Supporting Information

Gold nanorod assembly based approach to toxin detection by SERS

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Experimental Section

1. Materials and Apparatus.

Hydrogen tetrachloroaurate (III) hydrate, thioctic acid, 4-aminothiophenol and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich and used as received. MC-LR was purchased from Express Technology Co., Ltd. (China). All other reagents were available commercially. The antibodies to MC-LR were prepared in our laboratory following immunization of New Zealand rabbits (Nie et al., 2008). The antigen were prepared by coupling MC-LR to ovalbumin (OVA) using the glutaraldehyde (GDA) coupling method. Milli-Q ultra-pure water (18.2 MΩ) was used in all experiments.

UV-vis spectra were obtained using the Unico 2100PC UV-vis instrument. Transmission electron microscopy (TEM) was performed using a JEOL JEM-2100 transmission electron microscope. A typical sample was prepared by dropping 10 μL of the nanorods solution onto carbon/formvar coated copper grids. The grids were

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subsequently dried in air and imaged. Raman spectra were recorded 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 a confocal configuration with a holographic grating (600 g/mm) and an air-cooled He-Ne laser for 632.8 nm excitation with a power of ca. 8 mW.

2. Modification of the MC-LR antibody and antigen.

Modification of the antibody (m-Ab) to MC-LR and antigen (MC-LR-OVA) (m-Ag) were prepared as follows: The carboxyl group of thioctic acid (TA) was activated by 1-ethyl-3- (3-dimethylaminopropyl)-carbodiimide (EDC) and linked with the amine group of the antibody and antigen. Briefly, 0.15 mL of TA ethanol solution (100 mM) was mixed with 0.2 mL of sulfo-N-hydroxysuccinimide (sulfo-NHS) (100 mM) and 0.2 mL of EDC (100 mM). After reacting for 15 min, the mixture was added dropwise to 2.08 mL of Ab (7.47 mg/mL, 48 μM) and mixed gently by inversion. This solution was reacted at room temperature for 4 h. After this, the conjugate was dialyzed against PBS (0.01 M, pH 7.4) for the next 3 days at 4°C, changing the dialysate three times each day. The m-Ab was stored at 4°C. The method of preparing m-Ag was similar to the method used to modify the antibody.

3. Synthesis of Gold Nanorods.

All glassware used was cleaned with freshly prepared aqua regia and rinsed thoroughly in H_2O prior to use. GNRs with an aspect ratio of about 3 were prepared from a slightly modified seed-mediated growth procedure described by El-Sayed and Murphy. Cetyltrimethylammonium bromide (CTAB, 99%) and sodium borohydride, L-ascorbic acid, hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4$) (99%), and silver

nitrate were all purchased from Sigma-Aldrich. Milli-Q ultra-pure water (18.2 MΩ) was used in all experiments.

Initially, 0.1 mL of a 5 mM $HAuCl_4$ solution was added to 1 mL of 0.20 M CTAB solution which was kept at a constant temperature of 28.0°C. Immediately, a deep orange color appeared. Then, 0.12 mL of freshly prepared 0.01 M $NaBH_4$ solution was quickly added to the solution and mixed by inversion. The solution was rapidly stirred for 2 min, and the color of the solution turned pale brown. Upon seed production, the GNRs were fabricated. 5 mL of 0.005 M $HAuCl_4$ was added to 5 mL of 0.2 M CTAB solution and then 4 mL of water was added, which was the GNRs growth solution. To this mixture, 65 µL of 0.1 M ascorbic acid was added followed by 0.125 mL of 0.01 M $AgNO_3$ solution which was mixed by inversion for about 2 min. The solution became colorless. Finally, 0.05 mL of seed solution was added with gentle mixing by inversion for about 20 s. The GNRs were used after 4 h.

4. Fabrication of gold nanorod probes.

The GNRs were centrifuged at 7500 rpm for 15 min in order to remove excess CTAB, Vc, $AgNO_3$ and small spherical particles. The GNRs were resuspended in an equivalent volume of 0.005 M CTAB solution (containing 0.1% SDS) at pH 3.8. The pH of the solution was adjusted using 1M HCl.

