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View Article Online
View Journal | View Issue



Cite this: *Analyst*, 2013, **138**, 4732

# A novel aptasensor for the ultra-sensitive detection of adenosine triphosphate *via* aptamer/quantum dot based resonance energy transfer†

Received 5th March 2013
Accepted 8th June 2013

DOI: 10.1039/c3an00449j

www.rsc.org/analyst

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**We designed a novel aptamer based biosensor (aptasensor) for ultrasensitive detection of adenosine triphosphate (ATP) through resonance energy transfer (RET). The ATP aptamer was modified with Cy3 at the 3′ end, and a green quantum dot (525) was attached to the 5′ end of its complementary sequence respectively. The ATP aptamer and its complementary sequence could assemble into a duplex structure in the absence of target ATP, and then decrease the distance between the quantum dot and Cy3 which could produce significant RET signal. Upon ATP binding, the ATP aptamer could dissociate with its complementary sequence and then increase the distance between the quantum dot and Cy3 which would significantly decrease the RET signal. Therefore, the ATP detection could be easily achieved through detection of the fluorescence intensity ratio between 525 nm and 560 nm. The results show that the emission fluorescence intensity ratio of 525/560 is linearly related to the logarithmic concentration of ATP. The linear range of this aptasensor is from 0.1 nM to 1 μM, and the detection limit is lower down to 0.01 nM. Excellent selectivity of this aptasensor for ATP has been demonstrated through the detection of thymidine triphosphate (TTP), cytidine triphosphate (CTP), guanosine triphosphate (GTP) and adenosine diphosphate (ADP) respectively as control. The method we described here could easily detect ATP with excellent selectivity, linearity and sensitivity down to the nanomolar range, as well as avoid photobleaching.**

Aptamers are *in vitro* selected functional single-stranded DNAs (ssDNAs), RNAs, or even chemically modified nucleic acids[1] which could fold into special structures and possess high recognition ability to specific targets ranging from metal ions,[2] organic and inorganic small molecules,[3] proteins,[4] and even whole cells.[5] They have been extensively used to construct different biosensor systems and different detection strategies.[6]

Adenosine 5′-triphosphate (ATP) is the major energy source in living organisms. The development of a fast, sensitive and quantitative ATP detection strategy would have great importance in both basic studies as well as in the diagnostic field. ATP is usually detected by methods based on chromatography, bioluminescence or chemiluminescence.[7] However, the chromatography based methods suffer from the tedious separation of sample and low accuracy of detection. Although both chemiluminescence and bioluminescence based methods have good sensitivity, the stability and specificity are always a problem. High stability, specificity and sensitivity would always be the challenges in the ATP detection strategy development.

So far, various designs of aptamer based biosensors (aptasensor) for ATP detection have been developed based on fluorescence,[8] electrochemical,[9] and colorimetric detection.[10] Compared to other methods, fluorescent aptasensors are particularly interesting due to their high stability, high sensitivity and feasibility of quantification. Quantum dots (QDs) have the advantages of broad absorption with narrow emission spectra, high quantum yield, high chemical stability and resistance to photo-bleaching.[11] Recently, several groups have reported aptamer/QD based biosensors for ATP detection.[12] However, they still suffer from complicated preparation and purification procedures, as well as low sensitivity. In this paper, we developed a fast and simple aptamer/QD based biosensor for ultrasensitive ATP detection based on resonance energy transfer (RET).

The design principle for the RET detection of ATP is illustrated in Scheme 1. Here, the ATP aptamer (5′-ACCTGGGGGAGTATT GCGGAGGAAGGT) was synthesized and its 3′-end was labeled with Cy3 (Shanghai shenggong, China). The complementary sequence of the ATP aptamer was modified with an amino group at its 5′ end through a $(CH_2)_{12}$ linker (3′-TGGACCCCCTCATAA CGCCTCCTTCCA–$(CH_2)_{12}$–$NH_2$, Shanghai Shenggong, China).

