Electronic Supplementary Material (ESI) for Analyst.
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Supplementary Information

Materials

The following reagents do not require further purification and are used directly in the experiment. The solution in the experiment was prepared with ultrapure water (resistivity of 18.2 MΩ·cm) purified by Milliproe pure water system instrument. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 49.0%) and sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99.5%), silver nitrate ($AgNO_3$, 99.8%) purchased from Sigma-Aldrich(Shanghai, China), Ascorbic acid (AA) hydrochloric acid (HCl) sodium lauryl sulfate (SDS, 99.5%), sodium hydroxide (NaOH, 96.0%), sodium sulfate (Na_2SO_4, 99.0%), disodium phosphate (Na_2HPO_4, 98.0%), phosphoric acid (H_3PO_4, 85.0%), Boric acid (H_3BO_3, 99.8%), citric acid ($C_6H_8O_7$, 99.8%), acetic acid (CH_3COOH, 99.8%), ethanol (CH_3CH_2OH, 75.0%) were purchased from Sinopharm Chemical Reagent Co., LTD. (Shanghai, China).

Preparation of pH buffer solution: a. Britton-Robinson(B-R) buffer solution: First, prepare 0.04 M of mixed acid solution: Add 240 μL CH_3COOH, 400 μL H_3PO_4 and 0.248 g H_3BO_3 to the beaker, then add ultra-pure wat er to mix well and set the volume in a 100 mL volumetric bottle. Secondly, 0.2 M NaOH solution was prepared. Finally, the prepared NaOH solution and the mixed acid solution were mixed in different volume ratios to prepare the buffer solution with different pH values, and the pH value of the buffer solution was further calibrated by pH meter. b. Disodium hydrogen phosphate - citric acid buffer solution: First, prepare 0.2 M Na_2HPO_4 and 0.1 M citric acid solution respectively. Then, the prepared Na_2HPO_4 solution and citric acid solution were mixed in different volume ratios to prepare buffer solutions with different pH values, and the pH value of buffer solutions was further calibrated by pH meter.

Test instrumentation

UV-visible spectrophotometer Model: Shimadzu UV3600 purchased from Shimadzu Company; Transmission electron microscope model: Hitachi HT7700 purchased from Hitachi Corporation; Heating digital display Zili agitator Model :SHT-19T purchased from joanlab Company; Ultrasonic cleaner type: SK2200H purchased from Shanghai Science Guide; Model: ZetaPALS, Brookhaven; Electronic balance model: BSA124S from Sartorius; Centrifuge model: H1650 purchased from Xiangyi ; Concussion incubator model: ZQTY-50 purchased from Shanghai Zhichu Instrument. Experimental dark field microscopy (DFM) images and SERS spectra were measured by a −75 ℃ cooled CCD detector (PIXIS 400BR), monochromator (Acton SP2358) BLZ 750 nm 1200 grating, Recorded by Nikon DS-f2 true color digital camera and inverted microscope inspection instrument system with dark field condenser (0.8< numerical aperture (NA)<0.95) with 60× objective lens. At the same time, a stable 633 nm laser is selected as the excitation source, and the

resolution of the measured spectrum is about 0.816 cm^{-1}.

Examination methods

Synthesis of urchin AuNPs

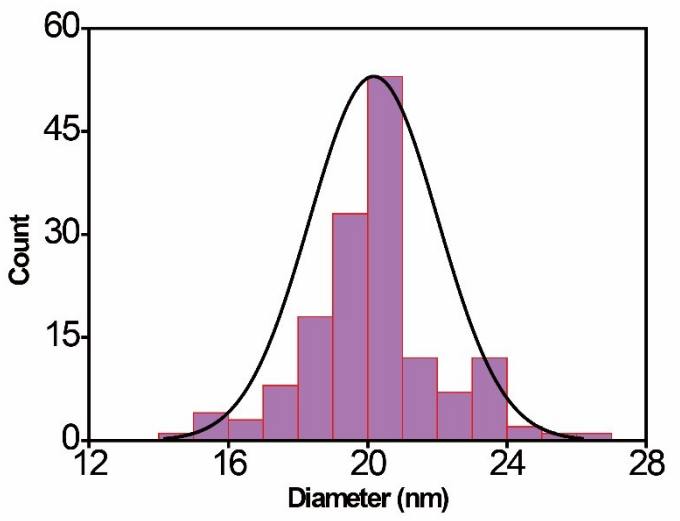

Figure S1 Statistical map of Au seed TEM particle size.

