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Supporting Information

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Side-by-Side and End-to-End Gold Nanorod Assemblies for Environmental Toxin Sensing**

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Supporting Information

Zeta Potential Measurements and Data

The corresponding zeta potential, ξ, results confirm the conjugation of ABs to NRs in both cases. In the electrostatic attachment method, the zeta potential of the NRs changed from ξ =+31.6±2.1 mV (pH 8.6) to ξ =+18.3±1.6 mV after the partial neutralization of NR surface charge by the negatively charged ABs (ξ =-11.3±1.7 mV) in the side-by-side way. Concurrently, in the end-to-end model, the zeta potential of NRs which were conjugated to ABs covalently, demonstrated only very modest change in zeta-potential from +42.7±1.8mV (pH 3.8) to ξ = +34.7±1.6mV of the TA-modified NRs and then to ξ =+29.3±1.3 mV after the conjugation of the ABs.

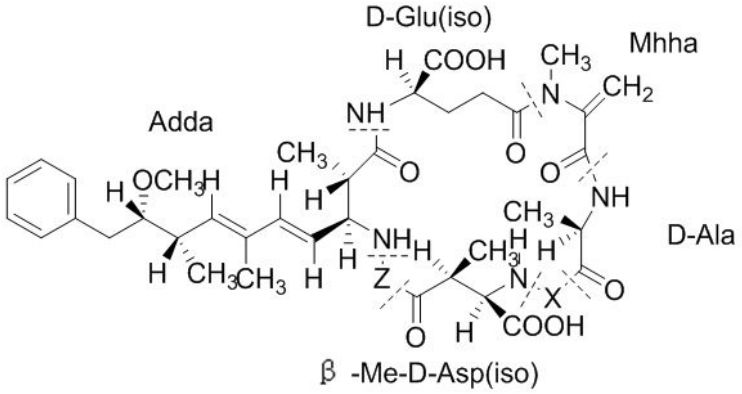

Figure S1. The chemical structure of the MC-LR toxin.

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a **b**

Figure S2. Comparison of UV spectra for monomer and assembled NRs in cases of side-by-side (a) and end-to-end (b) motifs.

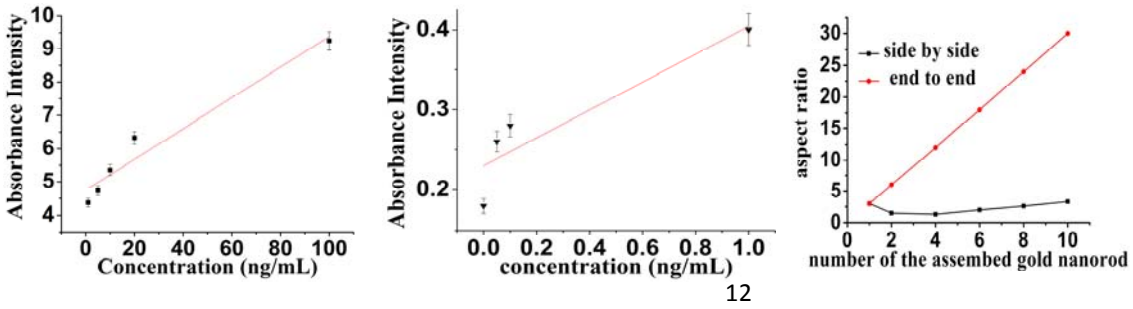

Figure S3. The calibration curve of the MC-LR detection based on the (a) side-by-side (b) end-to-end motifs of the assembly (c) calculations of the aspect ratio of gold NR in case of side-by-side and end-to-end assemblies.

The determination of the number of Abs n the surface of the Au NRs:

Different aliquots of Au NR solution were added drop-wise into 50 μL of 100μg/mL solution of AB. The mixture was stirred for 10 min and shaked gently for 30 min at room temperature, followed by incubation at 37°C for 30 min. Then the NR-AB monomers were purified by centrifugation for 15min at 6500g and 2 times washes with 1mL of washing buffer. The supernatant solution was collected and measured by UV-vis and used as the criterion according to the empirical equation:

$$C_{AB}(\mu g/mL)=183\ A_{230nm}-78.5\ A_{260nm} \quad (1)$$

The UV-vis spectrum of the supernatant decreased with the adding of the Au NR into

