Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

## Au nanoflowers-Ag nanoparticles assembled SERS-active substrates for sensitive MC-LR detection

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**Fig. S7** (A) The representative SERS spectra of Au NFs-Ag NPs core-satellite assemblies for the detection of MC-LR samples from Table S3. (B) Statistical analysis of the SERS peak at 1330 $cm^{-1}$ under different concentration of MC-LR.

**Fig. S8** HPLC chromatogram for the detection of MC-LR in Tai lake water. (A) Tai lake water, (B) 1μM MC-LR in ultrapure water, (C) 4.5 μM MC-LR spiked in Tai lake water, (D) 9.0 μM MC-LR spiked in Tai lake water.

**Table S4.** Detection of MC-LR in Tai lake water using HPLC and this method.

## Experimental section

### 1.1 Synthesis and characterization of Au NFs

An aliquot of 1.25 mL 5 mM $HAuCl_4$ solution was added into 23.75 mL Millipore-Q water. The mixture was heated at 100 ºC for 5 min under vigorous stirring. An amount of 500 μL 1% sodium citrate was quickly added and the reaction was kept for 10 min. And then, an amount of 500 μL the above solution was added into 1 mL 0.1 M PBS containing 500 μL 1% PVP under vigorous stirring. An amount of 300 μL 10 mM $HAuCl_4$ solution and 300 μL 10 mM $NH_2OH$-HCl solution were synchronously added. After 2 h, the mixture was centrifuged at 2400 g for 5 min, and the precipitates were dissolved in 100 μL Millipore-Q water. The plasmonic property of Au NFs was determined by UV-vis spectrometer, and the detailed structures were characterized through transmission electron microscope (TEM) and scanning electron microscope (SEM).

### 1.2 Preparation of Ag NPs

14 ± 2.5 nm Ag NPs were synthesized according to the previous reported methods.[1] Briefly, an amount of 20 mL Millipore-Q water was mixed with 5 mL 1% PVP in an ice-water bath. An aliquot of 600 μL freshly prepared 0.1 M $NaBH_4$ solution was quickly added into the above solution. And then, 5 mL 1% PVP and 5 mL 10 mM $AgNO_3$ solution were added drop wise to the above solution under agitation. The suspension was kept at 80 ºC for 2 h to remove the excess $NaBH_4$. An aliquot of 2 mL Ag NPs was centrifuged at 9600 g for 10 min, and the precipitates were dissolved in 200 μL Millipore-Q water.

The size of Ag NPs was chosen according to the assembly steric hindrance. The small sized Ag NPs could be to the maximum assembled around the larger Au NFs, resulting in the formation of more “hot spot” and the enhancement of SERS signal. Ag NPs with the size below 14 ± 2.5 nm

were difficult to centrifugal. Most of the small Ag NPs would be lost during the assembled process due to the hard manipulation. Ag NPs with the size above 14 ± 2.5 nm were difficult to synthesize and showed irregular sphere structure. Bigger sized Ag NPs were not stable and may lead to aggregations when used for assembly.

### 1.3 Surface functionalization of Au NFs and Ag NPs

An amount of 100 μL Au NFs was used to react with 10 μL 10 μM probe 1 through Au-SH covalent bond.[2, 3] The molar ratio of Au NFs and probe 1 was about 1:100 (Table S1, ESI†). The mixture of Au NFs and probe 1 was incubated in 0.5× TBE buffer containing 50 mM NaCl. After 12 h, the solution was centrifuged to remove the unreacted probe 1. The precipitates were washed for three times and finally dissolved in 100 μL 1× TBE buffer. Similarly, an amount of 200 μL above-prepared Ag NPs was modified by probe 2 with the molar ratios of 1:10 according to the procedure described above. Ag NPs-probe 2 conjugates were further modified by 4-nitrothiophenolate (4-NTP) as Raman label. The final concentration of 4-NTP was 2 μM.

