Electronic Supplementary Material (ESI) for ChemComm.

Recognition of Plastic Nanoparticles using Single Gold Nanopore Fabricated at the Tip of a Glass Nanopipette

Reagents and materials

Borosilicate glass capillary (0.58 mm I.D., 1.0 mm O.D., with filament) was purchased from Sutter Instrument Company (Novato, CA, USA). L-Ascorbic acid (99%) and Rhodamine 6G (95%) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroauric acid (Hydrogen tetrachloroaurate (III) trihydrate, 99.99%) and Tungsten wire (0.25mm diameter, 99.95%) were purchased from Alfa Aesar (Ward Hill, MA, USA). Potassium chloride (≥99.5%) and Sodium citrate (Trisodium citrate dihydrate, ≥99.0%) were purchased from Nanjing reagent company (Nanjing, China). Sodium borohydride (≥96.0%) and N, N-Dimethylformamide (DMF, ≥99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shang, China). 4-Mercaptobenzonitrile (4-Mbn, 95%) was purchased from Nanjing Norris Pharm Technology Co., Ltd. (Nanjing, China). Polystyrene nanosphere (PS, 3020A) with 20 nm diameter was purchased from Thermo Scientific (Fremont, CA, USA), zeta potential (the potential difference between the medium and the stationary layer of medium attached to the PS particle) measurement shows that the PS nanosphere is negatively charged. Poly(methyl methacrylate) (PMMA) with 10nm diameter was purchased from Suzhou Nanomicro Technology Co., Ltd(Suzhou, China).

All aqueous solutions were prepared from deionized water (resistivity ≥18.2 MΩ·cm).

Instruments

Glass nanopipette was pulled on a P-2000 CO_2-laser-based pipette puller system (Sutter Instrument Company, USA). SEM characterizations were carried out on FE-SEM S-4800 system (Hitachi High-Technologies, Japan). Raman experiments were conducted on a Renishaw InVia Reflex confocal microscope (Renishaw, UK) equipped with a high-resolution grating with 1800 grooves/cm, additional band-pass filter optics, and a CCD camera. All measurements were carried out using a He−Ne laser (633 nm; laser power: 17 mW). The laser was focused onto the sample by using a ×50 objective lenses with (N.A. 0.75), and the diameter of the laser spot was 1 μm. Wavelength calibration was performed by measuring silicon wafers through a ×50 objective, assessing the first-order phonon band of Si at 520 cm^{-1}. Deionized water used to prepare aqueous solutions was produced by a Synergy UV water purification system (Millipore, USA). The zeta potential measurements were performed with a 90plus particle size analyzer (Brookhaven, USA). The bias potential applied on the fluidic Raman measurement was powered by a CHI 760E electrochemical working station (CH Instruments, China). The high voltage electric pulse used for gold nanopore drilling was released from a modified KT-2511 electric lighter (KOVEA, Korea), and a HDO6104A oscilloscope (Teledyne Lecroy Inc., USA) was used to characterize this electric pulse. Besides, an AM412 micromanipulator system (Rayme, China) was used to control the distance between two electrodes in the nanopore drilling process.

Fabrication of gold nanopore

The fabrication process of gold nanopore can be divided into three parts.

First, glass nanopipette with 200 nm inner diameter was fabricated by the P-2000 pipette puller system from borosilicate glass capillary. The parameters used are shown below:

Heat = 320, Filament = 3, Velocity = 30, Delay = 200

Heat = 310, Filament = 2, Velocity = 27, Delay = 160, Pull = 150

Then, gold layer with cap-like structure was synthesized at the tip of glass nanopipette by an in situ chemical reduction method. Briefly, the glass nanopipette backfilled with 100 mM Ascorbic acid solution was immersed into 0.25 mM Chloroauric acid solution with Au seeds (around 7 nm, which was synthesized according to a described method [1]), these two solutions met at the tip and formed gold nanoparticles, which gradually gathered and grew into gold cap. Typical reaction time was 1 hr, slight variation of reaction time (±10 min) showed no obvious influence on subsequent fabrication.

