

A cascade amplification strategy based on rolling circle amplification and hydroxylamine amplified gold nanoparticles enables chemiluminescence detection of adenosine triphosphate

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Cite this: *Analyst*, 2014, 139, 3796

A highly sensitive and selective chemiluminescent (CL) biosensor for adenosine triphosphate (ATP) was developed by taking advantage of the ATP-dependent enzymatic reaction (ATP-DER), the powerful signal amplification capability of rolling circle amplification (RCA), and hydroxylamine-amplified gold nanoparticles (Au NPs). The strategy relies on the ability of ATP, a cofactor of T4 DNA ligase, to trigger the ligation-RCA reaction. In the presence of ATP, the T4 DNA ligase catalyzes the ligation reaction between the two ends of the padlock probe, producing a closed circular DNA template that initiates the RCA reaction with phi29 DNA polymerase and dNTP. Therein, many complementary copies of the circular template can be generated. The ATP-DER is eventually converted into a detectable CL signal after a series of processes, including gold probe hybridization, hydroxylamine amplification, and oxidative gold metal dissolution coupled with a simple and sensitive luminol CL reaction. The CL signal is directly proportional to the ATP level. The results showed that the detection limit of the assay is 100 pM of ATP, which compares favorably with those of other ATP detection techniques. In addition, by taking advantage of ATP-DER, the proposed CL sensing system exhibits extraordinary specificity towards ATP and could distinguish the target molecule ATP from its analogues. The proposed method provides a new and versatile platform for the design of novel DNA ligation reaction-based CL sensing systems for other cofactors. This novel ATP-DER based CL sensing system may find wide applications in clinical diagnosis as well as in environmental and biomedical fields.

Received 10th March 2014

Accepted 30th April 2014

DOI: 10.1039/c4an00458b

www.rsc.org/analyst

1. Introduction

ATP, as a biologically important molecule, is often used as a universal energy storage molecule in all living organisms and also as an indicator of living organisms for cell viability and cell injury.^{1–4} It is well known that ATP is the primary energy carrier of the living systems and the aberrant ATP levels are associated with a variety of genetic diseases such as hypoxia, hypoglycemia, Parkinson's disease and some malignant tumors.^{5–8} Therefore, the sensitive and selective detection of ATP is of great significance for biochemical studies and clinical diagnosis. In recent years, various aptamer-based biosensors for ATP have been developed using electrochemiluminescence (ECL),^{9–11} electrochemical,^{12,13} fluorescence^{14–18} and colorimetric methods.^{19,20} However, these methods show moderate sensitivity because the anti-ATP DNA aptamer is a low-affinity species with a relatively low association constant with ATP.²¹ Furthermore, most of

these methods cannot discriminate ATP from its analogues, such as AMP.

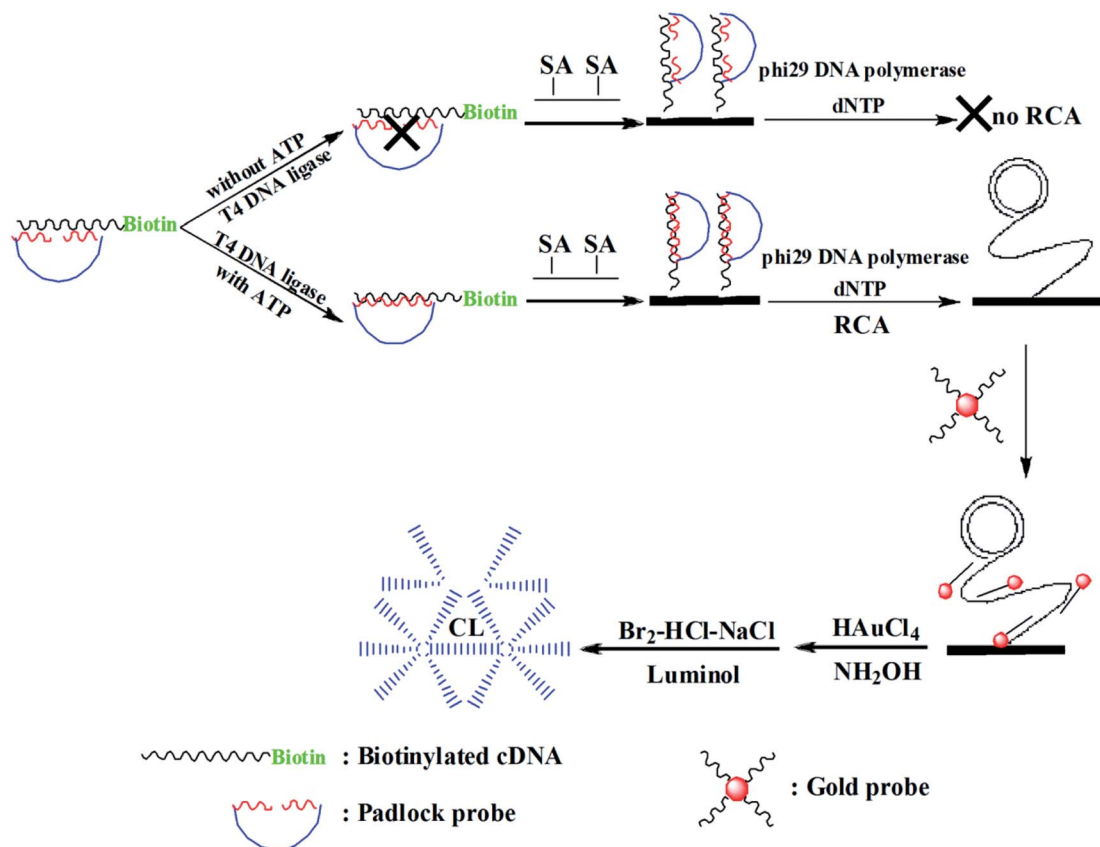
Instead of aptamer-based ATP sensors, some enzyme reactions show specific dependence on cofactor ATP, namely ATP-DER, providing an efficient platform for constructing highly selective sensing systems for ATP. Using ATP as a cofactor of T4 DNA ligase, several fluorescent and electrochemical sensing systems have been reported for the detection of ATP, most of which are based on dually labeled molecular beacon^{22,23} or molecular beacon-like DNA.²⁴ The dually labeled oligonucleotide probe brings about complexity and high cost, which may limit its practical use. Yu's group developed a highly selective fluorescent biosensor for ATP detection using a single-labeled probe based on ATP-DER and the different adsorption affinities between graphene oxide and DNA structures.²⁵ Wang's group developed a non-covalent labeling strategy for ATP based on inhibition of template-directed G-quadruplex assembling by ligation reaction.²⁶ T4 DNA ligase is one of the key enzymes involved in the RCA assays, in which the hybridized padlock probes are circularized by ATP-activated T4 DNA ligase and are then copied into linear concatemers by DNA polymerases under isothermal conditions.²⁷ These concatemers are frequently

