Supporting Information

Femtogram Level Competitive Immunoassay of Mercury (II) Based on Surface-enhanced Raman Spectroscopy

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1. Production and characterization of monoclonal antibody against Hg(II)

The details of the production and characterization of monoclonal antibody against Hg(II) were described in reference. [1]

2. Preparation of the MBA-labeled immunogold nanoparticles.

The gold nanoparticles were prepared according to Frens' method.[2] In a typical process, 100 mL of 0.01% $HAuCl_4$ aqueous solution was heated to boiling with vigorous stirring, to which 1 mL of a 1% trisodium citrate solution was added. The mixture was then kept boiling for 30 min. Afterward, the solution was allowed to cool to room temperature with continuous stirring. The resulting red gold colloidal was about 30 nm in diameter.

The Raman reporter-labeled immunoassay gold nanoparticles were prepared by following a procedure reported by Ni et al. with a slight modification.[3] A total of 2.5 μL of 1mM probe molecule (4-mercaptobenzoic acid, MBA) in ethanol was added to 1.0 mL of gold nanoparticles, and the resultant mixture was allowed to shake for 1 h. The reporter-labeled nanoparticles were then separated from the solution by centrifugation at 5000 g for 10 min and resuspended with 1.0 mL of borate buffer. Next, 15 μL of 1mg/mL mAb against Hg(II) was added to 1.0 mL of MBA-labeled

gold nanoparticles with gentle agitation. After incubation at room temperature for 1 h, the MBA-labeled immunogold nanoparticles were purified by centrifugation and resuspended with 1.0 mL of borate buffer. Then 10 μL of BSA (5%) was added to the above MBA-labeled immunogold nanoparticles to make sure that no bare sites on gold nanoparticles were left to eliminate nonspecific binding sites and to prevent the possible formation of Hg-Au alloys in presence of Hg(II) in citrate solution [4]. The mixture was incubated for 1 h at room temperature and then centrifuged and resuspended in 1.0 mL of borate buffer.

3. **Immobilization of coating antigen on the substrate.**

The substrates were microscopic glass slides coated with multiple layers of materials as described below and were donated by FullMoon BioSystems [5]. The slide surface was first coated with a buffer layer of Ni-Cr using a vacuum deposition process and then coated with a thin layer of silver. After being activated, the surface was covered with a polymer layer, which contains specifically designed functional groups (-COOH) that can bind to the -NH_2 groups of coating antigen. This particular binding arrangement allows coating antigen to be erected on the surface without compromising their biological activities.

Next, 50 μL of 500 ng/mL (i.e. 25ng) coating antigen was dropped onto the substrate. After being placed in a chamber with a relative humidity of 65-75% for over 12 h, the substrates were allowed to dry at room temperature for 30 min. The substrates were then incubated in 5% BSA for 1 h to block nonspecific active sites, rinsed with water, and dried under nitrogen.

4. Optimization of assay conditions.

To establish the SERS based competitive immunoassay, the optimal concentrations of coating antigen and the MBA labeled immunogold were determined. The SERS signals of the coating antigen at the concentrations of 5000, 500, 50 and 5 ng/mL were shown in Figure S1. It was seen that when the coating antigen at 500 ng/mL, the ratio of B_0/B was the biggest (B_0 and B was Raman signal of Hg(II) at zero concentration and at 10 ng/mL, respectively), thus the coating antigen at 500ng/mL was selected.

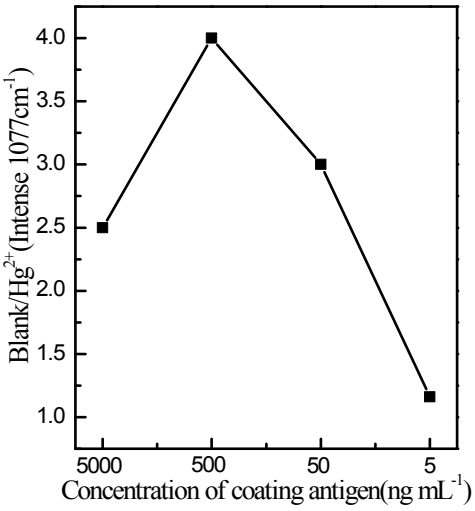

Figure S1. The optimization of coating antigen conditions.

At the conditions that MBA labeled immunogold was employed at the dilution of 1:5, 1:10, and 1:20, the SERS signals obtained at 0 ng/mL and 10 ng/mL of Hg^2 were shown in Figure S2. After comprehensively considering the values of B_0/B and SERS signals, we think the 1:10 dilution of MBA labeled immunogold to be used in the assay is appropriate.

