Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Analyst

RSCPublishing

PAPER

Multiple amplified detection of microRNA based on host-guest interaction between β-cyclodextrin polymer and pyrene

Xiaochen Guo,[a] Xiaohai Yang,*[a] Pei Liu,[b] Kemin Wang,*[a] Qing Wang,[a] Qiuping Guo,[a] Jin Huang,[a] Wenshan Li,[a] Fengzhou Xu[a] and Chunxia Song[a]

Cite this: DOI: 10.1039/x0xx00000x

Received 00th 00,2015
Accepted 00th 00,2015

DOI: 10.1039/x0xx00000x

www.rsc.org/

MicroRNAs (miRNAs) participate in various biological processes of life course. The levels of miRNAs can be useful biomarkers for cellular events or cancers diagnosis, thus sensitive and accurate analysis of miRNA expression is crucial for better understanding its functions and the early diagnosis of human disease. Here, we developed a multiple amplified detection for miRNA based on host-guest interaction between β-cyclodextrin polymer and pyrene, which takes advantage of the polymerase-aided strand displacement amplification and λ exonuclease-assisted cyclic enzymatic amplification. The proposed method allowed quantitative detection of miRNA-21 in a dynamic range from 1 pM to 5 nM with a detection limit of 0.3 pM and demonstrated good ability to discriminate the target sequence from single-base mismatched miRNA sequence. Moreover, the assay was applied successfully in complex biological matrix. We believe that this proposed sensitive and specific assay has great potential as a quantification method for miRNA detection in biomedical research and clinical diagnosis.

Introduction

MicroRNAs (miRNAs) are evolutionary conserved, single-stranded, short noncoding RNA molecules involved in the regulation of gene expression.[1-3] Increasing evidence has indicated that the expression levels of individual miRNAs may serve as reliable molecular biomarkers for cancer diagnosis and prognosis.[4-5] Therefore, sensitive and accurate detection of miRNAs is imperative for better understanding the biological functions of miRNAs and the early diagnosis of human cancers. However, quantitative detection of miRNA has always been a tough challenge due to its small size, low expression level, susceptibility to degradation and sequence similarity.[6-7] Conventional standard methods used for miRNA analysis are Northern blotting, real-time polymerase chain reaction (real-time PCR) and microarrays.[8-12] However, they are obsessed with many drawbacks, such as low sensitivity and throughput, complex design and tedious steps.[13-16] To overcome these shortcomings, many new and simple enzymes-aided target recycling methods have sprung up for miRNA analysis. Unfortunately, most of the methods are limited by its unsatisfactory sensitivity or the intrinsic properties of the enzymes used in miRNA detection. Thus, the development of fast, convenient, sensitive and specific methods for miRNA detection is still in great demand.[17-18]

Recently, many novel fluorescent probes have been designed for fluorescence technology application in various fields, including nucleic acid detection, protein detection, metal ions detection and tumor cell targeting.[19-24] Among them, many nanomaterials were applied for the novel fluorescent probes design due to their unique fluorescent property, like gold nanoparticles, carbon nanotubes and graphene. Several new miRNA detection methods were reported based on integration of the unique optical, electronic and catalytic characteristics of these nanomaterials.[25-27] At the same time, many new fluorescent methods based on supramolecular polymers have also been developed.[28-30] For example, a series of cyclodextrin polymers sensing systems based on the host-guest interaction have been fabricated for fluorescent sensing.[31-32] Previously, our group has developed several fluorescence analytical methods based on host-guest interaction between epichlorohydrin cross-linked β-cyclodextrin polymer (β-CDP) and pyrene.[33-36] Compared with β-CD monomer, this kind of β-CDP is far more soluble in water and could achieve more excellent fluorescence enhancement effect for pyrene. Conventional fluorescent methods based on fluorescence resonance energy transfer (FRET) are working in close proximity and therefore are limited by the distance between the donor and acceptor molecule, which practically makes the design of labeling strategies not easy as expected.[37-38] Since this signal amplifying mode based on host-guest inclusion between β-CDP and pyrene eliminates the previously demanding quencher, it not only could solve complex probe design but also achieve further improvement in sensitivity.