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thousands of nucleotides in length, leading to a large amplification of the initial circular "signal". Using ATP-triggered RCA, several fluorescent sensing schemes for detection of ATP have been developed.^{28,29} For example, Jiang's group developed highly sensitive detection of ATP based on dumbbell probe-mediated RCA-responsive G-quadruplex formation.²⁸ These ATP-DER-based methods are proven to be highly sensitive and more selective than the aptamer-based methods due to the unique role of ATP in the enzymatic reaction. However, we noticed that most of these methods focused on using fluorescent methods. As one of the most sensitive methods, CL promises high sensitivity, simplicity, and low cost. It has become an attractive bioanalytical method. In recent years, several CL mechanisms have been designed for the determination of ATP. For example, in previous work, our group developed a label-free CL aptasensor for the detection of ATP with a detection limit of 70 μM by using a graphene oxide nano-platform.³⁰ Based on chemiluminescence resonance energy transfer (CRET), Zhao's group developed an aptasensor for ATP with a detection limit of 185 nM.³¹ Because of the reliance on the anti-ATP DNA aptamer, the above designs for the CL determination of ATP suffered from low sensitivity as well as low discrimination of ATP and its analogues. To overcome these problems, the present work developed a novel CL assay for the determination of ATP based on ATP-DER, rolling circle amplification and hydroxylamine amplified Au NPs. To the best of our

knowledge, an ATP-DER-based CL strategy for ATP detection has not been reported yet.

Au NPs are particularly attractive in bioassay by virtue of their facile synthesis, large specific surface area, high chemical stability, and biocompatibility.^{32–34} As an amplification tag, Au NPs have been the subject of research directed at gene analysis and antibody or antigen detection.^{35–38} In current work, an ATP-DER-based CL assay using hydroxylamine amplified Au NPs as the label was developed for the determination of ATP with high sensitivity and specificity. Scheme 1 shows the sensing mechanism. The 28 3'- and 5'-terminal bases of the 81-mer padlock probe were designed to be complementary to the biotinylated cDNA. In the presence of T4 DNA ligase together with its cofactor ATP, the 3'-OH and 5'-PO₄ ends of the padlock probe could be ligated to produce a closed circular DNA template in an amount that is positively related to the concentration of cofactor ATP. The ligation products were then immobilized onto the surface of a 96-well plate *via* biotin-streptavidin reaction. The immobilized cDNA acted as primers to initiate the RCA reaction in the presence of phi29 DNA polymerases and dNTP. The RCA reaction process produced long single-stranded DNA by replicating the circular template hundreds to thousands of times, which could serve as an excellent template for the binding of a gold probe. Au NPs assembled on the surface of a 96-well plate were then enlarged through a redox reaction of HAuCl₄ and NH₂OH, in which gold metals were catalytically deposited onto the surfaces of the Au NPs.^{39,40} Finally, the gold



Scheme 1 The schematic diagram of ATP triggered ligation-RCA assay for CL detection of ATP.

metal contained in the bound phase was dissolved in an oxidative $\text{Br}_2\text{-HCl-NaCl}$ solution, and the gold ions thus released into the solution were quantitatively measured by the Au^{3+} catalyzed luminal CL reaction. The CL signal was proportionally correlated with the concentration of ATP in the standard or sample. In contrast, in the absence of ATP, the T4 DNA ligase was inactive and could not catalyze the ligation reaction of the padlock probe and subsequent RCA reaction. Therefore, no Au NPs could be immobilized on the surface of the 96-well plate. We successfully converted the ATP-DER into detectable CL signals in this way.

