Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Turning on Fluorescence by Plasmonic Assembly with Large Tunable Spacing: A New Observation and Its Biosensing Application

Shuo-Hui Cao, Wei-Peng Cai, Qian Liu, Kai-Xin Xie, Yu-Hua Weng, Yao-Qun Li*

Department of Chemistry and the MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, P. R. China

*yaoqunli@xmu.edu.cn

Chemicals and Materials. Human IgG, anti-human IgG, bovine serum albumin (BSA) and polyethylene glycol (PEG) were purchased from Xiamen Tagene Biotechnology Co., Ltd. 3-aminopropyltriethoxysiane was purchased from Acros Organics. 3-(trihydroxysilyl)-propylmethylphosphonate was purchased from Gelest. Cysteamine, 11-mercaptoundecanoic acid, Rhodamine B isothiocyanate, 2-morpholinoethanesulfonic hydrate (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), poly (sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) were purchased from Sigma-Aldrich. Siver nitrate ($AgNO_3$), chloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$) and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. All other reagents were of analytical grade and used without further purification. Ultrapure water was used throughout the study.

SPCE measurement. SPCE signal was detected by home-made multifunctional spectrofluorimeter. The sensing chip was attached with index-matching fluid to a semi-cylindrical prism positioned on a precise rotary stage. In reverse Kretschmann (RK) configuration, the light was incident from the front of sample interface. The SPCE signal was collected at a directional angle via the prism.

Dequenching model system. A 50 nm gold film was deposited on a quartz slide with a 2 nm adhesive layer of chromium, which provided a smooth surface with the roughness no more than 2 nm (Figure S1). The gold surface was immersed in 100 μM cysteamine and 1 mM 11-mercaptoundecanoic acid mixed together in ethanol for 12 h. After rising, a certain concentration of Rhodamine B isothiocyanate in carbonate buffer was added for 6 h immersion. After rising, the adhesion layer, such as 1.5 mg/mL PAH in 1M NaCl was added for 30 min immersion. And the silver nanoparticles were added following rising to get the dequenching signal. The blank experiments were done without fluorophores on the film. As shown in Figure S2, there was just low background signal before and after the adsorption of silver nanoparticles on the film.

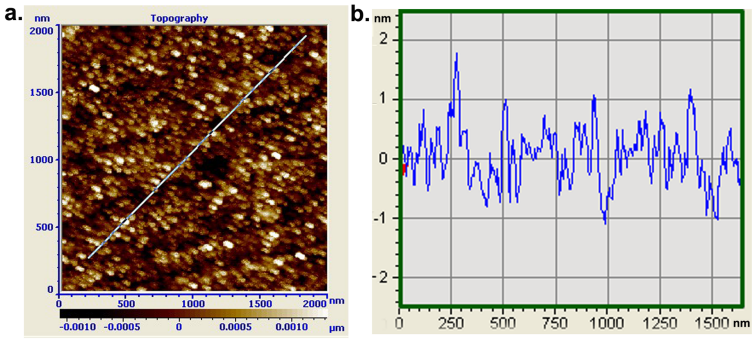

Figure S1. (a) AFM image of gold substrate (b) Depth analysis of the area shown in part (a) indicated by line.

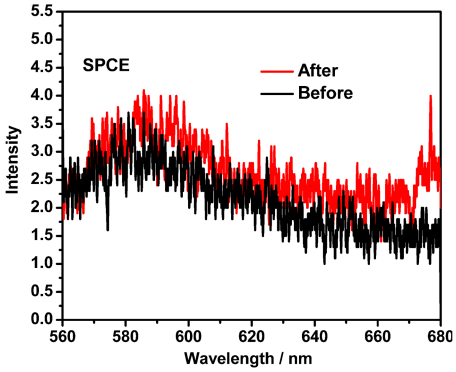

Figure S2. The signal before and after the adsorption of silver nanoparticles on the metal without fluorophores.

Testing dequenching ability under different conditions. The influences of the diameter of silver nanoparticles, the concentration of loaded dyes and the concentration of silver nanoparticles on the dequenching fold were tested (Figure S3). The detailed test procedures are introduced in the following parts.