the solution. Untill the added volume of the Au NR is 40μL, the value of the calculated absorption is constant. In order to ensure the saturation of the antibody on the surface of the Au NR, 50 μL is adopted as the optimal value for the following research. According the above equation, the calculated concentration of the AB in solution which are present on the surface of the Au NR is ca. 0.93 μg/mL. Considering that the average molecular weight of the AB is about 15kD and the concentration of Au NR is 0.2nmol/mL and the TEM dimensions (Fig. 2), the approximate number of ABs per one NR for side-by-side modification is N~ $(C_{AB(ug/mL)}/M_{AB(g/mol)})/C_{Au\ NR}(nmol/mL) = (0.93*1*10^{-6}/\ 1.5*10^{5})\ /(0.2*10^{-12}) = 31$. The similar measurements and calculations for the NRs for end-to end assembly leads to N ~ 10.

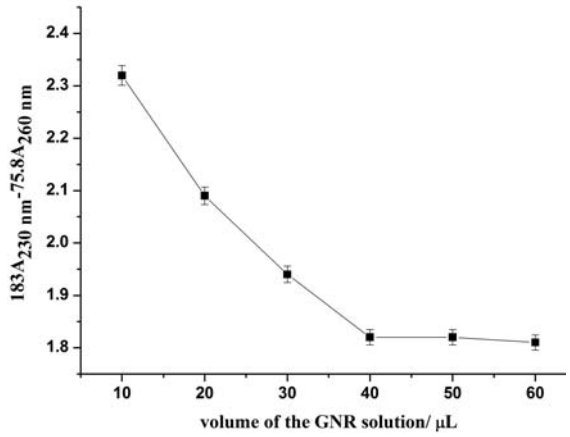

Figure S4. Quantification of the AB content on NRs using supernatant after modification of the NRs for side-by-side assemblies.

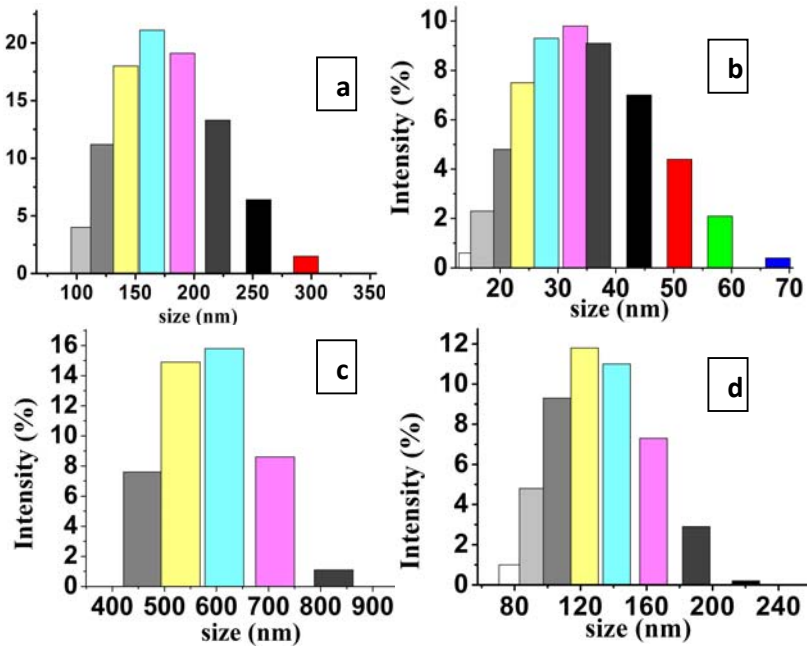

Figure S5. Additional DLS data for the side-by-side and end-to-end NR assembly motifs. (a-b): Detection with the side-by-side assembly. (a) Size distribution at 0 ng/mL of MC-LR (b) Size distribution at 100 ng/mL of MC-LR. (c-d) Detection with the end-to-end assembly. (c) Size distribution at 0 ng/mL of MC-LR. (d) Size distribution at 100ng/mL of MC-LR .

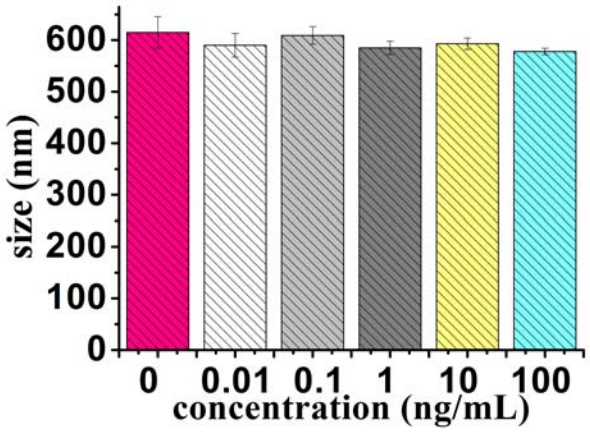

Figure S6. The hydrodynamic diameter of the assembled aggregates at different concentrations of control toxin (ochratoxin A).

