is shown in **Figure 9**, where the geometry bounding and mesh bounding boxes can be seen.

▲ **CRITICAL STEP** It is important that the mesh be appropriate for the object. The Yee cell size should not be greater than 2 nm × 2 nm × 2 nm.

(B) Set the material parameters

- (i) Use a Debye material type for Au. Set the values of ϵ_s , ϵ_∞ , σ and τ to 16,000, 9.012, 1.525×10^7 S m⁻¹ and 9.3×10^{-15} s, respectively.

▲ **CRITICAL STEP** A simple Drude model cannot accurately describe the frequency-dependent complex permittivity of a metal over a wide frequency range. This is especially true for Au, because its interband transitions have a large effect on its dielectric function. Therefore, a general Drude model (a Debye material type) should be adopted to simulate the complex permittivity of Au. It has the form

$$\epsilon(\omega) = \epsilon_\infty + \frac{\epsilon_s - \epsilon_\infty}{1 + i\omega\tau} + \frac{\sigma}{i\omega\epsilon_0}$$

where ϵ_s , ϵ_∞ , σ and τ represent static permittivity, infinite frequency permittivity, conductivity and relaxation time, respectively; ω is angular frequency and ϵ_0 is the permittivity of free space. The first four parameters can be adjusted through curve-fitting techniques to correctly match the complex permittivity derived from experimentally obtained optical constants through the relationships $\epsilon_r = n^2 - k^2$ and $\epsilon_i = 2nk$, where ϵ_r and ϵ_i are the real and imaginary parts of the dielectric function of the dispersive material, respectively. This model for the local dielectric function of metals will faithfully represent their optical response over a wide frequency range.

- (ii) Use a normal material type with a relative permittivity of 2.12 and a conductivity of 0 for silica.

(C) Set up the excitation source

- (i) Set the wavelength of the incident light to be 632.8 nm.
- (ii) Set the waveform to be sinusoidal with an amplitude of 1 V m⁻¹.
- (iii) Set the source type to be a plane wave.
- (iv) The incident monochromatic plane wave should approach from the top (along the z axis) with its polarization parallel to the y axis.
- (v) Set the number of periods of the incident sinusoidal plane wave to be 10.

▲ **CRITICAL STEP** The total number of periods (time steps) of the incident sinusoidal plane wave should be chosen carefully. A low number will terminate the calculation before convergence of the results. A high number will cause unnecessary calculations to be performed, but it will not result in errors.

(D) Request the results

- (i) Save the sinusoidal peak electric field magnitudes distributed in the Au substrate to view the calculation result.
- (ii) Save some near-zone electric field data at the hot spots to check the calculation convergence.

- (iii) Collect Raman signals from the orange peel using a Raman microscope and a portable Raman spectrometer.
 - (iv) Drop 10 μ l of concentrated SHIN solution onto the orange peel and dry it in a vacuum dryer for 15 min.
 - (v) Collect Raman signals from the orange peel again using the Raman microscope and the portable Raman spectrometer.
- ▲ **CRITICAL STEP** If the Raman microscope and the portable Raman spectrometer use different excitation wavelengths, SHINs with different SPRs may be required for the two sets of equipment. We used 55-nm Au sphere SHINs (SPR at ~600 nm) in our Raman microscope experiments (excitation $\lambda = 633$ nm), and 20-nm by 60-nm Au rod SHINs (SPR at ~770 nm) in our portable Raman spectrometer experiments (excitation $\lambda = 785$ nm).

