

Electronic Supplementary Information

Femtogram Cytokine Detection in a Direct Assay Using SERS Microspectroscopy with Hydrophilically Stabilized Au/Ag Nanoshells

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Experimental Section:

Reagents. 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), 4-mercaptobenzoic acid (MBA), N-(3-dimethyl-aminopropyl)-N'-ethyl-carbodiimide (EDC), and N-hydroxy-sulfosuccinimide sodium salt (s-NHS), AgNO₃, HAuCl₄, were purchased from Sigma/Aldrich/Fluka. NaHCO₃, NaCl, Na₂HPO₄, KH₂PO₄, sodium acetate hydrate, ethanol, anhydrous ethylene glycol, polyvinylpyrrolidone (PVP), bovine serum albumin (BSA), HEPES, and Tris (tris(hydroxymethyl)aminomethane) were purchased from Carl Roth, Germany. 2-nitro-5-thiobenzoate (NTB) was obtained by cleavage of DTNB with sodium borohydride. Raman reporter (NTB and 4-MBA) molecules attached by a short monoethylene glycol (MEG) unit with terminal –OH group and a longer triethylene glycol moiety (TEG) with terminal CO₂H group were synthesized according to our previous report.¹⁻³ Human IL-6 affinity purified polyclonal antibody (AF-206-NA), human CXCL8/IL-8 affinity purified polyclonal antibody (AF-208-NA), and recombinant human IL-6 (206-IL-010) and CXCL8/IL-8 (208-IL-050) were purchased from R&D system.

Preparation of SERS immuno-labels. Au/Ag nanoshells were prepared based on the template-engaged replacement reaction between silver nanoparticles and an aqueous HAuCl₄ according to the method reported by Xia and co-workers.⁴⁻⁵ As-prepared Au/Ag nanoshells were reacted with Raman reporters (the mixture of NTB-MEG-OH and NTB-TEG-CO₂H or 4-MBA-MEG-OH and 4-MBA-TEG-CO₂H, the ratio is 100:1) at room temperature (RT) under shaking for several hours to form the dual-SAM on nanoshells surface (as displayed in Figure 1B). After that nanoshells with Raman

reporters were centrifuged at 1190g for 15 min and then re-disperse into 500 μ L HEPES buffer (pH=5.9) to form the SERS labels. Afterwards, the SERS labels were activated by EDC/s-NHS (40 μ L 5 mg/1.5 mL EDC and 40 μ L, 3 mg/1.5 mL s-NHS) in HEPES buffer at RT for 20 min under shaking. Centrifuge the SERS labels to remove the excess EDC/s-NHS and re-disperse into 500 μ L HEPES buffer. 0.5 μ g poly-clonal antibody (PAb) was then added into 500 μ L activated SERS labels solution for reacting at RT for 1 h and 4°C overnight for the formation of SERS immuno-labels. Then 400 μ L TT (Tris-Tween 20)-0.2% BSA buffer were added into 500 μ L SERS immuno-labels solution and react at RT for 15-20 min under shaking to block the SERS immuno-labels surface and avoid the non-specific binding. After the reaction, the SERS immuno-labels were washed three times with TT-0.2% BSA buffer by centrifuge the nanoshells at 1190g for 15 min at 6°C. Then the SERS immuno-labels were re-dispersed into 500 μ L TT-0.2% BSA buffer and used for the binding on the nitrocellulose membrane surface. The maximum numbers of antibody bound on the Au/Ag nanoshells surface was roughly estimated to be 415 /nanoshell.⁶

Direct Immunoassay (Dot-blot). Antigen dot were formed on the nitrocellulose (NC) membrane by depositing 1 μ L standard samples containing different concentrations of IL-6, which were immobilized on NC surface through both the electrostatic and hydrophobic interactions between the protein and the nitrocellulose. And then the membrane was blocked by 2% BSA (in PBS buffer with 0.5% Tween-20) for 2 h at room temperature to block the rest sites of the nitrocellulose. Then NC membrane was immersed in the solution containing SERS immuno-labels (OD=2.5) and shaken at room temperature for 1 h. Afterwards, the NC membrane were incubated in the SERS immuno-labels solution at 4°C overnight. NC membrane was then washed by PBST buffer and soft MQ water to remove the excess probes. Duplex cytokine detection was obtained by depositing 1 μ L samples containing different ratio of IL-6 and IL-8 from 0:1, 1:10, 1:5 and 1:0. The starting concentration of IL-6 and IL-8 are kept the same at 1 nM. And then the mixture was obtained using different volume ratios of IL-6 and IL-8. After

blocking the surface by BSA, NC membrane was immersed into the solution containing the corresponding SERS immuno-labels responding to IL-6 and IL-8.

