Electronic Supplementary Material (ESI) for Analyst

Supporting Information

SERS performance of gold@silver core-shell nanorods (Au@Ag NRs) and gold nanorods (GNRs)

Sensitivity is one of the most important criteria for a well-performed immunoassay. In order to improve the sensitivity of SERS-based immunoassay, it is quite critical to choose an appropriate metal nanoparticle with a high SERS activity. In our experiment, we fabricate SERS probes using Au@Ag NRs, which shows an excellent enhancement to SERS signals. At the same time, GNRs were used as a control.

To demonstrate the higher SERS activity of Au@Ag NRs than GNRs, the same amount of Raman reporters should be added to an equal volume of these nanorods solutions with the same concentration. As mentioned in the experimental section, we got 2 mL of Au@Ag NRs solution from 2 mL of the prepared GNRs solution. That is to say, these two nanorods solutions were equal in volume. Meanwhile, it can be seen from Fig.1b that most of the gold nanorods were coated with silver shell. On the basis of these facts, it is reasonable that we assume the concentration of GNRs solution was approximately equivalent to that of Au@Ag NRs solution. In the experiments, 10 μL of 10 mM 4MBA was mixed with 1 mL of nanorods solution to react overnight, followed by purification and the

SERS measurements. It is clearly reflected in Fig.S3a that the SERS intensity of silver coated nanorods is much stronger than that of uncoated gold nanorods, suggesting that a silver shell indeed improve the SERS performance, which might be attributed to the higher extinction coefficient of silver than that of gold or the electronic ligand effect in bimetallic nanoparticles[1]. Moreover, by replacing 4MBA with DTNB, similar results were observed in Fig.S3b. Therefore, Au@Ag NRs with a higher SERS activity are expected to increase the sensitivity of the immunoassay.

SERS comparison before and after antibody conjugation

Fig.S4 shows the SERS spectra of our immuno-probe before (dark blue curve) and after (light blue curve) antibody conjugation. It can be calculated from Fig.S4a that peak intensity at 1585 cm^{-1} only decreased by 13% during the antibody conjugation process, which demonstrates that most of the 4MBA molecules remained on the nanorods surface. Similarly, peak intensity at 1333 cm^{-1} decreased by 10% after antibody conjugation (Fig.S4b), confirming that only a small portion of DTNB molecules were removed from the nanorods.

Fig.S1 EDX spectrum of Au@Ag NRs

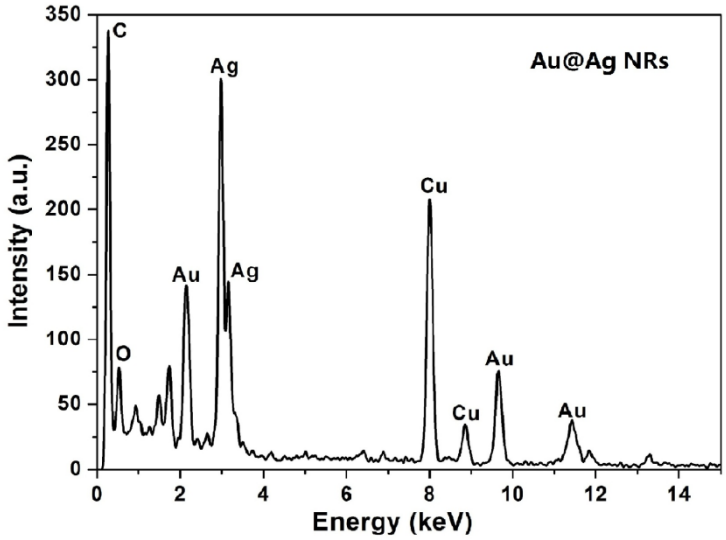


Fig.S2 Chemical structure of 4MBA and DTNB.