### 1.4 Fabrication of Au NFs-Ag NPs assembled SERS-active substrate

An amount of 50 μL Au NFs-probe 1 solution, 100 μL 4-NTP-Ag NPs-probe 2 solution and 100 μL 1 μM aptamers against MC-LR were mixed with 500 μL 1× TBE buffer. After 12 h, the solution was centrifuged at 2400 g for 5 min, and the precipitates were dissolved in 100 μL Millipore-Q water. Au NFs-Ag NPs core-satellite assemblies were fabricated depending on the formation of Y-shaped dsDNA hybrid configuration.

Au NFs-Ag NPs core-satellite assemblies employed as SERS-active substrates were mixed with 200 μL different concentration of MC-LR solutions, *i.e.*, 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 50 nM. The mixture was incubated in 20 mM Tris-HCl solution for 30 min at room temperature

and was centrifuged to remove the disassembled Ag NPs. The precipitates were finally dissolved and washed by 70% ethanol aqueous solution, in order to purify the residual Y-shaped dsDNA driven NP assemblies. SERS spectra of assemblies were determined through the LabRam-HR800 Micro-Raman spectrometer with the accumulation time of 10 s. SERS spectra were further managed through a Lab-spec 5.0 software. The surface charge density and hydrodynamic diameters of assemblies were measured through a Zetasizer Nano ZS system (ZetaPALS, Brookhaven Instruments Co. Ltd., UK).

**1.5 Evaluation of the selectivity and reproducibility of Raman aptasensors**

The selectivity of Raman aptasensors was determined in the presence of 5 nM other mycotoxin and proteins, involving MC-RR, aflatoxin B1 (AFB1), fumonisin B1 (FB1), ochratoxin A (OTA) and bovine serum albumin (BSA). The reproducibility of detection is investigated by detecting the different concentration of MC-LR standard solutions spiked in Tai lake water (Wuxi), including 0.10, 0.20, 0.50, 1.00, 2.00 and 5.00 ng/mL. The results were validated through HPLC equipped by UV array detector set at 238 nm. Applied analytical column was ultimate AQ C18 (4.6×250 mm). Volume of injected samples was 10 μL. The mobile phase was 36% methyl cyanide aqueous solution containing 0.05% trifluoroacetic acid. The flow rate was kept at 1.0 mL $min^{-1}$. Considering the low amount of MC-LR in Tai lake water and the LODs for HPLC method, different concentration of micromolar level MC-LR (0, 4.50, 9.00 μM) was spiked into the Tai lake water and was detected by HPLC. The samples were first diluted for 1000 times and then were detected by SERS-active substrates.

**Table S1.** Comparison of the LODs of different assay for MC-LR detection.

| Assay | MC-LR (pM) | Refs |
|---|---|---|
| Voltammetric Aptasensor | 1.9 | [4] |
| Surface-enhanced Fluorescence Immunosensor | 7.0 | [5] |
| Raman aptasensor | 8.6 | This work |
| Electrochemical Impedance Biosensor | 18.0 | [6] |
| Quartz Crystal Microbalance Sensor | 40.0 | [7] |
| Colorimetric Detection | 50.0 | [8] |
| Surface Plasmon Resonance Based Immunoassay | 190.0 | [9] |
| Immunosensor Based Nuclear Magnetic Resonance | 600.0 | [10] |
| Electrochemical Biosensor | 930.0 | [11] |
| Immunochromatographic assay | 1000.0 | [12] |

**Table S2.** The detail sequences of probes and aptamers of MC-LR.

| Name | Sequences (5´-3´) |
|---|---|
| Probe 1 | -SH-$(CH_2)_6$-CTG TGA CGG TAA TT |
| Probe 2 | -SH-$(CH_2)_6$-TGG TAT GGT CAC AG |
| Probe 3 | GCG GAG ATG GGG CAT AAT GAG GTG GTA TGG GTA ATT GTC ATG GTG GTC GTC TTT GGC GCC |
| Aptamers | GGC GCC AAA CAG GAC CAC CAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC |

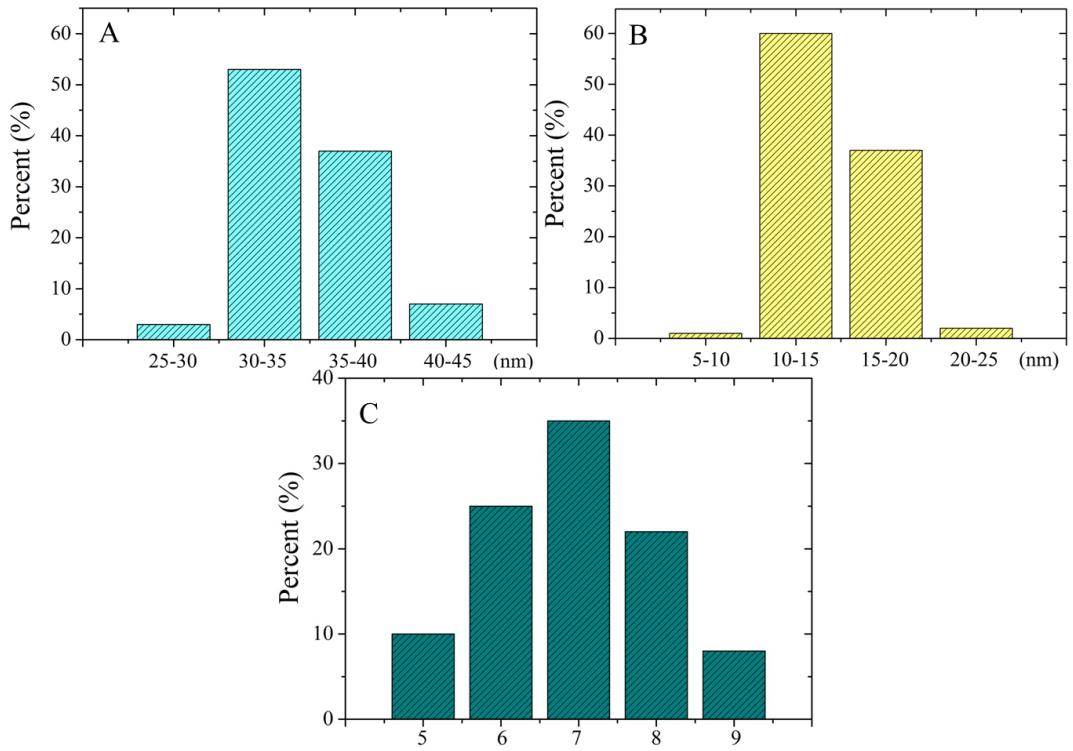


**Fig. S1** Statistical analysis of diameters for Au NFs (A), Ag NPs (B) and the number of Ag NPs on the surface of Au NFs (C).