Urchin AuNPs were synthesized by seed growth method. First, AuNPs seeds with a diameter of 20 nm were synthesized, and then urchin AuNPs was grown with AuNPs seeds of 20 nm. The gold nanoparticles are synthesized by adding 45 mL deionized water and 0.6 mL 1% $HAuCl_4$ into a 100 mL two-necked flask. Add another magneton and set the speed of magneton to 700 r/min, set the temperature of oil bath to 140 ℃, bring to a boil, add 5 mL (22 mM sodium citrate, 0.0324 g SC + 5 mL water), and add a condensing tube for reflux. About 10min, the solution changed from yellow to blue-gray, then to soft pink, and then to orange-red. When the color changes from soft powder to orange-red, turn off the heat source and cool down. After cooling to room temperature, the prepared Au seed colloidal solution is stored in 50 mL centrifuge tube and sealed in the refrigerator at 4 ℃ for later use. Add a magneton to a 20 mL glass bottle and place the glass bottle on a magnetic stirrer. At room temperature, 10 mL of ultra-pure water, 100 μL of 1% (w/v) $HAuCl_4$ solution, 10 μL of 1 mol of HCl, 20 μL of Au seed and 100 μL of 2 wt% sodium dodecyl sulfate were added to the glass bottle in sequence. Then, under intense agitation, 100 μL of $AgNO_3$ and 50 μL of 0.1 mol ascorbic acid solution were added at the same time, and the reaction was completed after continuous agitation for 10 min. Finally, the prepared AuNPs were sealed and kept away from light in a refrigerator at 4 ℃.

Preparation of SERS substrates

Two sets of 1 mL urchin AuNPs were centrifuged at 3000 rpm/min for 5 min to remove the supernatant, and then the precipitation was redissolved with ultra-pure water and sonicate for 5 min. After 3 repetitions, one set of urchin AuNPs with supernatant removed after centrifugation was enriched to 10 μL, and then dispersed by ultrasound for 5 min. The SERS base was prepared by solvent evaporation induction (sol film formation). First, the cleaned ITO glass sheet was placed in a clean petri dish, and then the enriched urchin AuNPs were dripped onto the ITO glass sheet with a pipette gun. After evaporation and drying at room

temperature, the first SERS base was prepared. The other urchin AuNPs mentioned above were redissolved by adding 1 mL ultra-pure water and sonicate for 5 min. Then the cleaned ITO glass sheet was put into the new centrifuge tube, and the urchin AuNPs after redissolved ultrasound was added into the centrifuge tube with a pipette gun, and the ITO glass sheet was immersed in ultra-pure water. After mixing, ultrasound was performed for 15 min. After the completion of ultrasound, the ITO glass sheet was removed and rinsed with ultra-pure water for several times to flush the unadsorbed urchin AuNPs. The cleaned ITO was blow-dried with N_2 bottle mouthpiece at 45° Angle, and the SERS substrate of single particles was prepared.