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3an00449j

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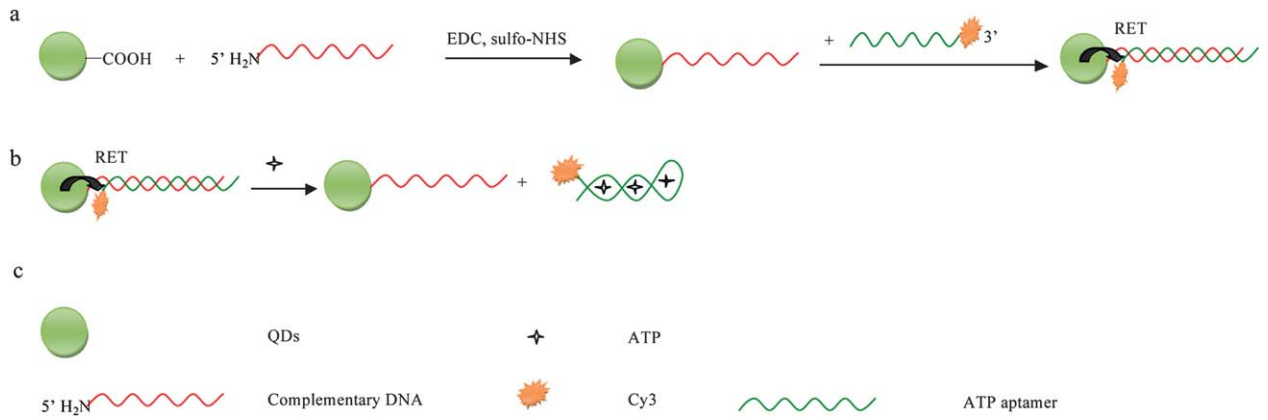


**Scheme 1** The schematic illustration of the design principle of the ATP aptasensor. (a) The coupling of the complementary sequence of the ATP aptamer onto the quantum dot surface, and the hybridization of the ATP aptamer to its complementary sequence; (b) the conformational change of the aptasensor in the presence of ATP; the ATP aptamer will dissociate with its complementary sequence in the presence of ATP, and then bind to ATP to form a stable complex; (c) the description of each component in the illustration.

The green CdTe quantum dots with carboxyl groups on their surface were synthesized according to the protocol described in the literature,[13] and their size, excitation and emission were characterized by TEM and fluorescence spectrometry.

The complementary sequence of the ATP aptamer was immobilized onto the QD surface through the EDC/sulfo-NHS chemistry. After the coupling reaction, the QDs were subjected to 1% agarose gel to confirm the successful modification of the QD surface. As shown in Fig. 1A, the successfully coupled QDs (lane 3) have a clear band shift compared to the original QDs (lane 1), or the mixture of QDs and the complementary sequence of the ATP aptamer (lane 2). Meanwhile, we could clearly see that we have less free nucleic acids in lane 3 compared to lane 2, which also means that the nucleic acids were successfully coupled to the QD surface in the presence of EDC/sulfo-NHS. Afterwards, the coupled QDs were dialyzed in PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM $KH_2PO_4$, 10 mM $Na_2HPO_4$, pH 7.4) for 24 h to remove the excess free nucleic acids. The UV spectra of the uncoupled QDs (Fig. 1B) and the dialyzed samples (Fig. 1C) were recorded. As shown in Fig. 1B