At last, a high voltage electric pulse was applied for drilling a single nanopore in the gold cap. To be specific, nanopipette backfilled with 0.5 mM KCl solution was dipped into a vessel containing the same electrolyte solution. As shown in Figure S2, two Tungsten wire electrodes connected with a modified electric lighter, to conduct electric current, were respectively inserted into the nanopipette and the external electrolyte. Micromanipulators were used to hold the nanopipette and the external Tungsten wire electrode to adjust them to align. Once after pulling the trigger of the modified electric lighter, a high voltage electric pulse with around 4.5 kV amplitude and 10 μs duration can be released to achieve the creation of nanopore.

In the following electrochemical characterization of created nanopore, the electrolyte solutions inside and outside the nanopipette were kept the same, 10 mM KCl. Two Ag/AgCl wires were used as electrodes and the bias voltage applied was defined as positive when the potential of the electrode inside the nanopipette was higher than the outside one.

Evaluation of Raman enhancement

To evaluate the Raman enhancement of resulting product, 4-Mbn was utilized as a Raman reporter. Briefly, nanopipette carrying gold nanopore was immersed into 10 mM solution of 4-Mbn in DMF for 3 h and then rinsed with DMF to remove unmodified reagents. After that, a confocal Raman microscope was used to characterize adsorbed 4-Mbn on nanopipette. For comparison, the Raman spectrum of 0.1 M 4-Mbn in DMF was also measured. All these Raman measurements were under 20 s integration time and 10% laser intensity. The acquired spectra are shown in Figure 2b and used to calculate the EF by the equation below:

$\mathrm{EF} = (\mathrm{I}_{SERS}/\mathrm{N}_{SERS})/\ (\mathrm{I}_{bulk}/\mathrm{N}_{bulk})$

Where $\mathrm{I}_{\mathrm{SERS}}$ is the intensity of the specific SERS band (herein, 2225 cm^{-1} Raman band, that correspond to cyano group of 4-MBN was selected for EF calculation) from the analyte adsorbed on a SERS active substrate, and N_{SERS} is the number of molecules contributing to I_{SERS}. Similarly, I_{bulk} is the intensity of the same Raman band from the bulk analyte, normalized with the laser power and acquisition time, and N_{bulk} is the number of molecules that yield I_{bulk}.

In SERS measurement, the I_{SERS} is around 1.15×10^4. Considering the outer diameter of glass nanopipette is 300 nm, the gold structure formed at the tip of nanopipette has an area of around 7.07×10^4 nm^2. Taking 4.5×10^{14} molecules/cm^2 for a monolayer of 4-MBN on Au [2], the number of N_{SERS} is approximately 3.18×10^5.

In bulk Raman measurement, the number of I_{bulk} is around 1.92×10^2. Given the fact that the diameter and the depth of the laser beam is 1 μm and 21 μm respectively, the effective excitation volume is about 16.49 μm^3. As the concentration of the 4-MBN solution is already known, the N_{bulk} would be around 9.93×10^8.

Based on the calculated parameters, the EF value is around 1.87×10^5.

Fluidic SERS measurement

To ensure that the gold nanopore can be totally shrouded under the laser beam in fluidic SERS measurement, a homemade cylindrical device as shown in Figure S5 was used to hold glass nanopipette vertically.

To investigate whether the electric driving force is sufficient to meet the actuation demand, an experiment around R6G was firstly carried out. The electrolyte solutions inside and outside of the nanopipette were kept the same, 10 mM KCl. In addition to that, the outside solution also contained 10^{-13} M R6G. A -0.5 V driving voltage was applied within this system by two Ag/AgCl wires to actuate R6G molecules translocating through nanopore. Under the same conditions (20 s integration time and 50% laser intensity), Raman spectra with driving voltage on and off were measured synchronously to verify the influence of electric driving force. For comparison, 10^{-3} M R6G was also measured under the same conditions, the acquired Raman spectrum was shown in Figure S6.

