Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic supplementary information

(ESI)

Antibody-labeled Gold Nanoparticles Based Resonance Rayleigh Scattering Detection of S100B

1. Comparison of absorption and resonance Rayleigh scattering spectra

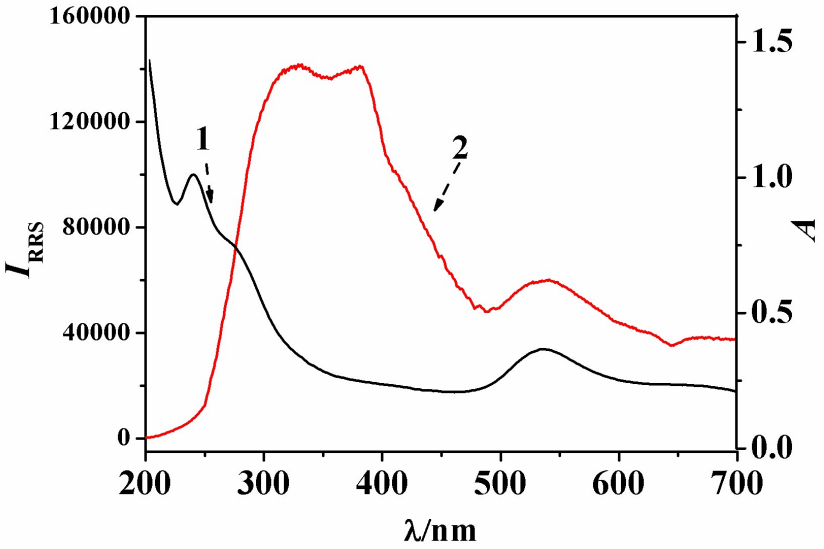

Fig. S1 Comparison of absorption (1) and resonance Rayleigh scattering (2) spectra of S100B-Ab-AuNP. S100B, 1.0 ng/mL

2. Optimization of Experimental Conditions

To optimize the condition for the S100B assay, various factors, such as the size distribution of gold nanoparticles, concentration of Ab, amount of Ab-AuNP, media pH, binding time and binding temperature were examined in detail.

We firstly optimize the size distribution of gold nanoparticles. As shown in Fig. S2, Ab-AuNP particles decorated with 13, 20, and 34 nm AuNP were successfully prepared. Fig.S2 shows ultraviolet–visible (UV–vis) spectra for aqueous dispersions of the S100B-Ab-AuNP.

The S100B-Ab-AuNP system had different absorption bands. S100B-Ab-AuNP system with 13 nm AuNP had only a small absorption around 520 nm. The absorption bands of S100B-Ab-AuNP system with 20 or 34 nm AuNP were red-shifted from 518 to 536 nm or from 528 to 550 nm, respectively. This red shift was induced by the coupling of plasmonic resonance among closely packed AuNP. Notably, as show in Fig. S2B, the RRS intensity was increased with increasing the size of AuNP. The red shift of UV-Vis spectra and RRS intensity increase of S100B-Ab-AuNP system with

34 nm AuNP were especially prominent compared to the others. Therefore, we used the 34nm AuNP for the immunoassay.

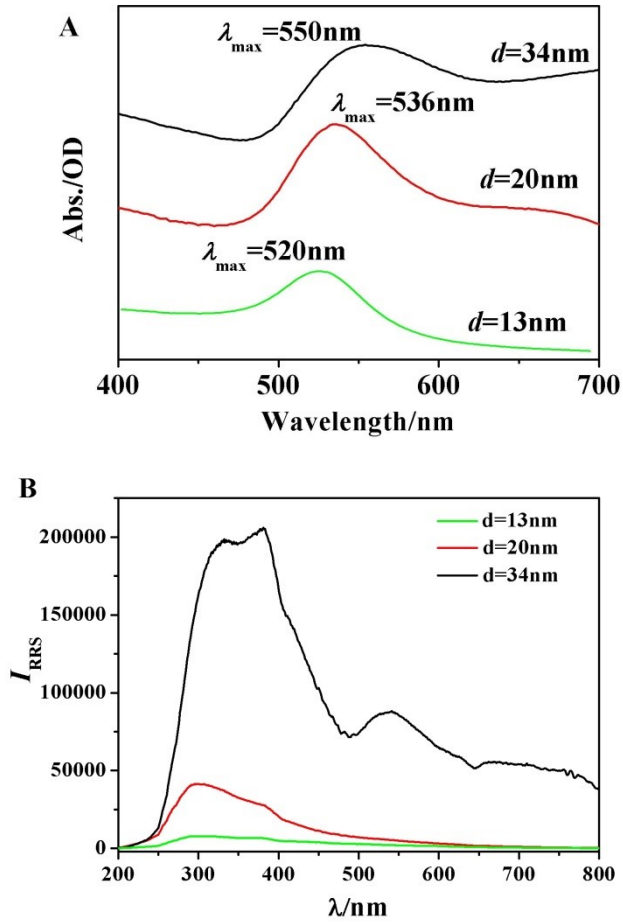

Fig. S2 (A) UV–vis spectra for S100B-Ab-AuNP with different size of AuNP. (B) RRS spectra for S100B-Ab-AuNP with different size of AuNP