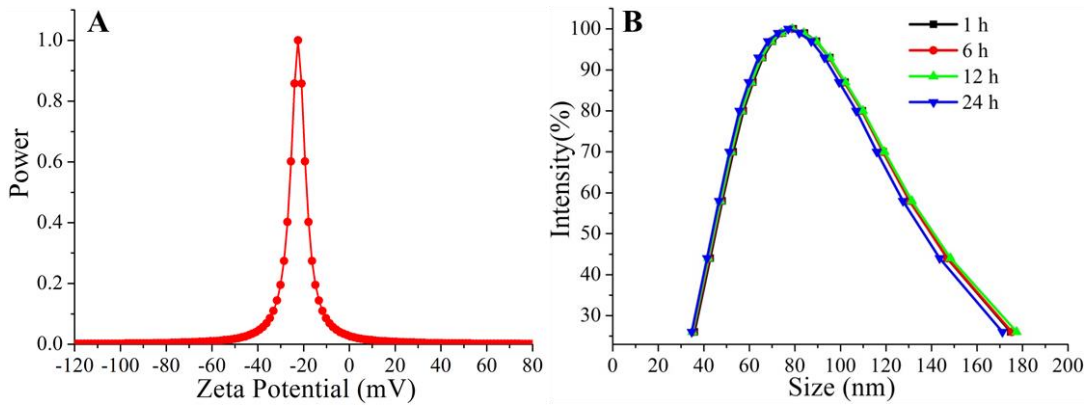


**Fig. S2** Zeta potential (A) and DLS spectra (B) of Au NFs-Ag NPs core-satellite assemblies.

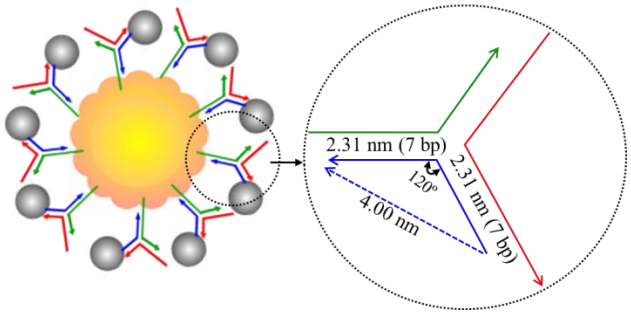


**Fig. S3** The enlarged Y-shaped DNA for the preparation of Au NFs-Ag NPs core-satellite assemblies. The distance between Au NFs and Ag NPs was calculated to 4 nm.