2. Experimental

2.1 Materials and chemicals

All chemicals were of analytical reagent grade and were used as received. Distilled water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was used throughout the current work. T4 DNA ligase, phi29 DNA polymerase, and deoxynucleotides (dNTPs) were obtained from Thermo Fisher Scientific (China) Co., Ltd. Bovine serum albumin (BSA), dithiothreitol (DTT), and human serum were obtained from Puboxin Biotechnology Co., Ltd (Beijing, China). Calmodulin was purchased from Sigma-Aldrich (St. Louis, MO). Hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), hydrogen tetrachloroaurate(III) tetrahydrate ($\text{HAuCl}_4\cdot 4\text{H}_2\text{O}$), Tween 20, and other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Luminol was purchased from Alfa Aesar (Tianjin, China). Citrate-stabilized Au NPs (13 nm diameter) were prepared according to the methods described in the literature.^{41,42} ATP, GTP, UTP, CTP, AMP and all oligonucleotides were acquired from Sangon Biological Engineering Technology & Service Co., Ltd (Shanghai, China). All oligonucleotides were purified by high-performance liquid chromatography (HPLC) and are listed in Table 1. A 96-well plate coated with a layer of reactive *N*-oxysuccinimide esters, referred to as the NOS group surface, was purchased from Corning Inc. (New York, United States).

2.2 Apparatus

CL measurements were performed with a BPCL chemiluminescence analyzer (Beijing, China). The hydrodynamic sizes of Au NPs were evaluated by using a Zetasizer Nano ZS system (Malvern Instruments Ltd., Worcestershire, UK).

2.3 Preparation of the reporter probe–Au NP conjugates (gold probes)

Streptavidin modified Au NPs were prepared *via* slightly modifying a previously published method.⁴³ Streptavidin (10% more

than the minimum amount, which was determined using a flocculation test) was added to a 1 mL colloidal gold suspension (pH 7.0, adjusted by 0.1 M K_2CO_3) followed by incubation at room temperature for 30 min. The conjugates were centrifuged at 4 °C (25 000g, 60 min). After carefully removing the supernatant, the red soft sediment was re-suspended in buffer A (20 mM Tris-HCl and 0.5 M NaCl, pH 8.0) containing 0.025% Tween 20 and 1% BSA. Subsequently, 3.09 μL of biotinylated reporter probe (16.2 μM) was added and incubated for 30 min under gentle mixing at 37 °C. The final conjugates were isolated by centrifugation (25 000g, 60 min). The red oily precipitate was re-suspended in 1 mL of buffer A. The addition of BSA with a final concentration of 1% allowed storage of the gold probe at 4 °C for several days.

2.4 Preparation of the streptavidin-bound 96-well plate

Streptavidin was diluted to 30 ng in 1 mL of coupling buffer (0.05 M $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$, pH 8.5). The diluted streptavidin solution was divided equally into the wells (50 μL per well) of a 96-well plate which had a NOS surface, and incubated at 37 °C for 60 min under gentle shaking. After removing the solution, 200 μL of Tris solution (10 mM in coupling buffer) was added into each well of the 96-well plate and incubated at 37 °C for another 60 min to block the remaining NOS groups. The wells were then washed twice with washing buffer (7 mM Tris, 0.17 M NaCl and 0.05% Tween 20, pH 8.0).

2.5 ATP-DER for ATP detection

For the ligation reaction with T4 DNA ligase, the reaction mixture consisted of ligation buffer (40 mM Tris-HCl, 10 mM MgCl_2 , 10 mM DTT, pH 7.8), 0.5 U of T4 DNA ligase, 0.25 pmol of padlock probe, 1.25 pmol of cDNA, and different concentrations of ATP in a reaction volume of 25 μL . Before the addition of T4 DNA ligase, ATP, and ligation buffer, the solution was heated to 90 °C and maintained for 3 min, and then cooled down to room temperature. After adding ligation buffer and a designed concentration of ATP, the mixture was incubated at 16 °C for 30 min under gentle shaking. T4 DNA ligase was then added and the reaction mixture was incubated at 16 °C for 90 min. T4 DNA ligase together with the cofactor ATP catalyzed the formation of a circular DNA template in an amount that is positively related to the concentration of the cofactor ATP. The reaction mixture was then heated at 65 °C for 10 min to denature the enzymes and cooled down to room temperature for the hybridization between cDNA and the circular DNA template.

The ligation products were transferred to each well of the streptavidin-bound 96-well plate, and then incubated at 16 °C for 30 min under gentle mixing. After washing in triplicate with $2 \times \text{SSC}$ buffer (0.3 M NaCl, 0.03 M $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot 2\text{H}_2\text{O}$, pH 7.0),

Table 1 Oligonucleotides synthesized in the present study^a

cDNA	5'-Biotin-AAAAAAAAATGTCCTGCTAGAGGAAACAGTTACCA-3'
Padlock probe	5'-PO ₄ -TAGCACGGACATATATGATGGTACCGCAGTATGAGTATCTCTCTATCACTACTAAGTGAAGAAATGGTAACTGTTTCCTTC-3'
Reporter probe	5'-Biotin-AAAAAAAAACGCAGTATGAGTATCTCC-3'

^a The underlined sequences in cDNA can hybridize the two ends of the padlock probe and the italic sequence in the padlock probe is similar to the end sequence of the detect probe.