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Step	Problem	Possible reason	Solution
2A(i)	Poor reproducibility of SHINERS intensity from different samples	The silica or alumina shell thickness was not precisely controlled during preparation (the Raman signal intensity depends crucially on the shell thickness)	The thickness of the silica shell should be carefully controlled during preparation, especially with regard to heating time and quickly stopping the reaction by cooling in an ice bath. The thickness of the alumina shell should be carefully controlled by adjusting the deposition parameters
2B(iv)	Observation of Raman peaks that are not from the probe molecule	There may be some pinholes in the shell. This is very likely the case if the shell is <2 nm in thickness. Then probe molecules can pass through the pinholes and adsorb on the Au; but other molecules in the solution or in the air can do so as well, thus causing the interference	Very carefully coat the Au NPs with the silica or alumina shell. Ensure that the coating is uniform and suitably thick so as to eliminate the occurrence of pinholes
3A(i)	The SHINERS signal is weak, even when SHINs are well prepared	If the SHIN overlayer is too thick (more than ~3 monolayers thick), it will block incident and scattered photons. If the SHIN overlayer is less than a full monolayer, the enhanced Raman signal will also be weak	Use a micropipette to add SHIN solution of the right volume and concentration, then dry to form about one monolayer of SHINs on the sample surface. One monolayer will generate the strongest SHINERS signal
3C(i)	Observation of Raman peaks from contamination	The prepared SHINs or solution/electrolyte are not clean	It is better to use freshly prepared SHINs. The longer time the SHINs are stored, the more contamination may stick on the SHINs surface. So, centrifuge SHINs as much times as possible. Normally, we centrifuge SHINs for 3–5 times to clean the surface. We also recommend using chemicals with ultrapure quality. The solution/electrolyte should be also freshly prepared with Milli-Q water

● TIMING

- Step 1A, preparation of 55-nm-diameter Au nanosphere seeds and associated Au@SiO₂ SHINs: 2 h
- Step 1B, preparation of Au@Al₂O₃ SHINs: 2 h
- Step 1C, preparation of 120-nm-diameter Au nanosphere seeds and associated Au@SiO₂ SHINs: 4 h
- Step 1D, preparation of nanorod SHINs: 2–3 d
- Step 1E, preparation of nanocube SHINs: 2–3 d
- Step 2A, test for the effect of shell thickness: 1 h
- Step 2B, test for pinholes in the silica shell: 1 h
- Step 2C, comparing the stability of SHINs and bare Au NPs: 10 d
- Step 3A, SHINERS experiment on Pt(111) single-crystal electrodes adsorbed with hydrogen: 2 h
- Step 3B, SHINERS experiment on Si(111) wafers adsorbed with hydrogen: 2 h
- Step 3C, SHINERS experiment on Au(111) and Au(100) single-crystal electrodes adsorbed with SCN⁻: 2 h
- Step 3D, SHINERS experiment on PATP adsorption using SHINs and bare Au NPs: 2 h
- Step 3E, Experiment on Pt(111) single-crystal electrodes adsorbed with CO using SHINs and bare Au NPs: 3 h
- Step 3F, SHINERS experiment on yeast cell walls: 2 d
- Step 3G, SHINERS detection of a pesticide residue on a fruit: 1 h
- Box 1, preparation of Au or Pt single crystals: 1 h
- Box 2, 3D-finite-difference time-domain (FDTD) calculations: 2–3 d

ANTICIPATED RESULTS

Overall, we anticipate good-quality, reproducible SHINERS spectra from surfaces of varying composition and morphology. Step 2A: The shell thickness test will show that Raman intensity decreases quite markedly with increasing shell thickness (Fig. 5a). Box 2: 3D-FDTD calculations (see Fig. 9) will confirm the experimental result obtained in Step 2A—electric field strength (and therefore Raman intensity) decreases rapidly with increasing shell thickness (Fig. 5b).

PROTOCOL

Step 2B: In the test for pinholes, a strong Raman signal will be obtained from pyridine adsorbed on Au SHIN cores if 1-nm silica shells (having pinholes) are used (**Fig. 6a**). No Raman signal will be obtained from pyridine if 4-nm silica shells (having no pinholes) are used, indicating that the Au SHIN cores are isolated (**Fig. 6b**).

Step 2C: During the 10-d stability test, the SHIN solution will not change color (**Fig. 7a**), and it will give a stable Raman signal (**Fig. 7c**). The bare Au NP solution will be far less stable (**Fig. 7b,d**).

Step 3A: In the SHINERS experiment on Pt(111) single-crystal electrodes adsorbed with hydrogen, a Pt(111)-H stretching band will be observed at $\sim 2,030\text{ cm}^{-1}$ (ref. 9 and **Supplementary Fig. 1**). We note that ordinary (unenhanced) Raman spectroscopy is not sensitive enough to detect hydrogen on a Pt(111) surface.

