Supporting Information

Immunoassay of Tumor Markers in Human Serum based on Si nanoparticles and SiC@Ag SERS-active substrate

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The surface functionalization of SiO_2-coated Si NPs

According to the surface modified process described in the section 2.2 and 3.1, the surface functionalization of SiO_2-coated Si NPs can be illustrated visually by Fig. S1.

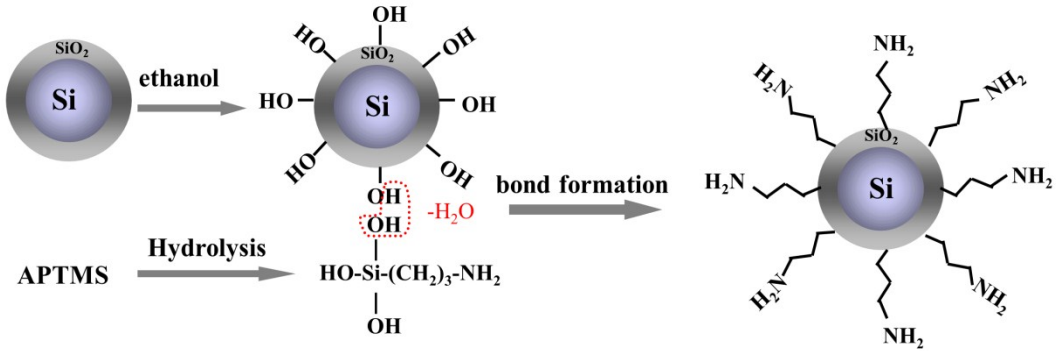

Fig. S1 Chemical modification of the amino group onto the surface of SiO_2-coated Si NPs.

The surface modification properties of SiO_2-coated Si NPs

In section 3.1, the surface modification properties of SiO_2-coated Si NPs have been analyzed by their ζ-potential measurements. Furthermore, after each modification step, the size distributions of the hydrated particleshad been measured and their DLS spectraare shown in Fig. S2. Concretely, the average hydrodynamic diameter of unmodified SiO_2-coated Si NPs is 218.6 nm (PDI = 0.188). After the SiO_2-coated Si NPs was modified, the average hydrodynamic diameter of APTMS-modified SiO_2-coated Si NPs is about 625.7 nm (PDI = 0.244), which meant that

SiO_2-coated Si NPs had aggregated into larger structures. However, after linking antibodies with APTMS-modified SiO_2-coated Si NPs, the average hydrodynamic diameters of three immue probes have a little increase, respectively. That is, the PSA immue probes ~ 640.3 nm (PDI = 0.349), the AFP immue probes ~ 641.8 nm (PDI = 0.410), and the CA19-9 immue probes ~ 637.3 nm (PDI =0.369). Therefore, the variations of the hydrated particles sizes verify that the APTMS-modified SiO_2-coated Si NPs and immune probes were successfully prepared, respectively.

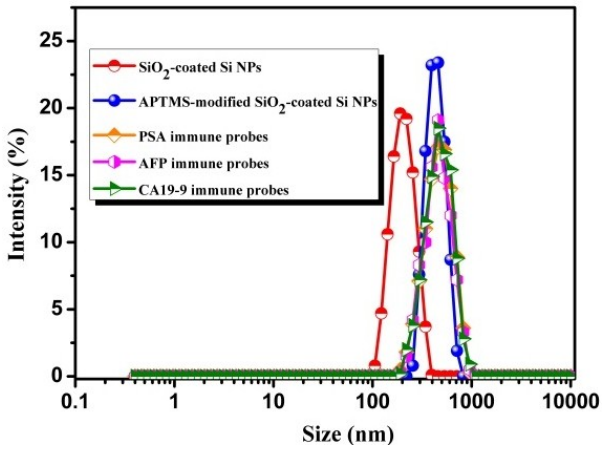

Fig. S2 Size distributions of the hydrated particles after each modification step.

Saturated antibody concentration

Taking anti-PSA antibody as an example, the steps of obtained the saturated antibody concentration are listed as follows. In our experiments, the different concentrations of anti-PSA antibodies (5, 2.5, 2, 1.25, 0.625, 0.3125, 0.15625 $mg \cdot mL^{-1}$) were firstly dropped onto the as-prepared SERS-active substrates, and then incubated at 37 ˚C for 2 h. After immobilized the antibody, the substrate was rinsed successively with TBS/0.05% Tween@ 20 buffer solution, PBS, and the deionized water to remove any residual antibody that was not linked with the SERS-active substrate. Next, the substrate was covered with 20 μL (10 mM) of the 4-MBA solution and dried in a gentle flow of argon gas. Then, the SERS spectra of 4-MBA were measured to estimate whether the captured antibody reached to saturation. Concretely, the weaker the SERS signal of 4-MBA is, the higher the concentration of antibody. When the SERS signal of 4-MBA is the weakest, the saturated antibody concentration captured on substrate is obtained due to the least 4-MBA moleculars are linked with the SERS-active substrate. As showin in Fig. S3(a) and S3(d), the intensities of SERS peaks of 4-MAB are decreased with increasing of the concentrtions of anti-PSA antibody and arrives a minimum at the saturated antibody concentration of ~ 2.5 $mg \cdot mL^{-1}$.

