Supplementary information for

Facilely synthesized polydopamine encapsulated surface-enhanced Raman scattering (SERS) probes for multiplex tumor associated cell surface antigen detection using SERS imaging

Experimental section

Cell culture and chemical reagents

Three prostate cancer cell lines LnCAP, PC-3, and DU145 were acquired from FuDan IBS Cell Center and cultured under recommended conditions. Antibody Rabbit Anti-Human VEGF and Mouse Anti-Human Vimentin were ordered from PeproTech (Rocky Hill, U.S.A.) and Alpha Diagnostic International (San Antonio, U.S.A.) respectively. Antibody Mouse Anti-Human EpCAM-CD326 and Mouse Anti-Human EGFR were ordered from Biolegend (San Diego, U.S.A.). Dopamine hydrochloride, bovine serum albumin (BSA), *p*-mercaptobenzoic acid [*p*MBA], and *p* -nitrothiophenol [*p*NTP] were obtained from Sigma-Aldrich Inc. (St. Louis, MO, U.S.A.) *p*-aminothiophenol [*p*ATP], Tetrachloroaurate (HAuCl4•3H2O), sodium citrate and other chemical reagents were purchased from Sinopharm Chemical Reagents Co., Ltd (Shanghai, China).

Synthesis of polydopamine encapsulated SERS probes

60 nm Au nanoparticles (Au NPs) were synthesized according to previous reports [19]

and used as the substrate of surface enhanced Raman scattering in this study. Briefly, 100 mL $HAuCl_4$ (0.1 mg/mL) was reduced by 0.75 mL sodium citrate (10 mg/mL) under vigorous stirring and boiling condition. Au NPs in 1 mL of Au colloid were collected by centrifugation and re-dispersed in 1 mL of boric acid buffer (2 mM). Three thiophenol derivatives were dissolved in ethanol with desired concentration (*p*MBA 1mM, *p*ATP 0.05mM and *p*NTP 1mM). To 1 mL of Au NPs solution, 10 μL of Raman reporter solution were added and resultant mixtures were allowed to shake at 37 °C for 20 min to get SERS active Au NPs. After shaking, the mixtures were rinsed by centrifugation at 8000 rpm for 5 min using water for one time and the precipitates were re-dispersed in 1 mL of 10 mM Tris-HCl buffer (pH 8.5). 20μL of 1 mg/mL dopamine hydrochloride solution were added to each of these samples, followed by 1 hour incubation at 37 °C under shaking. Polydopamine encapsulated Au NPs were rinsed by water and resuspended in 100 μL of deionized water. Antibodies were immobilized on to the surface of polydopamine encapsulated Au NPs after incubating with 20 μL of antibody solution at 4°C for 12h. Then, 10 μL of 10 mg/mL BSA was added and incubated at 37 °C for 1 hour to block unreacted catechol groups.

SERS measurement

Three prostate cancer cell lines (LnCAP, PC-3 and DU145) were seed in glass cover sides individually and cultured overnight in complete growth medium for attachment. After cell culture, cancer cells were washed three times using PBS buffer and then

supplemented with 500 μL fresh PBS buffer. 300 μL of three SERS probes mixture (100μL for each probes) were added and incubated at 37 °C for 1h with gentle shake. Post incubation, nonspecific bounded SERS probes were discarded by three time washes. Then, cancer cells were fixed using 2% formaldehyde for 20min. After washes with PBS and DI water, SERS probe labeled cancer cells were supplemented with 100 μL of fresh PBS buffer and store at 4 °C before SERS imaging.

SERS imaging measurement were performed using a Renihaw Invia Raman microscope system assembled with a Diode Laser (λ = 785 nm) with a power of 3 mW. A combined spectral resolution of 2 cm^{-1} was achieved by coupling a charge-coupled device (CCD) to a spectrograph. Using a Raman point-mapping method, Raman images of individual cell were obtained with the help of a computer-controlled x-y translation stage in a 2 μm step size. The Raman images of each pixel were decoded using characteristic Raman peak intensity of three SERS probes using WiRE software V 1.3 (Renihaw, U.K.).