Synthesis of urchin AuNPs-6-MPN probe

Firstly, 0.001 g of 6-MPN was dissolved in 1ml anhydrous ethanol, and then diluted with ultra-pure water to prepare 6-MPN molecular solutions of different concentrations. A drop of 100 μL molecular solution was added to the SERS substrate and incubated at room temperature for 2 h, then the unadsorbed molecules were rinsed with ultra-pure water and dried with N_2. In addition, 1 mL of centrifuged urchin AuNPs were placed in a centrifuge tube, and 20 μL of 6-MPN solution was added to the centrifuge and incubated in a shaking table at 25 ℃ and 300 rpm/min for 4 h. After the shaking incubation, centrifuge for 5 min at a rotating speed of 3000 rpm/min, remove the supernatant, then add ultra-pure water to redissolve the precipitation and ultrasound for 5 min. After repeated three times, the modified urchin AuNPs were added to the new centrifuge tube, and a clean ITO glass sheet was added at the same time. Then ITO glass sheet was added with ultra-pure water, the centrifugal tube was placed in the ultrasonic machine for 10 min, removed with clean tweezers and rinsed with ultra-pure water at 45° Angle for several times, and finally dried with N_2 mouthpiece at 45° Angle for testing.

pH response feasibility test of urchin AuNPs-6-MPN probe

Probe test for preparation of SERS substrate induced by solvent evaporation. In order to test whether 6-MPN molecules were successfully modified on SERS substrate, in the experiment, the unmodified SERS substrate was first placed on Raman spectrometer for blank base spectrum detection and SERS base spectrum was collected. Secondly, a drop of 6-MPN molecular solution with a concentration of 100 μL 10^{-6} M was added to the blank SERS substrate for incubation for 2 h, and the SERS probe spectrum after the modified was collected. Then the operation was repeated to collect 6-MPN molecular solutions at 10^{-5} M, 10^{-4} M and 10^{-3} M in order to modify them on SERS base, and collect spectra successively. In addition, drops of 100 μL 6-MPN solution were added to SERS substrate, and after incubation for 2 h, the solution was absorbed with pipette gun and rinsed with ultra-pure water for 3 times, drops of 100 μL acidic solution were added, and then the spectrum of the probe was collected. After cleaning the probe with ultra-pure water for 3 times, add 100 μL alkaline solution, stand for 3 min, and then collect the spectrum of the probe. The spectra were acquired five times in parallel at 633 nm laser excitation wavelength at 29 μW laser power and 20 s exposure time.

Probing test of the SERS base prepared by AuNPs-6-MPN of single particle sea urchin. On the basis of probe detection based on SERS substrate induced by solvent evaporation, the experiment further explored whether 6-MPN was successfully modified on single particle urchin AuNPs. First, the prepared SERS substrate was placed on the test platform, and the optical scattering of the single particle before the unmodified molecule was characterized by dark field microscopy, and the LSPR scattering peak was measured. Then, the single particle substrate was kept unchanged, a drop of 100μL 6-MPN molecular solution was added, and after standing for 2 h, the optical scattering of the modified single particle was characterized by dark field microscopy, and the LSPR scattering peak of the modified single particle was tested, and the SERS spectrum after modification was tested. pH response test of AuNPs-6-MPN probe with

single particle urchin. Since the 6-MPN molecule has a pKa of 6.46, the N atom on 6-MPN is protonated under acidic conditions and is better responsive to pH. Therefore, the pH response time is explored first, with a pH = 9 buffer. In the experiment, 6-MPN molecular solution with pH = 4 was prepared, and after co-incubation with AuNPs like sea urchin, the single particle probe was constructed according to the operation. After the probe was constructed on ITO, the single particle to be tested was found under a dark field microscope, and 100 μL buffer solution with pH = 9 was added. Test the Raman spectrum immediately. The pH response of single particle SERS probes was investigated. First, the ITO glass of the constructed single-particle probe was placed on the test platform of Raman spectrometer, and the particles to be measured were found under the dark-field microscope. First, a buffer of pH = 4 was added to the probe, and after the reaction was left for 180 s, the exposure time was 20 s. At excitation wavelength of 633 nm and laser power of 29 μW, the spectrum of the probe was collected in parallel for 5 times, and then the buffer was removed by pipette gun and cleaned by drops of ultra-pure water for 3 times. Then, the above operations were repeated, and the buffering solution with pH = 5, 6, 7, 8 and 9 were successively used to collect SERS spectrum of the single particle urchin AuNPs-6-MPN probe, and the data were processed and analyzed.