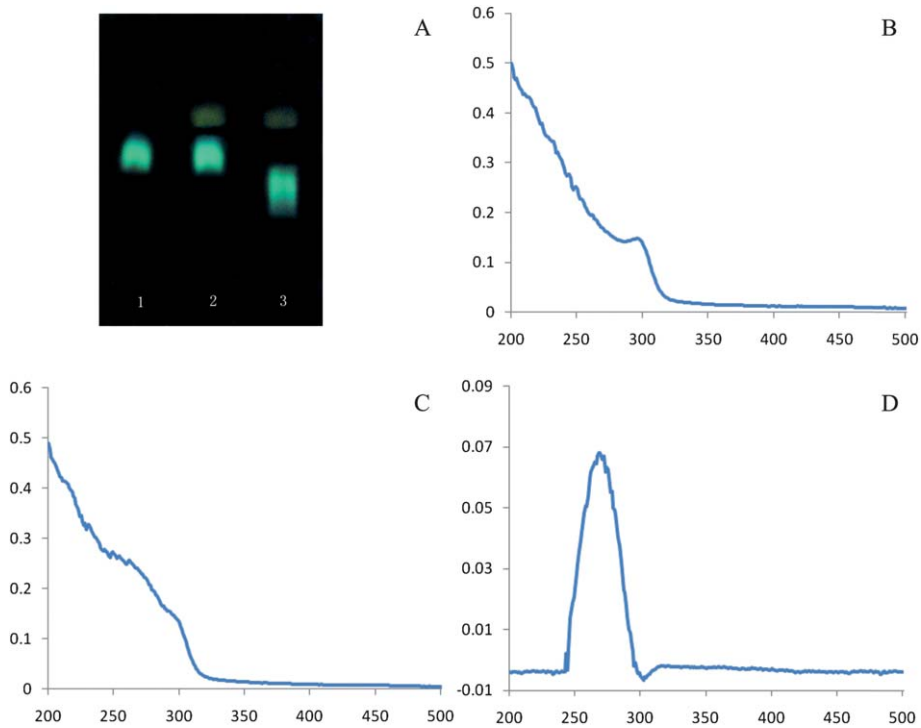


**Fig. 1** (A) The agarose electrophoresis results of the coupling between the QDs and the complementary sequence of the ATP aptamer; (lane 1): quantum dots only; (lane 2): before coupling, and (lane 3): after coupling of the complementary sequence to the quantum dots; (B) the UV absorption spectrum of the quantum dots; (C) the UV spectrum of complementary sequence coupled quantum dots; (D) the subtraction result of (B) to (C), which proved the successful coupling.

and C, although we already had very strong background absorption of the uncoupled QDs at 260 nm, we still have a clearly different absorption profile between the coupled and uncoupled QDs. Here, we have used the uncoupled QDs' spectrum (Fig. 1B) as the background to correct the absorption spectrum of the coupled QDs (Fig. 1C). The result is shown in Fig. 1D. As shown in Fig. 1D, we could see a clear single absorption peak at 260 nm, which is right according to the nucleic acid absorption. This means that the QD surface was successfully modified by the complementary sequence of the ATP aptamer through our coupling protocol.

To construct the ATP aptasensor, the Cy3 modified ATP aptamer was hybridized to its complementary sequence coupled QDs, which has been described above. The ATP aptamer would assemble into a duplex structure with its complementary sequence, which has been attached to the QD in the absence of target ATP. Afterwards, the un-hybridized ATP aptamer was removed through dialysis in PBS buffer for another 24 h. Then, the duplex aptasensor was constructed and collected for use. The successful hybridization of the ATP aptamer was confirmed by recording the Cy3 spectrum (Fig. S1A†), as well as the circular dichroism (Fig. S1B†). As shown in Fig. S1A,† we could detect a very strong Cy3 signal even after the dialysis, which means that the ATP aptamer was successfully hybridized to its complementary sequence. To further confirm the hybridization, the circular dichroism spectrum was recorded. As shown in Fig. S1B,† we could see a clear shift of the CD spectrum of the hybridized QDs compared to the none-hybridized QDs, which is well fitted with the classic CD spectrum of ds-DNA.

Since the ATP aptamer and its complementary sequence have assembled into a duplex structure in the absence of ATP, the distance between the green quantum dot and the Cy3 dye would be decreased and give a significant RET signal after the excitation of the QDs. Therefore, the absorption and emission spectra of the duplex aptasensor have also been recorded. As shown in Fig. S1C,† we could see a good double peak emission spectrum from 500 nm to 580 nm upon excitation of QDs at 480 nm without adding ATP. The first peak (525 nm) represents the quantum dot emission upon 480 nm excitation, and the second peak (560 nm) represents the Cy3 emission that was excited by the energy transfer from the QD emission.