2. Supplemental experiment results

Optimization of dopamine concentration.

SERS probe with strong and characteristic Raman signal is very favorable for SERS imaging since it can not only increase the sensitivity but also shorten the acquisition time. The Raman intensity of synthesized SERS probes along the concentration of dopamine used for polydopamine encapsulation is first investigated. *p*MBA modified

Au NPs were selected as model SERS nano tags. Dopamine hydrochloride was added into the SERS active nano tags suspension at the concentration ranging from 0.01mg/mL to 1 mg/mL and incubated at 37 °C for 1 hour. After rinsing by water, Raman spectra of synthesized probes were measured in the same condition. As shown in Fig. S1 (A), the intensity of the characteristic Raman peaks of *p*MBA (1080 cm^{-1}) decrease gently with the increase of dopamine concentration.

The reason of this phenomenon was further investigated. As shown in Fig. S2(A), with the increase of dopamine concentration from 0.02 mg/mL to 1 mg/mL, the thickness of polydopamine increase from 5 nm to 70 nm. The increase of the thickness of polydopamine shell may be one reason that cause the decrease of Raman intensity. What's more, when the dopamine concentration increase, increased self-polymerization dopamine particles were formed. These self-polymerization dopamine particles may absorb most of excitation light (Fig. S2(B)). As a result, the excitation light intensity applied on SERS probes decrease, which leads to the decrease of Raman intensity. As shown in Fig. S2(C), when self-polymerization dopamine particles were added into SERS active nano tags, Raman intensity of SERS active nano tags decrease dramatically. Hence, we speculate that the decrease of Raman intensity under high dopamine concentration may be generated by two reasons. First, the increase of the thickness of polydopamine shell may weaken the Raman signal of SERS active nano tags. Second, the increase of self-polymerization polydopamine particles may absorb most of excitation light, which leads to the decrease of Raman intensity. As a compromise of Raman intensity and encapsulation efficiency, 0.02

mg/mL was choose as the optimal concentration of dopamine.