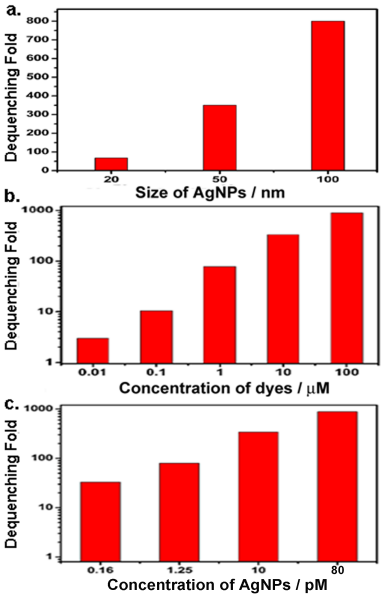

Figure S3 (a) SPCE dequenching folds responding to sizes of adsorbed silver nanoparticles (AgNPs). (b) SPCE dequenching folds responding to the concentrations of dyes added on the gold film. 100 nm AgNPs were used. (c) SPCE dequenching folds responding to the concentrations of AgNPs added onto the surface. The concentrations of dyes were 100 μM.

The silver and gold nanoparticles with different diameters were synthesized separately. The initial concentrations of the metal ions were kept to be similar. For the fabrication of 20 nm silver nanoparticles, 1 mL 10 mM $AgNO_3$ were dropped into 10 mL 6 mM $NaBH_4$ solution under stirring and ice bath. For 50 nm silver nanoparticles, 1 mM $AgNO_3$ and 7 mM trisodium citrate were mixed, and the pH was modulated into 6.1; under stirring and ice bath, $NaBH_4$ was quickly added with the final concentration of 0.4 μM; after 1 min, the solution was heated in a reflux for 1 h. For 100 nm silver nanoparticles, 1 mM $AgNO_3$ was sired to boil and then trisodium citrate was added into the boiling solution with the final concentration of 3.4 mM. The heating continued 1 h. For the fabrication of 14 nm gold nanoparticles, 1 mM $HAuCl_4$ was stirred to boil and then trisodium citrate was added into the boiling solution with the final concentration of 4 mM. Another 15 min boiling with stirring was needed. For 40 nm gold nanoparticles, 1 mM $HAuCl_4$ was stirred to boil and then trisodium citrate was added into the boiling solution with the final concentration of 1.3 mM. Another

15 min boiling with stirring was needed. For the fabrication of 120 nm gold nanoparticles, 40 nm gold nanoparticles were used as the seeds for growth. 4 mL 40 nm gold nanparticles solution was mixed with 4 mL 24 mM $HAuCl_4$ and 4 mL 34 mM trisodium citrate, and diluted with water to 100 mL. After the stirring of 15 min, 5.6 mL 10 mM hydroxylamine hydrochloride was dropped and the stirring continued for further 2 h. And the dequenched ability under different nanoparticles was tested. Figure S6 shows the dequenching SPCE responding to sizes of adsorbed gold nanoparticles. The results were lower than those from silver nanoparticles adsorbed surface.

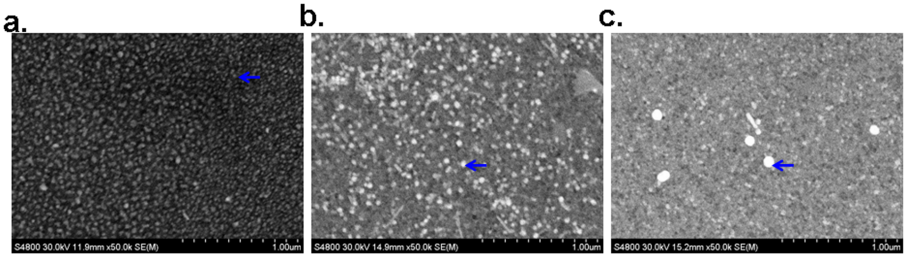

Figure S4. SEM images of (a) 20 nm, (b) 50 nm and (c) 100 nm silver nanoparticles. Arrows point to silver nanoparticles

