Simple and sensitive aptamer-based colorimetric sensing of protein using unmodified gold nanoparticle probes†

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Received (in Cambridge, UK) 22nd May 2007, Accepted 19th June 2007
First published as an Advance Article on the web 29th June 2007
DOI: 10.1039/b707642h

We describe herein simple and sensitive aptamer-based colorimetric sensing of protein (alpha-thrombin in this work) using unmodified gold nanoparticle probes.

Aptamers are novel *in-vitro* selected functional DNA or RNA structures that possess high recognition ability towards specific molecular targets ranging from small inorganic and organic substances to even a protein or cell.[1–3] Recently, a variety of aptamer-based analytical methods have been developed for molecular recognition and detection, including electrochemistry,[4] fluorescence,[5–8] SPR,[9] AFM,[10] quartz crystal microbalance[11] and so on. Simple colorimetric sensors have the potential to eliminate the use of analytical instruments and are attracting more and more attention.[12] Now, a number of aptamer-based colorimetric sensors have been developed, most of which use gold nanoparticles (AuNPs) as sensing elements.[12–19] However, most of these colorimetric methods need steps such as modifying aptamers onto the AuNPs and separating the modified AuNPs from the unmodified AuNPs or surplus aptamers. These steps, firstly, led to complication and relatively high cost of the experiments. What's more, the target binding sites and conformational changes of the aptamers after binding were not all known precisely, so labeling sites were not only difficult to design, but labeling also could weaken the affinity between the target and the aptamer. Therefore, developing modification-free AuNPs colorimetric sensors to simplify the detection process would be important and attractive. Rothberg's group reported a novel DNA sensor using unmodified AuNPs colorimetric sensing based on the discriminated effects of different DNA structures on the nanoparticles.[18] Recently, Fan *et al.* extended the targets of the unmodified methods to metal ion K^+.[19]

In this communication, we report a simple and sensitive aptamer-based sensor for protein detection using unmodified AuNPs based on the same principle,[18,19] which could extend the unmodified colorimetric approaches to complex protein molecules. With our method the aptamer structure change during the binding process could be detected by the color change of AuNPs, thereby protein recognition is realized. At the same time, common steps such as modification and separation could be successfully avoided, which could potentially broaden the applicability of AuNPs colorimetric sensors in the future.

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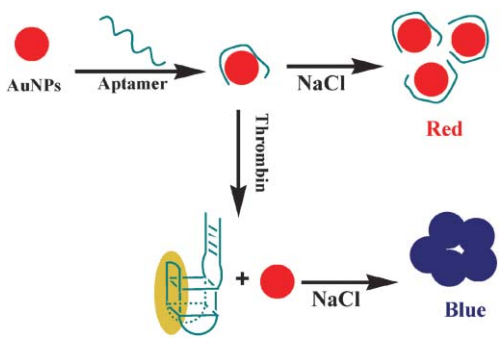
† Electronic supplementary information (ESI) available: Experimental details and additional figures. See DOI: 10.1039/b707642h

Here thrombin and its 29-mer binding aptamer (herein referred to as TBA) were taken as a model. When thrombin interacts with its aptamer, the aptamer is much more inclined (than the unfolded conformation) to fold into a structure of G-quadruplex/duplex.[20] This structure could not be affected by K^+ and gave a very high affinity of $K_d \sim 0.5$ nM. It had been reported that unfolded ssDNA could stabilize the AuNPs in the presence of a given high concentration of salt, while the quadruplex/duplex structure could destroy this protecting function and made the AuNPs aggregate.[18,19] We just make use of this property to design our sensor. As shown in Scheme 1, by exploiting interactions between the AuNPs and the DNA sequences, color changes of the AuNPs could sensitively differentiate the conformational change of TBA before and after adding the target thrombin.

Shown in Fig. 1 is a typical colorimetric detection of thrombin using unmodified 13 nm AuNPs probes (17 nM).[21] The AuNPs colloidal solution with TBA/thrombin changed from red to purple as soon as 100 μL 0.5 M NaCl were added, while the one with only TBA remained red after adding the same amount of salt. Through this color change phenomenon, the change of TBA conformation from the unfolded one to G-quadruplex/duplex could be directly observed with the naked eye, realizing the detection of protein thrombin in a very convenient way.