the RCA reaction was performed at 30 °C for 90 min in a volume of 50 μ L of solution containing 33 mM Tris-acetate, 10 mM Mg(Ac)₂, 66 mM KAc, 1 mM dNTPs, 1 U phi29 DNA polymerase, and 1 mM DTT. The wells were then washed three times with buffer AT (buffer A containing 0.05% Tween 20). An aliquot of 50 μ L of gold probe (0.928 nM in buffer A containing 1% BSA) was hybridized with the RCA products for 40 min at 30 °C. The excess gold probe was washed away with washing buffer, and the Au NPs assembled on the surface of the 96-well plate were catalytically enlarged in the presence of 0.5 mM NH₂OH and 0.05 mM HAuCl₄ at room temperature for 4 min. Then, the wells were rinsed three times with the washing buffer. After carefully removing the rinsing solution, 200 μ L of gold metal oxidative solution (the final concentration was 0.25 mM Br₂-2.5 mM HCl-0.15 M NaCl) was pipetted into the wells and made to react for 10 min. The resultant mixture was placed in the water bath at 60 °C for 4 min. Finally, 60 μ L of the solution was transferred into 14 $\times$ 40 mm glass tubes containing 100 μ L of 5×10^{-6} M luminol (in 0.75 M NaOH) and the CL signal was then displayed in the CL analyzer ($n = 3$).

3. Results and discussion

3.1 Feasibility of the CL biosensor for ATP detection

Firstly, the feasibility of the CL biosensor was verified by agarose gel electrophoresis. The electrophoresis experiment was performed with 1.0% agarose gels and run in $1 \times$ TAE buffer (40 mM Tris, 20 mM HAc, 10 mM EDTA, pH 8.0) at room temperature under a constant voltage of 100 V for 50 min with loading of 10 μ L of each sample into the lanes. As shown in the inset of Fig. 1, a long DNA product of ligation-RCA triggered by 5 nM ATP was observed in lane Fig. 1B1, in which the RCA product showed extremely low mobility, meaning that the length of the RCA product was more than 10 000 bp. In addition, a blurry band, suggesting the length of DNA is about 1000 bp, is also seen in Fig. 1B1. The above results indicated that the length of RCA products ranged from $\sim$ 1000 to >10 000 bp, and most of the products were longer than 10 000 bp. In contrast, there is no band in the absence of ATP (data not shown). We further reduced the electrophoresis time to 20 min. As shown in the inset of Fig. 1, a band between 100 and 250 bp, which is consistent with the total length of cDNA and padlock probe, is observed in lane Fig. 1A1, indicating that no RCA product was generated in the absence of ATP. Secondly, we investigated the corresponding CL intensities of the sensing strategy in the absence and presence of ATP, respectively. As shown in Fig. 1, the control experiment without ATP only exhibited a low CL intensity, while 5 nM ATP showed a significant CL intensity enhancement. These results indicated that ATP acted as a trigger of the ligation-RCA reaction. In the presence of ATP, the two ends of the padlock probe could be ligated and the subsequent RCA reaction could be initiated to produce lots of complementary copies of the circular template. Therefore, more gold probes could be assembled on the surface of the 96-well plate resulting in an enhanced CL signal. In contrast, in the absence of ATP, the DNA ligase was inactive and could not catalyze the ligation reaction of the padlock probe and initiate the subsequent RCA reaction.

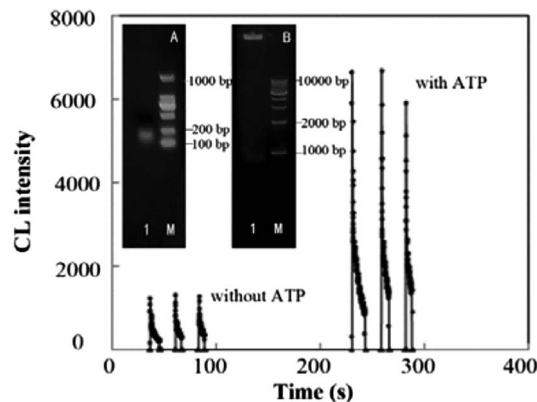


Fig. 1 CL intensity in the absence (A) and presence (B) of ATP; the corresponding agarose gel electrophoretic experiments were depicted in the inset. The marker was indicated by M.

3.2 Optimization of assay conditions for the determination of ATP

In order to achieve the best sensing performance, several experimental parameters were investigated systematically. Firstly, ATP dependent ligation reaction, a crucial step for ATP sensing, has a significant effect on the RCA process. Experimental conditions which affect ligation reaction, such as the amount of T4 DNA ligase and cDNA, and the molar ratio of cDNA to padlock probe were optimized first. As shown in Fig. 2A, the relative CL intensity (CL_1 deducted CL_0 , where CL_1 and CL_0 are the CL intensities in the presence and absence of ATP, respectively) increased rapidly with the increase of T4 DNA ligase from 0.1 to 0.5 U, and then reached a plateau between 0.5 and 1 U. Hence, 0.5 U of T4 DNA ligase was selected for the following studies. The full hybridization equilibrium between the cDNA and the padlock probe is a key factor for ensuring efficient ligation reaction. It is essential to optimize the molar ratio of cDNA to padlock probe. Using a fixed amount of cDNA (1.25 pmol), the molar ratio of cDNA to padlock probe was investigated. As shown in Fig. 2B, when the molar ratio increased to 5 : 1, the maximum relative CL intensity was achieved. A higher molar ratio brought a gradual decrease in relative CL intensity. This phenomenon may be attributed to that a large excess of the padlock probe may disturb its hybridization with cDNA in a head-to-tail fashion and subsequent RCA reaction. Therefore, the optimum molar ratio of cDNA to padlock probe was 5 : 1. Fig. 2C shows the effect of cDNA amount on the relative CL intensity. The strongest relative CL intensity was obtained by using 1.25 pmol of cDNA. Hence, 1.25 pmol cDNA was selected for further study.