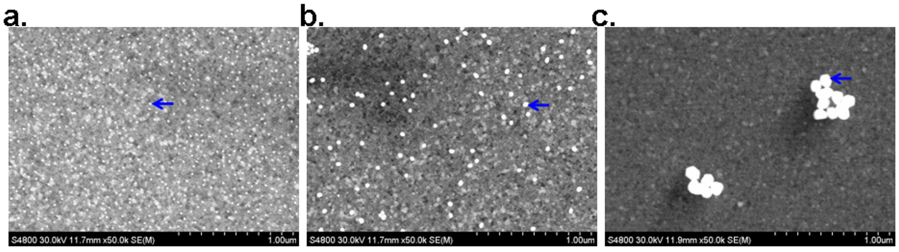

Figure S5. SEM images of (a) 14 nm, (b) 40 nm and (c) 120 nm gold nanoparticles. Arrows point to gold nanoparticles.

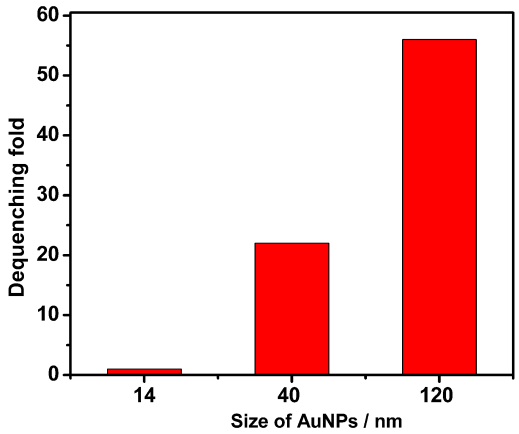

Figure S6. The dequenching SPCE responding to sizes of adsorbed gold nanoparticles

The concentrations of dyes used for the modification of surface were changed to investigate the dequenching phenomenon influenced by the loading of dyes. Glass substrate with amino group (Fabrication is through immersing the slide in the water mixture of 100 μM 3-aminopropyltriethoxysiane and 1 mM 3-(trihydroxysilyl)- propylmethylphosphonate for 1h, following by rising and drying at 110 ℃.) was used for the contrast test. 0.01-100 μM Rhodamine B isothiocyanate in carbonate buffer was added to immerse both gold and glass substrates for 6 h. After rising, the fluorescence was detected. Figure S7 shows that, the fluorescence first increases

with the concentration until to 1 μM, and the intensity rapidly decreases due to the self-quenching from numerous fluorophores. But the intensity was still increasing in dequenching-SPCE.

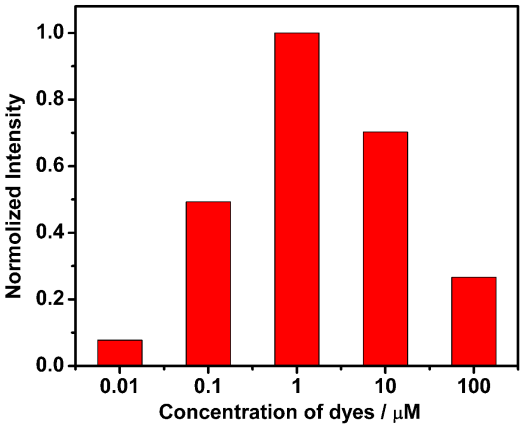

Figure S7. The normalized intensity responding to the concentrations of dyes used for the modification of glass substrate.

The concentrations of silver nanoparticles added to metal surface were changed. As shown in Figure S8, the quantities of adsorbed nanoparticles were increased with increasing the concentrations of added silver nanoparticles. Different concentrations of nanoparticles were obtained through diluting, and the initial concentration was estimated by the average size of nanoparticle and the addition of metal ions in synthesis.