In the further fluidic SERS measurement based on PS nanosphere, the mass fraction of PS nanosphere outside of the nanopipette was set to 0.5%. Some changes were made to facilitate PS nanosphere translocating through nanopore, for example, the concentration of electrolyte solution was raised to 100 mM and the driving voltage was raised to -1 V. Multiple Raman spectra measured at 0.03 s integration time were continuously acquired at a 0.3 s time interval to achieve direct and real-time single particle analysis. The same measurement without applying bias potential was also carried out for comparison, the acquired featureless waterfall plot was shown in Figure S7.

References：

1. J. S. DuChene, W. Niu, J. M. Abendroth, Q. Sun, W. Zhao, F. Huo and W. D. Wei, Chemistry of Materials, 2012, 25, 1392-1399.

2. E. C. Le Ru, E. Blackie, M. Meyer and P. G. Etchegoin, J. Phy. Chem. C, 2007, 111, 13794-13803.

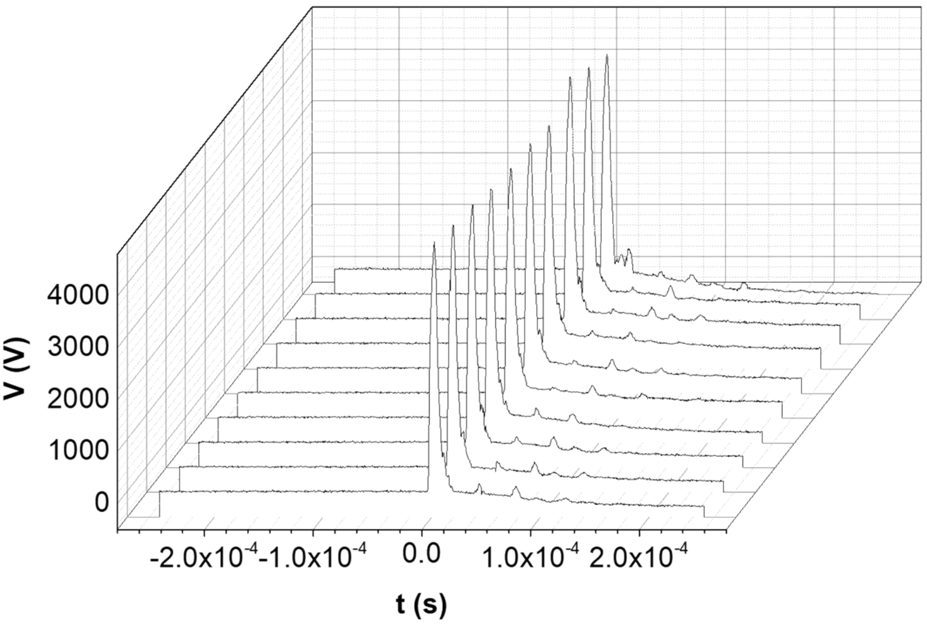

Figure S1. Characterization of the high voltage electric pulses used for nanopore drilling. At this measurement (repeated 10 times), electric pulses have an average amplitude of 4.5 kV and an average duration of 10 μs.