Similarly, corresponding to anti-AFP antibody and anti-CA19-9 antibody, the SERS spectra of 4-MBA at different antibody concentrations are shown in Fig. S3(b) and S3(c), respectively. The intensities of SERS peaks at 1078 cm^{-1} *vs* the concentrations of anti-AFP antibody and anti-CA19-9 antibody are also ploted in Fig. S3(d), respectively. From Fig. S3(d), we can estimate the saturate concentration of anti-AFP antibody and anti-CA19-9 antibody to be 2.0 $mg \cdot mL^{-1}$ and 1.5 $mg \cdot mL^{-1}$, respectively.

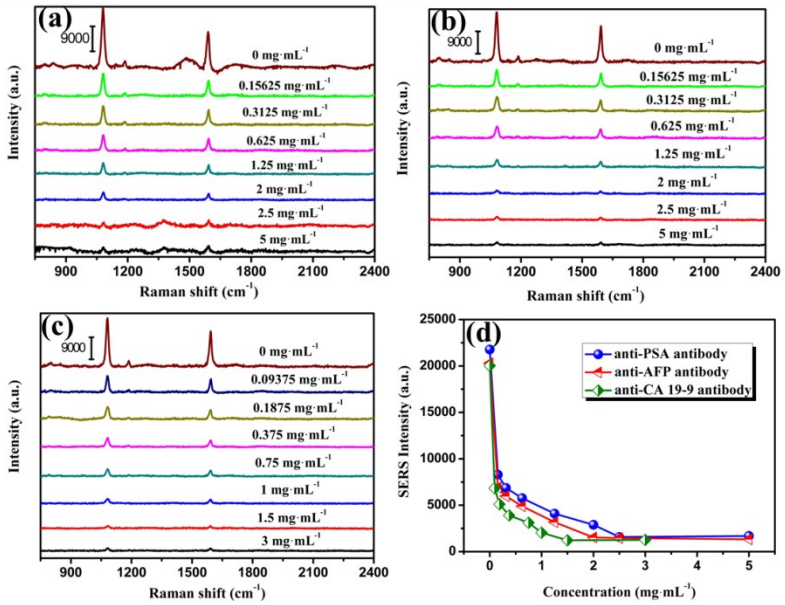

Fig. S3 SERS spectra of 4-MBA at the different concentrations of (a) anti-PSA antibody, (b) anti-AFP antibody and (c) anti-CA19-9 antibody; (d) the intensities of peak at 1078 cm^{-1} dependence on the different concentrations of three antibodies.

Upper detection limitation of the immunoassay

According to the proposed immunoassay protocol, the upper detection limitation of PSA and AFP have been experimentally obtained. Fig. S4(a) and S4(b) give the SERS spectra of Si NPs with different concentrations of PSA and AFP, respectively. And the intensities of SERS peaks at 520 cm^{-1} dependence on the concentration of PSA and AFP are ploted in Fig. S4(c), respectively. It can be found that the SERS intensities of Si NPs firstly climb up with increasing of the concentration of the antigens and then tend to invariant. Finally, the upper detection limitations of PSA and AFP, 71.87 $ng \cdot mL^{-1}$ and 95.75 $ng \cdot mL^{-1}$, can be determined from the standard dose-response curves in Fig. 6, respectively.

In our experiments, as represented in Fig. 6(f), the dose of CA19-9 shows a maxmum of 1000 $U \cdot mL^{-1}$. And, as described in section 3.3, the maximal dose of

CA19-9 in the serum samples is measured to be 2.69013 $U \cdot mL^{-1}$, which is in the range of the standard dose-response curves and much less than the maximal dose of 1000 $U \cdot mL^{-1}$ in Fig. 6(f). Thus, the upper detection limitation of CA19-9 could be set as 1000 $U \cdot mL^{-1}$.

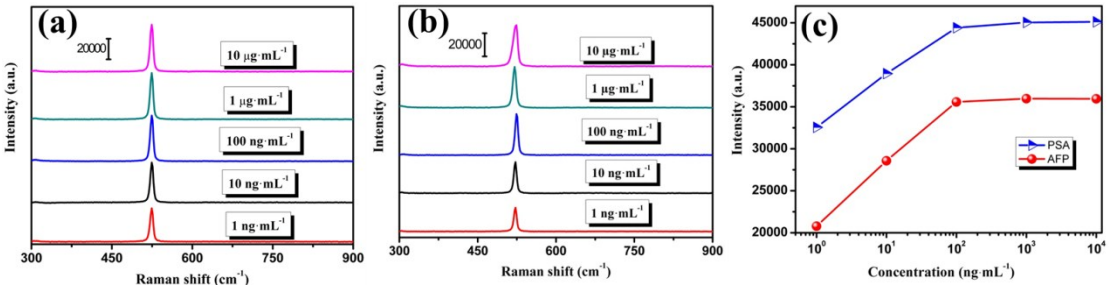

Fig. S4. SERS spectra of Si NPs for the different concentrations of (a) PSA and (b) AFP; (c) the intensity of SERS peak at 520 cm^{-1} dependence on the concentration of PSA and AFP.