Upon binding of ATP, the ATP aptamer and its complementary sequence will be dissociated from each other and the distance between the QD and Cy3 will be increased, which causes a significant decrease of the RET signal. This means that the emission signal (525 nm) of QDs will be increased, and the Cy3 emission signal (560 nm) will be decreased upon ATP binding. Therefore, the ATP detection could be easily achieved through detecting the fluorescence intensity ratio between 525 nm and 560 nm. To verify the ATP detection ability of our developed aptasensor, ATP of different concentrations was added into the aptasensor in PBS buffer (137 mM NaCl, 2.7 mM KCl, 2 mM $KH_2PO_4$, 10 mM $Na_2HPO_4$, pH 7.4), and the ratio between 525 nm and 560 nm was calculated. Upon adding 0.1 nM ATP into our aptasensor system, we could clearly see the increase in fluorescence intensity of the first peak and a decrease in the fluorescence intensity of the second peak (Fig. 2, curves a and b). Therefore, it is clearly demonstrated the dissociation of the ATP aptamer and its complementary sequence, which induced the decrease of the RET signal upon ATP binding. To investigate the linear range of our aptasensor, 1 nM, 10 nM, 100 nM and 1 μM ATP were further added into the aptasensor system. As shown in Fig. 2(a–e), we could clearly see the sequentially increased fluorescence intensity of the first peak and decreased fluorescence intensity of the second peak along with the increase of ATP concentration up to 100 nM. However, we could only see the increase in fluorescence intensity of the first peak, but not the decrease in fluorescence intensity of the second peak (sometimes even slightly increased) with 1 μM ATP or even high concentration (Fig. S2†). One possibility would be that the emission peak of quantum dots and Cy3 were not well separated. These two peaks still had huge

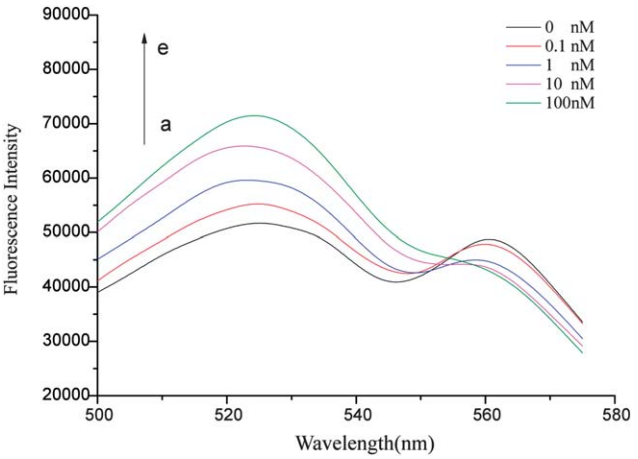


**Fig. 2** The spectra of the constructed ATP aptasensor from 500 nm to 580 nm ($\lambda_{ex}$ = 480 nm, the width of the slits for both excitation and emission was 4 nm) in the presence of different concentrations of ATP. (a–e) The responses of the aptasensor to different concentrations of ATP, 0 nM, 0.1 nM, 1 nM, 10 nM and 100 nM respectively.

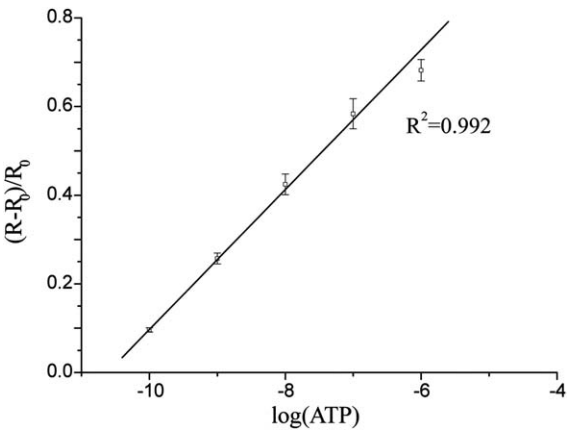


**Fig. 3** The calculated responses of the aptasensor and the linear fit of the responses to the logarithmic concentration of ATP, 0 nM, 0.1 nM, 1 nM, 10 nM, 100 nM and 1 μM ATP respectively. $R_0$ represents the ratio of 525/560 in the absence of ATP, and $R$ represents the ratio of 525/560 in the presence of ATP.