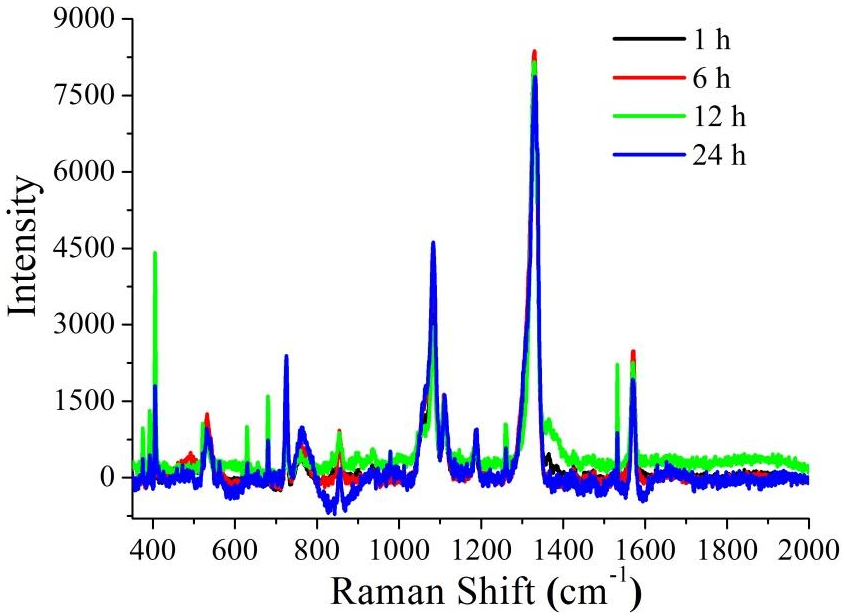


**Fig. S4** SERS spectra of Au NFs-Ag NPs core-satellite assemblies.

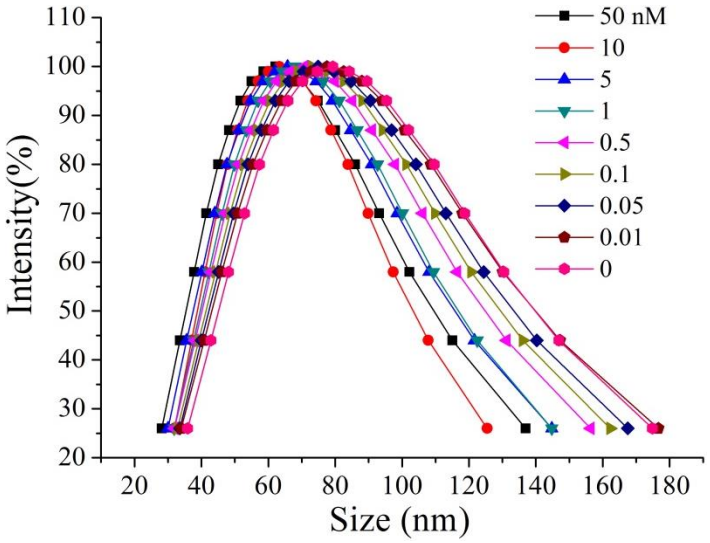


**Fig. S5** DLS data of Au NFs-Ag NPs core-satellite assemblies in the present of different concentration of MC-LR.

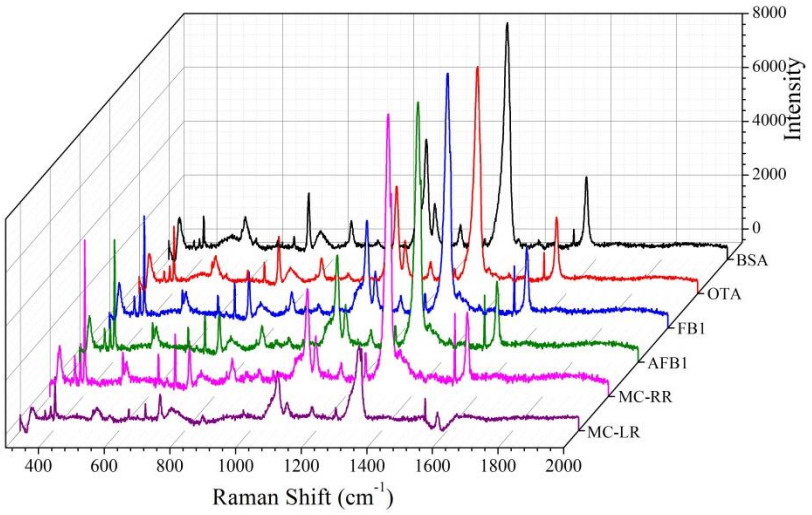


**Fig. S6** SERS spectra of Au NFs-Ag NPs core-satellite assemblies after embedding in 5 nM MC-LR, MC-RR, AFB1, FB1, OTA and BSA solution.

**Table S3.** Detection of MC-LR spiked in Tai lake water.

| Polluted water[a] | Original concentration (ng/mL)[b] | Spiked concentration (ng/mL) | Detected concentration (mean[c] ± SD[d], ng/mL) | Recovery (mean[c] ± SD[d]) |
|---|---|---|---|---|
| 1 | 0.58 | 0.10 | 0.65 ± 0.07 | 95.59 ± 2.85 |
| 2 | 0.73 | 0.20 | 0.89 ± 0.05 | 95.70 ± 2.63 |
| 3 | 0.26 | 0.50 | 0.74 ± 0.04 | 97.37 ± 3.07 |
| 4 | 0.33 | 1.00 | 1.28 ± 0.21 | 96.24 ± 3.18 |
| 5 | 1.54 | 2.00 | 3.38 ± 0.43 | 95.48 ± 3.42 |
| 6 | 1.06 | 5.00 | 5.92 ± 0.76 | 97.70 ± 3.56 |

[a]Polluted water samples from six different areas of Wuxi city.
[b]MC-LR was concentrated and then detected by HPLC.
[c]The mean of three experiments. [d]SD = standard deviation.

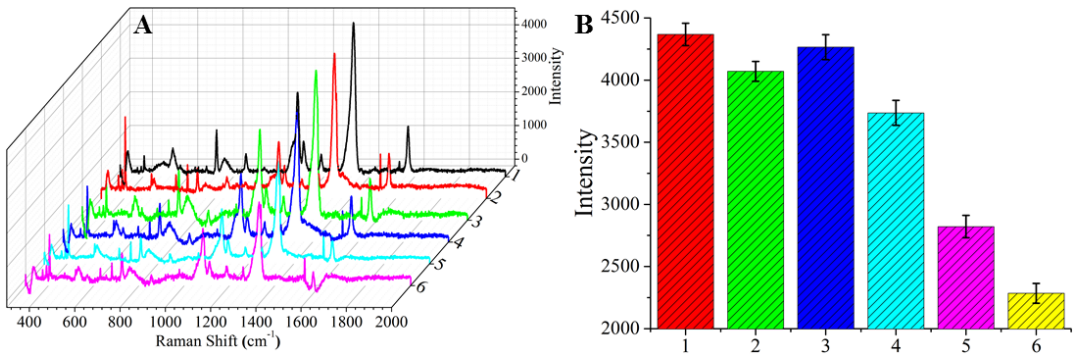


**Fig. S7** (A) The representative SERS spectra of Au NFs-Ag NPs core-satellite assemblies for the detection of MC-LR samples from Table S3. (B) Statistical analysis of the SERS peak at 1330 $cm^{-1}$ under different concentration of MC-LR.