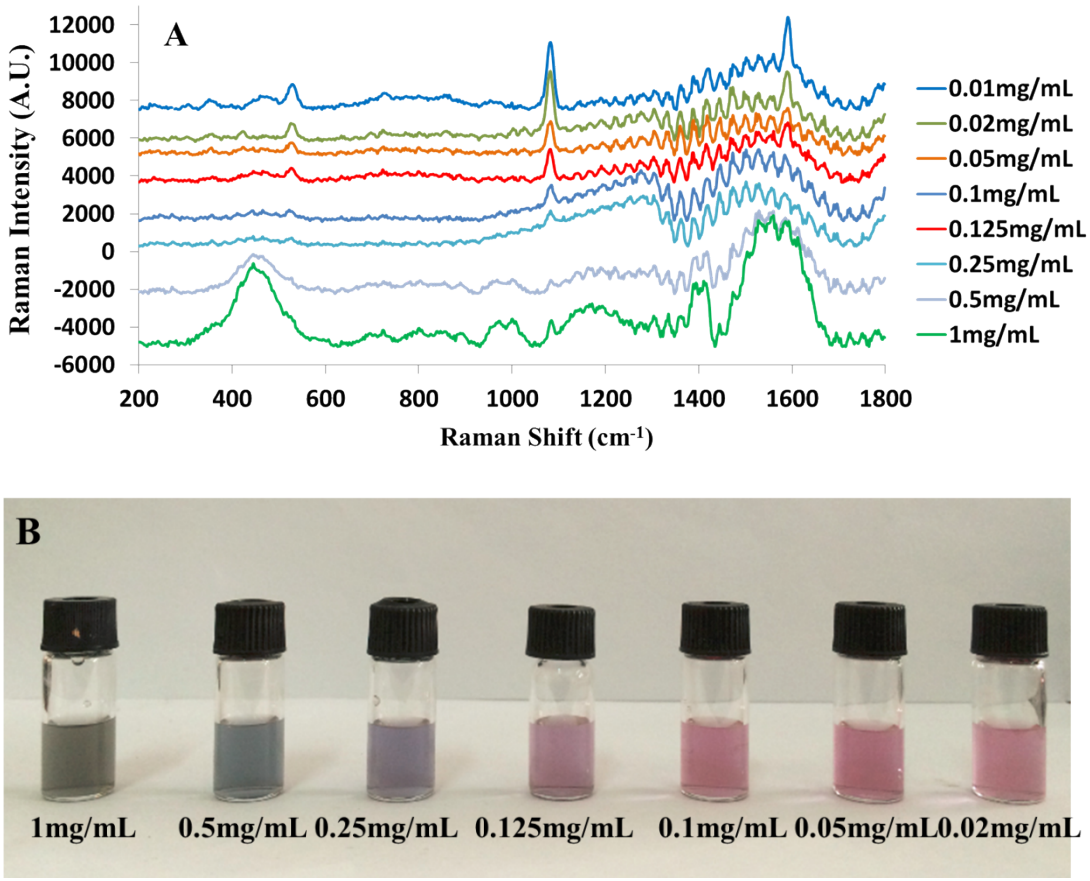

Fig. S1 (A) SERS spectrum of polydopamine coated SERS nano tags synthesized under different dopamine concentration. **(B)** Photo of polydopamine coated SERS nano tags synthesized under different dopamine concentration.

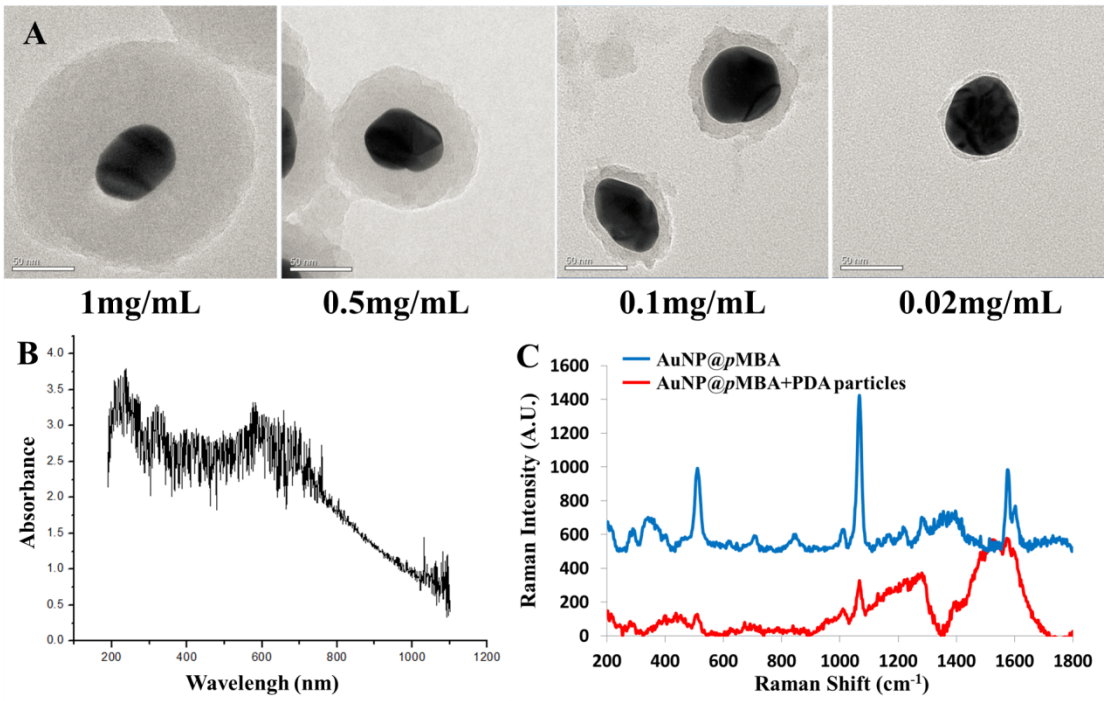

Fig. S2 (A) TEM images of polydopamine encapsulated SERS probes under different concentrations of dopamine. **(B)** UV/Vis spectra of self-polymerization dopamine particles generated by the self-polymerization of 1 mg/mL dopamine in 10 mM Tris-HCl buffer (pH 8.5). **(C)** Raman spectra of SERS active nano tags (Au NPs@*p*MBA) before and after self-polymerization dopamine particles addition.

