Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Simultaneous Immunoassay for the Detection of Two Lung Cancer Markers Using Functionalized SERS Nanoprobes†

Hyangah Chon, Sangyeop Lee, Soo Young Yoon, Soo-Ik Chang, Dong Woo Lim and Jaebum Choo*

List of contents

Experimental details

In this experiment, anti-CEA conjugated magnetic beads (M_{CEA}) and anti-AFP conjugated magnetic beads (M_{AFP}) were prepared. A 0.1 mg/mL anti-CEA monoclonal antibody (Abcam, UK) and 0.1 mg/mL anti-AFP monoclonal antibody (Abcam, UK) were conjugated on the surface of 1.0 μm carboxylic-activated magnetic beads at 0.5 mg/mL (Dynabeads® MyOne™; Invitrogen, Eugene, OR, USA) through 0.1 M 1-ethyl,3-(3-dimethylaminopropyl) carbodiimide (EDC, Sigma-Aldrich) and 0.1 M 4-(4-maleimidophenyl)butyric acid N-succinimidyl ester (NHS, Sigma-Aldrich) coupling. MGITC-labeled and anti-CEA polyclonal antibody-conjugated HGN probes ($H_{MGITC-CEA}$) and XRITC-labeled and anti-AFP polyclonal antibody conjugated HGN probes ($H_{XRITC-AFP}$) were prepared. Hollow gold nanospheres (HGNs) were synthesized as previously described and a simple protocol is provided in the Supporting Information (Fig. S3 in ESI). The antibody conjugation procedure was reported in our previous paper.[10c] Briefly, 1.0 μL 10 mM MGITC and 1.0 μL 50 mM XRITC were added to 1.0 mL 0.7 nM HGN colloid. The surface of the HGNs was modified using 2.0 μL 2.5 mM dihydrolipoic acid (DHLA, Sigma, St. Louis, MO, USA) for 2 h then unreacted chemical reagents were removed by centrifugation. Here, 1.0 mL PBS (pH 7.4) was added to disperse the HGNs. Carboxyl groups on the surface of the HGNs were activated with 1.0 μL 0.1 mM EDC and NHS solution for 1 h. After centrifugation, the solution was replaced with PBS for coupling the antibody to the particles and the solution was then redispersed. Subsequently, 10 μL 1.0 mg/mL of the anti-CEA polyclonal antibody solution was added to the MGITC-labeled HGN colloid, and 10 μL 1.0 mg/mL anti-AFP polyclonal antibody solution was added to the XRITC-labeled HGN colloid. Each mixture was reacted overnight at 4 °C. After removing unreacted polyclonal antibody, each probe was dispersed in PBS and deactivated by 2.0 μL 2.5 mM ethanolamine for 3 h. Each HGN colloid was washed once.

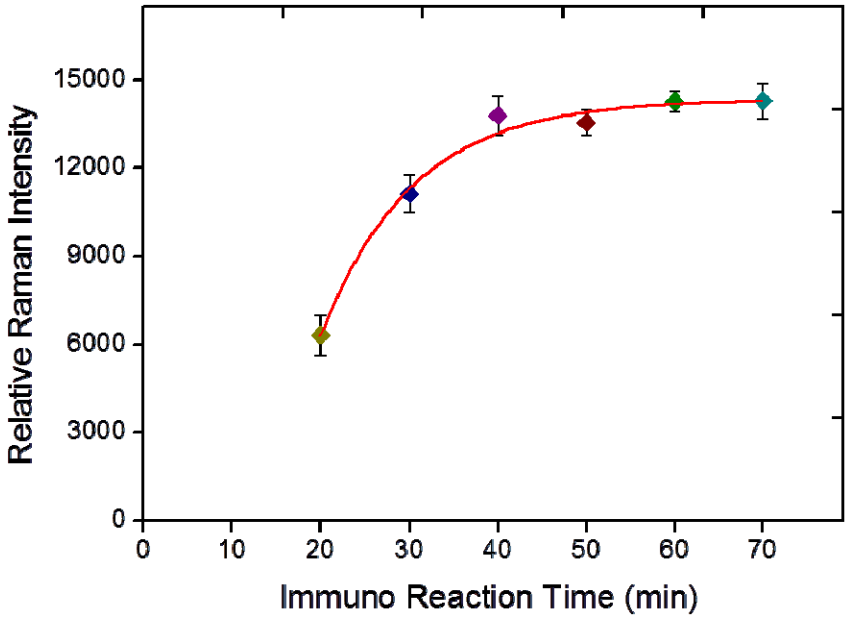

Figure S1. Monitoring of Raman intensity for the 1:1 mixture between CEA and AFP at room temperature. This experiment was done to find an optimal reaction time.