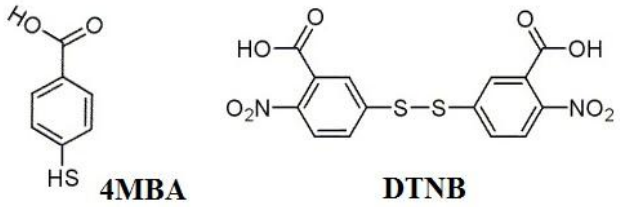


Fig.S3 SERS spectra of **(a)** 4MBA-labeled GNRs and 4MBA-labeled Au@Ag NRs; **(b)** DTNB-labeled GNRs and DTNB-labeled Au@Ag NRs.

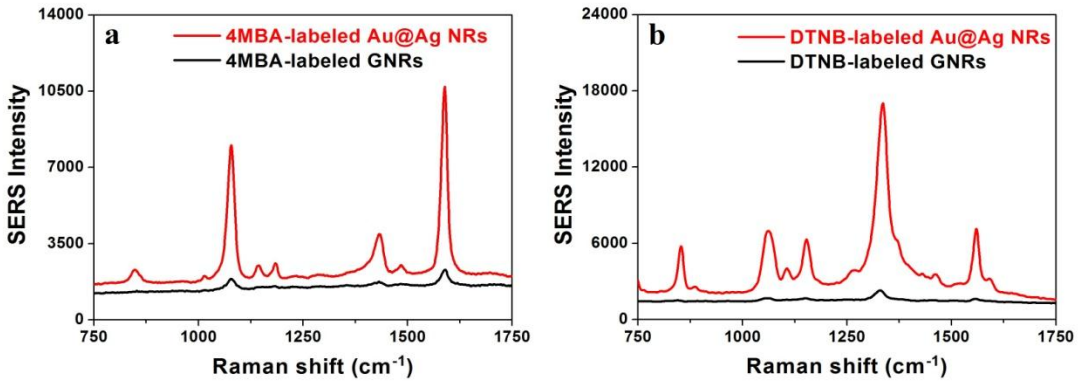


Fig.S4 SERS spectra of the functionalized nanorods before and after antibody conjugation. **(a)** 4MBA-labeled Au@Ag NRs; **(b)** DTNB-labeled Au@Ag NRs.

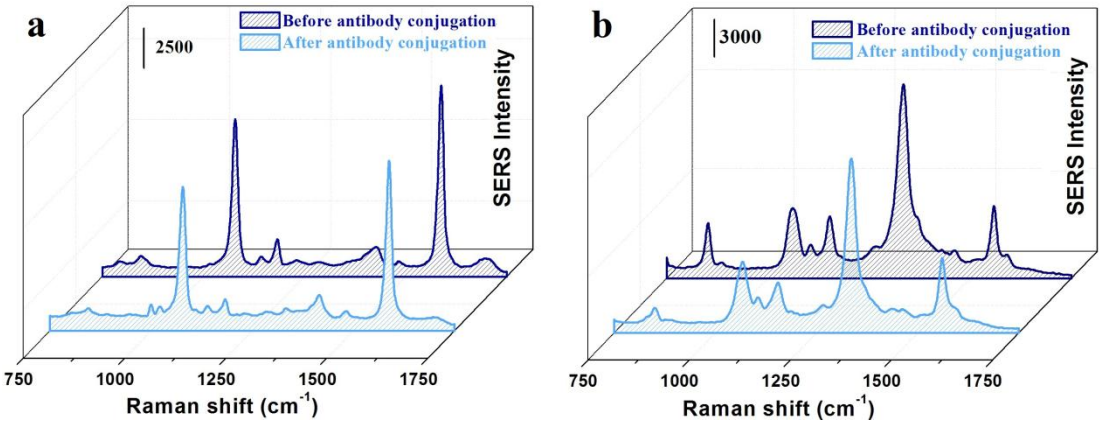


Fig.S5 (a) SERS spectra of Probe A exposed p53 and other nonspecific biomolecules including bovine serum albumin (BSA), lysine, human IgG, rabbit IgG and bovine IgG; **(b)** SERS spectra of Probe B exposed p21 and other nonspecific biomolecules including bovine serum albumin (BSA), lysine, human IgG, rabbit IgG and bovine IgG.