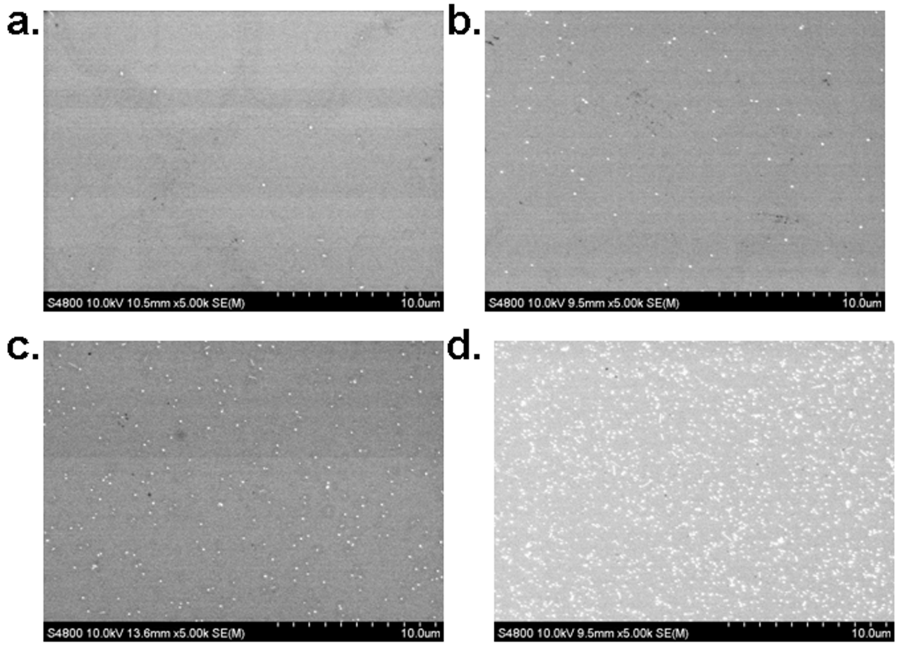

Figure S8. (a) 0.16 pM, (b) 1.25 pM, (c) 10 pM and (d) 80 pM 100 nm silver nanoparticles added to gold surface to form NPs-Film structure tested by SEM image.

Changing the distance between nanoparticles and film. The distance between nanoparticles and film was changed by inserting polyelectrolyte layers as the separation. The carboxyl groups modified on gold film provided the negative charged surface, so the positive charged PAH and negative charged PSS could be deposited through layer-by-layer assembly. 1 mg/mL PSS and 1.5 mg/mL PAH were respectively dissolved in 1 M NaCl solution. The substrates were immersed in the PAH solution for 20 min, followed a wash for 5 min, and immersed again in a PSS solution for 20 min, followed by a wash in water for 5 min. The one assembly of PAH and PSS was evaluated to be 5 nm.

Then the assembly is repeated. PAH was always exposed on the top layer to adsorb the negative charged trisodium citrate on the surface of silver nanoparticles.

Scattering spectra of AgNPs assembled on surface. 100 nm AgNPs were assembled on glass slide and gold film respectively. Dark-field optical microscopy was used to collect the scattering spectra. As shown in Figure S9, there is a significant shift in spectra for the assembling of AgNPs from on glass slide to on gold film. It indicates that the plasmon coupling between AgNPs and gold film can have an enormous influence in the system.

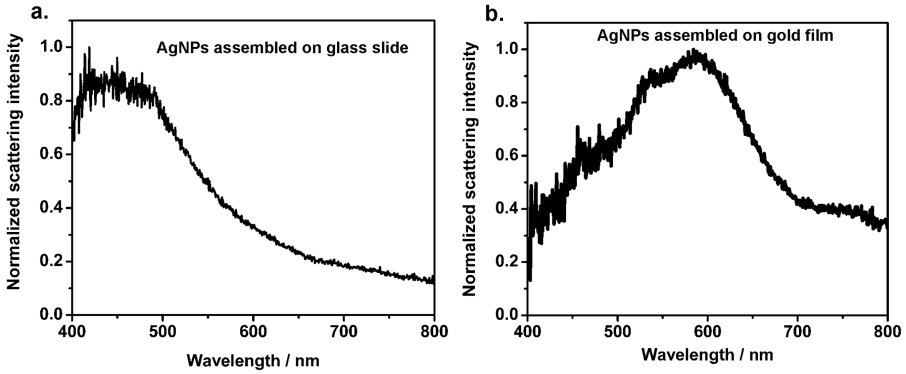

Figure S9 (a) normalized scattering spectrum of 100 nm AgNPs assembled on glass slide. (b) normalized scattering spectrum of 100 nm AgNPs assembled on gold film.