Stability of polydopamine encapsulated SERS probe.

SERS probes without protection layers tend to aggregated randomly under high saline concentration, leading to enhanced but non-reproducible Raman signal. Hence, the stability of SERS probes in saline solution is crucial for SERS imaging applications. Therefore, the stability of our polydopamine encapsulated SERS probes under different salt conditions was also investigated. After the encapsulation of polydopamine under optimized condition, synthesized polydopamine encapsulated SERS probes were re-dispersed in various concentrations of saline solution (10 mM –

40 mM NaCl). As is shown in Fig. S3, with the increased concentration of sodium chloride, bare nano tags without polydopamine encapsulation immediately change from red to colorless dark, an indication of nanoparticle aggregation. In contrast, the color of polydopamine encapsulated nano tags barely changed even at high saline concentration, indicating an enhanced stability of nano tags after the encapsulation of polydopamine. This enhanced stability of polydopamine encapsulated nano tags was further verified by UV/Vis spectral, in which the polydopamine encapsulated nano tags remains unchanged even in 40 mM salt solution (Fig. S3).

The time-dependent stability of our polydopamine encapsulated SERS probes, which is crucial for cell incubation, was also investigated. 1 mL of polydopamine encapsulated SERS probes was re-dispersed in water and PBS buffer respectively. These SERS probes were stored at room temperature and their stability was investigated as described above. As is shown in Fig. S4, our polydopamine encapsulated SERS probes were stable after 2 hours storage both in water and PBS buffers. These results confirmed that our polydopamine encapsulated SERS probes have very good time-dependent stability.

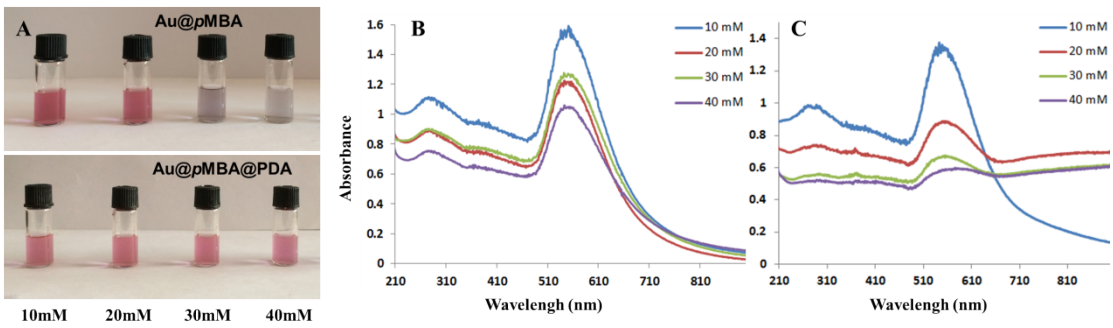

Fig. S3. (A) Color changes of Au colloid with and without the encapsulation of polydopamine in various salt concentration. (B) UV/Vis spectra of polydopamine

encapsulated nano tags in various salt concentration. (C) UV/Vis spectra of bare nano tags without polydopamine encapsulation in various salt concentration.