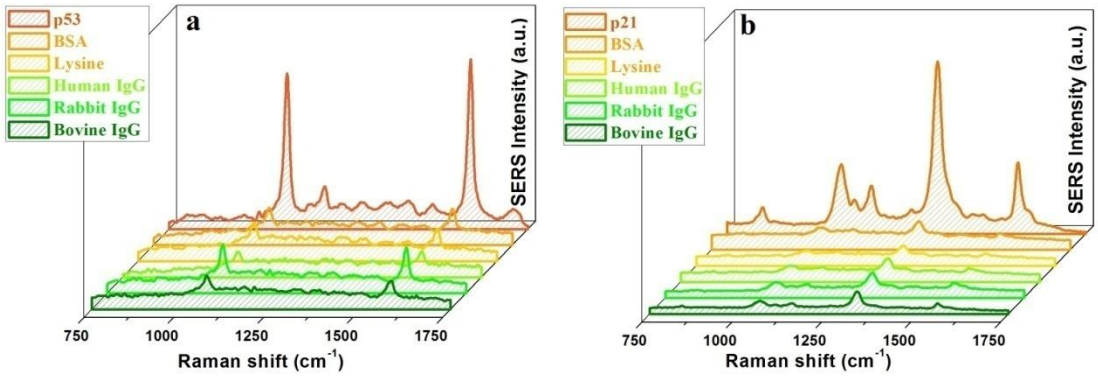


Fig.S6

(a) Concentration-dependent SERS spectra for p53 detection using GNRs probes. The concentrations of p53 ranges from 5 μg/mL to 500 fg/mL and the blank spectrum was obtained by replacing p53 with PBS;

(b) Plot of peak intensity at 1585 cm^{-1} as a function of p53 concentration. The linear calibration equation is y = 217.9.2 log (c) + 2673.5 (r = -0.9712, n = 3, y represents peak intensity at 1585 cm^{-1}, c stands for the concentration of p53) and each error bar indicates the standard deviation of five different readings The dashed pink line represents peak intensity of the control group.

(c) Concentration-dependent SERS spectra for p21 detection using GNRs probes. The concentrations of p21 ranges from 5 μg/mL to 500 fg/mL and the blank spectrum was obtained by replacing p21 with PBS;

(d) Plot of peak intensity at 1333 cm^{-1} as a function of p21 concentration. The linear calibration equation is y = 182.6 log (c) + 2389.5 (r = -0.9604, n = 4, y represents peak intensity at 1585 cm^{-1}, c stands for the concentration of p21) and each error bar indicates the standard deviation of five different readings. The dashed pink line represents peak intensity of the control group.

