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Communication

Disassembly of gold nanoparticle dimers for colorimetric determination of ochratoxin A

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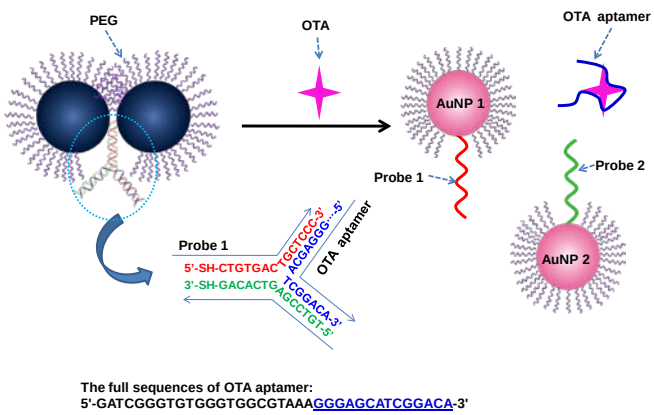
Colorimetric sensors based on assembly or disassembly of isotropic gold nanoparticle (AuNP) aggregates have been frequently reported. Herein we demonstrate the first colorimetric aptasensor for sensitive determination of ochratoxin A, one of the most-abundant food-contaminating mycotoxins, based on disassembly of orient-aggregated AuNP dimers.

On-site determination of food and environmental contaminates is vital important regarding the serious food and environmental safety situation all over the world.[1-2] In view of the scattered sites distribution of these contaminates, methods based on the use of sophisticated instruments in laboratories are not suitable for this purpose. Instead, analytical approaches that utilize portable instruments or instrument-free could be desirable. On this occasion, colorimetric sensors could be a good alternative because normally colorimetric sensors can be inspected with naked eye, or detected with a simple portable spectrometer.[3-5]

Among diverse sensing strategies, colorimetric sensors based on analytes induced aggregation of gold nanoparticles (AuNPs) have been frequently reported.[6-11] This sensing strategy is based on the change of localized surface plasmon resonance (LSPR) property of AuNPs in the presence and absence of analytes. In the absence of analytes, AuNPs distributed in the solution individually, and the solution is in red color; in the presence of analytes, analytes would induce the aggregation of AuNPs, thus LSPR coupling would occur. As a result, the solution color turns from red to purple. In addition, it is also reported that the reverse process of this sensing mechanism, that is, the disassembly of the preformed AuNP aggregates by analytes is also effective for the detection of target molecules.[12-17] It is worth noting that the sensitivities of sensors based on either assembly or disassembly of AuNP aggregates are determined not only by the interparticle distances, but also by the number of nanoparticles that consists of the aggregates.[18] Usually aggregates consist with thousands of AuNPs are necessary to generate significant color variations. The necessity for the formation of macroscopic AuNP aggregates brings at least two limitations to these colorimetric sensors: first, the macroscopic AuNP aggregates tend to sedimentation in aqueous solution. Thus the LSPR spectra and the color of the solution is time-dependant, which seriously affects the accuracy of the sensor; second, the sensitivities of these sensors are limited because a molecular excess of targets versus AuNPs is necessary to assemble or disassemble these microscopic aggregates.

In order to overcome the above limitations, recently we developed a colorimetric sensor based on analytes induced assembly of oriented AuNP dimers.[19] DNA asymmetrically functionalized AuNPs were used to avoid the formation of large AuNP aggregates, and a Y-shaped DNA duplex was utilized to minimize the interparticle distance. These innovations brought significant improvements of the analytical figures of merit, e.g. the sensitivity was improved by 10,000 times and the dynamic range was improved by more than two orders of magnitude comparing with a sensor fabricated using conventional strategies.

Herein, we investigate the reverse process of the assembly of AuNP dimers, namely, we demonstrate the disassembly of orient-aggregated AuNP dimers by target molecules (Scheme 1). This enables us to expend the analytes from DNAs to other molecules, e.g. the detection of ochratoxin A (OTA) is selected as a demonstration. OTA is one of the most-abundant food-contaminating mycotoxins.[20] It is produced by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum*. A large variety of food product commodities may contaminate by OTA, e.g. dried fruits, coffee, cereals, beans, wine, beer and meat. In recent years, a number of methods have been developed for the determination of OTA.[21-25] However, sensitive and rapid visual sensors suitable for on-site determination of OTA are still strongly in demand.