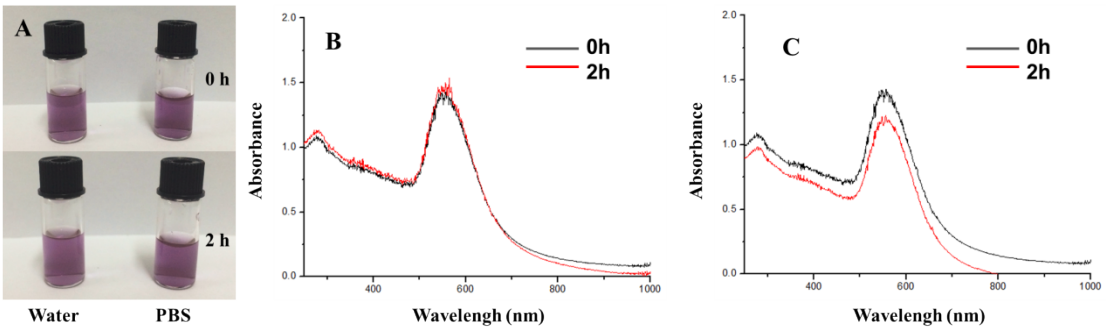

Fig. S4. (A) Photo of polydopamine encapsulated SERS probes before and after 2 hours store. (B) UV/Vis spectra of polydopamine encapsulated SERS probes before and after 2 hours store in water. (C) UV/Vis spectra of polydopamine encapsulated SERS probes before and after 2 hours store in PBS buffers.

Synthesize of EpCAM specific SERS probes.

To render SERS probes with specificity to EpCAM, polydopamine encapsulated SERS active nano tags were modified by monoclonal Anti-EpCAM antibody. As is shown in Scheme 1A, antibody can be immobilized onto polydopamine film facilely using the Michael addition or Schiff base reactions between the catechol groups of polydopamine film and the amino group of antibody. Then, unreacted catechol groups of polydopamine were blocked by BSA to enhance the specificity of synthesized SERS probes. The successful immobilization of antibodies was verified by SDS-PAGE using sliver staining and UV/Vis analysis (Fig. S5).

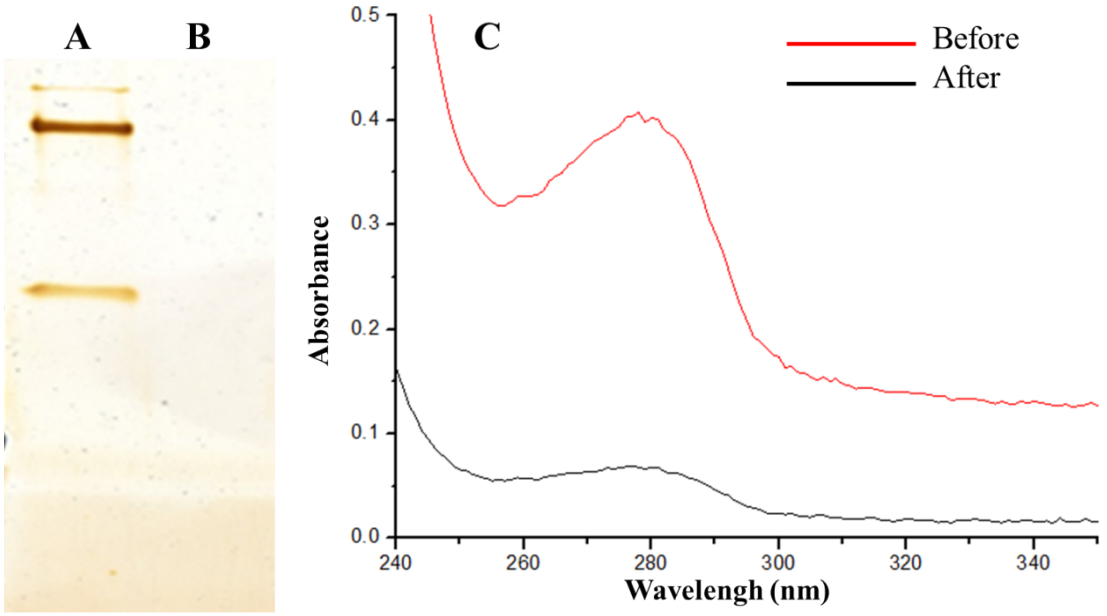

Fig. S5. SDS-PAGE analysis of antibody solution before (A) and after (B) reacting with polydopamine encapsulated SERS nano tags**.** UV/Vis analysis of antibody solution before and after reacting with polydopamine encapsulated SERS nano tags (C)**.**

Regression analysis of the SERS intensity of SERS probes versus the SERS probes concentration.