Performance test of urchin AuNPs-6-MPN probe

Loop test

In the experiment, acidic buffer with pH = 4 and alkaline buffer with pH = 9 is selected to carry out cyclic test on the probe. Firstly, the ITO glass sheet of 6-MPN-modified urchin AuNPs prepared under acidic conditions was placed on the test platform of Raman spectrometer, and the particles to be tested were found under the dark field microscope. The buffer solution with pH = 9 was first added to the probe, and then stood for 140 s. The spectrum of the probe was collected in parallel for 5 times at the exposure time of 20 s and the excitation wavelength of 633 nm. Then, the buffer was absorbed by pipetting gun and cleaned with drops of ultra-pure water for 3 times. The ITO glass was kept stationary, and a buffer of pH = 4 was added to the probe. The spectra of the probe were collected in parallel for 5 times with an exposure time of 20 s and an excitation wavelength of 633 nm, and then the buffer was removed with a pipette gun and cleaned with ultra-pure water for 3 times. The above process was repeated, and the SERS spectra under the condition of pH = 9 for three times and pH = 4 for two times were collected. Finally, the acquired spectral data were processed and further analyzed.

Stability test

In the experiment, 10^{-5} M NaCl, KCl, CsCl, $MgCl_2$, NaBr, NaI and Na_2SO_4 solutions were used to test the ion interference of a single granular urchin AuNPs-6-MPN probe to verify the stability of the probe. The experimental procedure was consistent with the cyclic test in the previous section. The single-particle probe to be tested was first found under a dark field microscope, then NaCl solution was added with a pipette gun, and after the reaction was left for 140 s, the spectral collection of the probe was carried out five times in parallel under a laser with an exposure time of 20 s and excitation wavelength of 633 nm. Then absorb the buffer and rinse with ultra-pure water for 3 times. The process is repeated, and the spectra of the probe in KCl, CsCl, $MgCl_2$, NaBr, NaI and Na_2SO_4 solutions are collected successively. Finally, the collected spectral data are processed and further analyzed.

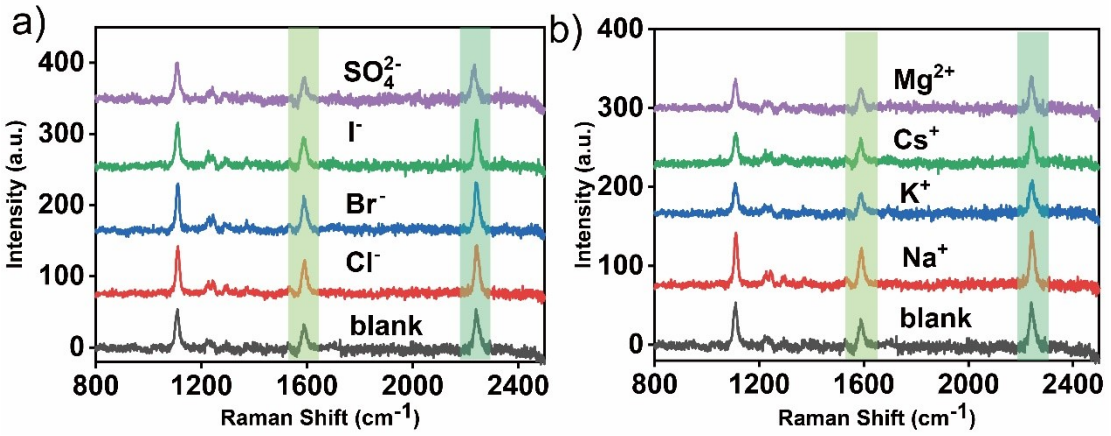

Figure S2 （a）SERS spectra of urchin AuNPs-6-MPN SERS probe in the presence of Cl^-, Br^-, $I^-$and SO_4^{2-}.（b）SERS spectra acquired in the presence of Na^+, K^+, Cs^+ and Mg^{2+}.