In principle, the more complementary copies of the circular template are produced, the stronger CL intensity will be generated. So the effects of phi29 DNA polymerase concentration and RCA reaction time were examined. As shown in Fig. 3A, the relative CL intensity increased with the increase of phi29 DNA polymerase from 0.25 to 1 U and then reached a plateau between 1 and 2 U. Thus, 1 U of phi29 DNA polymerase was selected for the following experiments. To obtain a maximum response using a minimal concentration of the gold probe, we then optimized the molar ratio of the biotinylated reporter probe to streptavidin-gold and the concentration of the gold

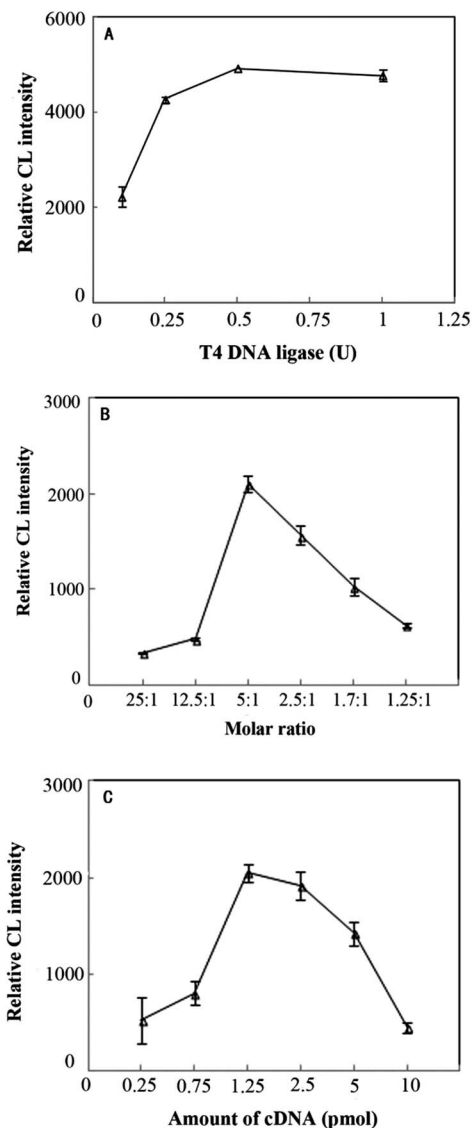


Fig. 2 (A) The effect of T4 DNA ligase amount on the relative CL intensity of the ATP sensing system, experimental conditions: cDNA, 1.25 pmol; padlock probe, 80 fmol; ATP, 50 nM; dNTPs, 1 mM; phi29 DNA polymerase, 0.5 U; RCA time, 2 h; gold probe, 0.928 nM; HAuCl₄, 0.05 mM; NH₂OH, 0.5 mM; T_R , 4 min. (B) The effect of molar ratio of cDNA to padlock probe on the relative CL intensity of the ATP sensing system, experimental conditions: ATP, 5 nM; T4 DNA ligase, 0.5 U; other experimental conditions were the same as (A). (C) The effect of cDNA amount on the relative CL intensity of the ATP sensing system. Experimental conditions: the molar ratio of cDNA to padlock probe, 5 : 1; other experimental conditions were the same as (B). The error bars show the standard deviations for three replicate determinations.

probe. The molar ratio of the biotinylated reporter probe to streptavidin-gold was evaluated using a fixed concentration of streptavidin-gold (0.928 nM). As shown in Fig. 3B, the relative CL intensity reached a maximum by using 2.5 pmol of biotinylated reporter DNA. Finally, we optimized the concentration of the gold probe by investigating the relative CL intensity of the CL biosensor with different concentrations of the gold probe (Fig. 3C). The relative CL intensity increased rapidly by raising the concentration of the gold probe at the beginning, leveled off

between 0.928 and 2.32 nM, and then decreased, due to the greatly enhanced background signal. Hence, the 0.928 nM gold probe was selected for the following experiments.

3.3 Assay performance of the ATP-DER CL sensing system for ATP detection

The quantitative behavior of the CL assay was assessed with different concentrations of ATP under optimized conditions. Fig. 4 shows the relative CL intensity of the sensing system upon addition of different concentrations of ATP. The relative CL

