SERS Active Gold Nanostar Dimer for Mercury Ion Detection

EXPERIMENTAL SECTIONS

Material

Thiolated DNA oligonucleotides, purified by high performance liquid chromatography (HPLC), were manufactured by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, P.R. China). These were dissolved in deionized (DI) water to give a final concentration of 100 μM. Unless stated otherwise, all other chemicals used in this work were purchased from Sigma-Aldrich. DI water from a Milli-Q device (18.2 M Ω, Millipore, Molsheim, France) was used in all experiments. Cu\(^{2+}\), Hg\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Cr\(^{3+}\), Mn\(^{2+}\), Co\(^{2+}\), Fe\(^{3+}\), Zn\(^{2+}\), Al\(^{3+}\), Mg\(^{2+}\), and Ca\(^{2+}\) (1000 µg mL\(^{-1}\) in 1% HNO\(_3\) or 5% HCL) were purchased from the National Institute of Metrology P.R China (Beijing, China).

The detailed sequences of the oligonucleotide are:

DNA 1: 5’-SH-AAAAAGTGACCATTTTTGCAGTG-3’
DNA 2: 5’-SH-AAAAAACACTGCTTTTTGTGCAC-3’

Instrumentation

Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100 operating at an acceleration voltage of 200 kV. The size distribution of the GNS was measured using a Zetasizer Nano ZS system (Malvern). A 633 nm laser was used for the DLS characterization. All UV-Vis spectra were acquired using a UNICO 2100 PC UV-Vis spectrophotometer and processed with Origin Lab software. Raman spectra were measured using a LabRam-HR800 Micro-Raman spectrometer with Lab-spec 5.0 software attached to a liquid cell. The slit and pinhole were set at 100 and 400 mm, respectively, in the confocal configuration, with a holographic grating (600 g/mm) and an air-cooled He-Ne laser giving 785 nm excitation with a power of ~ 8 mW.
Gold Nanostar synthesis

Gold nanostars were synthesized by a seed-mediated growth method. Initially, the gold seed was prepared by adding 15 mL of 1% citrate solution to 100 mL of boiling 1 mM HAuCl₄ solution under vigorous stirring. After cooling to room temperature, 200 µL 1-2 mM AgNO₃ (for 50nm GNS is 1mM and 2 mM for 60 nm GNS) and 100 µL 0.1 M ascorbic acid were mixed together quickly into 20 mL of 0.25 mM HAuCl₄ with 200 µL seeds, the pH is kept at 3. The colloidal solution was resuspended into 0.05% tween-20 by centrifugation at 3000 rpm for 15 min to prevent further reaction.

Preparation of ssDNA-Functionalized GNS

Briefly, 2 µL 100µM thiolated modified DNA 1 or DNA 2 solution was added to 100 µL of just prepared GNS and incubated for 3h at ambient temperature. Subsequently, 0.05 M NaCl was mixed and then the mixture was incubated for 12 h with constant shaking. The excess DNA was removed by two centrifugations at 3000 rpm for 10 min. The mixtures were denoted as GNS-DNA 1 and GNS-DNA 2.

Synthesis of GNS dimer structure

To prepare 50 nm GNS dimers, 50 nm GNS-DNA 1 and GNS-DNA 2 were mixed with a ratio of 1:1. A similar procedure was used for the 60 nm GNS dimer. The heterodimers were formed by adding equal quantities of 50 nm GNS-DNA 1 to 60 nm GNS-DNA 2. Then, 4-ATP ethanol solution, with a final concentration of 1 µM, was added to all three mixtures and incubated at room temperature overnight. After redispersion in DI water, Hg²⁺ solution, with a final concentration 1 ng mL⁻¹, was mixed with all three samples and shaken for 3 h.

Fabrication of GNS sensors

800 µL 60 nm GNS-DNA 1 and GNS-DNA 2 in the ratio of 1:1 were mixed with 1 µM 4-ATP ethanol solution and incubated for 12 h with constant shaking. This mixture is denoted as the sensor solution. Samples inoculated with different concentrations of Hg²⁺ (0, 0.5, 0.1, 0.05, 0.01,
0.005, 0.002 ng mL$^{-1}$) were separately added to 100 μL of sensor solution in separate tubes. After reacting for 3 h, the samples were processed by TEM, UV-Visible spectrophotometry and SERS.

**Figure S1.** Uv-vis spectrum of different GNS dimers and particles (A), DLS of 50, heterodimer, 60 nm GNS sensor before and after addition 1 ng mL$^{-1}$ Hg$^{2+}$ (B, C, D).

**Figure S2.** Uv-vis spectrum of 60 nm GNS dimer sensors in diverse Hg$^{2+}$ samples (A), SERS intensity of different heavy metal irons based on 60 nm GNS dimer sensor (B)
Figure S3. Lower magnification images of GNS dimer sensor with different Hg$^{2+}$ addition
(A) 0 ng mL$^{-1}$, (B) 0.005 ng mL$^{-1}$, (C) 0.01 ng mL$^{-1}$, (D) 0.05 ng mL$^{-1}$