Concentration of AuNP: 0.47 nM; Concentration of S100B: 1.0 ng/mL

During the preparation of antibody-AuNP (Ab-AuNP) bioconjugates by physisorption, several parameters should be considered: (a) the isoelectric point (pI) of the antibody, (b) the pH, and (c) the added quantity of antibody [1]. The Ab concentration was optimized. As shown in Fig. S3, ΔI_{RRS} improves with the increasing amount of Ab from 0 ug to 10 ug and then remains unchanged beyond the amount of 10 ug, probably due to the balance of the reaction between AuNP and Ab. Therefore, 10 ug of Ab is used in the following experiments.

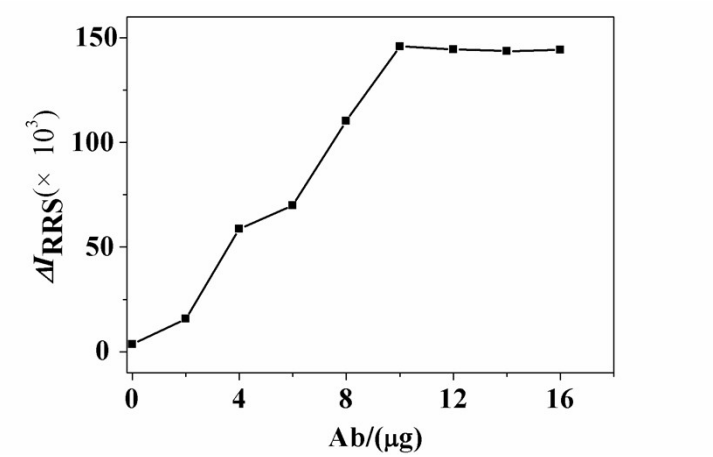

Fig. S3 Effect of Ab amount

Concentration of Cy-AuNP: 0.47 nM; Concentration of S100B: 1.0 ng/mL

It is generally agreed that maximal adsorption of proteins occurs when the pH is close to or slightly above their pI. [2] For many proteins, especially antiserum-derived immunoglobulins, the average pI spans over a broad range of pH values. The optimal coupling pH value for a given antibody should be determined through measurement of its relative pI range.

The PBS was used as reaction media, the effect of the pH is shown in Fig. S4. The ΔI_{RRS} of the system reached a maximum in the pH 5.8. Since the pI of Ab used in this study is 5.3, the experimental conditions are conducive to the modification and adsorption of antibodies. Therefore, PBS of pH 5.8 was taken as the reaction medium in the following experiments.

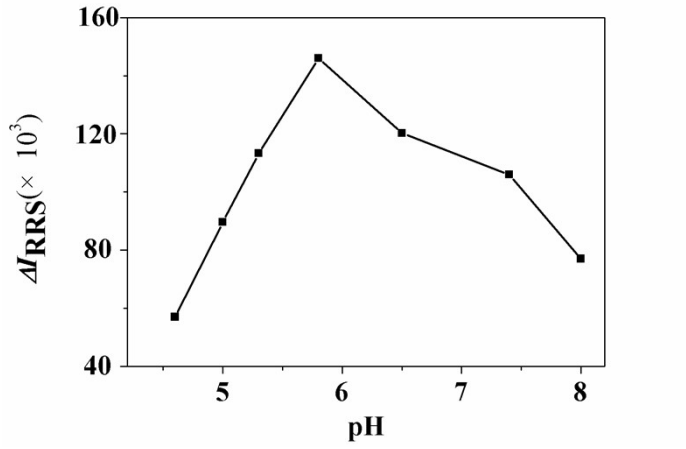

Fig. S4 Effect of pH

Concentration of S100B: 1.0 ng/mL; reaction temperature: 20~25 °C;

reaction time: 30 min

The effect of Ab-AuNP amount on the intensity was studied. According to the experimental results, ΔI_{RRS} reached the maximum when using 1.0 mL Ab-AuNP (Fig.S5).Without enough probe, the reaction was incomplete, when the probe was excessive, the scatting intensity would be reduced, because the reagent blank would enlarge. Therefore, we choose 1.0 mL as a suitable probe amount in the experiment.