Step 3B: In the SHINERS experiment on Si(111) wafers adsorbed with hydrogen (ref. 9 and **Supplementary Fig. 2**), a Si-H band will be observed at $\sim 2,150\text{ cm}^{-1}$ after the sample is treated with HF solution. This peak will disappear after the sample is further treated with an oxygen plasma, indicating that the surface hydrogen has been removed.

Step 3C: Different spectral characteristics will be observed for SCN^- adsorbed on Au(100) and Au(111) single-crystal electrodes (ref. 9 and **Supplementary Fig. 3**). The Au-S stretching band for S-bound SCN^- at 234 cm^{-1} will be much stronger for Au(100) than for Au(111). The C-N stretching band for S-bound SCN^- at $2,119\text{ cm}^{-1}$ will be much sharper and stronger for Au(100) than for Au(111), and it is known that this peak becomes sharper and stronger as the S-bound fraction increases and the N-bound fraction decreases. These spectral characteristics indicate that a greater fraction of SCN^- is S-bound, and a smaller fraction of SCN^- is N-bound, on Au(100) compared with Au(111). Here we demonstrate that SHINERS can be used to distinguish different adsorbate orientations on different single-crystal facets.

Step 3D: SERS and SHINERS experiments on PATP adsorption using bare Au NPs and SHINs, respectively (ref. 9 and **Supplementary Fig. 4**), reveal that photocatalytic dimerization of PATP will lead to additional Raman signals at $1,139$, $1,387$ and $1,433\text{ cm}^{-1}$ when bare Au NPs are used but not when SHINs are used. In contrast to bare Au NPs, SHINs will give the true spectrum of PATP on Au and ZnO nanorods.

Step 3E: When bare Au NPs are used to detect CO on Pt(111), a Raman signal from CO on Au will be observed at $2,125\text{ cm}^{-1}$ and the CO on Pt(111) frequency will shift from $2,072$ to $2,060\text{ cm}^{-1}$ because charge is transferred from Au to Pt. When the Au NPs are surrounded by a chemically and electrically inert shell, the true spectrum of CO on Pt(111) can be obtained. This is shown in ref. 9 and in **Supplementary Figure 5**.

Step 3F: SHINERS spectra and ordinary Raman spectra collected from yeast cells (ref. 9 and **Supplementary Fig. 6**) will be quite different. SHINERS spectra will be similar to the SERS spectrum of mannoproteins, which are considered to be the main components of the yeast cell walls.

Step 3G: Raman peaks indicating parathion contamination will be detected on the orange peel at $\sim 1,108$ and $\sim 1,340\text{ cm}^{-1}$ when SHINs are present but not when they are absent (ref. 9 and **Supplementary Fig. 7**). This result can be obtained using either a Raman microscope or a portable Raman spectrometer.

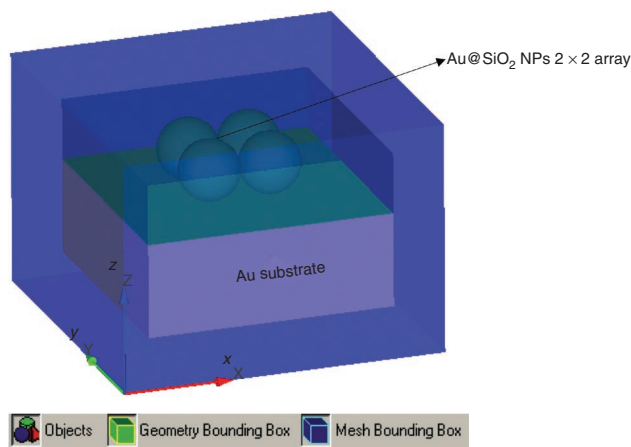


Figure 9 | 3D-FDTD modeling of four SHINs on an Au substrate. The objects are surrounded by a geometry bounding box, which in turn is surrounded by a mesh bounding box.

Note: Supplementary information is available in the online version of the paper.

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Corrigendum: Surface analysis using shell-isolated nanoparticle-enhanced Raman spectroscopy

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