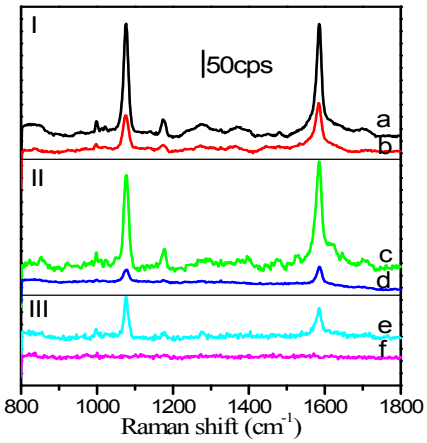

Figure S2. The optimization of MBA labeled immunogold. The SERS signals at dilution of MBA labeled immunogold at 1:5, 1:10 and 1:20 were shown in I, II and III. a, c, e are the signals at 0 ng/mL Hg(II) and b, d, f are the signals at 10 ng/mL Hg(II).

5. The effect of coating antigen on the assay

Because some amino-acid residues in the protein of the coating antigen might be Raman active species, thus whether the coating antigen (i.e. antigen-protein conjugate) will produce somewhat background SERS signals should be investigated. We prepared two conjugates, e.g. MBA-gold nanoparticles and mAb-gold nanoparticles,

and applied them to SERS procedures. As shown in Figure S3, there was no Raman signals appeared in these two cases, which indicated that the amino-acid residues in the coating antigen made no contribution to the Raman signals, e.g. all signals in the SERS based immunoassay were generated from MBA bound on immunogold.

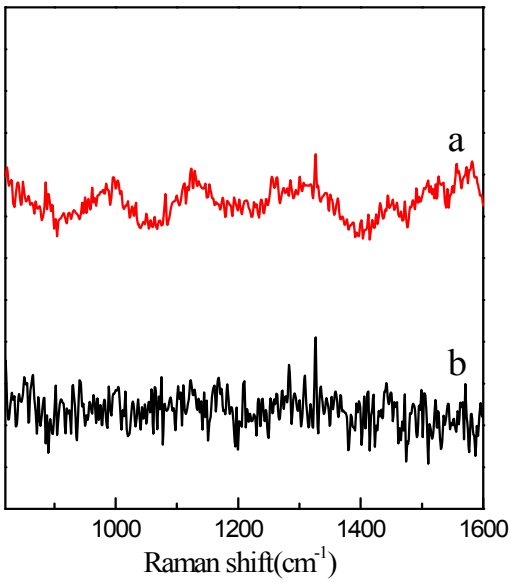

Figure S3. The effect of coating antigen of the assay. (a) the SERS signal when mAb-gold nanoparticles was applied; (b) the SERS signal when MBA-gold nanoparticles was applied.

6. The specificity of the SERS based immunoassay

Besides Hg(II), six other species including Pb(II), Cd(II), Ag(I), MNA, $CH_3Hg(I)$ and CH_3HgMNA were applied to SERS immunoassay. As shown in Figure S4, the SERS signals of Pb(II), Cd(II), Ag(I), MNA, $CH_3Hg(I)$ and CH_3Hg-MNA at concentration of 100 ng/mL (c-h curves) are almost the same as that when Hg(II) is at 0 ng/mL (a curve). When Hg(II) is at 100 ng/mL (b curve), the signal is greatly decreased. The SERS spectra of the cross reaction tests with Pb^{2+},Cd^{2+},Ag^{+},MNA, CH_3ClHg and MNA-CH_3ClHg are shown as Figure S4b. (For Pb^{2+},Cd^{2+} and Ag^{+}, a:0.1ng/mL; b:1ng/mL; c:10 ng/mL; d:100 ng/mL; For MNA, CH_3ClHg and MNA-CH_3ClHg, a:1ng/mL; b:100 ng/mlL).

The results clearly indicated that the SERS based immunoassay is highly specific to Hg(II).