CTAB preferentially bound along the {100} facet of the longitudinal side of the nanorods. The {111} facets at the ends of the GNRs bound fewer CTAB, so they could conjugate the thiol-modified antibody and antigen. In order to conjugate antibody to GNRs, 0.5 mL of the redispersed GNRs solution was added dropwise into 1.00 mL of diluted antibody solution, which was prepared from 50 µL of modified Ab solution at 7.47 mg/mL. This solution was reacted at room temperature for 4 h with

gentle shaking. After that, the probe was subsequently collected following centrifugation at 6000 rpm for 10 min (3 times). The washing buffer was 0.005 M CTAB solution (containing 0.1% SDS) at pH 3.8. The GNRs were redispersed in 50 μL of washing buffer. The probe was stored at 4°C. Conjugation of the GNRs and modified antigen was carried out in a similar way. The only difference was that the concentration of modified antigen was prepared from a dilution of 60 μL of modified antigen solution at 5.0 mg/mL with 940 μL 0.005 M CTAB solution (containing 0.1% SDS) at pH 3.8.

Subsequently, 4-ATP ethanol solution (10 μM) was added to the GNR-Ab and GNR-Ag solutions and incubated for 12 h, respectively. After that, the GNR-Ab and GNR-Ag solutions were centrifuged and redispersed in 50 μL of washing buffer for the next step.

5. Competitive immunoassay of MC-LR using GNR-Ab and GNR-Ag.

A volume of 20 μL of GNR-Ag was mixed with 20 μL of MC-LR standard solution at different concentrations with gentle shaking for 5 min. To each mixed solution, a 20 μL volume of GNR-Ab was added and mixed well. Each mixture was reacted at 37°C for 10 min. The concentrations of the MC-LR standard were 0, 0.01, 0.05, 0.1, 1 and 5 ng/mL. After reacting, the sample was analyzed by TEM, UV-Visible spectrophotometry and SERS. A standard curve was established by plotting the Raman signal intensity against concentrations of MC-LR standard. The limit of detection (LOD) was calculated as LOD = 3.3SD/S, where SD is the standard deviation of the response and S is the slope of the calibration curve.

6. Finite integration technique (FIT) Simulations of Plasmonic Properties:

Field enhancement properties of nanorod assemblies were calculated taking advantage of recently developed Finite integration technique (FIT) method. An FIT package from CST STUDIO SUITETM 2010 utilizing the Drude model was used for the calculation in the vicinity of the plasmonic for media with electrical permittivity of ε=1 and magnetic permeability of μe=1. The end to end assemblies were modeled by 16 nm (diameter) x 50 nm (length) NR by a gap of 15 nm. The electrical permittivity (ε) and plasma frequency (ωp) of gold employed in the calculations were taken to be constant and equal to 1.0 and 1.37 x 1016 rad/s, respectively. The local electromagnetic fields were calculated for 632.8 nm excitation and parallel orientation of rods to the external field.

