Electronic Supplementary Information

Enzyme-free catalytic DNA circuit for amplified detection of aflatoxin B1 using gold nanoparticles as colorimetric indicators

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Experimental Section

Chemicals and materials

AFB1, streptavidin, trisodium citrate, HAuCl₄·3H₂O, Bovine Serum Albumin (BSA), tris-(hydroxymethyl)aminomethane (Tris), and methanol were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents and chemicals were of analytical grade and used without further purification. All solution was prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA).

All oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed in Table S1 (ESI†).

Table S1. Sequences of oligonucleotides used in the present work⁹.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences (from 5’ to 3’)</th>
<th>AFB1 aptamer</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>GCATACCTCCCTAAACTCTCAAGCGTGGGACGTTGTGTCTCTCTGTGTCTCCTGGCCTTCAGCTAGGCCC</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>AGACAAACGTTGCCCAACGCCTTG</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>GCTTGAGATGTAGGGGCTGATGCTCCAATCAACACGCACTAAGCTCCCTAATACACATC-biotin</td>
<td>b²</td>
</tr>
<tr>
<td>H2</td>
<td>AGGGAGTCTGCATTGGAAACATCTCAAGCTCCAACTCAACACGCACTA-biotin</td>
<td>a²</td>
</tr>
<tr>
<td>H3</td>
<td>GTTGTGATTTGGAAGCTTGATGTTGCTACTCCCTAATACATCTCAAGCTCCAAAT-biotin</td>
<td>a³</td>
</tr>
</tbody>
</table>

*The sequences are colored in the same way as in Scheme 1. Domains are represented here by numbers; a starred domain denotes a domain complementary in sequence to the domain without a star (for example, domain a* is complementary to domain a).

Preparation of gold nanoparticles (AuNPs)

AuNPs with an average diameter of 20±3 nm were synthesized according to the reported method with slight modifications.ⁱ⁻³ All glassware was thoroughly cleaned in aqua regia (HCl/HNO₃=1:3), rinsed with ultrapure H₂O, and oven-dried prior to use. In a 500 mL round-bottom flask, 300 mL of 0.01% HAuCl₄ in doubly distilled water were brought to boil with vigorous stirring, followed by the addition of 13 mL of 1% trisodium citrate. The solution turned deep blue within 20 s, and the final color changed to wine-red within about 90 s. The resulting solution was boiled for an additional 15 min, allowed to cool to room temperature with stirring, and filtered through a 0.22 µm syringe filter. The final AuNPs solution was stored in dark bottles at 4 °C and used to prepare the AuNPs-streptavidin conjugate.
Preparation of AuNPs-streptavidin (Au-SA)

1 mL of AuNPs solution was adjusted to pH 6.0 with 0.1 M K$_2$CO$_3$, then 15 μg of SA was added to mix with the AuNPs solution and the mixture was shaken gently at room temperature for 1 h. 100 μL of 10% Bovine Serum Albumin (BSA) was added to react for 30 min to block the nonspecific sites on the surface of AuNPs. The solution was centrifuged at $12 \times 10^3$ g for 20 min at 4 °C, and the Au-SA precipitates were suspended with 10 mM phosphate buffer (PB) containing 1% BSA, 0.2% Tween-20, and 0.05% NaN$_3$. The resulting Au-SA conjugates were stored at 4 °C for use.

Detection procedure

Each hairpin DNA (H1, H2, and H3) was heated at 95 °C for 5 min and then gradually cooled to room temperature before use. 100 nM DNA T was first incubated with 300 nM DNA B in the reaction buffer (10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 5 mM MgCl$_2$, pH = 7.4) for 30 min to form the T-B duplex. AFB1 was mixed with the T-B solution and incubated at room temperature for 45 min. Subsequently, H1, H2, and H3 (each was 600 nM) were added and incubated at room temperature for 90 min. Finally, 100 μL of the above reaction mixture was added into 500 μL of the prepared Au-SA solution. After thorough shaking and incubating for about 45 min, the color changes of the solutions and their corresponding UV-vis absorption spectra were recorded.

Instrumentation

The absorption spectra were recorded on a TU-1902 UV-vis spectrophotometer (Persee, China) at room temperature. Transmission electron microscopy (TEM) measurements were performed on a JEM-3010 transmission electron microscope (Hitachi, Japan). The samples for TEM characterization were prepared by placing a drop of colloidal solution (10 μL) on a carbon-coated copper grid and drying at room temperature. Dynamic light scattering (DLS) tests were performed using a Nano ZS/Mastersizer 2000E (Malvern Instruments Ltd., Malvern, UK), which was operated under the following conditions: temperature 25 °C, detector angle 90°, incident laser wavelength 633 nm. The sample was diluted to 2 mL with ultrapure water and measured three times.

Specificity analysis

To evaluate the specificity of the colorimetric assay for AFB1, several other mycotoxins such as ochratoxin A (OTA), aflatoxin M1 (AFM1), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin B2 (AFB2), zearalenone (ZEN), deoxynivalenol (DON), and fumonisin (FB1) at the concentration of 1 μM were selected for control testing. All other analysis conditions were
identical to those used in the AFB1 detection procedures described above.

**Analysis of AFB1 in rice samples**

AFB1-free rice samples were purchased from local market and finely ground in a household blender. 1 g of the pulverizing real rice samples were spiked with AFB1 at different concentrations (0.1, 1, 10, 50, and 100 nM) and mixed thoroughly in a vortex mixer. The spiked samples were extracted with 5 mL of methanol-water (8:2, v/v) by vortex sharking for 15 min at room temperature. After centrifugation at 6000 g for 10 min, the supernatant was filtered through a 0.22 µM syringe filter and diluted 20-fold with the reaction buffer for recovery studies. The procedures of AFB1 detection in rice samples were similar to those mentioned above for the detection of AFB1 standards.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked level (nM)</th>
<th>Detected concentration a (nM)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.09</td>
<td>3.6</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.12</td>
<td>2.3</td>
<td>112</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9.6</td>
<td>5.2</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>46.8</td>
<td>6.7</td>
<td>93.6</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>108.4</td>
<td>4.5</td>
<td>108.4</td>
</tr>
</tbody>
</table>

*aEach sample was analyzed in triplicate, and the results are the average values.*
A search was also conducted to identify the suitable buffer composition for AFB1 monitoring. Several buffers such as phosphate buffer saline (PBS, 10 mM, pH 7.4), borate buffer (BB, 10 mM, pH 7.4), and Tris-HCl buffer (10 mM, pH 7.4) were tested in this study as reaction buffers. As shown in Fig. S1, Tris-HCl exhibited the best sensing performance for AFB1 detection. To facilitate the aptamer-target binding\textsuperscript{4,6} and accelerate the toehold-mediated hairpin DNA assembly,\textsuperscript{7} 120 mM NaCl, 5 mM KCl, 5 mM MgCl\textsubscript{2} were selected as the working ionic strength conditions.

![Fig. S1 Selection of an optimal buffer for the colorimetric aptamer assay. AFB1 concentration: 10 nM. T: 100 nM, B: 300 nM, hairpin DNA: 600 nM. The experiments were performed at room temperature (~25°C).](image)

References