Instruments. Nanoshells were characterized by TEM (Zeiss, EM 902) and UV-Vis absorption/extinction spectroscopy (Perkin Elmer, Lambda 35). SEM measurements were conducted with a Zeiss Supra 50 electron microscope on NC membrane, which were cut of the sample area with and without antigen deposited. SERS spectra were recorded with a *WITec* alpha 300 R Raman Microscopy. The 632.8 nm line from a HeNe laser was used for excitation of Raman scattering. SERS images and spectra were obtained at 2 sec integration with laser power (6 mW) by mapping an area of $100\ \mu\text{m} \times 100\ \mu\text{m}$ with 20×20 points with a long distance objective (40 x, NA=0.60).

References:

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Supporting Figures

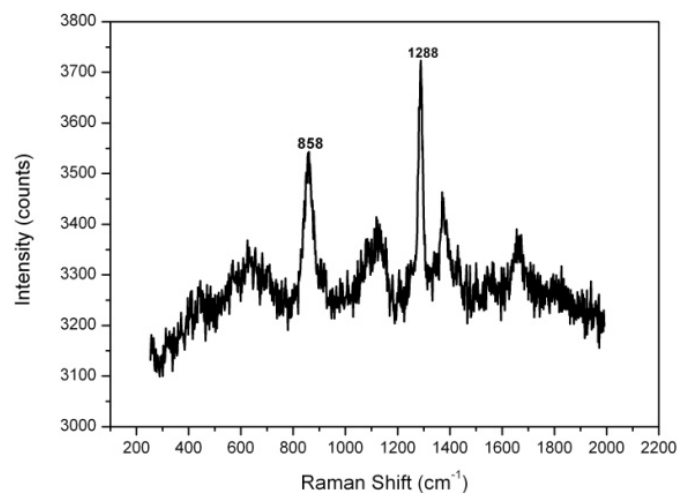


Fig. S1. Normal Raman spectrum of nitrocellulose membrane.

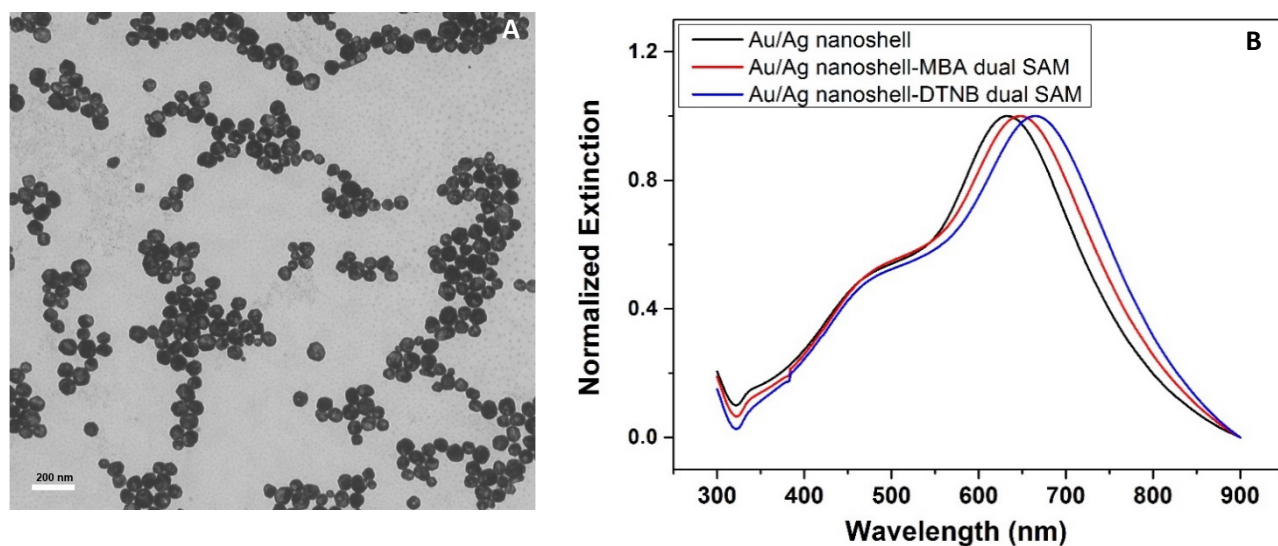


Fig. S2. TEM image of Au/Ag nanoshells (A) and extinction spectra (B) of Au/Ag nanoshell, Au/Ag nanoshell with MBA and NTB dual SAM, respectively.