 

overlapping between each other, which could be clearly seen from the spectrum (Fig. 2 and S1C†). Therefore, the strong increasing fluorescence of the QD emission (the first peak) might compensate for the decreasing fluorescence of the Cy3 emission (the second peak). In such case, there might be no change or increase of the second peak even when the emission of the Cy3 has been decreased.

The ratio of 525/560 was calculated and fitted with the logarithmic concentration of ATP. As shown in Fig. 3, we could clearly see that the increasing ratio of 525/560 is linearly related to the logarithmic concentration of ATP from 0.1 nM to 1 μM with a relation coefficient ($R^2$) of 0.992. Since the increase of the quantum dot emission (the first emission peak) could only be due to less energy transfer from QDs to Cy3, we could only take the increase of the first peak fluorescence intensity into consideration. In such a case, we could even further detect ATP in a wider range from 0.1 nM to 10 μM (Fig. S3†), with an acceptable linear relation coefficient ($R^2$) of 0.908. To test the stability of our constructed aptasensor, the duplex was stored at 4 °C and the ratio of 525/560 was measured in the absence of ATP over days. As shown in Fig. S4,† the ratio of 525/560 was relatively stable over 11 days with an average ratio of 1.015 ± 0.015. It means that the detected ratio change in the presence of ATP is not due to the automatic dissociation of the duplex, but due to the much more stronger binding of the ATP to its aptamer, which then causes dissociation of the duplex.

Since our constructed aptasensor was linearly related to the logarithmic concentration of ATP, the resolution might be a problem. That means we might not be able to distinguish ATP in very closed concentrations, which would further decrease the reliability of our aptasensor in practical applications. To test the resolution of our aptasensor, the ATP concentrations from 0.1 nM to 0.5 nM were tested by our aptasensor. As shown in Fig. S5,† our duplex aptasensor actually could nicely distinguish these relatively closed concentrations (Fig. S5A†) with an increase at the 525 nm peak and a decrease at the 560 nm peak, as well as an acceptable linear relation coefficient ($R^2$) of 0.913. Therefore, our duplex aptasensor not only has a very wide detection range but also has a great resolution.

To verify the specificity and selectivity of our aptasensor, 1 mM ADP, CTP, GTP, TTP and 1 μM ATP were added into the aptasensor respectively. As shown in Fig. 4, the response of our aptasensor to all of the none specific targets such as ADP, CTP, TTP and GTP is almost minimizing to the background level (in the absence of ATP), which is much lower than the response of ATP, although we have 1000 times higher concentration of ADP, CTP, TTP and GTP than ATP. These results clearly proved that our aptasensor has great specificity and selectivity to ATP.

The detection limit of our aptasensor could be further pushed down to 0.01 nM, although it is out of the linear range. As shown in Fig. 5A, we could clearly distinguish the decrease of the 525 nm peak and the increase of the 560 nm peak in the presence of only 0.01 nM ATP. The ratio of 525/560 was calculated; as shown in Fig. 5B, the ratio is significantly different in

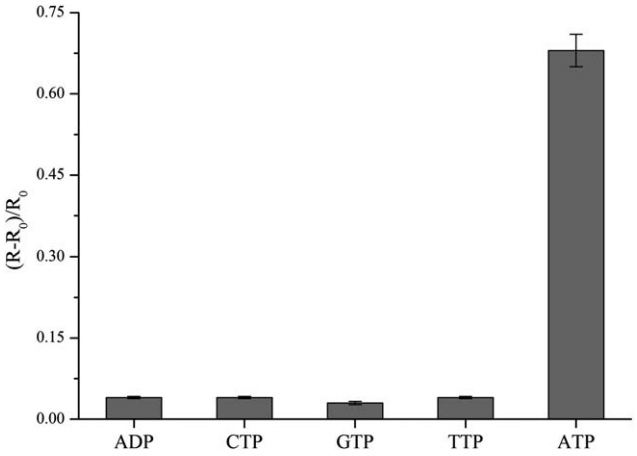


**Fig. 4** The selectivity and specificity of the constructed ATP aptasensor. *R* represents the ratio of 525/560 in the presence of target, and $R_0$ represents the ratio of 525/560 in the absence of any target. The concentration of ADP, CTP, GTP and TTP was 1 mM, and the concentration of ATP was 1 μM, respectively.