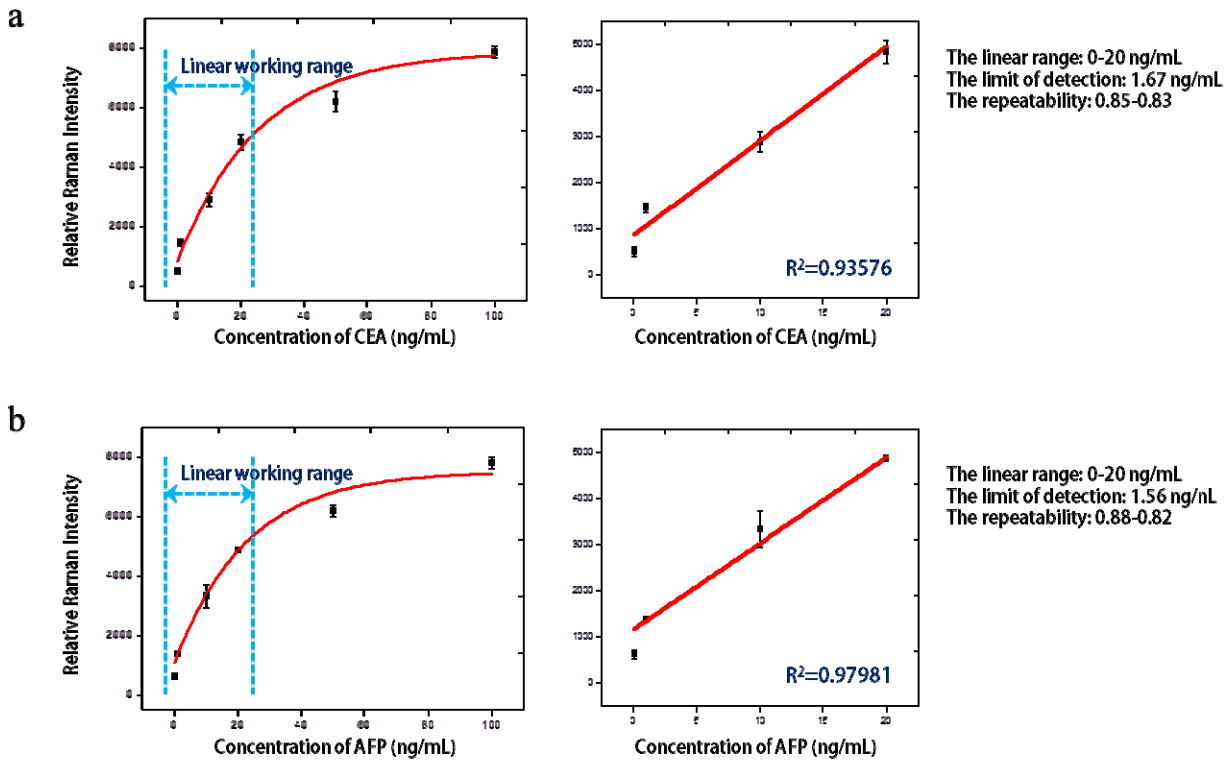

Figure S2. SERS-based assay curve for (a) CEA and (b) AFP in FBST for known concentrations.

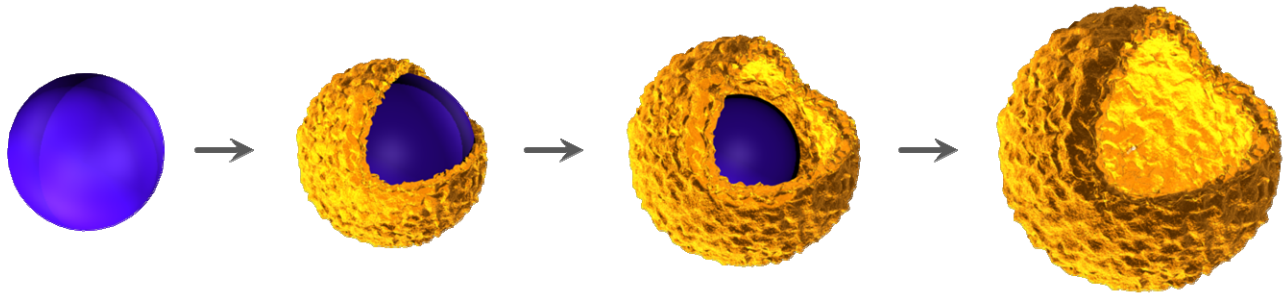

Figure S3. Schematic illustration of synthesizing HGNs for SERS immunoassay. A hollow interior is formed by dissolving the cobalt nanoparticle in the core.