To demonstrate that the stability of synthesized SERS probe is not disturbed in high particle concentration, regression analysis of the Raman intensity of SERS probes versus the SERS probes concentration was conducted. Briefly, synthesized SERS probes were diluted into a series of concentration by water and PBS, respectively. Then, Raman spectra of these SERS probe solution were measured under the same condition. Regression analysis of the intensity of the characteristic Raman peaks of *p*MBA (1080 cm^{-1}) versus the SERS probes concentration was conducted and displayed in Fig. S6.

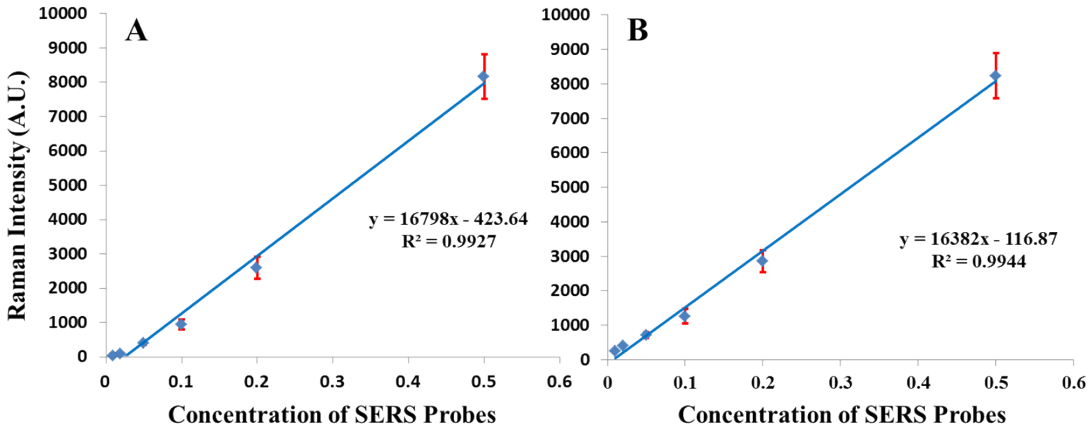

Fig. S6. Regression analysis of the SERS intensity of SERS probes (at 1080 cm^{-1}) versus the SERS probes concentration in water (A) and PBS (B).

Specificity of EpCAM specific SERS probe.

To investigate the specificity of our SERS probes, DU145 cells (high EpCAM expression) and U251 cells (negative for EpCAM) were incubated with our SERS probes and measured by Raman microscope, respectively. As shown in Fig. S7, characteristic Raman peaks of *p*MBA were detected in all DU145 cells after incubation, indicating the successful label of SERS probes. In contrast, negligible Raman intensity was detected for U251 cells due to the low expression of EpCAM. Raman images of random selected DU145 and U251 cells were obtained using a Raman point-mapping method. Bright-field image and SERS image in the same microscope field were displayed in Fig. S8. As shown in Fig. S8, every DU145 cell was labeled by SERS probes uniformly and can be form clear SERS image using the characteristic Raman peaks of SERS probes by SERS imaging techniques. As expect, all the U251 cells exhibit negligible Raman signals and can't produce clear SERS

images as shown in Fig. S8D. Negative control SERS probes (Au@pMBA@PDA) was also synthesized and used to label DU145 cell. In contrast to DU145 cells incubated with Au@*p*MBA@PDA@Anti-EpCAM, none of these DU145 cells exhibit distinct Raman signals after 1 hour incubation (Fig.S7). This result confirmed that the interaction between our SERS probes and tumor cells was derived from the specific interaction between antibodies and cell surface antigens. It also confirmed that antibodies were immobilized on the surface of SERS probes successfully.