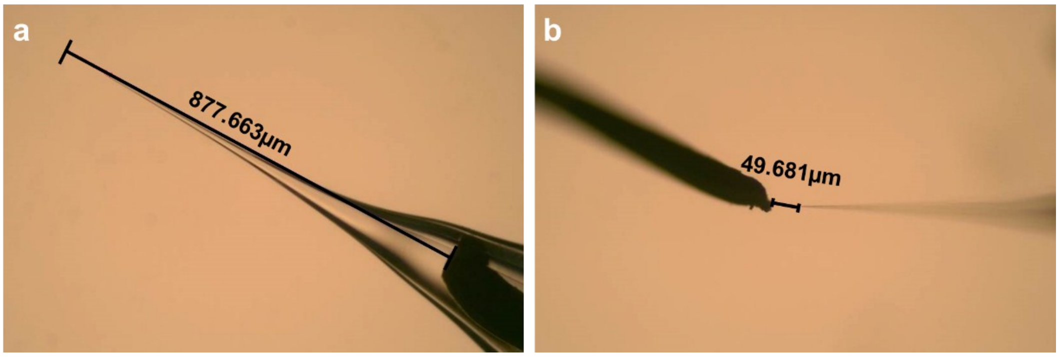

Figure S2. Optical images of nanopipette prepare for electric pulse treatment. (a) The distance between the orifice to the internal Tungsten wire is around 1 mm. (b) The distance between the orifice to the external Tungsten wire is around 50 μm. It is clear that the solution between the orifice and inside tungsten wire shown in figure a determines the overall resistance. However the distance between the orifice and the inside tungsten is hard to adjust. We have to push the inside tungsten wire close to the orifice as possible in every nanopore drilling experiment. Because the neck length of lass nanopipettes are very close, the overall resistance is solely determined by the salt concentration.

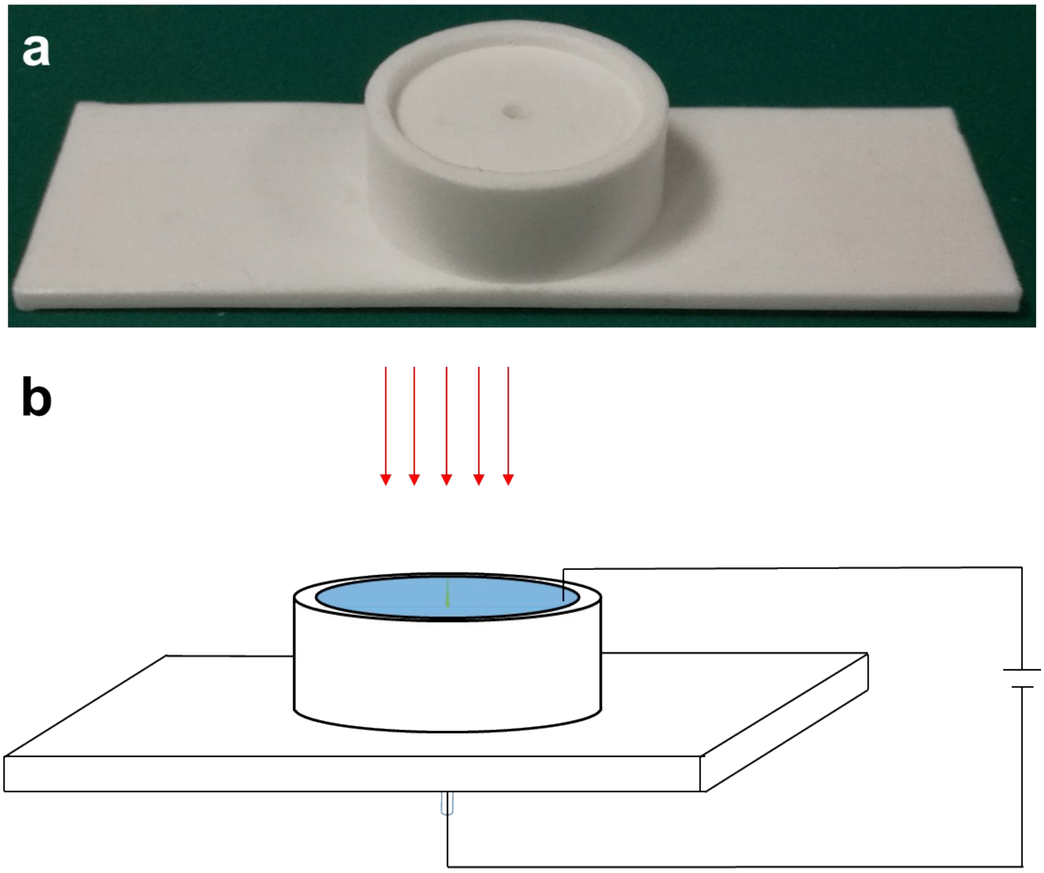

Figure S3. (a) Optical image of the homemade cylindrical device used to hold glass nanopipette during fluidic SERS measurement. (b) Schematic diagram for the role of this device in fluidic SERS measurement.