Herein, we reported a multiple amplification fluorescent method based on host-guest interaction between β-CDP and pyrene. This amplification strategy integrates two reactions operating in series. The first reaction is the polymerase-aided isothermal strand displacement amplification activated by the target miRNA. The second reaction is the λ exonuclease (λ exo)-assisted cyclic enzymatic amplification.[39-41] By combining strand displacement amplification, cyclic enzymatic amplification and the excellent fluorescence enhancement effect of β-CDP for pyrene, this proposed method allows for sensitive detection of miRNAs in a dynamic range from 1 pM to 5 nM with a detection limit of 0.3 pM. Moreover, this method also demonstrates good ability for discriminating the target miRNA sequence from other mismatched miRNA sequences, and achieved successful performance in a complex biological matrix. In addition, the fluorescent probe involved in the proposed method is very convenient to design and use which can reduce the analytical cost and achieve simplicity for miRNA detection.

Experimental section

Materials

Klenow fragment polymerase (exo$^-$), λ exonuclease (λ exo), Deoxynucleotide (dNTP) and 10×NEB buffer (100 mM Tris-HCl, 100 mM $MgCl_2$, 500 mM NaCl and 10 mM DL-Dithiothreito, pH 7.9) were purchased from New England Biolabs (NEB, UK). DNA marker and loading buffer were purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (USA). All the tips and tubes were sterilized and purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China). All the solutions were treated with 0.1% diethyl pyrocarbonate (DEPC) and autoclaved to protect from RNase degradation. DEPC was purchased from Sinopharm Chemical Reagent Co., Ltd. RNase inhibitor was purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China). β-cyclodextrin (β-CD) (97%) was purchased from Sigma-Aldrich Co. Serum samples were collected from healthy volunteers. All other chemicals, obtained from Reagent and Glass Apparatus Corporation of Changsha, were of the highest analytical grade and used without further purification or treatment. All the solutions were prepared with ultrapure water obtained from a Millipore water purification system (>18.2 MΩ•cm). The RNA sequences and mono-pyrene-labeled molecular beacon were synthesized by TaKaRa Bio Inc. (Dalian, China) and purified by HPLC. The DNA sequence was synthesized and purified through HPLC by Shanghai Sangon Biotechnology Co. (Shanghai, China). All the sequences used in this work are listed in Table S1.

Apparatus

All fluorescence spectra were measured using a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan) controlled by FL Solution software equipped with aqueous thermostat (Amersham) accurated to 0.1 °C. Excitation and emission slits were all set for a 5.0 nm band-pass with a 700 V PMT voltage. The excitation wavelength was set at 345 nm and the emission spectra from 365 to 480 nm were collected with a 0.2×1 cm^2 quartz cuvette containing 100 μL of solution. The fluorescence intensity at 380 nm was used to evaluate the performance of the proposed assay strategy.

Preparation of β-CDP

The β-CDP can be easily synthesized *via* a reaction of β-CD and epichlorohydrin in a sodium hydroxide solution according to the procedure reported previously.[42] The purified β-CDP powder was re-dispersed in aqueous solution and stored at 4 ℃ for use. The structure of β-CDP was confirmed by FTIR spectra and ^1H NMR spectra (Fig. S1). The molecular weight of β-CDP (Mn~94,400) was measured by using gel permeation chromatography (GPC, waters-515).

Agarose electrophoresis analysis

A 3% agarose gel was prepared by using 0.5× tris-borate-EDTA (TBE) (pH 8.0). SYBR gold was used as the DNA stain and mixed with the samples. The gel was run at 100 V for 45 min at room temperature with loading of 10 μL of sample into each lane, and then photographed in Gel Imaging (Tanon 2500 R, Tianneng Ltd., Shanghai, China).

miRNA-21 detection procedures

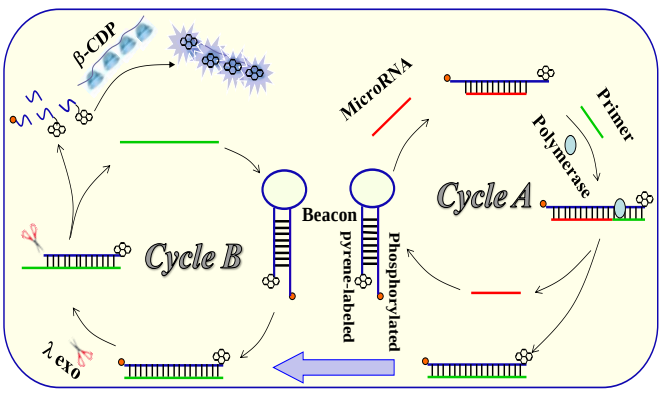
The experiments were performed in 100 μL solution consisting of miRNA-21 at different concentrations, 100 nM probe, 200 nM primer, 25 U mL^{-1} polymerase, 50 μM dNTPs and 1×reaction buffer. Initially, the prepared solution was incubated for 40 min at 37 ℃ for the isothermal strand-displacement polymerization reaction. Following that, 5 U mL^{-1} λ exo was added for incubation about 25 min for the cyclic enzymatic reaction. Subsequently, the resultant mixture was annealed and mixed with 1.5 mg mL^{-1} β-CDP for the fluorescence measurements.