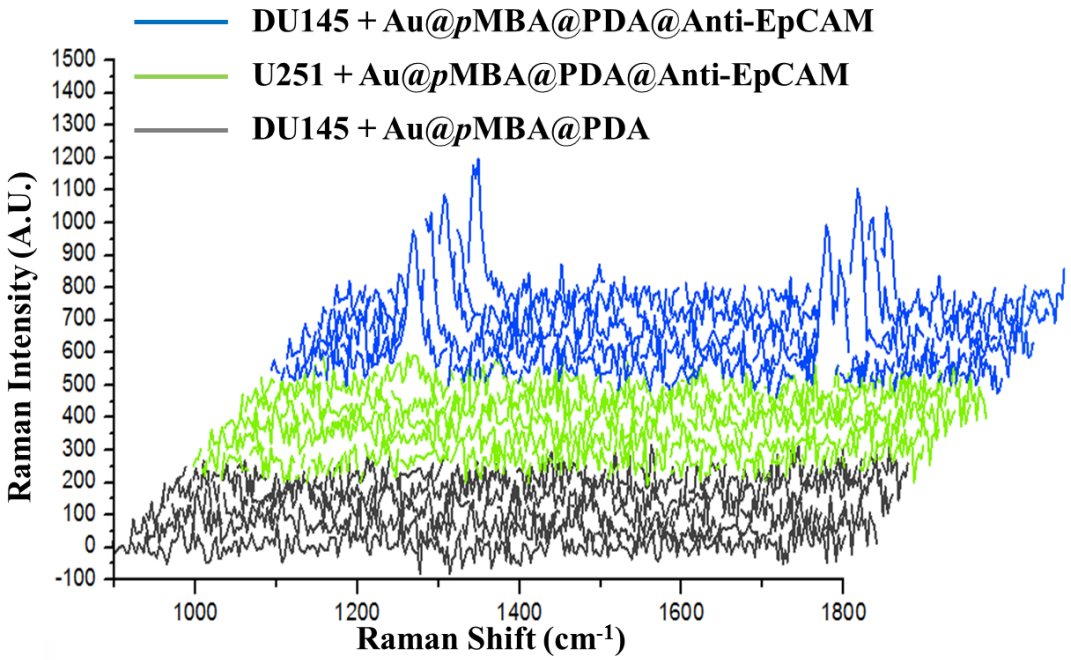

Fig. S7. SERS spectra of DU145 cells incubated with antibody modified SERS probes (blue), U251 cells incubated with antibody modified SERS probes (green) and DU145 cells incubated with negative control SERS probes (gray).

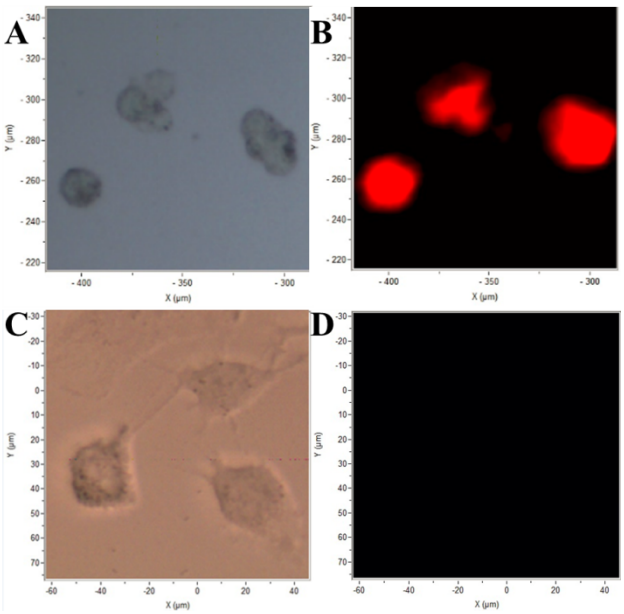

Fig. S8. Bright field image and SERS image of DU145 cells (A and B) and U251 cells (C and D) after the incubation with SERS probes in the same microscope field.

Synthesize of three kinds of SERS probes.