Dequenching-SPCE in immunoassay. The antibody-nanoparticle conjugates were prepared by the simple adsorption process. 100 μg IgG antibody was mixed with 2mL 5-fold diluted 100 nm silver nanoparticles solution with pH 9.0 adjusted by 0.1 M K_2CO_3. And the mixture was incubated at room temperature with gentle shaking for 30 min, followed by the incubation of 200 μL 1% PEG solution for 10 min. The mixture was then centrifuged at 8000 rpm for 10 min. After that, the sediment was washed and resuspended in PBS buffer solution containing 1.0 mg/ mL BSA for two times. The gold surface was modified with amine and carboxyl groups: the amine groups were used to connect Rhodamine B isothiocyanate; the carboxyl groups were activated by EDC/NHS to react with the amino groups of antibody. The substrate after modification of dyes was immersed in aqueous solution containing 0.1 M MES, 10 mM NHS and 10 mM EDC for 30 min. After rising, 20 μg/mL anti-human IgG was added on the surface with the incubation for 3h at 4 ℃. After the excess was rinsed, 1% BSA was used to block the surface for 1 h, followed by rising. Then the chips were incubated with a certain concentration of antigen at 37 ℃ for 1 h. After rising, the antibody-nanoparticle conjugates were added for incubation in a certain time. After the excess was rinsed, the chip was used for dequenching-SPCE detection.

The immune conditions were tested. The signal from the immunoassay of 50 ng/mL IgG was compared to the detected intensity from 1% BSA as the antigen in immunoassay, and the ratio was defined as the signal to noise ratio in this study to evaluate the conditions. First, the concentration ratio of cysteamine to 11-mercaptoundecanoic acid used to modify the gold surface was tested, and the concentration of cysteamine was kept as 100 μM. As shown in Figure S10, the signal to noise ratio reaches to the maximum at the concentration ratio of 1: 100. It can be explained that: for more cysteamine in the ratio, the subsequent capacity of connecting to antibody was low, leading to the immunoassay not efficient enough; for less cysteamine in the ratio, the loading of fluorophores will be not enough to report the signal. Second, the incubation time of antibody-nanoparticle conjugates in the immunoassay was tested. Figure S11 shows that 20 min is the best time for the incubation of the nanoparticles. Shorter time is not enough for the immunoassay, but longer time more than 20 min should cause the nonspecific adsorption to increase noise level.

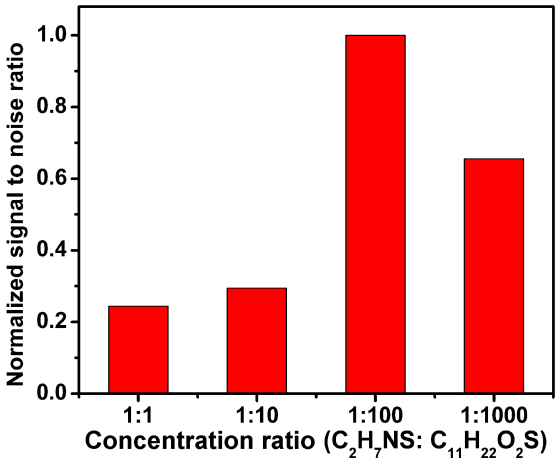

Figure S10. The normalized signal to noise ratio responding to different concentration ratio of cysteamine (100 μM) to 11-Mercaptoundecanoic acid used to modify the gold surface.

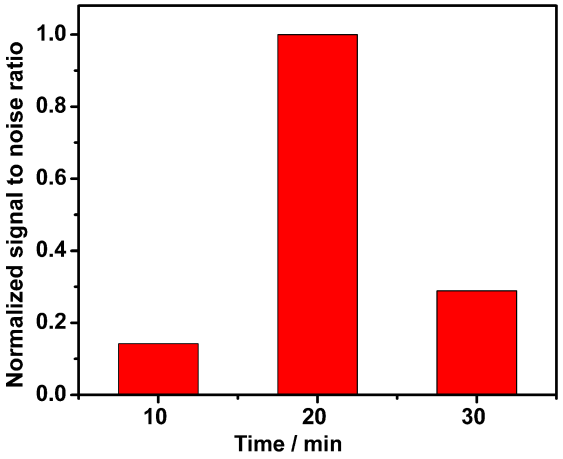

Figure S11. The normalized signal to noise ratio responding to the incubation time of antibody-nanoparticle conjugates in the immunoassay.