The miRNA-21 analysis in complex biological matrices were also detected using the proposed multiple amplification method. The miRNA-21 spiked serum samples were prepared by adding different concentration of miRNA-21 in the treated human normal serums (which was treated using centrifugal filtration device (30K) to remove macromolecules,[43] and additional 0.2 U μL^{-1} ribonuclease inhibitor was added to create an RNase-free environment). Note: unless noted otherwise, all the experiments for measurements were repeated three times at least in this study.

Results and Discussion

Design strategy for miRNA detection

As a proof-of-concept of our approach, miRNA-21 was chosen as the model, and the mono-pyrene-labeled molecular beacon acted as the signal report probe, which was phosphorylated at the 5′ end and labelled with a pyrene at the 3′ end (Scheme 1). In addition, λ exo was introduced in this proposed method, which processively degrades the removal of phosphorylated 5′ end from duplex DNA to yield mononucleotides and single stranded DNA.[44]



Scheme 1 (a) Schematic of the multiple amplified detection based on host-guest interaction between β-CDP and pyrene by taking advantage of the polymerase-aided strand displacement amplification and λ exo-assisted cyclic enzymatic amplification.

Scheme 1 outlines the mechanism of our amplified strategy. In the presence of target miRNA, the strand-displacement amplification reaction is initiated by the hybridization of the target miRNA with the loop region of the molecular beacon, which makes the molecular beacon be opened. Next, the free primer annealed to the end of the molecular beacon and a primer extension reaction happens in the presence of polymerase and dNTPs, which displaces the target miRNA and synthesizes a single stranded DNA complementary to the molecular beacon. The displaced miRNA subsequently bound to a new molecular beacon, and a new extension cycle then occurred (Cycle A in Scheme 1). With each reaction cycle, the target miRNA is regenerated and DNA duplexs are formed. In the presence of λ exo, the DNA duplex produced from Cycle A is digested from the phosphorylated 5′ end of the beacon, yielding pyrene-labeled mononucleotides and single stranded DNA. The released single strand DNA will perfectly match with the molecular beacon and act as another target to trigger the second cycle (Cycle B in Scheme 1).

The lipophilic microenvironment of β–CDP could increase the fluorescence intensity of pyrene. However, when the pyrene is labeled on the DNA strand, there will be some steric hindrance for pyrene to enter in the cavities of β–CDP because of the rigid conformation of DNA strand. In other words, the steric hindrance hinders pyrene from entering the cavities of β–CDP freely and thus affects the fluorescence enhancement.[34] Because of very weak steric hindrance of mononucleotides, the pyrene-labeled mononucleotides are easily trapped into the hydrophobic cavity of β-CDP, and accompanied with significant fluorescence enhancement.

In contrast, the molecular beacon will not be opened in the absence of target miRNA and can not be digested by λ exo.[45] Therefore, it is difficult for pyrene attached onto the hybridized stem to enter the cavity of β-CDP because of strong steric hindrance, which will lead to a weak fluorescence signal.

Verification of the multiple amplification method

At first, the polymerase-aided strand-displacement reaction and λ exo assisted cyclic enzymatic reaction were proved by gel electrophoresis. As shown in Fig. 1A, when polymerase was alone added, a band higher than that in Lane 2 was formed (Lane 3), suggesting that polymerase start the circular strand-displacement reaction and produce DNA duplex. As expected, when λ exo was added, the DNA duplex disappeared compared with that in the presence of only polymerase. It was because that λ exo digested one of the strands of the duplex DNA. The progress of the reaction was also monitored via the fluorescence emitted from DNA duplex and the digested molecular beacon as shown in Fig. 1B. In the absence of miRNA-21, the strand-displacement amplification reaction and cyclic enzymatic reaction was not initiated after the addition of polymerase and λ exo. The pyrene attached onto the hybridized stem could not enter the cavity of β-CDP because of strong steric hindrance, which led to the weak fluorescence signal. Therefore, apparent increase in fluorescence intensity was neither observed after the addition of polymerase and λ exo (Fig. 1B (b)). In the presence of miRNA-21, the strand-displacement amplification reaction was initiated after the addition of polymerase. Pyrene at the end of the formed DNA duplex could enter the cavity of β–CDP reluctantly with some steric hindrance, accompanying with a certain degree of fluorescence enhancement. Then, cyclic enzymatic reaction was initiated after the addition of λ exo. The pyrene-labeled mononucleotides could enter the cavity of β-CDP freely with very weak steric hindrance, accompanying with a quick rise in fluorescence intensity (Fig. 1B (a)). These results confirmed that the polymerase and λ exo assisted quencher-free multiple amplification fluorescent method indeed took place as expected.