To evaluated the expression of three kinds tumor associated antigens in prostate cancer cell lines by SERS imaging, three kinds of SERS probes, each of which exhibit distinct characteristic Raman peaks, were synthesized by the absorption of three thiophenol derivatives (*p*MBA, *p*ATP, *p*NTP). After the encapsulation of polydopamine, three kinds of antibodies were immobilized as described above to render SERS probes with specificity to corresponding antigens. SERS spectra of three synthesized SERS probes are displayed in Fig. S9, from which we can designate at least one Raman peak as the characteristic peak for each SERS probe (533 cm^{-1} for Au@*p*MBA@PDA@Anti-VEGF, 401 cm^{-1} for Au@*p*ATP@PDA@Anti-EGFR, 865 cm^{-1} for Au@*p*NTP@PDA@Anti-Vimentin). Since the total Raman spectral of SERS

probes is not necessary for SERS imaging, these characteristic Raman peaks are sufficient to distinguish individual SERS probes. SERS spectra of a series of tertiary mixture with different SERS probe ratios were also measured and displayed in Fig. S10.

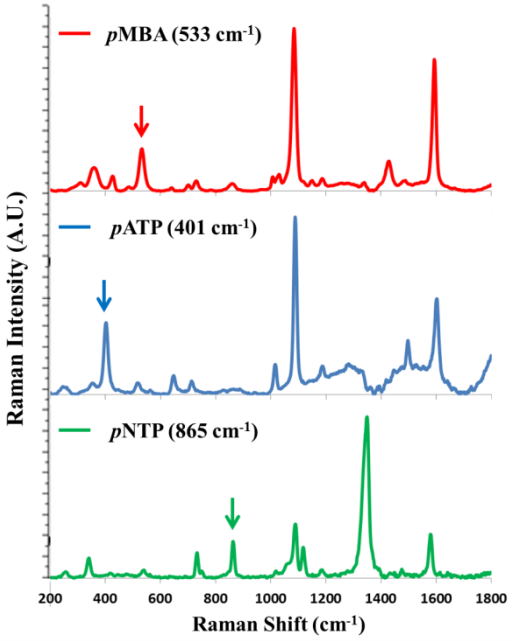

Fig. S9. Raman spectra of *p*MBA, pATP and pNTP modified SERS nano tags.

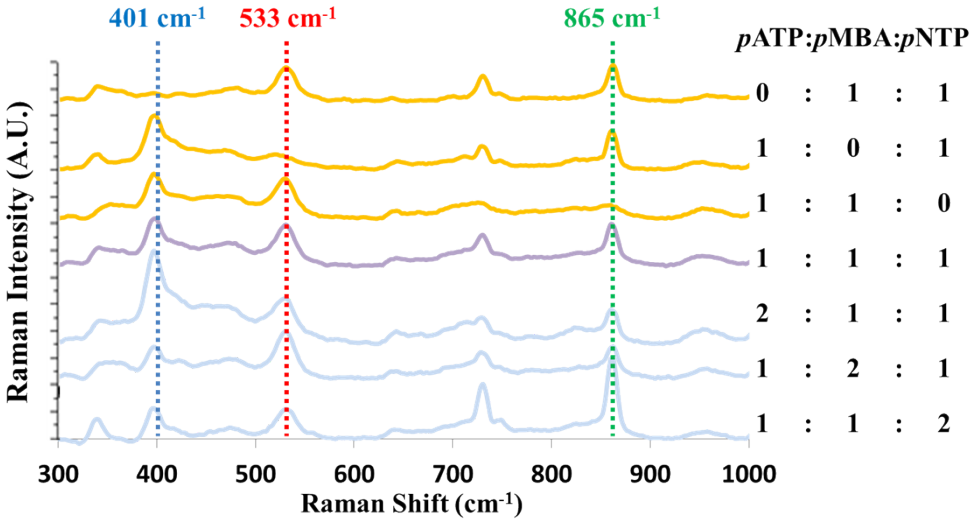

Fig. S10. SERS spectra of a series of tertiary mixture with different SERS probe ratios.