Ultrasensitive colorimetric detection of protein by aptamer–Au nanoparticles conjugates based on a dot-blot assay†

Yuling Wang, Dan Li, Wen Ren, Zuojia Liu, Shaojun Dong* and Erkang Wang

Received (in Cambridge, UK) 23rd January 2008, Accepted 28th February 2008
First published as an Advance Article on the web 31st March 2008
DOI: 10.1039/b801055b

A simple, rapid and ultrasensitive colorimetric detection of protein using aptamer–Au nanoparticles (AuNPs) conjugates based on a dot-blot array has been developed, which was combined with the unique optical properties of AuNPs, enabling the visual detection of protein within minutes without any instrument.

Aptamers are short single-stranded nucleic acids that are selected *in vitro* from a huge combinatorial library using the "systematic evolution of ligands by exponential enrichment" (SELEX) procedure based on their affinity and specificity for the targets ranging from small inorganic or organic substances to proteins or cells.[1–3] Since their first discovery in the 1990s, aptamers are starting to appear in a series of applications in biosensors,[4] imaging probes[5] and drugs[6] due to their high binding ability. However, a key challenge to their successful applications is how to transform the aptamer-binding events into physically detectable signal. Optical or electrochemical aptamer biosensors based on enzyme,[7] fluorophore,[8] nanoparticle labels or on a binding-induced label-free detection[9] have been developed to meet this challenge. Even though, only a few of them can be used in practical applications such as in home and clinical tests due to the high requirements for the personal experience or the expensive measurement instrument. Gold nanoparticles (AuNPs) have been widely used in immunoassay,[10] biochip[11] and aptasensor[12] read out techniques due to their long-term stability, friendly biocompatibility with biomolecules such as antigen, antibody and DNA, and their unique optical properties. Recently, Lu's group has developed a colorimetric sensor for the detection of small molecules based on aptamer,[13] and our group also developed a kind of colorimetric sensor for detection of α-thrombin based on AuNPs through the interaction of the aptamer to α-thrombin.[14] However, one big disadvantage of the colorimetric sensors based on AuNPs for the detection of small molecules or proteins in solution is the interference from the color of background, resulting in the decrease in detection sensitivity of the sensors. Based on this idea, Liu *et al.* have developed a simple and sensitive dipstick test for small molecules based on lateral flow separation of aptamer-linked nanostructures,[15] which is a positive step towards improving the user-friendliness and sensitivity.

Herein, we developed a simple, rapid and ultrasensitive colorimetric detection of protein based on aptamer–AuNPs conjugates using a dot-blot assay, taking α-thrombin as a model protein. Based on the fact that AuNPs have the unique chemical and physical properties, when AuNPs were functionalized with α-thrombin-binding-aptamer (TBA), it can be bound on the active site of α-thrombin immobilized on the membrane with high affinity to form the red dots, which can be observed within minutes with the naked eye without any instrument. Taking advantage of the color transfer that is from colorless to red, the aptasensor is anticipated to have high sensitivity and selectivity for protein detection due to the high affinity of aptamer to the target. Another has to be pointed out that during our submission we found Jana *et al.* used the Au–SiO_2 nanoparticles as the probe to detect protein based on a dot-blot assay,[16] further indicating this method has gained more attentions in bioanalysis. More importantly, we have extended this method to detect the targets in a complex biological sample such as human plasma, providing great promise of this device in clinical application.

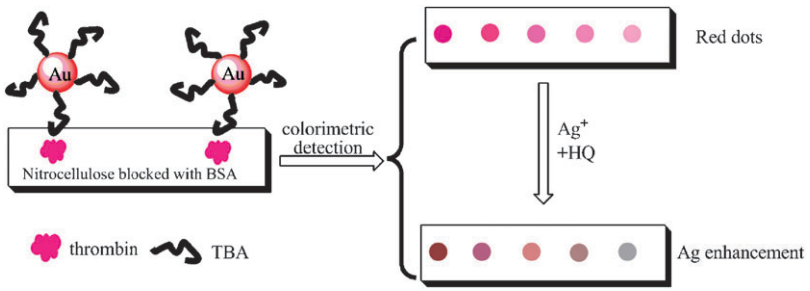
The overall process of fabricating the device is shown in Scheme 1. Standard samples containing different concentrations of α-thrombin were firstly immobilized on the nitrocellulose (NC) membrane through both the electrostatic and hydrophobic interactions between the protein and the nitrocellulose.[17] And then the membrane was blocked by 10% BSA for 2 h at the room temperature to block the rest sites of the nitrocellulose. The well blocked membrane was then incubated with the TBA-labeled AuNPs at 37 °C for 20 min. Then the red dots will appear on the membrane clearly. The color intensity of the dots will differ with the different concentrations of the protein due to the optical properties of AuNPs, as shown in the Scheme 1. To increase the detection sensitivity, silver enhancement solution was used to cover AuNPs and intensify the color of AuNPs.