The mechanism of the colorimetric effects between AuNPs/TBA and AuNPs/TBA/thrombin can be explained as follows. The as-prepared AuNPs are stable due to the negative capping agent's (*i.e.* citrate) electrostatic repulsion against van der Waals attraction between AuNPs.[18,21] Therefore, the addition of enough salt would screen the repulsion between the unmodified negative-charged AuNPs and lead to the aggregation of the AuNPs which provokes a corresponding red-to-blue color change. As previously reported, there is stronger coordination interaction between the nitrogen atoms of the unfolded ssDNA and the AuNPs than electrostatic repulsion between the negative-charged phosphate backbone and



Scheme 1 AuNPs colorimetric strategy for thrombin detection.

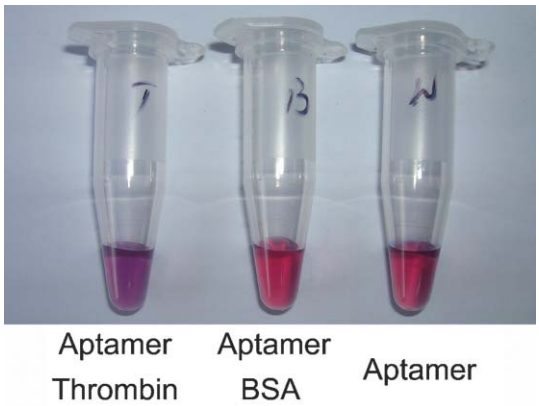
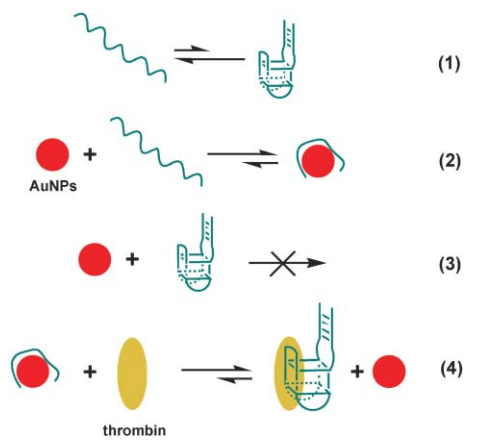


Fig. 1 Colorimetric detection of thrombin. Photographs of 200 μL 13 nm AuNPs stabilized by the aptamer (30 μL 4.59 μM) after the addition of salt (100 μL 0.5 M NaCl) in the presence and absence of target protein (from left to right: 83 nM thrombin, 83 nM BSA and water as control).

the negative-charged AuNPs.[18,19] Thus, the unfolded TBA (ssDNA) would adsorb onto the AuNPs and help to enhance the AuNPs' stability against salt-induced aggregation. As for dsDNA or folded ssDNA (*e.g.* G-quadruplex), the relatively rigid structure prevents the exposure of the DNA bases to the AuNPs and the high density of negative charges increases the repulsion between the DNA and the AuNPs.[18,19] Thus the two DNA structures could not adsorb on the AuNPs and lose the ability to protect the AuNPs.

The TBA used in this work is known to form a G-quadruplex/duplex without binding to thrombin but the presence of thrombin is favorable for this conformation.[20] Thus TBA should be different from ssDNA of random sequence. As shown in Scheme 2, there is, indeed, a mixture of unfolded and folded conformations of TBA. However, in the absence of thrombin, there should be an equilibrium between unfolded and folded conformations of TBA. According to the previous report, the unfolded TBA (ssDNA) would adsorb onto the AuNPs and help to enhance the AuNPs' stability against salt-induced aggregation. Meanwhile the folded TBA (G-quadruplex/duplex here) could not adsorb onto the AuNPs and thus could not protect the AuNPs against salt-induced aggregation.[18,19] In the case of our study, when the AuNPs were mixed with TBA, unfolded TBA would adsorb onto the AuNPs, and thus cause an equilibrium shift toward unfolded TBA. After the addition of thrombin, thrombin would interact with TBA and induce TBA to fold into a G-quadruplex/duplex structure, thus losing the protection against salt-induced AuNPs aggregation. Therefore once enough thrombin is added, the formation of the G-quadruplex/duplex structure could decrease the ssDNA adsorbed onto the AuNPs thereafter leading to the aggregation of the AuNPs under the high salt conditions (Scheme 1).



Scheme 2 The differentiation ability of AuNPs towards unfolded TBA (ssDNA) and folded TBA (the G-quadruplex/duplex structure).