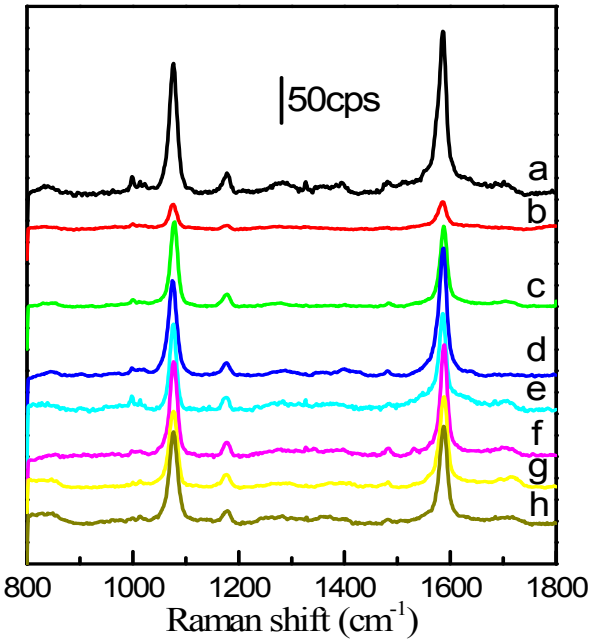

Figure S4a. The SERS signals of Hg(II) and cross-reactivity testing compounds. (a) 0 ng/mL of Hg^{2+}; (b-h) 100ng/mL of Hg^{2+}, Ag^{+}, Cd^{2+}, Pb^{2+}, MNA, $CH_3Hg(I)$ and CH_3Hg-MNA.

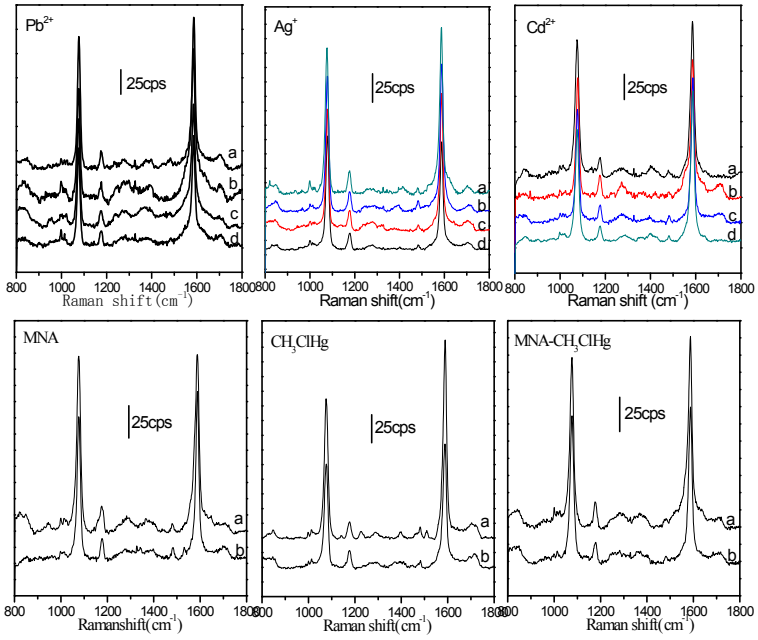

Figure S4b The SERS spectra of the measurements on cross reaction with different species. For Pb^{2+}, Cd^{2+} and Ag^{+}, a:0.1ng/mL; b:1ng/mL; c:10 ng/mL; d:100 ng/mL; For MNA, CH_3ClHg and MNA-CH_3ClHg, a:1ng/mL; b:100 ng/mlL.

7. The comparison of the sensitivity achieved from different analytical methods for the detection of Hg(II)

In the last years, many analytical methods have been reported for the detection of Hg(II). The comparison of the sensitivity achieved from different analytical methods for the detection of Hg(II) is summarized in Table S1.

Table S1 The comparison of the sensitivity achieved by different analytical methods for the detection of Hg(II)

Analytical method	Limit of detection
Colorimetry	1 nM [6]
Colorimetry	100 nM [7]
Colorimetry	50 nM [8]
Colorimetry	4.5 nM [9]
Colorimetry	20 bbp [10]
Fluorescent/Colorimetric	1 bbp [11]
Fluorescence detection	3 pM [12]
Fluorescence detection	32 nM [13]
Fluorescence detection	10 nM [14]
Fluorescence detection	80 nM [15]
Fluorescence detection	50 nM [16]
Fluorescence detection	10 nM [17]
Fluorescence detection	1ppb [18]
UV-vis	19 nM [19]
Resonance scattering	1.3 nM [20]
Hyper-Rayleigh Scattering	5 bbp [21]
Electrochemical	100 pM [22]
SERS	0.34 nM [23]
SERS based immunoassay (this work)	0.4 pM

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