AuNPs used in this system are stabilized by citrate and synthesized according to the method reported by Daniel *et al.* and Storhoff *et al.*[18] The modification of AuNPs by TBA was performed according to the procedures in the literature.[19] TEM image shows that the diameter of the AuNPs is ~13 nm with the surface plasmon absorption at 519 nm (shown in Fig. S1 in ESI†).

The optical images of the red dots with different levels of α-thrombin on NC membrane are given in Fig. 1A from spot 1

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, and Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Changchun, Jilin, 130022, P.R. China. E-mail: dongsj@ciac.jl.cn; Fax: +86-431-85689711

† Electronic supplementary information (ESI) available: The experimental details and the TEM and UV-Vis absorbance of the AuNPs and control experiments. See DOI: 10.1039/b801055b



Scheme 1 Description of colorimetric detection of protein by aptamer–AuNPs conjugates based on a dot-blot assay.

to 7, respectively, from which the color change is evident with the level of α-thrombin. With the increase of the level of α-thrombin, the spot intensity of a dot is improved and no color can be seen when the level of the α-thrombin was decreased to 0.115 pmole without silver enhancement.

The levels of α-thrombin on NC membrane were able to be quantified using Adobe Photoshop software as proposed by Nam *et al.*,[20] each spot was selected and the selected area was quantified using the histogram function with the brightness channel option of the Adobe Photoshop. The mean value from the histogram window was used to calculate the spot intensity of each spot. It has to be pointed out that the spot intensity herein is defined as the mean channel value of a control spot (with no red dot) divided by that of a given sample spot. Fig. 1C gives the spot intensity response to the level of α-thrombin, from which the dynamic range of this assay ranging from 0.115 pmole to 3.7 pmole can be obtained.

To improve the detection sensitivity, silver enhancement solution was used as reported by other groups.[21] The dot images were given in Fig. 1B and the dot can be seen clearly even the level of α-thrombin was decreased to 0.115 pmole, further indicating silver enhancement can improve the detection limit. Much lower levels of α-thrombin can be detected upon silver enhancement and the detection limit is 14 fmole as shown in Fig. S2 (ESI†). The detection curve for α-thrombin corresponding to red dots with silver enhancement in Fig. 1B is given in Fig. 1D and the dynamic range of this assay ranging from 14 fmole to 1.85 pmole can be obtained.

Control experiments were conducted to reveal the selectivity and specificity of the recognition reaction for the detection of BSA, β and γ-thrombin as shown from spots 8 to 10 in Fig. 1. Upon the interaction of the TBA–AuNPs with foreign proteins, no color change was detected, even under the enhancement of silver solution, indicating almost no binding of the TBA modified AuNPs to the other proteins occurred. When the level of β-thrombin is increased to 10 pmole the red dot can be seen on the NC membrane, indicating a higher level of β-thrombin may give little interference to the detection of α-thrombin as shown in Fig. S3 (ESI†).

Finally, the possibility of using this device for the detection of the analyte in a complex biological sample such as human plasma was investigated. Healthy human plasma was pretreated firstly as reported by Mascini and co-workers[22] and diluted to 1%. Then, α-thrombin was added into the