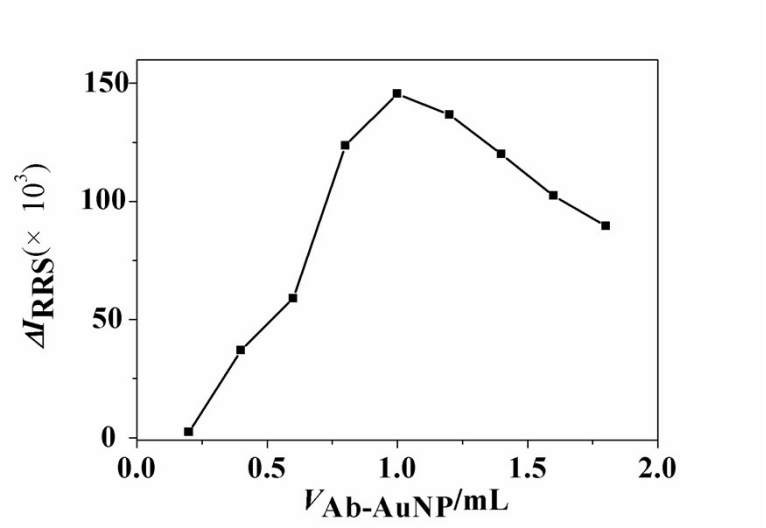

Fig. S5 Effect of Ab-AuNP amount

Concentration of EGFR: 1.0 ng/mL; pH 5.8; reaction temperature: 20~25 °C;

reaction time: 30 min

Additionally, we optimized the time Ab-AuNP probe incubated with S100B protein. As shown in Fig. S6, the ΔI_{RRS} improves dramatically and reaches a plateau within 30 min due to the higher binging of the probe to S100B. Thus, 30 min is selected as the optimal reaction time.

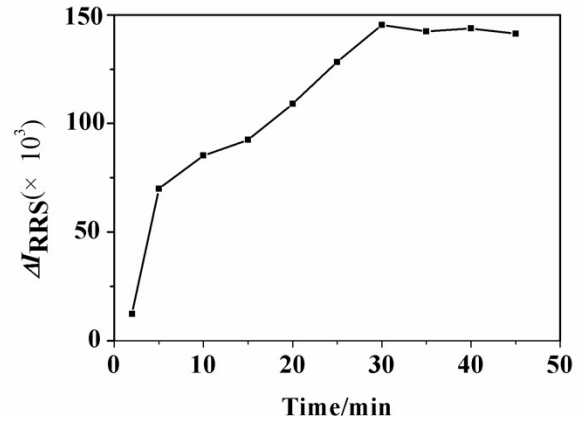

Fig. S6 Effects of reaction time

Concentration of S100B: 1.0 ng/mL; pH 5.8; reaction temperature: 20~25 °C

The effect of temperature on the immune-recognition reaction is shown in Fig. S7. The ΔI_{RRS} increases with decreasing temperature. . The RRS intensity changes little at temperature of 20-25 °C. Although the rate of chemical reactions usually increases with temperature, the immune-recognition reaction involves a negative change in entropy $\Delta S < 0$. Therefore, the increase of the temperature results in an increase in the free energy, and a decrease in the stability of the binding products. It is also found that decreasing the temperature to 15 °C increases the rate of nonspecific aggregation.

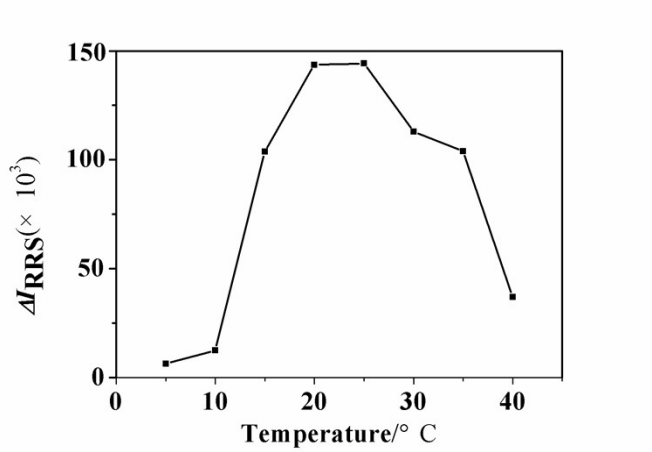

Fig. S7 Effect of reaction temperature

Concentration of EGFR: 100 ng·mL^{-1}; pH7.4; reaction time: 15 min

Reference

[1] Geoghegan WD. The effect of three variables on adsorption of rabbit IgG to colloidal gold. Journal of Histochemistry & Cytochemistry. 1988;36(4):401-407. doi:10.1177/36.4.3346540

[2] Demanèche S, Chapel JP, Monrozier LJ, Quiquampoix H. Dissimilar pH-dependent adsorption features of bovine serum albumin and alpha-chymotrypsin on mica probed by AFM. Colloids Surf B Biointerfaces. 2009;70(2):226-231. doi:10.1016/j.colsurfb.2008.12.036