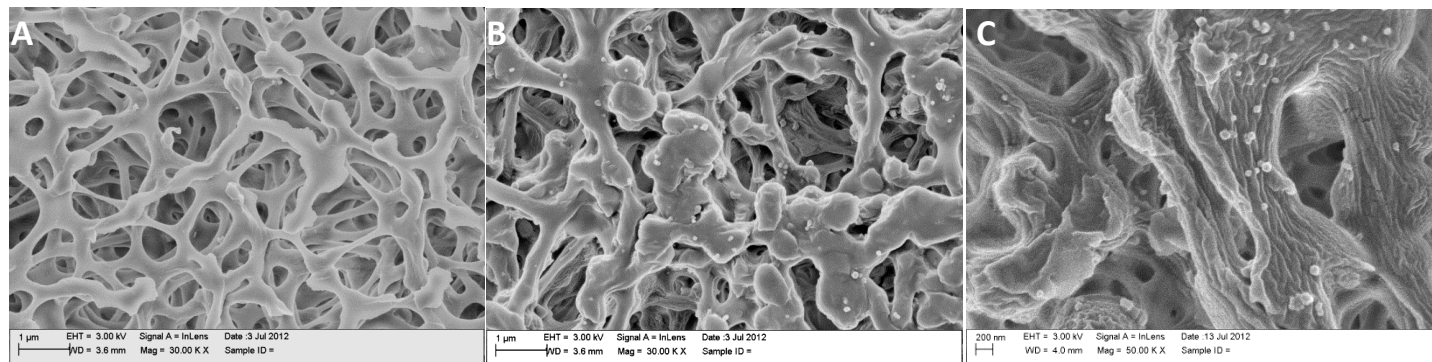


Fig. S3. SEM images of Au/Ag nanoshells on NC membrane with negative control (A) and IL-6 at the concentration of 100 pg/mL (B) and (C).

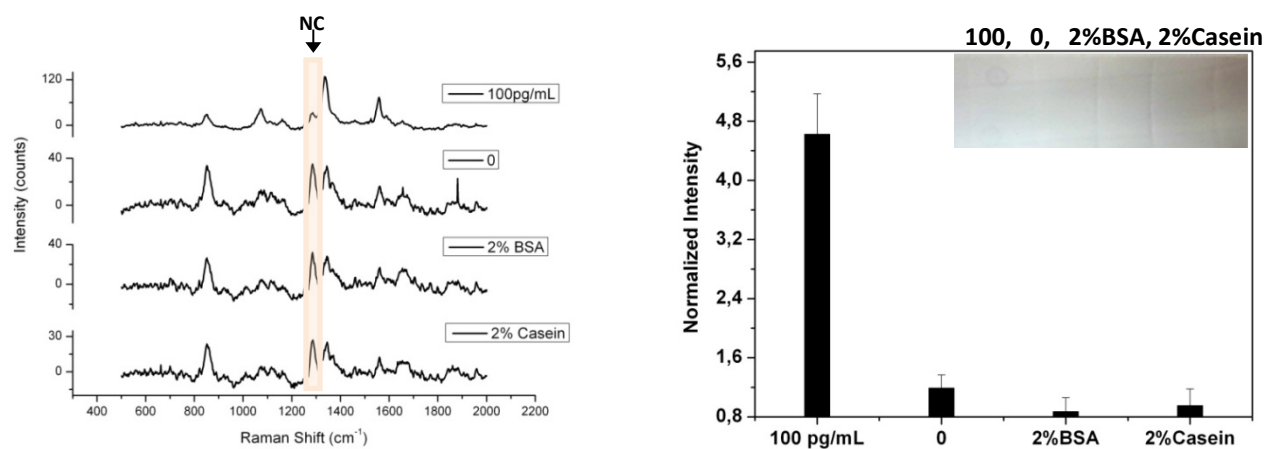


Fig.S4. SERS detection for IL-6 with BSA, casein and PBS as control (specificity).