Scheme 1 Sensing mechanism of the AuNP dimer-based colorimetric aptasensor

The AuNP dimers were prepared via an approach developed previously.[19] Briefly, AuNPs (average diameter ~48 nm) were firstly asymmetrically functionalized with poly(ethylene glycol) 2-thioethyl ether acetic acid (PEG, average M_n 1,000) and DNA probe 1 and 2, respectively. In the presence of OTA aptamers, the two AuNPs modified with probe 1 and probe 2 were linked with a Y-shaped DNA duplex consisted with three oligonucleotides as shown in Scheme 1; two of the oligonucleotides were asymmetrically modified on the surface of AuNP-1 and AuNP-2, respectively. Another oligonucleotide that consisted of the Y-shaped DNA duplex was specifically designed to be the sequences of OTA aptamer. Thus, in the absence of OTA, this oligonucleotide acted as a linker to assemble the formation of the AuNP dimers. In the presence of OTA, OTA would compete with DNA probe 1 and probe 2 to bind with the aptamer. As a result, the preformed AuNP dimers would be disassembled, and the solution color would turn from blue to red (Scheme 1).

Figure 1a shows the extinction spectra of the sensor upon the addition of different amount of OTA. In the absence of OTA, a twin-peak spectrum was observed. One peak located at ~539 nm, and the other peak located at ~606 nm. According to our previous works,[19, 26-27] this is a typical spectrum of AuNP dimers. With the increase of OTA concentrations, the peak intensity at 606 nm decreased, while the one at 539 nm increased. This kind of spectra variations indicated the disassembly of the pre-formed AuNP dimers. The response dynamic range was found to be 0.2 nM to 250 nM (see the inset at the top right of Figure 1a), with an experimental determined LOD of 0.05 nM (S/N=3.3). Figure 1 b and c show representative SEM images of the preformed AuNP dimers in the absence (b) and presence (c) of OTA (250 nM). It can be clearly seen that in the absence of OTA, most of the AuNPs are formed into dimers; while in the presence of OTA, most of the AuNP dimers are redispersed into monomers.

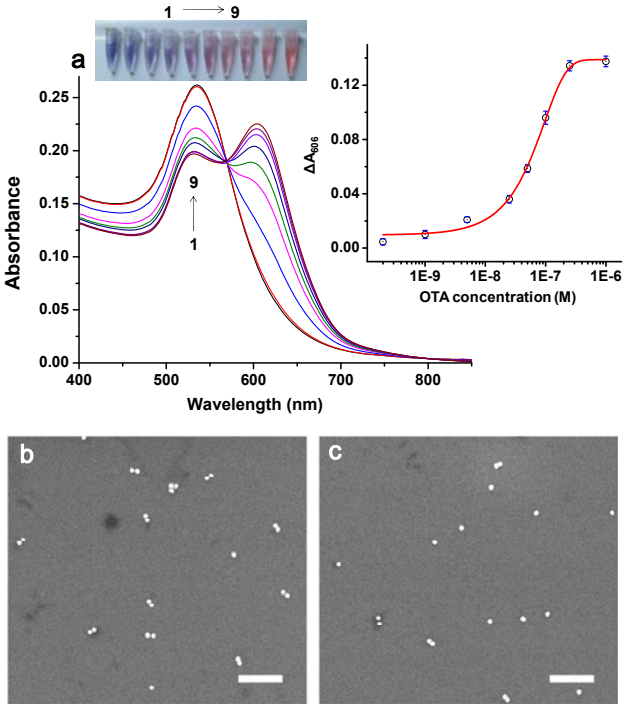


Figure 1 a. Extinction spectra of the sensor in different concentration of OTA. OTA concentrations of sample number 1 to 9 were 0, 0.2, 1, 5, 25, 50, 100, 250, 1000 nM, respectively. The inset at the top left is the images of the corresponding solutions. The inset at the top right shows the calibration curve for OTA detection. (b) and (c) are representative SEM images of the preformed AuNP dimers in the absence (b) and presence (c) of 250 nM OTA. The scale bars represent 500 nm.

Besides the simplicity, the other concern for an on-site colorimetric sensor is the analytical efficiency of the method. Thus the kinetics of the color change was investigated (Figure 2). The results showed that stable absorbencies at 606 nm were observed within 3 min. This indicated the disassembly of the preformed AuNP dimers by OTA was a fast process. It is worth to note that our previous investigation revealed that the assembly of oriented AuNP dimers cost ~1 h to obtain stable response.[19] Thus we concluded that the colorimetric sensor based on the disassembly of AuNP dimers was more efficient than the one based on the assembly of AuNP dimers. This kind of fast response property of the sensor is preferable for outdoor on-site food and environmental pollutes assays.