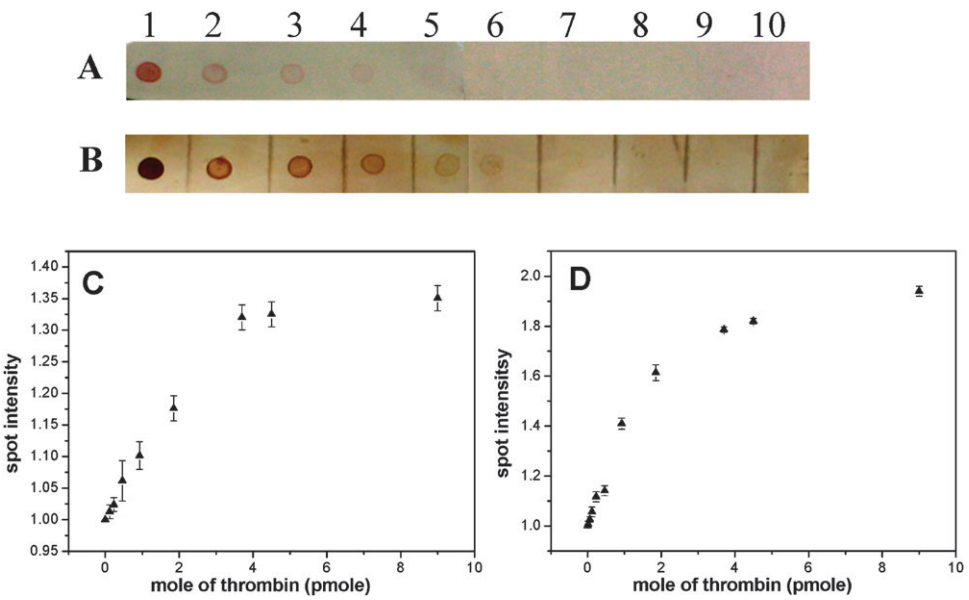


Fig. 1 Dot images for colorimetric detection of α-thrombin without (A) and with silver enhancement (B) for different levels of α-thrombin. The level of α-thrombin from 1 to 7 is 18.5, 3.7, 1.85, 0.925, 0.463, 0.230, and 0.115 pmole, respectively. Control experiments were conducted on 8, 9, 10 for 1.0 pmole of BSA, β-thrombin and γ-thrombin, respectively. (C) Detection curve for α-thrombin corresponding to red dots in Fig. 1A and (D) detection curve for α-thrombin corresponding to red dots (with silver enhancement) in Fig. 1B.

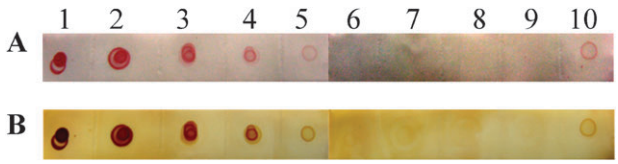


Fig. 2 Dot images for colorimetric detection of α-thrombin in 1% human plasma without (A) and with silver enhancement (B). The level of α-thrombin in 1% plasma from 1 to 7 is 9.25, 3.7, 1.85, 0.925, 0.463, 0.230, and 0.115 pmole, respectively. Control experiments were conducted on 8, 9, 10 for 1% human plasma, 1.0 pmole of BSA, and 10% human plasma, respectively.

pretreated diluted plasma to test the performance of TBA–AuNPs conjugates to the analyte as shown in Fig. 2. The recorded signals increased with the level of added α-thrombin in plasma and the 1% plasma and 1 pmole BSA have no interference to the results, demonstrating the device was able to operate in this complex matrix. It should be pointed out that different concentrations of plasma have been conducted as shown in Fig. S4 (ESI†), which indicates that 8% plasma has little interference on the results with silver enhancement while 10% plasma has interference on the detection as shown in spot 10 of Fig. 2. That is to say, over and above 8% plasma will interfere with the sensor working. In addition, different concentrations of BSA have also been done as shown in Fig. S5 (ESI†). The results indicate that 5% BSA (corresponds to 735 μM) has no interference on the detection, and over and above 10% BSA will interfere with the sensor working (data not shown).

In summary, we have developed a simple, rapid and ultrasensitive colorimetric detection for protein using a dot-blot AuNPs assay. Due to the strong affinity between aptamer and protein, red dots can be visible to the naked eye without any instrument. The aptasensor has high sensitivity with the detection limit of 14 fmole with silver enhancement (such detection limit corresponds to the concentration of 14 pM in 1 mL sample). Based on the high binding activity of aptamer with the protein, the aptasensor also shows very high selectivity. More importantly, the overall process is amenable for extension to detect targets in complex biological sample such as human plasma.

This work was supported by the National Natural Science Foundation of China (Nos. 20427003, 20575064).

