

Disassembly of gold nanoparticle dimers for colorimetric determination of ochratoxin A

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Experimental Details

Materials and Reagents. All oligonucleotides and modifications were conducted by Sangon Biotech Co., Ltd. (Shanghai). Detail sequences and modifications of the oligonucleotides were shown in Table S1. Sodium citrate, poly(ethylene glycol) (average M_n 8,000), poly(ethylene glycol) 2-thioethyl ether acetic acid (thiol-PEG-acid, average M_n 1,000), gold(III) chloride trihydrate (99.9%), ochratoxin (OTA), and ochratoxin B (OTB) were obtained from Sigma-Aldrich. MaxSignal[®] Ochratoxin A ELISA Test Kit was purchased from Bioo Scientific Corporation (Austin TX, USA). Tris(hydroxymethyl)aminomethane (Tris) and Ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Aladdin (Shanghai, China). All other reagents were of analytical purity and used as received. Ultrapure water used in all experiments was obtained from a Milli-Q water purification system ($\geq 18\text{M}\Omega$).

Oligonucleotides stock solutions (100 μM) were prepared in TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and diluted to the requested concentrations with ultrapure water shortly before use. 10 \times binding buffer for OTA: 100 mM Tris, pH 8.5, 1.2 M NaCl, 50 mM KCl, and 0.2 M CaCl_2 .

Apparatus. Scanning electron microscopy (SEM) images were obtained using an FE-SEM (Nova NanoSEM 230, FEI, USA). The samples were prepared by dropping the AuNP solution on silicon wafers and were observed under FE-SEM without coating. All absorbance spectra were measured with a portable Maya2000 Pro UV-Vis spectrometer (Ocean Optics, Dunedin, Florida, USA). The spectra were taken in a transmission mode using a pair of optical fiber bundles. The concentration of AuNPs

was calculated by the absorbance at 543 nm and 450 nm, respectively, with a method reported previously.¹

Preparation of the preformed AuNP dimers. DNA probe 1 and probe 2 asymmetrically modified AuNPs were prepared according to our previous reports.²⁻³ AuNP dimers were synthesized as follows:

- 1) 0.5 mL AuNPs modified with probe 1 (0.3 nM) was mixed with 0.5 mL AuNPs modified with probe 2 (0.3 nM);
- 2) 10 μ L of aptamer stock solution (0.3 μ M) were added to the mixture solution;
- 3) 0.2 mL 10 \times binding buffer was added to the above mixture solution, and the solution was finally diluted to a final volume of 2.0 mL with ultrapure water;
- 4) The solution was incubated for 1 hour in room temperature to allow the hybridization between the aptamer and the DNA probes.

Determination of OTA. AuNP dimers were formed at an aptamer-to-AuNP ratio of 10 for OTA detection. The following procedures were used for the set up of the calibration curves:

- 1) 0.1 mL the pre-formed AuNP-dimer solution (0.15 nM calculated by the concentration of AuNPs), 0.1 mL 10 \times binding buffer and 0.3 mL ultrapure water was mixed together;
- 2) 0.5 mL OTA standard solution with a concentration of 0, 2.0×10^{-10} , 1.0×10^{-9} , 5.0×10^{-9} , 2.5×10^{-8} , 5.0×10^{-8} , 1.0×10^{-7} , 2.5×10^{-7} , and 1.0×10^{-6} M, respectively, was then added to the above mixture solution;
- 3) the solution was mildly mixed and incubated for 5 min at room temperature;

4) the absorbance spectra of the solutions were measured with a spectrometer. The photos of the solutions were taken with a CCD camera (Canon EOS 600D).

For the detection of OTA in red wine, standard OTA solution was added to the wine sample (Imported from Chile, 12.5% alcohol) to a final OTA concentration of 5 nM. Then the sample solution was pretreated with an OTA extraction solution as follows: 1.0 mL extraction solution (50 g/L NaHCO₃ and 10 g/L PEG) was mixed with 1.0 mL wine sample. The solution was vigorously shaken with a vortex mixer for 1 minute. Then the solution was centrifuged at 10,000 ×g for 5 minutes. 0.5 mL of the supernatant was used for OTA detection. The analysis procedures were the same as the method used for the setting up of the calibration curve except for the replacement of the 0.5 mL OTA standard solution with 0.5 mL of the supernatant.

ELISA determination of OTA was conducted using a commercially available Kit (MaxSignal® Ochratoxin A ELISA Test Kit, Bioo Scientific Corporation, USA). Experiments were carried out followed by the procedures listed in the specification of the Kit.

References:

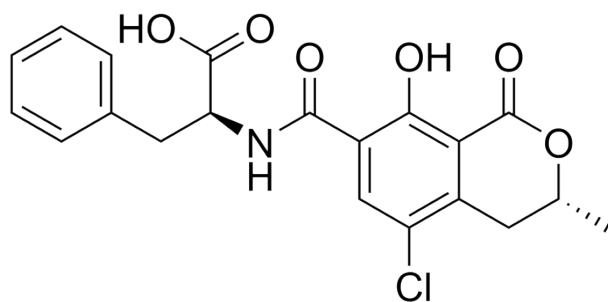
1. W. Haiss, N. T. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215.
2. L. Guo, Y. Xu, A. R. Ferhan, G. Chen and D.-H. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 12338.
3. Y. Xu, L. Guo, L. Huang, K. Palanisamy, D. Kim and G. Chen, *Journal of colloid and interface science*, 2013, **409**, 32.

Table S1 Oligonucleotides and modifications used in this work

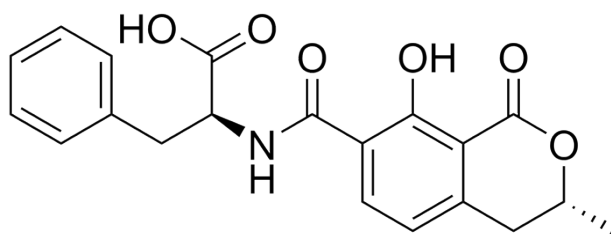
Type	Modifications and sequences*
Probe 1	5'-SH-CTGTGACTGCTCCC-3'
Probe 2	5'-TGTCCGAGTCACAG-SH-3'
Aptamer	5'-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3'
control oligonucleotides	5'-CGTACGGTTAACGTTTCGGCGTACACTTCAACTACGG-3'

* The underlined aptamer sequences are used for the formation of the Y-shaped DNA duplex in the absence of OTA.

Figure S1 structural formula of ochratoxin A and ochratoxin B



Ochratoxin A



Ochratoxin B