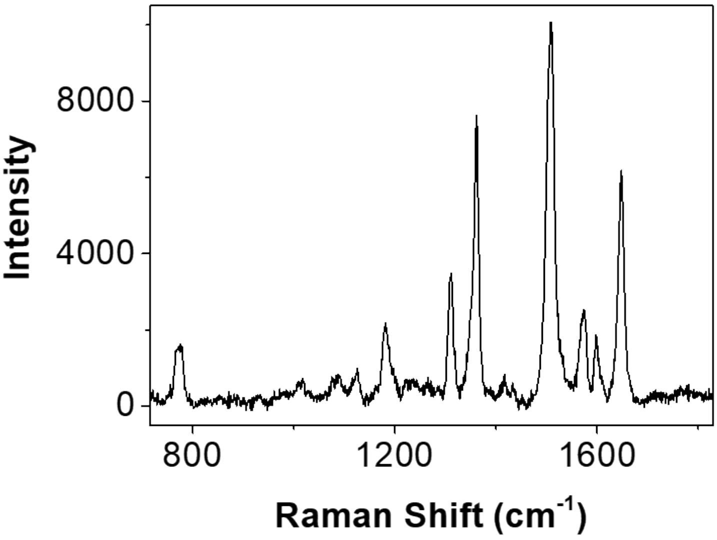

Figure S4. The SERS spectra of 10^{-9} M R6Gin 10 mM KCl with the bias potential of -0.5 V. Integration time: 20 s, laser intensity: 50%.

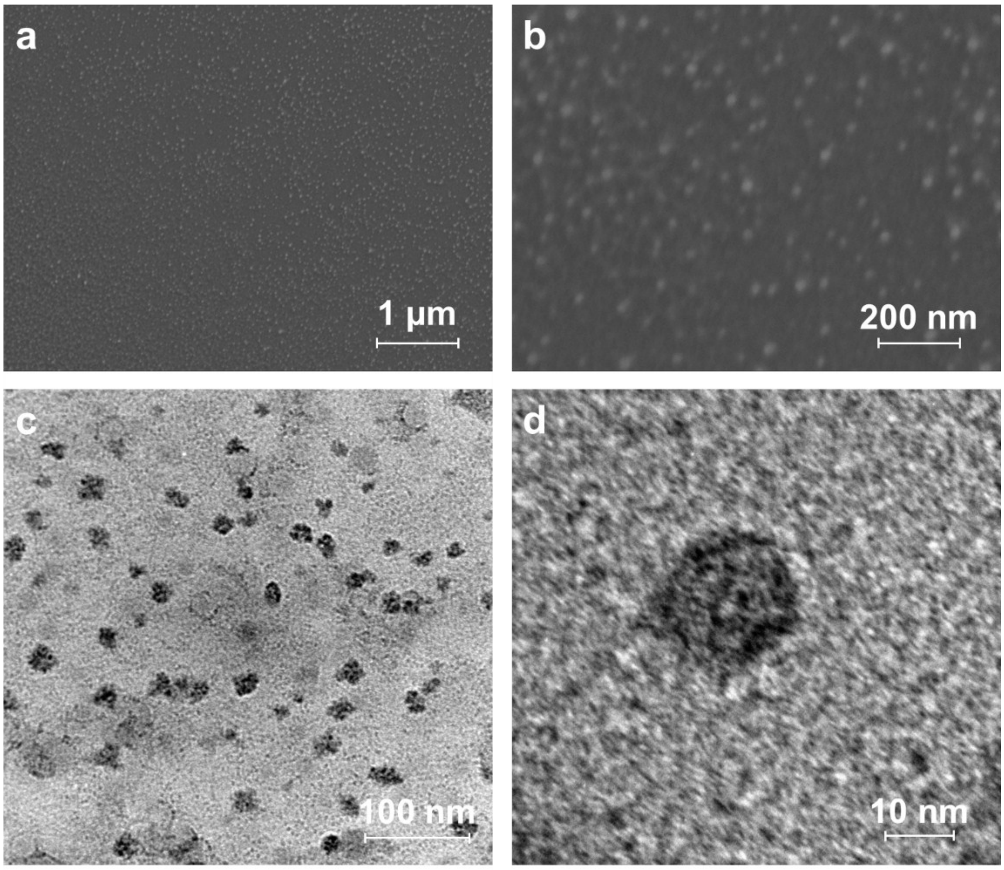

Figure S5. SEM (a, b) and TEM (c, d) characterization of purchased PS nanosphere at different magnifications, (a) 1 μm scale bar, (b) 200 nm scale bar, (c)100 nm scale bar, (d) 10 nm scale bar.