To quantitatively detect thrombin using our AuNPs colorimetric aptasensor, UV–visible spectra of 200 μL AuNPs stabilized by TBA (30 μL 4.59 μM) in the absence and presence of different concentrations of thrombin after addition of 100 μL 0.5 M NaCl were recorded. As shown in Fig. 2, once the salt is added, the A620/A520 values of solutions with thrombin display an increasing trend during the 30 minutes, which illustrates that the AuNPs/TBA/thrombin system gradually loses the stability protected by the unfolded TBA. The destabilization trend is obviously dependent on the concentration of thrombin. A linear range from 0 to 167 nM is obtained with a detection limit of 0.83 nM for thrombin, which is as low as the most sensitive methods.[4] Significant color change is visible at as low as 83 nM, suggesting that the AuNPs are sensitive for thrombin sensing (Fig. 1).

To provide evidence that the recognition of thrombin by TBA is specific, control experiments were performed using BSA and the other random ssDNA sequence. When BSA displaces thrombin, no color change of the AuNPs solution takes place under the same amount of salt (Fig. 1, 3 and S3). The random sequence used here could provide no significantly lower ability than TBA to stabilize the AuNPs but without any significant response to thrombin under the same experimental conditions, which is enough to prove the specific recognition of thrombin by TBA (Fig. 3).

In conclusion, simple and sensitive aptamer-based colorimetric sensing of protein is realized originally using unmodified gold nanoparticle probes. The method we developed shows multifaceted advantages for analytical recognition. First, protein detection is finished through the simple color change of the AuNPs probes using only commercially available materials; any usual steps such as modification and separation are therefore successfully avoided. Second, the absence of modification can ensure the original conformation of the aptamer when interacting with its target, thereby leading to high binding affinity and very sensitive detection. What's more, as the method is based on the conformational change of aptamer before and after binding with its target, the design itself could provide another technique for ssDNA structure detection besides traditional ones such as fluorescence. In

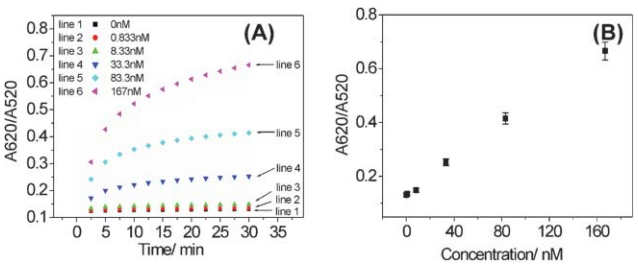


Fig. 2 (A) Plots of changes in the absorption ratio (A620/A520) over 30 min after the addition of varying concentrations of thrombin. (B) Plot of the absorption ratio (A620/A520) at 30 min *vs.* thrombin concentration.

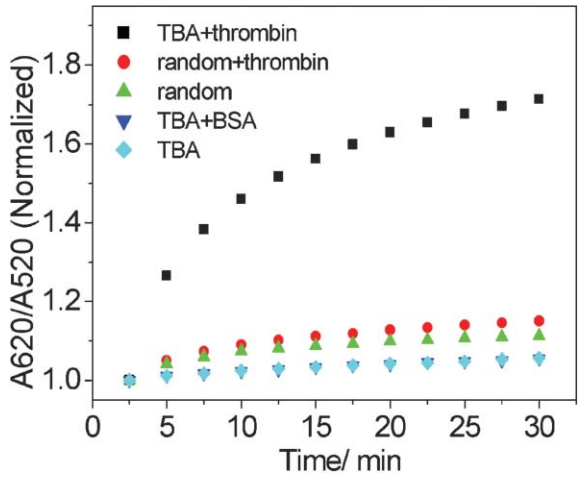


Fig. 3 Plots of changes in the absorption ratio (A620/A520) over 30 min of 200 μL 13 nm AuNPs stabilized by the aptamer or the random control sequence (30 μL 4.59 μM) after the addition of salt (100 μL 0.5 M NaCl) in the absence and presence of target protein (83 nM thrombin or 83 nM BSA).

summary, though some limits exist, the method developed here has extended the applications of colorimetric sensors using unmodified AuNPs, and due to its sensitivity, convenience and low cost, the sensor is promising in a lot of fields such as high throughput assays in microwell-based plates and even automated analysis by using microfluidics.

This work is supported by the National Natural Science Foundation of China with Grants 20335040, 20575063 and 20427003, and the Chinese Academy of Sciences (KJCX2-YW-H09).

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