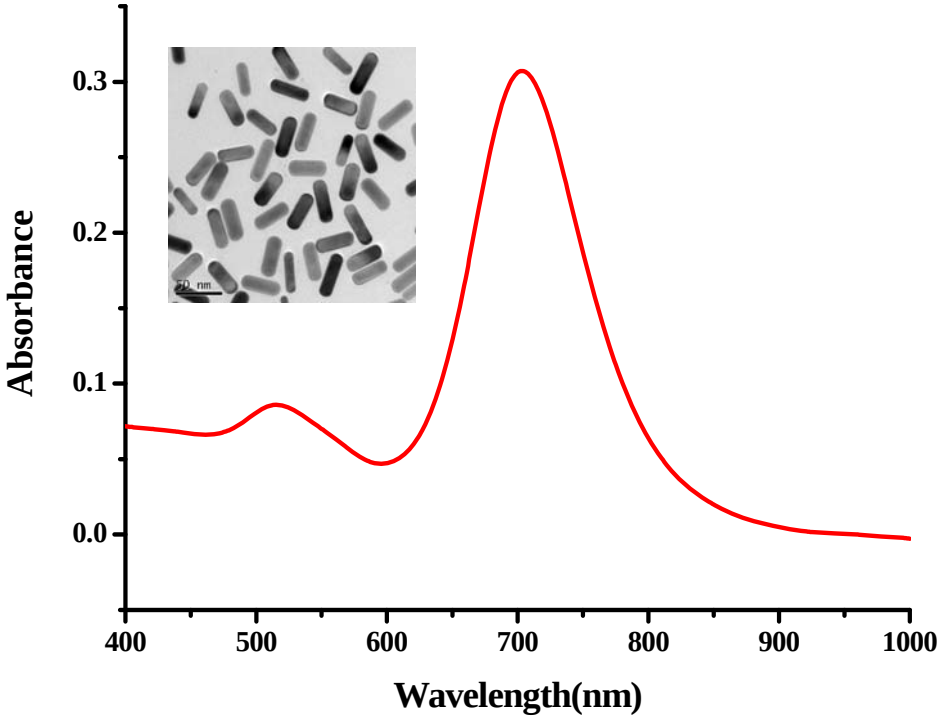

Figure S1. UV-Vis spectra of gold nanorods, representative TEM images of as-prepared gold nanorods. Scale bars represent 50nm.

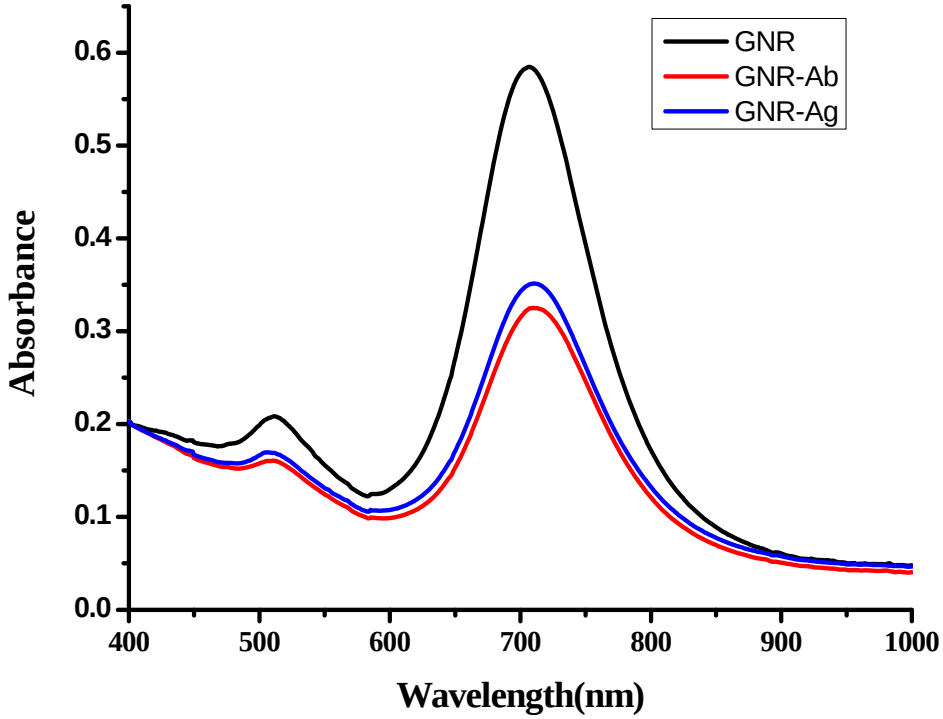

Figure S2. UV-vis spectra of gold nanorods, before and after the GNRs probes formation.