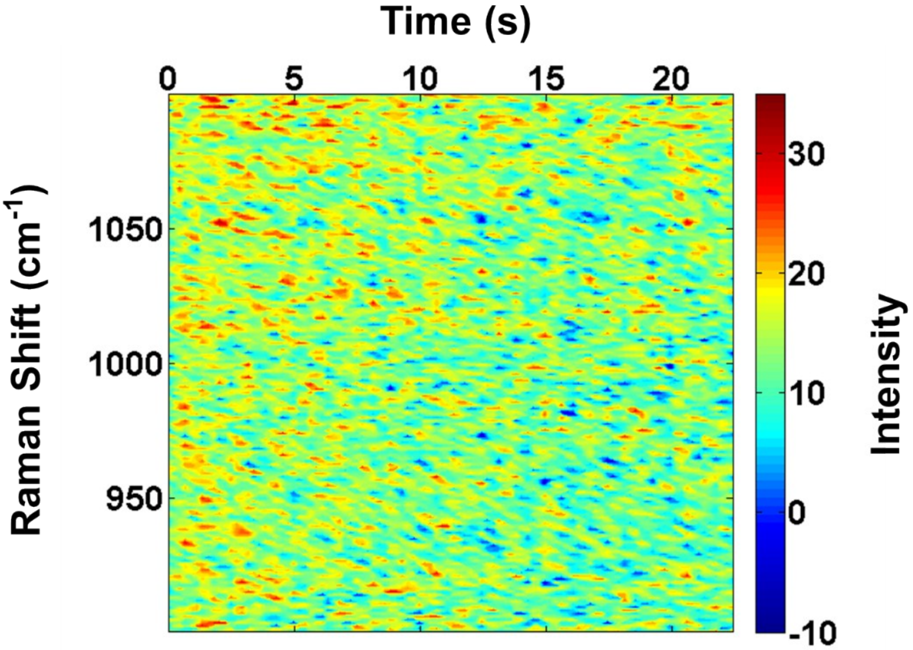

Figure S6. The waterfall plot of continuously acquired SERS spectra of 0.5% PS in 100mM KCl without applying bias potential. The spectra were measured under 30m s integration time and 50% laser intensity, the time interval between each Raman measurement was 0.3s.

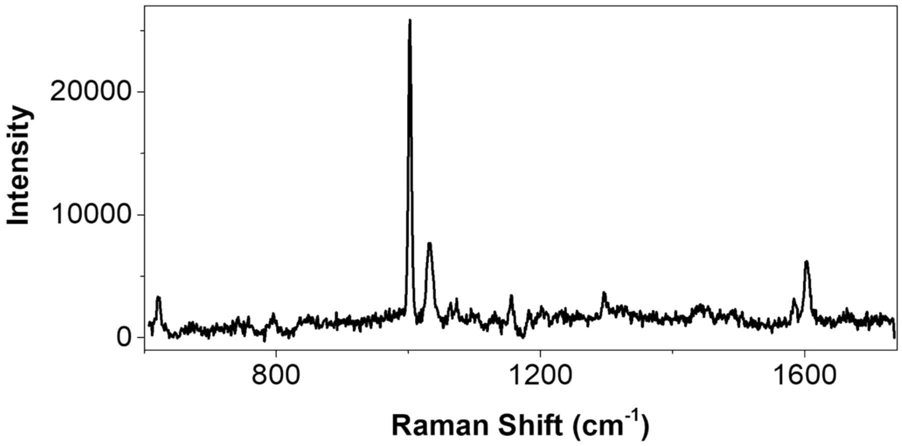

Figure S7. Raman spectrum of purchased PS nanosphere, this spectrum was measured under 20 s integration time and 10% laser intensity.