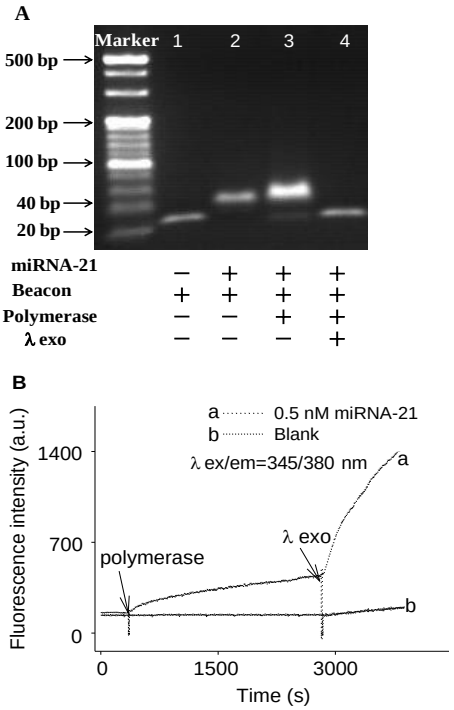


Fig. 1 (A) Gel electrophoretic analysis of the products by the polymerase-aided strand displacement amplification and λ exo-assisted cyclic enzymatic amplification. miRNA-21 (Lane 2): 200 nM; miRNA-21 (Lane 3, 4): 50 nM; beacon: 200 nM; primer: 400nM; polymerase: 50 U mL^{-1}, λ exo: 10 U mL^{-1}. (+ means in the presence of, - means in the absence of). (B) Time course study of the polymerase and λ exo-aided multiple amplification fluorescent method. The concentrations of miRNA-21, beacon, primer, polymerase, λ exo and β-CDP were 0.5 nM, 100 nM, 200 nM, 25 U mL^{-1}, 5 U mL^{-1} and 1.5 mg mL^{-1}, respectively.

As the basis of our strategy, the excellent fluorescence enhancement effect of β-CDP for pyrene was then proved. As shown in Fig. 2A, the fluorescence intensity enhanced greatly with the increasing concentration of β-CDP in the presence of 0.5 nM miRNA-21. In the absence of miRNA-21, the fluorescence intensity kept very faint with the increasing concentration of β-CDP due to the steric hindrance of pyrene-labeled molecular beacon. To further explore the amplification effect of different methods, we evaluated their performance with control experiments under the same target miRNA-21 concentration of 0.5 nM. In the presence of β-CDP, some increase in fluorescence intensity was observed with the assistance of polymerase-aided strand displacement amplification (Fig. 2B (c)). It is worth noting that a significant increase in fluorescence intensity was obtained by combining the polymerase-aided strand displacement amplification and λ exo-assisted cyclic enzymatic amplification. (Fig. 2B (d)). It is clear that this strategy is not only dependent on the polymerase-aided strand displacement amplification but also obviously relies on the λ exo-assisted cyclic enzymatic amplification.