Notes and references

1 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818.
2 C. Tuerk and L. Gold, *Science*, 1990, **249**, 505.
3 M. P. Robertson and G. F. Joyce, *Nature*, 1990, **344**, 467.
4 B. Basnar, R. Elnathan and I. Willner, *Anal. Chem.*, 2006, **78**, 3638; V. Pavlov, B. Shlyahovsky and I. Willner, *J. Am. Chem. Soc.*, 2005, **127**, 6522; Y. Xiao, B. D. Piorek, K. W. Plaxco and A. J. Heeger, *J. Am. Chem. Soc.*, 2005, **121**, 17990; B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger and K. W. Plaxco, *J. Am. Chem. Soc.*, 2006, **128**, 3138.
5 J. W. J. Li, X. H. Fang and W. H. Tan, *Biochem. Biophys. Res. Commun.*, 2002, **292**, 31; X. H. Fang, Y. M. Mi, J. W. J. Li, T. Beck, S. Schuster and W. H. Tan, *Cell Biochem. Biophys.*, 2002, **37**, 71.
6 V. Bagalkot, O. C. Farokhzad, R. Langer and S. Jon, *Angew. Chem., Int. Ed.*, 2006, **45**, 8149.
7 K. Ikebukuro, C. Kiyohara and K. Sode, *Biosens. Bioelectron.*, 2005, **20**, 2168; E. Baldrich, J. L. Acero, G. Reekmans, W. Laureyn and C. K. O'Sullivan, *Anal. Chem.*, 2004, **76**, 7053.
8 R. Nutio and Y. Li, *J. Am. Chem. Soc.*, 2003, **125**, 4771; B. L. Li, H. Wei and S. J. Dong, *Chem. Commun.*, 2007, 73.
9 V. Pavlov, Y. Xiao, B. Shlyabovsky and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 11768; Y. Xiao, A. A. Lubin, A. J. Heeger and K. W. Plaxco, *Angew. Chem., Int. Ed.*, 2005, **44**, 5592; D. K. Xu, D. W. Xu, X. B. Yu, Z. Liu, W. He and Z. Ma, *Anal. Chem.*, 2004, **76**, 5107; M. C. Rodriguez, A. Kawde and J. Wang, *Chem. Commun.*, 2005, **34**, 4267.
10 C. Cao and S. J. Sim, *Biosens. Bioelectron.*, 2007, **22**, 1874.
11 A. K. R. Lytton-Jean, M. S. Han and C. A. Mirkin, *Anal. Chem.*, 2007, **79**, 6037; Y. W. C. Cao, R. C. Jin and C. A. Mirkin, *Science*, 2002, **297**, 1536.
12 C. C. Huang, Y. F. Huang, Z. H. Cao, W. H. Tan and H. T. Chang, *Anal. Chem.*, 2005, **77**, 5735.
13 J. W. Liu and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 90; J. W. Liu and Y. Lu, *Anal. Chem.*, 2004, **76**, 1627.
14 H. Wei, B. L. Li, J. Li, E. K. Wang and S. J. Dong, *Chem. Commun.*, 2007, 3735.
15 J. W. Liu, D. Mazumdar and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 7955.
16 N. R. Jana and J. Y. Ying, *Adv. Mater.*, 2008, **20**, 430.
17 S. Y. Hou, H. K. Chen, S. C. Cheng and C. Y. Huang, *Anal. Chem.*, 2007, **79**, 980.
18 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959; M. C. Daniel and D. Astruc, *Chem. Rev.*, 2004, **104**, 293.
19 J. W. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246; Y. L. Wang, H. Wei, B. L. Li, W. Ren, S. J. Dong and E. K. Wang, *Chem. Commun.*, 2007, 5220.
20 J. M. N. Nam, A. R. Wise and J. T. Groves, *Anal. Chem.*, 2005, **77**, 6985.
21 S. Gupta, S. Huda, P. K. Kilpatrick and O. D. Velev, *Anal. Chem.*, 2007, **79**, 3810; Z. X. Wang, J. Lee, A. R. Cossins and M. Brust, *Anal. Chem.*, 2005, **77**, 5770.
22 A. Bini, M. Minunni, S. Tombelli, S. Centi and M. Mascini, *Anal. Chem.*, 2007, **79**, 3016; S. Centi, S. Tombelli, M. Minunni and M. Mascini, *Anal. Chem.*, 2007, **79**, 1466.