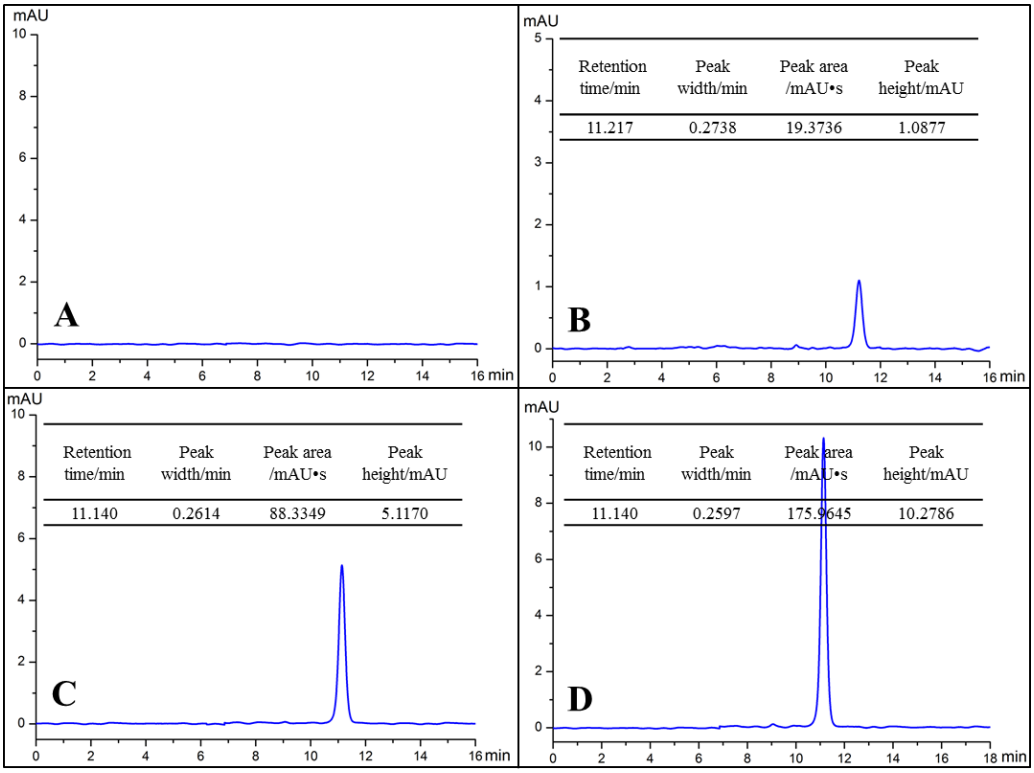


**Fig. S8** HPLC chromatogram for the detection of MC-LR in Tai lake water. (A) Tai lake water, (B) 1μM MC-LR in ultrapure water, (C) 4.5 μM MC-LR spiked in Tai lake water, (D) 9.0 μM MC-LR spiked in Tai lake water.

**Table S4.** Detection of MC-LR in Tai lake water using HPLC and this method.

| Samples | Spiked concentration (μM) | Detected concentration by HPLC (mean[a] ± SD[b], μM) | Detected concentration by this method (mean[a] ± SD[b], μM) |
|---|---|---|---|
| Tai lake water | 0 | 0 | $(0.58 \pm 0.15)\times10^{-3}$ |
| | 4.50 | 4.56 ± 0.62 | 4.52 ± 0.24 |
| | 9.00 | 9.08 ± 1.27 | 9.03 ± 0.57 |

[a]The mean of three experiments. [b]SD = standard deviation.

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