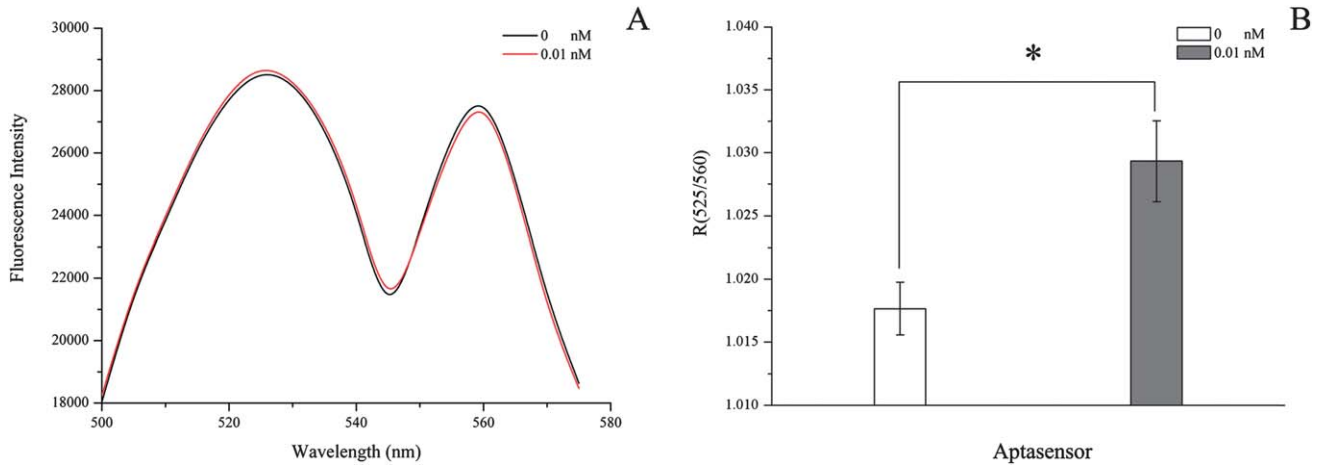


**Fig. 5** The detection limit of the constructed ATP aptasensor. (A) The emission spectra of the aptasensor from 500 nm to 580 nm ($\lambda_{ex}$ = 480 nm, the width of the slits for both excitation and emission was 4 nm) in the absence and in the presence of 0.01 nM ATP; (B) the calculated ratio of 525/560 in the absence and in the presence of ATP (*$p$ < 0.05, Student's $t$-test).

the absence and in the presence of 0.01 nM ATP. It is clearly demonstrated that our aptasensor has a much lower detection limit (0.01 nM) compared to the reported ATP sensor strategies.

In summary, we have reported an ultrasensitive ATP aptasensor in this paper. The aptasensor reported here is based on the ATP aptamer through the resonance energy transfer principle. Our aptasensor has a linear range from 0.1 nM to 1 μM. The detection limit of our aptasensor is lower down to 0.01 nM, which is much lower than other reported ATP sensors. Meanwhile, our aptasensor has very good selectivity and could nicely distinguish ATP and its different analogues. The method we described here has excellent selectivity, linearity and sensitivity, which could be further developed into an *in vivo* ATP imaging sensor.

## Acknowledgements

This work is supported by the National Natural Science Foundation of China (Grant no. 31201008), the University Natural Science Foundation of Jiangsu Province (Grant no. 12KJB180013), the National Key Technology R&D Program of the Ministry of Science and Technology (Grant no. 2012BAI19B05), the National High-tech R&D Program of China (863 Program, Grant no. 2012AA040504) and the Special Fund for Basic Research on Scientific Instruments of the National Nature Science Foundation of China (Grant no. 612278043).

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