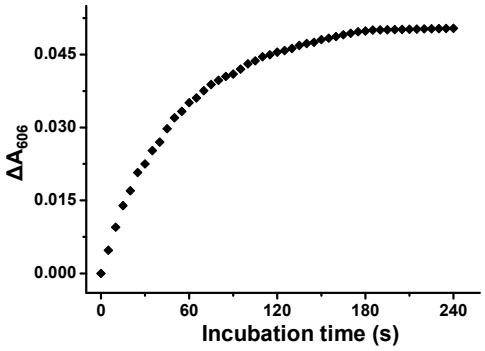


Figure 2 Kinetics of the color change at an OTA concentration of 50 nM.

The selectivity of a sensor is essential for real sample analysis. Figure 3 shows the selectivity of the sensor to OTA, ochratoxin B (OTB) and a control DNA. OTB is structural similarity with OTA (see Figure S1 for the structural formula of the two molecules), and they are always coexistent in a sample. OTB is less toxic than OTA, thus the discrimination of these molecules is important to evaluate the toxicity of a sample. The results shown in Figure 3 indicated that the proposed sensor was highly specific to OTA.

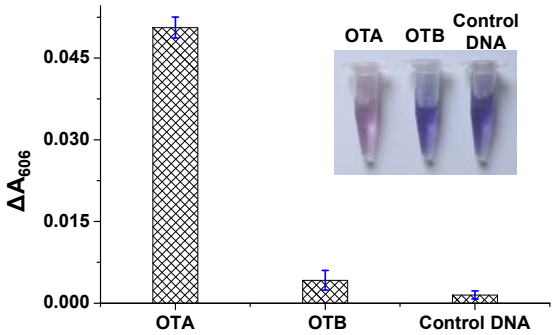


Figure 3 Responses of the sensor to 50 nM of OTA, OTB and the control DNA.

Finally, we demonstrated the application of the proposed sensor for OTA detection in red wine. No OTA was detected in the original wine sample, thus we used standard addition method to evaluate the feasibility of the sensor for OTA detection in complex sample matrix. Standard OTA solution was added to red wine to result in a final OTA concentration of 5 nM. Before analysis, the wine sample was pretreated with an extraction solution and then centrifuged. The supernatant was then used for OTA detection. Figure 4 shows the extinction spectra of the sensor before and after the standard addition of OTA. The OTA concentration determined with this method is 5.17 nM, which was consistent with the result of 5.16 nM obtained from a commercially available ELISA kits. Additionally, it is worth noting that this kind of trace amount of OTA could generate visual distinguishable color change to the sensing solution (see the inset in Figure 4). It is worth noting that the European Food safety Authority set a maximum level for OTA of 5 nmol/Kg for all types of wines.[28] Thus visual inspection of the proposed sensor by naked eye could satisfy the need for OTA detection in all types of wines. These results strongly indicated the potential utility of the present method for OTA detection in real samples.

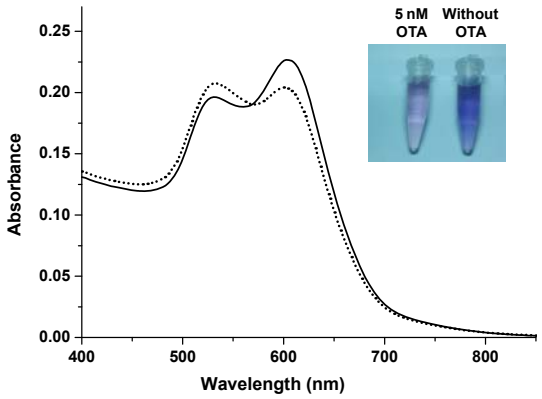


Figure 4 Extinction spectra of the sensor with (black dots) and without (black line) the addition of 5 nM OTA into red wine samples. The inset shows the corresponding images of the two solutions.

In conclusion, we demonstrate the first colorimetric aptasensor for sensitive determination of OTA based on disassembly of the preformed AuNP dimers. This AuNP dimer-based sensor predominated over conventional colorimetric sensors based on the disassembly of large AuNP aggregates in terms of stability, sensitivity and detection dynamic range. In addition, the response speed of the proposed sensor is much faster than our recent reported sensor based on the assembly of oriented AuNP dimers.

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Notes and references

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