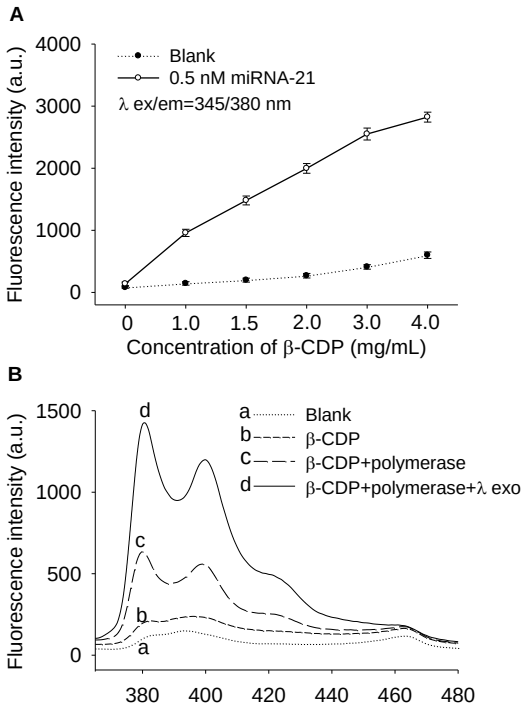


Fig. 2 (A) Fluorescence enhancement of various concentrations of β-CDP: 0, 1, 1.5, 2, 3 and 4 mg mL^{-1}. The concentrations of miRNA-21, beacon, primer, polymerase and λ exo were 0.5 nM, 100 nM, 200 nM, 25 U mL^{-1} and 5 U mL^{-1}, respectively. (B) Fluorescence spectra of different amplification methods. a: miRNA-21 + beacon + primer; b: miRNA-21 + beacon + primer + β-CDP; c: miRNA-21 + beacon + primer + polymerase + β-CDP; d: miRNA-21 + beacon + primer + polymerase + λ exo + β-CDP. Error bars indicated the standard deviations of three experiments.

Optimization of experimental conditions

The fluorescence increasing factor used in this work was $(F-F_0)/F_0$, where F and F_0 were the fluorescence intensities of the detection system with and without target miRNA-21 respectively.

Some factors, such as the concentration of mono-pyrene-labeled molecular beacon, polymerase and λ exo, and the reaction time of polymerase-aided strand displacement amplification and cyclic enzymatic amplification would affect the performance of the detection method. To ensure a better performance, these factors were investigated to establish the optimal condition for our detection system.

The mono-pyrene-labeled molecular beacon plays a crucial role in the performance of this assay. Enough beacon needed to be added to carry out enough rounds of cycles, but an obvious background signal would be observed at a relatively high concentration as shown in Fig. S2 (A), which could be resulted from the intrinsic fluorescence of the pyrene-labeled beacon and a bit of digestion by λ exo.

The concentration of λ exo and the reaction time of enzymatic recycling were optimized to avoid unwanted background signal mainly resulted from the non-specific cleavage by λ exo for the pyrene-labeled beacon as shown in Fig. S2 (B) and Fig. S2 (C).

As shown in Fig. S2 (D) and Fig. S2 (E), higher concentration of polymerase and longer amplification time did not obviously change the signal. Hence, 25 U mL^{-1} and 40 min were chosen for the strand displacement polymerization amplification reaction.

Sensitivity of miRNA detection

Under the above optimal experimental conditions and according to the experimental protocol described in the Experimental section, we investigate the detection limit of this strategy by measuring the fluorescence intensities upon addition of various concentrations of miRNA-21. As shown in Fig. 3A, a gradual increase in the fluorescent peaks at 380 nm and 400 nm was clearly observed in the miRNA-21 concentrations ranged from 0 pM to 5 nM. Fig. 3B illustrates that fluorescence intensity exhibited a good positive linear correlation towards miRNA-21 in the dynamic concentrations from 1 pM to 25 pM with a detection limit of 0.3 pM (three times the standard deviation of the blank solution). The sensitivity achieved in this proposed method attributes to the polymerase-aided strand displacement amplification, the λ exo-assisted cyclic enzymatic amplification and the excellent fluorescence enhancement effect of β-CDP for pyrene.