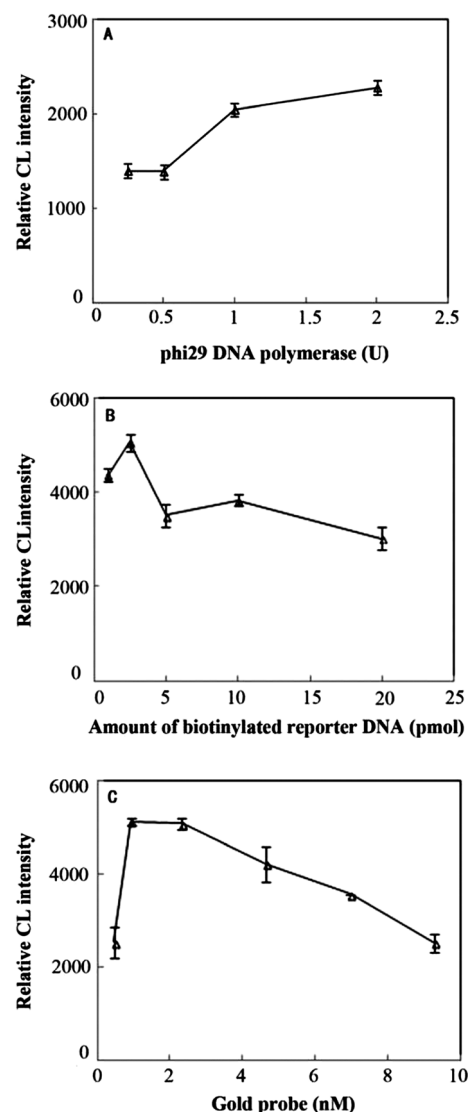


Fig. 3 (A) The effect of phi29 DNA polymerase amount on the relative CL intensity of the ATP sensing system; experimental conditions: cDNA, 1.25 pmol; other experimental conditions were the same as denoted in Fig. 2(C). (B) The effect of biotinylated reporter DNA on the relative CL intensity of the ATP sensing system; experimental conditions: phi29 DNA polymerase 1 U; RCA time, 1.5 h; other experimental conditions were the same as (A). (C) The effect of gold probe on the relative CL intensity of the ATP sensing system; experimental conditions: biotinylated reporter DNA, 2.5 pmol; other experimental conditions were the same as (B). The error bars show the standard deviations for three replicate determinations.

intensity increased remarkably with the increasing concentration of ATP from 0.1 to 5 nM, indicating that the formation of RCA products (or the capture of gold probes on the surface of the 96-well plate) was highly dependent on the concentration of target ATP. A calibration graph in the concentration range of 0.1–5 nM showed a linear correlation ($R^2 = 0.9808$) between the concentration of ATP (nM) and the CL intensity, represented by $I = 1009.1C + 281.4$. Based on the 3σ rule, as low as 100 pM of target ATP could be sensitively detected using this method, which exhibited 70 000 times enhancement of sensitivity over previous label-free CL detection of ATP³⁰ and 1850 times over the CRET format.³¹ In addition, the amplified CL signal was coupled to a relatively good reproducibility. A series of eight repetitive measurements of ATP solution (5 nM) yielded reproducible signals with a relative standard deviation of 4.6%.

Compared to previously reported anti-ATP DNA aptamer-based sensing systems, the ATP-DER based CL sensing system exhibits high specificity for ATP detection, because the ligation-RCA cannot occur in the absence of the cofactor ATP. The specificity of the CL sensing strategy was examined by challenging it with several possible interfering targets, including UTP, CTP, GTP, AMP, BSA and calmodulin. Fig. 5 illustrates the histograms of the relative CL intensity in the presence of ATP and other possible interfering targets. It could be observed that negligible signals were obtained in the presence of 5 nM of UTP, CTP, GTP and AMP, and 500 nM of BSA and calmodulin. The experimental results demonstrated that the ATP-DER based CL assay developed here showed high selectively toward ATP detection, which makes it promising for practical applications.

3.4 Analysis of ATP in serum samples

To demonstrate the feasibility of the approach in complex biological matrices, we applied this CL sensing system to detect the ATP spiked human serum. The addition of serum would lead to the matrix effect, so the dilution factor of serum was optimized in the preliminary experiment. And a better performance in terms of higher relative CL intensity was obtained

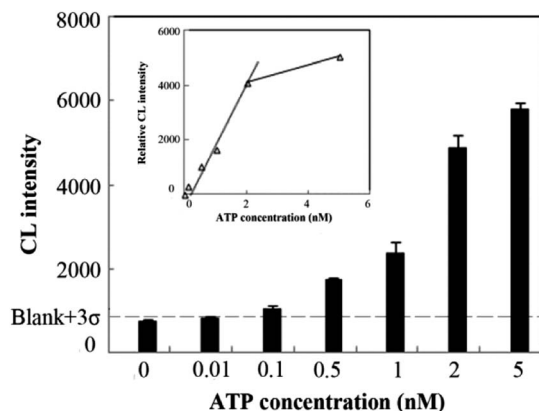


Fig. 4 CL intensity vs. different concentrations of ATP. The inset displays the linear relationship between relative CL intensity and ATP concentration in the range from 0.1 to 5 nM. The error bars show the standard deviations for three replicate determinations.

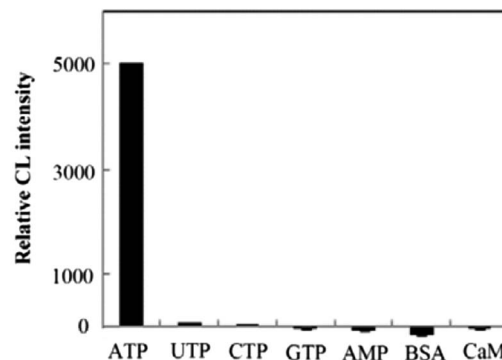


Fig. 5 Selectivity of the CL sensing system for ATP compared to other possible interfering targets. The concentration of ATP, UTP, CTP, GTP and AMP is 5 nM. The concentration of BSA and CaM is 500 nM. Other experimental conditions were the same as denoted in Fig. 4. The error bars show the standard deviations for three replicate determinations.