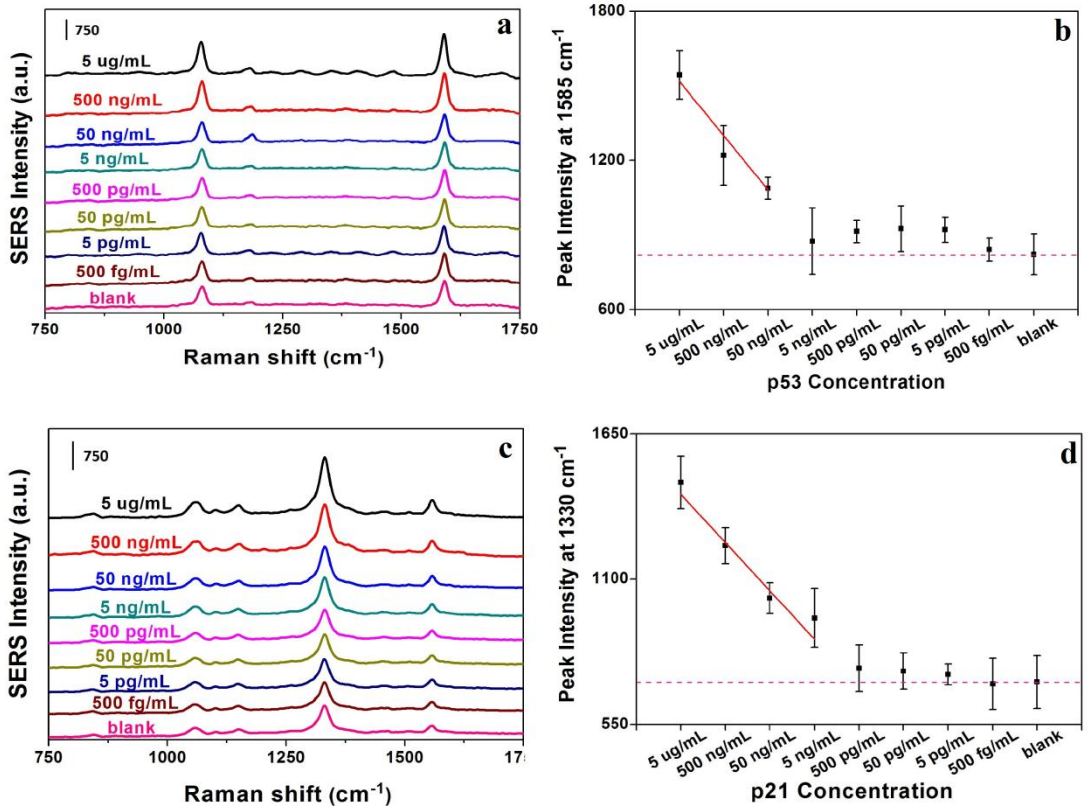
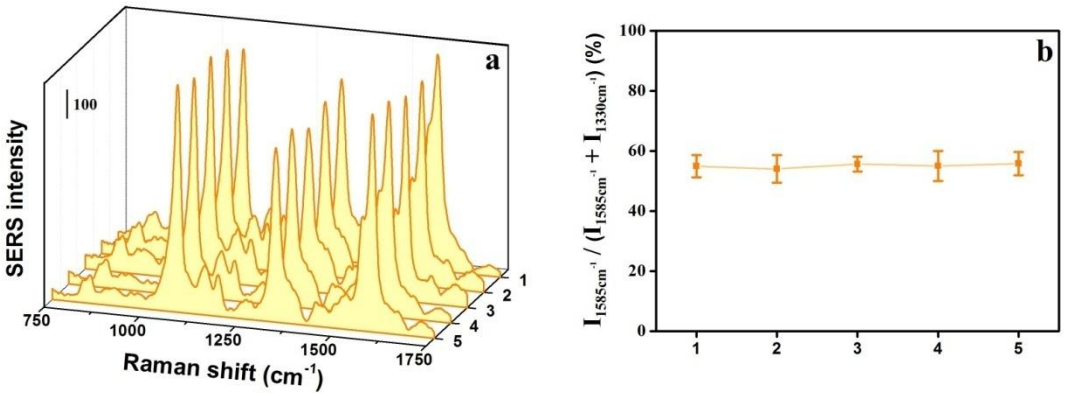


Fig. S7 (a) SERS spectra for simultaneous detection of p53 and p21 with a concentration ratio of 1:1 (the exact concentration of p53 and p21 are both 10^{-10} g/mL). The five spectra represent the results of five independently performed assays and each spectrum was obtained by averaging 5 individual measurements at randomly selected spots. **(b)** Plots of relative peak intensity ratio $I_{1585\ cm\text{-}1} / (I_{1585\ cm\text{-}1} + I_{1333\ cm\text{-}1})$ for five independent assays, each error bar indicates the standard derivation of 5 different readings.



Reference

1. Pande, S., Ghosh, S.K., Praharaj, S., Panigrahi, S., Basu, S., Jana, S., Pal, A., Tsukuda, T., Pal, T., 2007. Synthesis of normal and inverted gold-silver core-shell architectures in beta-cyclodextrin and their applications in SERS. *Journal of Physical Chemistry C* 111(29), 10806-10813.