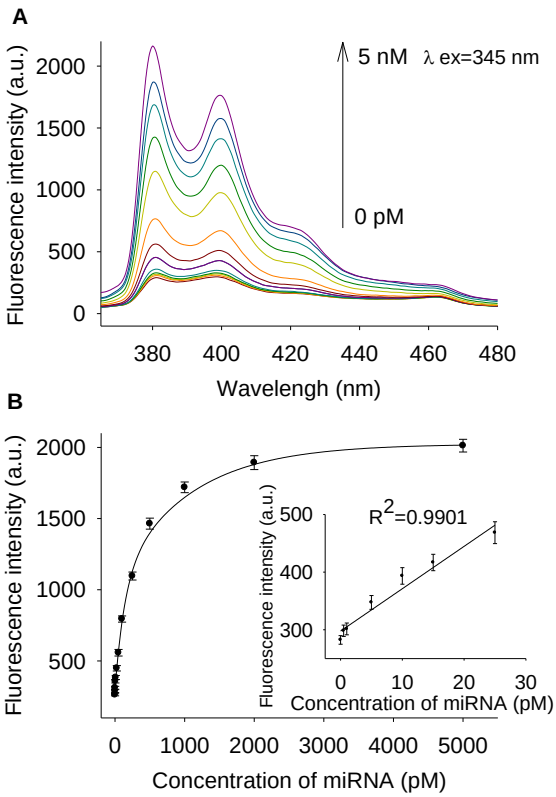


Fig. 3 (A) Fluorescence spectra of the multiple amplified fluorescent method over a range of miRNA-21 concentrations. (B) Standard curve for the relationship between the fluorescence intensity at 380 nm and concentration of miRNA-21. Inset is the calibration curve for the concentrations of miRNA-21 from 0 to 25 pM. The concentrations of beacon, primer, polymerase, λ exo and β-CDP were 100 nM, 200 nM, 25 U mL^{-1}, 5 U mL^{-1} and 1.5 mg mL^{-1}, respectively. Error bars indicated the standard deviations of three experiments.

Specificity of miRNA detection

Distinguishing among members from other homologous sequences is of great importance for better understanding the biological functions of individual miRNAs. Since miRNA families often possess closely related sequences with high homology (1- or 2-base difference), it is a great challenge for distinguishing individual miRNAs.[46-47] To validate the specificity of the detection system, the same concentration of miRNA sequences including miRNA-21, single-base mismatched miRNA-21 (smRNA), three-base mismatched miRNA-21 (tmRNA) and Random RNA were prepared for analysis

(Sequences were listed in Table S1, ESI). As shown in Fig. 4A, the fluorescence intensity of miRNA-21 is 2.5-fold higher than that of smRNA and 4.3-fold higher than that of tmRNA, suggesting the good specificity of the proposed method for miRNA assay, which further supports the feasibility of the proposed method.

Detection of miRNA in human serum

Considering the significance of miRNA analysis in complex biological matrices,[48] we challenged our method for detecting miRNA levels in serum samples. As demonstrated in Fig. 4B, the calibration curve revealed a dynamic correlation between the fluorescence intensity at 380 nm and the miRNA concentrations in the range from 5 pM to 10 nM, which was almost consistent with that obtained in pure buffer solution. With the addition of increasing concentrations of miRNA, the fluorescence intensity also showed a dynamic increase as obtained in pure buffer solution (see Fig. S3, ESI). The results strongly confirmed the adaptability of our multiple amplification method for miRNA detection in complex biological matrices.

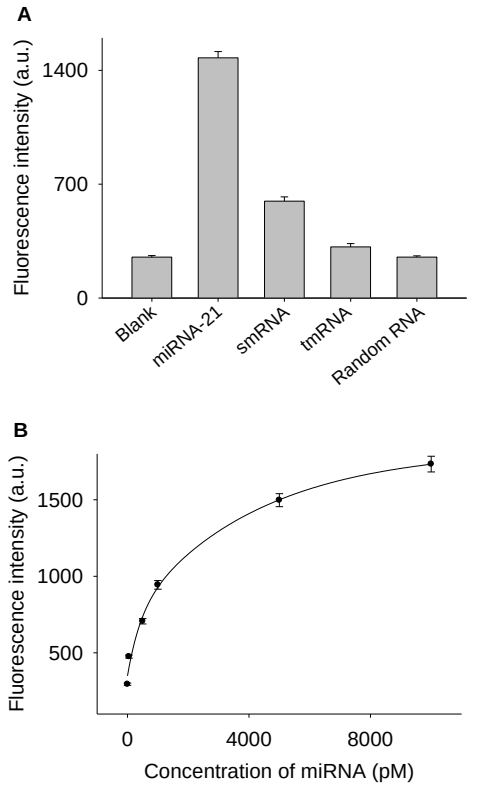


Fig. 4 (A) Specificity evaluation of the proposed method for miRNA-21, concentrations of RNA were all 0.5 nM. (B) Calibration curve for the relationship between the fluorescence intensity at 380 nm and concentration of miRNA-21 for detection in human serum. The concentrations of beacon, primer, polymerase, λ exo and β-CDP were 100 nM, 200 nM, 25 U mL^{-1}, 5 U mL^{-1} and 1.5 mg mL^{-1}, respectively. Error bars indicated the standard deviations of three experiments.