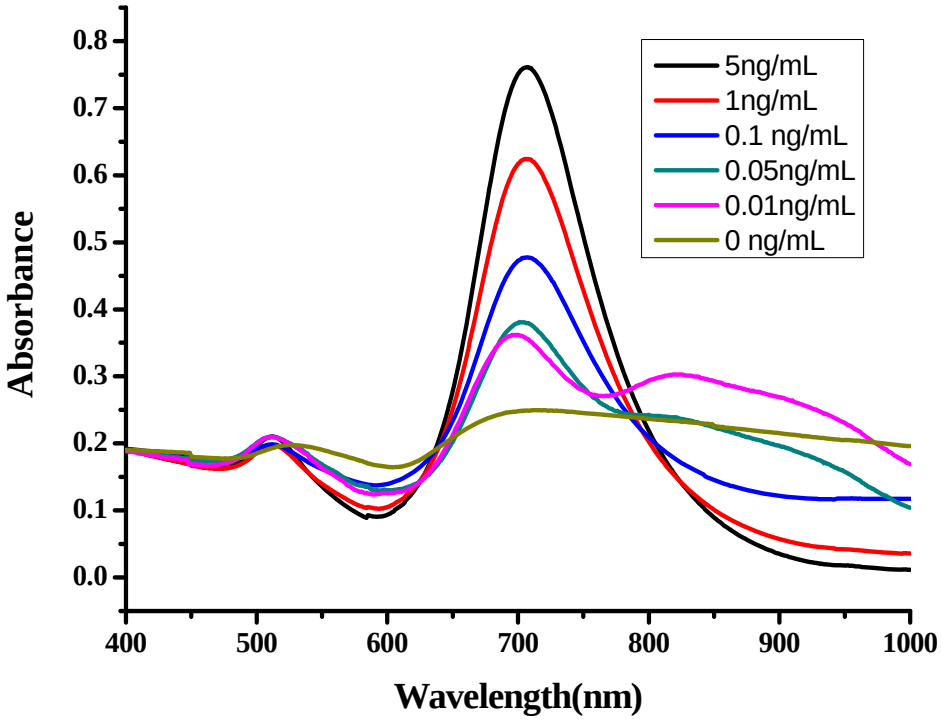

Figure S3. UV-vis spectra of assembly of GNRs in solution that includes different concentrations of MC-LR at 37 °C.