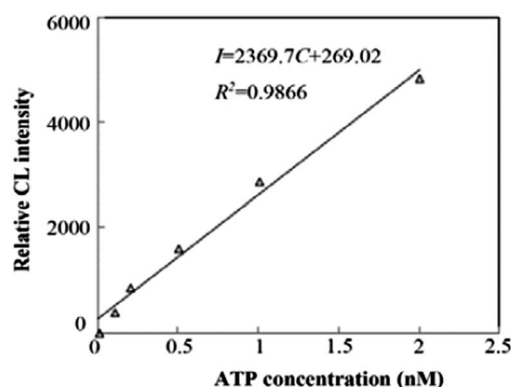


Fig. 6 Correlation between the results measured by the proposed method for ATP detection. It displays the linear relationship between relative CL intensity and ATP concentration in the range from 0.1 to 2 nM.

when 100-fold diluted serum was employed, and so 100-fold diluted serum was applied in the following experiment. Firstly, the dose-response curve generated from a representative serum sample was investigated. We noticed that the CL signals obtained in serum samples were similar to those in buffer solutions. As shown in Fig. 6, the relative CL intensity increased upon increasing the concentration of the spiked ATP, with linearity ($R^2 = 0.9866$) existing between the relative CL intensity and the concentration of ATP over the range from 0.1 to 2 nM. Secondly, the ability of the ATP-DER-based CL assay to detect ATP in the real sample was tested by using a standard addition method. Different amounts of ATP were added into 100-fold

Table 2 Recovery of ATP spiked into human serum samples

Amount added (nM)	Recovery (%)
0.5	113.1 ± 8.6
1	93.0 ± 9.6
2	111.4 ± 19.8

Table 3 Comparison of sensitivity for different ATP assay methods

Analytical method	Label	Detection limit	Linearity range	Method	Reference
Electrochemistry	[Fe(CN) ₆] ^{3-/4-}	0.05 nM	0.1–1000 nM	ATP-DER	24
Fluorescence	QDs	24 μM	0.1–1 mM	Aptamer-based	16
Fluorescence	FAM	2 μM	5–2500 μM	Aptamer-based	14
Fluorescence	QDs	3.7 μM	10–350 μM	Aptamer-based	21
Fluorescence	Cy3	1000 nM	10–100 μM	ATP-DER	27
Fluorescence	Label free	0.5 pM	0.001–100 nM	ATP-DER	28
Fluorescence	NMM	50 nM	0.05–0.6 μM	ATP-DER	26
Fluorescence	FAM	0.1 nM	0.1–300 nM	ATP-DER	22
CL	Label free	70 μM	0.1–4 mM	Aptamer-based	30
CL	QDs	185 nM	50–231 μM	Aptamer-based	31
ECL	Ru(phen) ₃ ²⁺	0.2 nM	0.5–1000 nM	Aptamer-based	10
CL	Au NPs	100 pM	0.1–5 nM	ATP-DER	This work

diluted serum samples and determined under optimal experimental conditions. The data for the quantization of ATP are shown in Table 2, which indicated recoveries ranging from 93.0% to 113.1%. The result indicated very little interference of complex matrices in the designed strategy, and thus the proposed method could be used in the real sample analysis.

4. Conclusion

In summary, a novel CL sensing strategy for ATP detection was developed by taking advantage of ATP-DER for increased specificity, and the powerful signal amplification capability of RCA and hydroxylamine-amplified Au NPs. The proposed ATP-DER-based CL sensing system demonstrated high sensitivity that can detect the presence of ATP at a concentration as low as 100 pM (corresponding to 5 fmol in a 50 μL volume), which compares favorably with those of other aptamer-based ATP detection techniques (Table 3). In addition, this ATP-DER based CL biosensor demonstrated extraordinary specificity towards ATP and could distinguish the target molecule ATP from its analogues. ATP has also been determined in diluted serum, indicating the applicability of this assay. In addition, it is worthwhile to point out that no sophisticated equipment is required. The proposed method provides a new and universal platform for design of a novel DNA ligation reaction-based CL sensing system for other cofactors, monitoring activity of DNA ligase, and may find wide applications in clinical diagnosis of ATP relative diseases as well as in environmental and biomedical fields.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (21105071), the Specialized Research Fund for the Doctoral Program of Higher Education of China (20110032120079) and the Elite Scholar Program of Tianjin University.

Notes and references

- 1 M. Erecinska and D. F. Wilson, *J. Membr. Biol.*, 1982, **70**, 1–14.