Conclusions

In summary, we have successfully demonstrated a homogeneous, sensitive and rapid multiple amplification method for miRNA detection based on host-guest interaction between β-CDP and pyrene. Our strategy offered several unique advantages: first, the host-guest interaction between epichlorohydrin cross-linked β-CDP and pyrene is an effective and simple fluorescence enhancement mode; second, the mono-pyrene-labeled reporter beacon eliminates the previously demanding quencher, which is very convenient to design and use; last, this multiple amplified detection method not only exhibits competitive analytical characteristics compared with previously reported multiple amplification methods (Table S2 in ESI), but also displays fine reproducibility and practical utility in complex biological samples. On the basis of the advantages presented here, we believe that this proposed assay holds great promise for future advances in research on the biological roles of miRNA and application of clinical diagnosis with miRNA as target, and might be further applied for other sensitive detection of small molecules, metal ions and proteins in combination with specific aptamers.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21190044 and 21175035), National Basic Research Program (2011CB911002), and International Science & Technology operation Program of China (2010DFB30300).

Notes and references

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, China.*
[b] *Hunan Province Environmental Monitoring Centre, Changsha 410014, China.*
E-mail: kmwang@hnu.edu.cn, yangxiaohai@hnu.edu.cn.
Tex/Fax: +86 731 88821566.

1 J. A. Chan, A. M. Krichevsky and K. S. Kosik, *Cancer Res.*, 2005, **65**, 6029.
2 B. C. Schanen and X. Li, *Genomics*, 2011, **97**, 1.
3 R. Siegel, D. Naishadham and A. Jemal, *Cancer Statistics*, 2012, **62**, 10.
4 K. A. Cissell, Y. Rahimi, S. Shrestha, E. A. Hunt and S. K. Deo, *Anal. Chem.*, 2008, **80**, 2319.
5 Y. Huang, Y. Yang, X. Zhang, X. Yu, Z. Wang and X. Cheng, *Med. Oncol*., 2013, **30**, 376.
6 C. K. Raymond, B. S. Roberts, P. Garrett-Engele, L. P. Lim and J. M. Johnson, *RNA*, 2005, **11**, 1737.
7 C. L. Bartels and G. J. Tsongalis, *Clin. Chem.*, 2009, **55**, 623.
8 L. He and G. J. Hannon, *Nat. Rev. Genet*., 2004, **5**, 522.
9 J. Lee, H. Cho and Y. Jung, *Angew. Chem., Int. Ed.*, 2010, **49**, 8662.
10 I. Lee, S. S. Ajay, H. Chen, A. Maruyama, N. Wang, M. G. MacInnis and B. D. Athey, *Nucleic Acids Res.*, 2008, **36**, e27.
11 H. Jia, Z. Li, C. Liu and Y. Cheng, *Angew. Chem., Int. Ed*., 2010, **49**, 5498.
12 J. Li, S. Schachermeyer, Y. Wang, Y. Yin and W. Zhong, *Anal. Chem.*, 2009, **81**, 9723.
13 R. Fu, T. Li, S. S. Lee and H. G. Park, *Anal. Chem*., 2011, **83**, 494.
14 S. Bi, L. Li and Y. Cui, *Chem. Commun*., 2012, **48**, 1018.
15 Y. Jiang, B. Li, X. Chen and A. D. Ellington, *Molecules*, 2012, **17**, 13211.
16 Y. Long, X. Zhou and D. Xing, *Biosens. Bioelectron.*, 2013, **46**, 102.
17 P. Miao, B. Wang, Z. Yu, J. Zhao and Y. Tang, *Biosens. Bioelectron*., 2015, **63**, 365.
18 L. Jiang, Y. Shen, K. Zheng and J. Li, *Biosens. Bioelectron*., 2014, **61**, 222.
19 Q. Xu, P. Pu, J. Zhao, C. Dong, C. Gao, Y. Chen, J. Chen, Y. Liu and H. Zhou, *J. Mater. Chem. A.*, 2015, **3**, 542.
20 K. H. Leung, H. He, P. Ma, H. Zhong, D. S. Chan, J. Zhou, J. L. Mergny, C. H. Leung and D. L. Ma, *Chem. Commun*., 2013, **49**, 5630.
21 H. He, K. H. Leung, W. Wang, D. S. Chan, C. H. Leung and D. L. Ma, *Chem. Commun*., 2014, **50**, 5313.
22 H. He, P. Ma, K. H. Leung, D. S. Chan, H. Yang, Z. Cheng, C. H. Leung and D. L. Ma, *Analyst*, 2012, **137**, 1538.
23 H. He, D. S. Chan, C. H. Leung and D. L. Ma, *Nucleic Acids Res*., 2013, **41**, 4345.
24 Z. Yu, R. M. Schmaltz, T. C. Bozeman, R. Paul, M. J. Rishel, K. S. Tsosie and S. M. Hecht, *J. Am. Chem. Soc*., 2013, **135**, 2883.