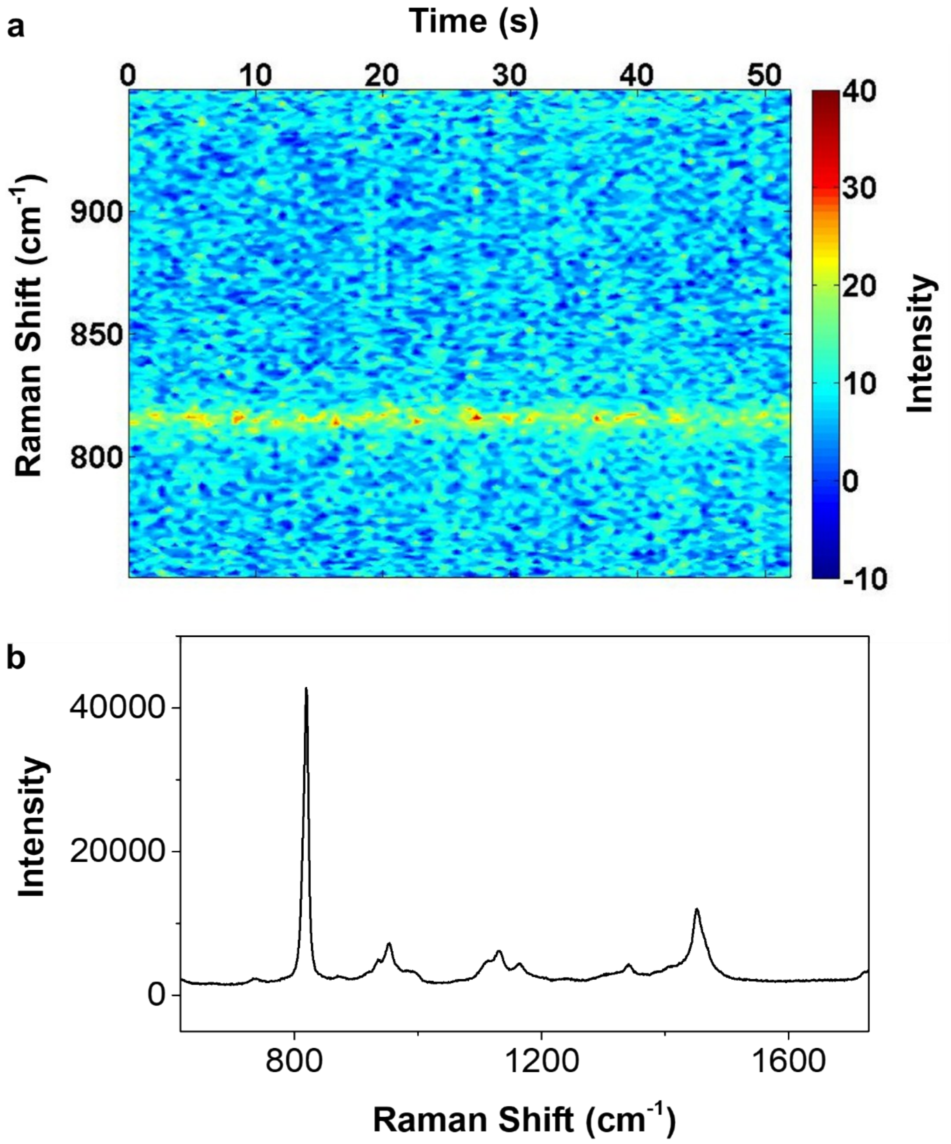

Figure S8. (a) The waterfall plot of continuously acquired SERS spectra of 0.5% PMMA in 100mM KCl, the bias potential was -1 V, the spectra were measured under 0.2s integration time and 50% laser intensity, the time interval between each Raman measurement was 0.3s. (b) Raman spectrum of purchased PMMA nanosphere, this spectrum was measured under 20 s integration time and 10% laser intensity.

Table S1. Zeta potential of purchased PS nanosphere in different salt solutions.

Concentration of KCl/Mm	**Zeta potential of PS nanosphere/mV**
0	-44.62
0.1	-39.19
1	-28.42
10	-15.48
100	-11.90