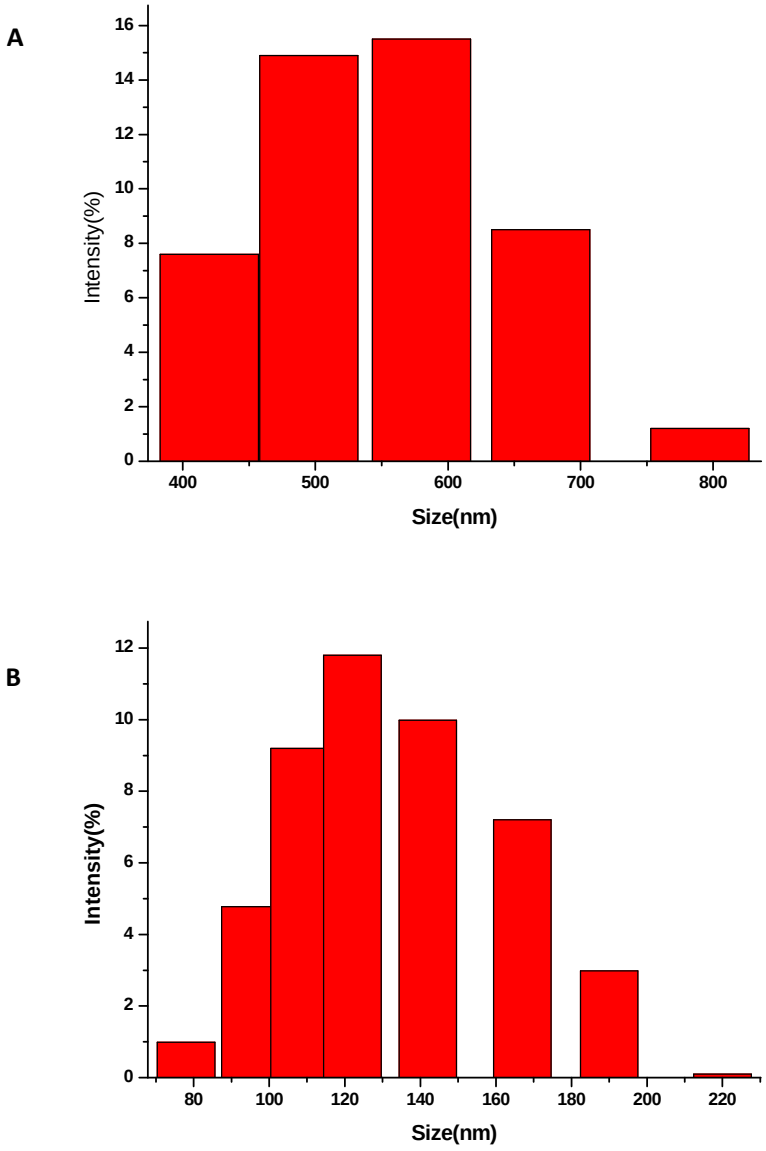

Figure S4. Typical size distributions of end-to-end assembly of Gold nanorods (A) without the addition of MC-LR and (B) with the addition of MC-LR at the concentration of 5.0 ng/mL.

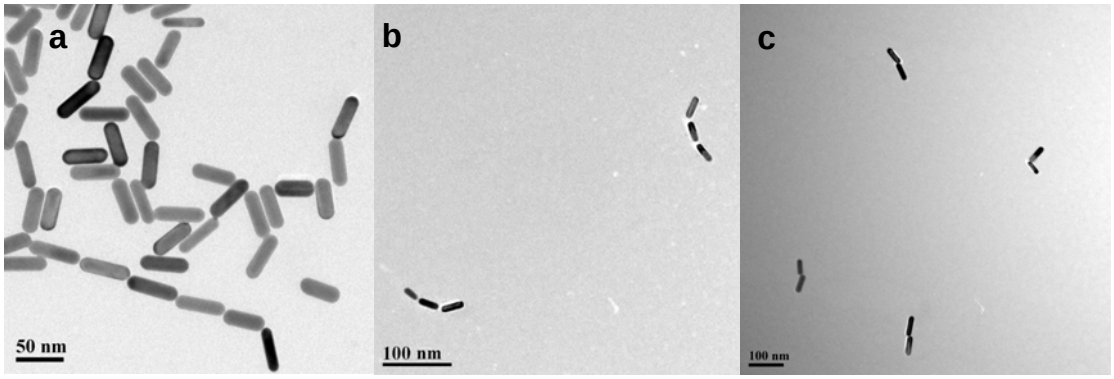

Figure S5. Lower magnification images of nanorods chains on addition of different concentrations of MC-LR. (a) 0ng/mL, (b) 1ng/mL, (c) 5ng/mL.

Table S1 Detection results of real water samples

Polluted water [a]	Original concentration (ng/mL)	Spiked concentration (ng/mL)	Detected concentration Mean±SD (ng/mL)	Recovery (%) Mean±SD[b]
1	0.27	0.1	0.34±0.03	93.21±3.08
2	1.06	0.2	1.22±0.04	101.23±3.53
3	0.76	0.5	1.32±0.04	105.19±2.89
4	1.04	1	1. 96±0.06	96.81±3.75
5	1.69	5.0	6.42±0.14	96.07±3.85

[a] Different origin of water samples from six different areas of the Tai Lake

[b] SD was calculated based on three experiment for each sample.

The detailed processes of the ELISA are as follows:

1. Coat each well in a 96-well plate (Costar #9018) with 100 μL of a coating antigen solution.
2. Cover and rock overnight in an incubator at 4 ℃.
3. Wash 3X with PBS-Tween 20 in vacuum-apparatus and pat dry.
4. The plate was blocked with 100μL (0.5%, w/v) OVA solution in PBS solution for 2 h at 37 ℃.
5. Wash 3X with PBS-Tween 20.
6. Add 100 μL/well MC-LR at different dilutions or samples with 100μL/well pAb then incubate for 0.5 hour at 37 ℃.
7. Wash 3X with PBS-Tween 20 and pat dry.
8. Dilute horseradish peroxidase-conjugated goat anti-rabbit IgG 1 : 3000 in PBS-Tween 20 for 0.5 hour and incubate as before.
9. Wash 6X as before and pat dry.
10. Prepare color substrate (TMB) and add 100 μL/well for 15 min in dark at room

temperature.

11. H_2SO_4 (2 mol/L) was added to stop the reaction and record the absorption in a micro plate reader at 450nm.