25 L. Cui, X. Y. Lin, N. H. Lin, Y. L. Song, Z. Zhu, X. Chen and C. J. Yang, *Chem. Commun.*, 2012, **48**, 194.
26 Y. Gao and B. Li, *Anal. Chem.*, 2014, **86**, 8881.
27 B. Zou, Y. Ma, H. Wu and G. Zhou, *Angew. Chem., Int. Ed*., 2011, **50**, 7395.
28 L. Cui, G. Ke, W. Zhang and C. J. Yang, *Biosens. Bioelectron*., 2011, **26**, 2796.
29 Z. Li, W. Zhu, J. Zhang, J. Jiang, G. Shen and R. Yu, *Analyst*, 2013, **138**, 3616.
30 J. Zhou and H. Ritter, *Polym. Chem.*, 2010, **1**, 1552.
31 F. Manakker, T. Vermonden, C. F. Nostrum and W. E. Hennink, *Biomacromolecules*, 2009, **10**, 3157.
32 N. Morin-Crini and G. Crini, *Progress in Polymer Science*, 2013, **38**, 344.
33 C. Song, X. Yang, K. Wang, Q. Wang, J. Liu, J. Huang, L. He, P. Liu, Z. Qing and W. Liu, *Chem. Commun*., 2015, **51**, 1815.
34 P. Liu, S. Sun, X. Guo, X. Yang, J. Huang, K. Wang, Q. Wang, J. Liu and L. He, *Anal. Chem*., 2015, **87**, 2665.
35 X. Guo, P. Liu, X. Yang, K. Wang, Q. Wang, Q. Guo, J. Huang, J. Liu, C. Song and W. Li, *Analyst*, 2015, **140**, 2016.
36 X. Ma and Y. Zhao, *Chem. Rev.*, 2015, DOI: 10.1021/cr500392w.
37 T. Tian, H. Xiao, X. Zhang, S. Peng, X. Zhang, S. Guo, S. Wang, S. Liu, X. Zhou, C. Meyers and X. Zhou, *Chem. Commun*., 2013, **49**, 75.
38 N. Venkatesan, Y. J. Seo and B. H. Kim, *Chem. Soc. Rev*., 2008, **37**, 648.
39 R. Duan, X. Zuo, S. Wang, X. Quan, D. Chen, Z. Chen, L. Jiang, C. Fan and F. Xia, *J. Am. Chem. Soc.*, 2013, **135**, 4604.
40 G. Zhu, L. Liang and C. Zhang, *Anal. Chem*., 2014, **86**, 11410.
41 Q. Guo, X. Yang, K. Wang, W. Tan, W. Li, H. Tang and H. Li, *Nucleic Acids Res*., 2009, **37**, e20.
42 C. Koopmans and H. Ritter, *Macromolecules*, 2008, **41**, 7418.
43 Q. Wang, F. Liu, X. Yang, K. Wang, H. Wang and X. Deng, *Biosens. Bioelectron*., 2015, **64**, 161.
44 C. Song and M. Zhao, *Anal. Chem*., 2009, **81**, 1383.
45 P. G. Mitsis and J. G. Kwagh, *Nucleic Acids Res.*, 1999, **27**, 3057.
46 F. Yu, H. Yao, P. C. Zhu, X. Q. Zhang, Q. H. Pan, C. Gong, Y. J. Huang, X. Q. Hu, F. X. Su, J. Lieberman and E. Song, *Cell*, 2007, **131**, 1109.
47 J. R. Buchan and R. Parker, *Science*, 2007, **318**, 187.
48 S. Gilad, E. Meiri, Y. Yogev, S. Benjamin, D. Lebanony, N. Yerushalmi, H. Benjamin, M. Kushnir, H. Cholakh, N. Melamed, Z. Bentwich, M. Hod, Y. Goren and A. Chajut, *Plos One*, 2008, **3**, e3148.