Experimental Procedures and Methods

Synthesis of Au NRs: All glassware used was cleaned with freshly prepared aqua fortis and rinsed thoroughly in H_2O prior to use. NRs with aspect ratio about 3 were prepared from a slightly modified seed-mediated growth procedure. [44-45]

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

Preparation of the side-by-side monomers: The NR with electrostatically

attached polyclonal MC-LR antibody to the sides. [46-47] Briefly, the pH of NR solution was adjusted to ~8.5-8.8 500μL of this solution was added drop-wise into 500 μL of 100μg/mL solution of AB to MC-LR. The mixture was stirred for 10 min and shaked gently for 30 min at room temperature, followed by incubation at 37°C for 30 min. Then the NR-AB monomers were purified by centrifugation for 15min at 6500g and 2 times washes with 1mL of washing buffer (washing buffer : pH 7.4 0.01M PBS included 0.1% PEG 20000). Finally, the NR-AB were re-dispersed in the same buffer (500μL) and stored at 4°C.

NR-ML-LR-OVA probes were prepared by the similar way used for NR-AB. The main difference was pH control of the solution. The conjugation process was carried out by adding 500μL coating antigen to the appropriate NR solution (500μL) and adjusted to pH ~7.0-7.2. The subsequent processing was the same as for the preparation of NR-AB.

Preparation of the end-to-end monomers: The carboxyl group of the thioctic acid (TA) was activated by EDC and linked with the amine group of the AB and MC-LR-OVA. Briefly, 0.3mL of ethanol (containing 1.625mg of TA) was mixed with 0.4mL of AB. 75μL of EDC was added dropwise to the mixture by gentle mixing by inversion and reacted for 4 h at room temperature. After the reaction, 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 everyday. The modified AB was stored at 4°C. The method to conjugation of MC-LR-OVA was the same.

The NRs were centrifuged at 7500 rpm for 15 min in order to remove the excess of ascorbic acid, $AgNO_3$ and small spherical particles. The gold NRs were resuspensed in an equivalent volume of 0.005M CTAB (pH 3.8) containing 0.1%PEG solution. The pH of the solution was adjusted by 1M HCl. The redispersied NRs were used in all of the flowing experiments and characterizations. In order to conjugate NRs and antibody, 0.5mL of the redispersed NRs solution was added dropwise into a 1.00 mL of diluted AB solution at 7.47 mg/mL. The mixture reacted at room temperature for 4 h with gentle shaking. After reaction, the conjugated NRs were collected by centrifugation for 10 min at 6000 rpm for 3 times. The washing buffer is 0.005M CTAB containing 0.1% PEG solution. The modified NRs were re-dispersed in 50μL of 0.005M CTAB containing 0.1% PEG solution and stored at 4^0C. The

conjugation of the NRs and MC-LR-OVA followed the same protocol. The only difference was the concentration of MC-LR-OVA, which was prepared by dilution of 60μL of MC-LR-OVA solution at 5.0 mg/mL with 940 μL of 0.005M CTAB (pH 3.8) containing 0.1% PEG solution.

Instrumental Analysis. The characterization of TEM was obtained with FEI Tecnai G2 (FEI Co. Ltd., USA). The size determination was read out by the Zetasizer Nano ZS system (Malvern Co. Ltd., UK). The 633 nm laser was adopted for the DLS characterization. The DLS data was processed using the DTS5.1 software. All UV-vis results were recorded with the Unico 2800 PCS UV-vis instrument (Beijing, China) and processed with the OriginLab software.

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 ^{0}C.
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 at 37 ^{0}C for 2 h.
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 at 37 ^{0}C for 0.5 hour.
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 at room temperature for 15 min in dark.
11. H_2SO_4 (2 mol/L) was added to stop the reaction and record the absorption in a micro plate reader at 450 nm.

The samples for the determination by the developed method in this manuscript were spiked with the MC-LR stock solution at different concentrations into the negative water samples. Then these samples were added in the NRs probes system and cultured for 15 min. Then, the DLS and UV-vis were recorded respectively.