Electronic Supplementary Information

Plasmonic metal scattering immunoassay by total internal reflection scattering microscopy with nanoscale lateral resolution

Seungal Lee,a Hyunung Yu,b and Seong Ho Kang*a

aDepartment of Applied Chemistry, College of Applied Science, Kyung Hee University, Yongin-si, Gyunggi-do 446-701, Republic of Korea

bNanobio Fusion Research Center Korea Research Institute of Standards and Science, Daejeon 305-600, Republic of Korea

*Corresponding Author.

Tel.: +82 31 201 3349; fax: +82 31 201 2340; email: shkang@khu.ac.kr (S.H. Kang).
The AVI movies show the lateral intensity distribution of the TIRS imaging before (M1) and after (M2) immunoreaction with TSH antibody-silver nanoparticles.
Experimental section

Reagent preparation. 11-mercaptoundecanoic acid (MUA, 95%), 6-mercapto-1-hexanol (MCH, 97%), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC), dimethyl sulfoxide (DMSO, 99.5%), 2-(morphozlino)ethanesulfonic acid (MES), glycine, and phosphate buffered saline (PBS) were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Sulfo-NHS (N-hydroxysulfosuccinimide, NHSS), dithiobis(succinimidyl propionate) (DSP), and Protein A/G were purchased from Pierce (Rockford, IL, USA). Tris(base) was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). StabilGuard was purchased from SurModics (Eden Prairie, MN, USA). Two-site immunoassays require a pair of monoclonal antibodies to be generated against two different epitopes of hTSH. One antibody, referred to as the “capture” antibody, is bound to a solid phase (gold spot), and the other antibody, referred to as the “detector” antibody, is bioconjugated with the 20 nm silver nanoparticles (SNPs, 7.0 \times 10^{10} \text{ particles/mL}) obtained from BBI Life Sciences (Cardiff, UK), as previously described (Fig. 1A) [Chem. Commun., 2013, 49, 8335-8337]. Monoclonal mouse anti-human thyroid stimulating hormone (hTSH-antibody, 2TS11-10C7 and 2TS11-5E8) was purchased from HyTest (Turku, Finland) and thyroid
stimulating hormone protein (TSH-antigen, 30-AT09) was purchased from Fitzgerald (North Acton, MA, USA).

**Fabrication of the gold-nanopatterned chips.** The gold-nanopatterned chip was manufactured by the National Nanofab Center (Daejeon, South Korea) as previously reported [*Analyst*, 2013, **138**, 3478-3482]. The four-inch glass wafer was cleaned using piranha solution (1:1 = H$_2$SO$_4$:30% H$_2$O$_2$). Polymethylmethacrylate (PMMA) was spin-coated on top of the glass substrate. In the next step, a 50 nm-thick PMMA layer was patterned *via* e-beam lithography (Elionix E-beam system, 100 KeV/100 pA). Then, Au/Cr (25/5 nm thickness) was deposited to form array patterns with a pitch of 10 μm and a diameter of 500 nm. The pattern consisted of $4 \times 5$ Au spots on a 10 mm$^2$ glass wafer. After deposition, dichloromethane was used for lift-off. The chips were immersed in acetone (99.5% purity) for 30 s, isopropyl alcohol (99.9% purity) for 30 s, and in piranha solution for 30 min. Between each wash step, the chips were thoroughly rinsed with ultra-pure water. The cleaned chips were dried under a nitrogen stream and stored in a desiccator.
The TIRS detection system. The schematic representation and physical layout of the apparatus were modified from previously published configurations (Fig. S1) [Talanta, 2013, 104, 32-38]. An upright Olympus BX51 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with a NA 0.6-1.3 objective lens (UPLANFLN, ×100) was used. The wavelength selection was accomplished with a set of filters purchased from Semrock (Rochester, NY, USA) with central wavelengths of 406/15 nm and 670/30 nm. A 20 mW, 405 nm laser (SOL-405-LM-020T, Shanghai Laser & Optics Century Co., Ltd., China) and a 30 mW, 671 nm laser (SDL-671-040T, Shanghai Laser & Optics Century Co., Ltd.) were used as the TIRS excitation source. TIRS images were acquired with an electron-multiplying cooled charge-coupled device (EM-CCD) camera (512 × 512 pixel imaging array, QuantEM 512SC, Photometrics, AZ, USA) along a 10 nm stepwise movement of the z-nanopositioner after the sandwich immunoreaction of hTSH on the gold spot was prepared following a previously reported procedure [Biosens. Bioelectron., 2014, 60, 45-51]. Through a shutter control, exposure time was set at 100 ms. Images were acquired and the scattered intensity was calculated as the difference between the intensities of the selected signal regions and the intensities of the background regions with the same area of the CCD image using MetaMorph 7.1 software.
Fig. S1 (A) The physical setup and (B) schematic diagram of the lab-made TIRS detection system. Circle: Evanescent field intensity ($I_z$) with perpendicular distance ($z$) by sinusoidal light waves on the gold-nanopatterned chip. Indicators: L1, 405 nm laser; L2, 671 nm laser; MS, mechanical shutter; DC, dichroic mirror; M, mirror; DP, dove prism; OL, objective lens; FC, filter cube; CCD, charge coupled device; Z, $z$-nanopositioner.
**Fig. S2** AFM images of (A) before and (B) after the sandwich immunoreactions with antibody-labeled SNPs on a 500 nm gold-nanopatterned chip (GNC).
Fig. S3 Total internal reflection scattering imaging after the immunoreaction of serial dilution concentrations (from 800 zM to 100 pM) of standard TSH on a gold-nanopatterned chip, including the use of a bandpass filter of 406 to match the 405 nm illumination source.