Electronic supplementary information

Bioidentification of Biotin/Avidin Using Surface Plasmon Resonance and Surface-Enhanced Raman Scattering (SPR-SERS) Spectroscopy

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1. The setup of the SPR-SERS spectrometer and its working way.

The configuration of the SPR-SERS microspectrometer was reported in our previous work.[i] It is composed of three main functional parts: an incident light system, an SPR detection system and a SERS detection system.

The incident light system is mounted on one arm of a two-arm goniometer, which is comprised of a laser (532 nm, Changchun New Industries Optoelectronics Tech. Co. Ltd), two lenses (Lens 1 has a numerical aperture of 0.18 and a focal length of 25 mm, Lens 2 has a numerical aperture of 0.15 and a focal length of 10 mm) and a polarizer. The SPR detection system on the other arm of the goniometer contains Lens 3 and a photodiode. SPR data acquisition and precise angular rotation of the goniometer are both controlled via a program written by LABVIEW software (National Instruments Co.). The arms of the goniometer move with a resolution of <0.005°.

The SERS detection system consists of three parts: an inverted microscope (with a 20× object lens, NA=0.35, focal length=20.5 mm)), a CCD imaging camera with a display screen, and a spectrometer (iHR320, Jobin-Yvon Co.) with a CCD (Synapse, Jobin-Yvon Co.). A mobile mirror can switch the light to a CCD imaging camera or a spectrometer. An edge filter (λ=532nm, Semrock Inc.) was fixed on the light path to remove the Rayleigh scattering.

A semi-cylindrical prism (K9 glass with the refractive index is 1.52 at 532 nm) was fixed in the center of the goniometer. On the bottom of the prism, a 45nm thickness of silver film was modified by the vacuum evaporation deposition method. The deposition rate was 0.1nm/s. A flow cell with an injector was fixed above the silver film, which was used for the layer-by-layer assembly of analytes.

The working way of this SPR-SERS spectrometer was as follows. Two arms rotate at θ° degree in the opposite direction. When the incident angle is beyond the critical angle, the light is totally reflected at the interface of the prism/silver film. The reflected light is collected by Lens 3 and then detected by a photodiode. On the same spot of the silver film, the SERS spectra of adsorbed analytes were simultaneously excited under the evanescent field. The SERS spectra were focused into the inverted microscope and detected spectrometer. We adjusted the incident and reflected angles to obtain the angle-dependent SPR and SERS spectra.

2. The interference experiment

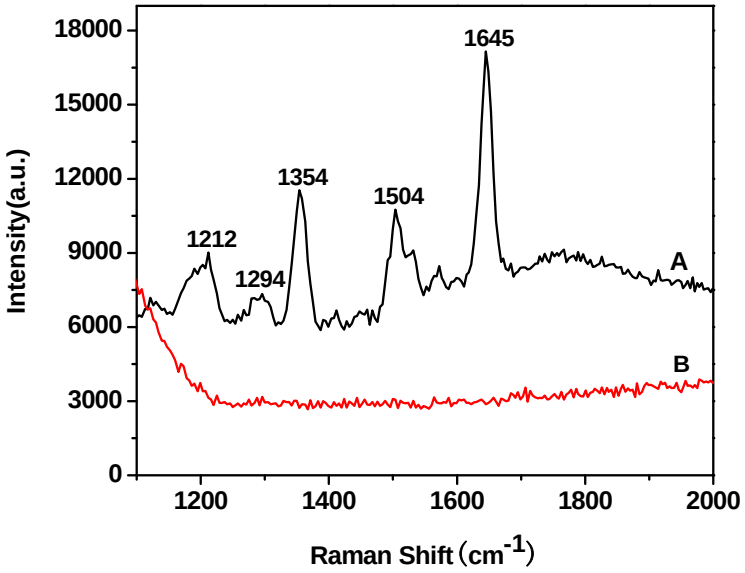

Figure S1. (A) SERS spectrum of TRITC- goat anti-rabbit IgG with silver colloid enhancement. (B) SERS spectrum of TRITC-goat anti-rabbit IgG on the avidin modified silver film by the SPR-SERS spectrometer.

The SERS spectrum of 6-tetramethylrhodamine isothiocyanate (TRITC) - goat anti-rabbit IgG was recorded (Figure S1 A). A silver colloid prepared by Lee's method [ii] was used to enhance the signal of TRITC. 100 μL of TRITC- goat anti-rabbit IgG was mixed with 0.9 mL of silver colloid. And then, we measured the SERS spectra of the mixed solution by a portable Raman spectrometer of 532 nm (B&WTek, Inc). The integration time was 6 s and integral number was 1. The laser power was 1.12 mW. The marked bands in Figure S1 A are all attributed to TRITC.

The interference experiment was carried out by using TRITC-goat anti-rabbit IgG instead of Atto610-biotin for the bioidentification of biotin/avidin. All the assembly steps on the silver film were as same as the procedures stated in the main manuscript except the last one, in which a 10^{-3}

mg/mL of TRITC-goat anti-rabbit IgG was injected into the flow cell. After 20 min, SERS spectrum of TRITC-goat anti-rabbit IgG/avidin complex by the SPR-SERS spectrometer (shown in Figure S1 B). The excitation wavelength was 532nm. The integration time was 3s.The laser power was 8mW.

References:

[i] Liu,Y.; Xu, S. P.; Tang, B.; Wang, Y.; Zhou, J.; Zheng, X. L.; Zhao, B.; Xu, W. Q. *Rev. Sci. Instrum.* **2010**, *81*, 036105.

[ii] Lee, P. V.; Meisel, D. *J. Phys. Chem.* **1982**, *86*, 3391–3395.