- 2 L. Kong, J. Xu, Y. Y. Xu, Y. Xiang, R. Yuan and Y. Q. Chai, *Biosens. Bioelectron.*, 2013, **42**, 193–197.
- 3 Y. Eguchi, S. Shimizu and Y. Tsujimoto, *Cancer Res.*, 1997, **57**, 1835–1840.
- 4 E. H. Abraham, K. M. Sterling, R. J. Kim, A. Y. Salikhova, H. B. Huffman, M. A. Crockett, N. Johnston, H. W. Parker, W. E. Boyle, A. Hartov, E. Demidenko, J. Efrid, J. Kahn, S. A. Grubman, D. M. Jefferson, S. C. Robson, J. H. Thakar, A. Lorico, G. Rappa, A. C. Sartorelli and P. Okunieff, *Blood Cells, Mol., Dis.*, 2001, **27**, 165–180.
- 5 J. A. Cruz-Aguado, Y. Chen, Z. Zhang, N. H. Elowe, M. A. Brook and J. D. Brennan, *J. Am. Chem. Soc.*, 2004, **126**, 6878–6879.
- 6 K. T. Bush, S. H. Keller and S. K. Nigam, *J. Clin. Invest.*, 2000, **106**, 621–626.
- 7 S. Przedborski and M. Vila, *Clin. Neurosci. Res.*, 2001, **1**, 407–418.
- 8 R. A. Harkness and O. D. Saugstad, *Scand. J. Clin. Lab. Invest.*, 1997, **57**, 655–672.
- 9 J. J. Lu, M. Yan, L. Ge, S. G. Ge, S. W. Wang, J. X. Yan and J. H. Yu, *Biosens. Bioelectron.*, 2013, **47**, 271–277.
- 10 N. N. Bu, A. Gao, X. W. He and X. B. Yin, *Biosens. Bioelectron.*, 2013, **43**, 200–204.
- 11 G. F. Jie, J. X. Yuan and J. Zhang, *Biosens. Bioelectron.*, 2012, **31**, 69–76.
- 12 B. C. Yin, Y. M. Guan and B. C. Ye, *Chem. Commun.*, 2012, **48**, 4208–4210.
- 13 B. Q. Liu, Y. L. Cui, D. P. Tang, H. H. Yang and G. N. Chen, *Chem. Commun.*, 2012, **48**, 2624–2626.
- 14 Y. He, Z. G. Wang, H. W. Tang and D. W. Pang, *Biosens. Bioelectron.*, 2011, **29**, 76–81.
- 15 J. M. Liu and X. P. Yan, *Biosens. Bioelectron.*, 2012, **36**, 135–141.
- 16 Z. Chen, G. Li, L. Zhang, J. F. Jiang, Z. Li, Z. H. Peng and L. Deng, *Anal. Bioanal. Chem.*, 2008, **392**, 1185–1188.
- 17 Y. Y. Xu, J. Xu, Y. Xiang, R. Yuan and Y. Q. Chai, *Biosens. Bioelectron.*, 2014, **51**, 293–296.
- 18 M. Zhang, S. M. Guo, Y. R. Li, P. Zuo and B. C. Ye, *Chem. Commun.*, 2012, **48**, 5488–5490.
- 19 A. H. Liang, H. X. Ouyang and Z. L. Jiang, *Analyst*, 2011, **136**, 4514–4519.

- 20 F. Liu, J. Zhang, R. Chen, L. L. Chen and L. Deng, *Chem. Biodiversity*, 2011, **8**, 311–316.
- 21 J. N. Tian, Y. Wang, S. Chen, Y. X. Jiang, Y. C. Zhao and S. L. Zhao, *Microchim. Acta*, 2013, **180**, 203–209.
- 22 L. M. Lu, X. B. Zhang, R. M. Kong, B. Yang and W. H. Tan, *J. Am. Chem. Soc.*, 2011, **133**, 11686–11691.
- 23 C. B. Ma, Z. W. Tang, K. M. Wang, X. H. Yang and W. H. Tan, *Analyst*, 2013, **138**, 3013–3017.
- 24 Y. H. Wang, X. X. He, K. M. Wang and X. Q. Ni, *Biosens. Bioelectron.*, 2010, **25**, 2101–2106.
- 25 W. P. Zhu, Z. W. Zhao, Z. Li, J. H. Jiang, G. L. Shen and R. Q. Yu, *Analyst*, 2012, **137**, 5506–5509.
- 26 J. T. Ren, J. H. Wang, J. Wang and E. Wang, *Biosens. Bioelectron.*, 2014, **51**, 336–342.
- 27 S. L. Daubendiek, K. Ryan and E. T. Kool, *J. Am. Chem. Soc.*, 1995, **117**, 7818–7819.
- 28 Q. W. Xue, L. Wang and W. Jiang, *Chem. Commun.*, 2013, **49**, 2640–2642.
- 29 E. J. Cho, L. T. Yang, M. Levy and A. D. Ellington, *J. Am. Chem. Soc.*, 2005, **127**, 2022–2023.
- 30 Y. H. Song, X. Yang, Z. Q. Li, Y. J. Zhao and A. P. Fan, *Biosens. Bioelectron.*, 2014, **51**, 232–237.
- 31 Z. M. Zhou, Y. Yu and Y. D. Zhao, *Analyst*, 2012, **137**, 4262–4266.
- 32 C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2001, **40**, 4128–4158.
- 33 A. Merkoci, M. Aldavert, S. Marin and S. Alegret, *Trends Anal. Chem.*, 2005, **24**, 341–349.
- 34 S. Bi, Y. M. Yan, X. Y. Yang and S. S. Zhang, *Chem.–Eur. J.*, 2009, **15**, 4704–4709.
- 35 L. M. Zanolli, R. D'Agata and G. Spoto, *Anal. Bioanal. Chem.*, 2012, **402**, 1759–1771.
- 36 D. B. Zhu, X. M. Zhou and D. Xing, *Anal. Chim. Acta*, 2012, **25**, 39–43.
- 37 D. J. Lin, J. Wu, M. Wang, F. Yan and H. X. Ju, *Anal. Chem.*, 2012, **84**, 3662–3668.
- 38 Y. J. Niu, P. Wang, Y. J. Zhao and A. P. Fan, *Analyst*, 2013, **138**, 1475–1482.
- 39 A. P. Fan, C. W. Lau and J. Z. Lu, *Analyst*, 2009, **134**, 497–503.
- 40 P. Wang, Y. H. Song, Y. J. Zhao and A. P. Fan, *Talanta*, 2013, **103**, 392–397.
- 41 G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20–22.
- 42 C. Guarise, L. Pasquato, V. De Filippis and P. Scrimin, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 3978–3982.
- 43 Y. J. Niu, Y. J. Zhao and A. P. Fan, *Anal. Chem.*, 2011, **83**, 7500–7506.