Accurate and sensitive fluorescence detection of DNA based on G-quadruplex hairpin DNA

Yi Liu, Rong Liao, Huan Wang, Hang Gong, Chunyan Chen, Xiaoming Chen, Changqun Cai

www.elsevier.com/locate/talanta

PII: S0039-9140(17)30821-4
DOI: http://dx.doi.org/10.1016/j.talanta.2017.08.003
Reference: TAL17799

To appear in: *Talanta*

Received date: 1 June 2017
Revised date: 28 July 2017
Accepted date: 1 August 2017

Cite this article as: Yi Liu, Rong Liao, Huan Wang, Hang Gong, Chunyan Chen, Xiaoming Chen and Changqun Cai, Accurate and sensitive fluorescence detection of DNA based on G-quadruplex hairpin DNA, *Talanta*, http://dx.doi.org/10.1016/j.talanta.2017.08.003

Accurate and sensitive fluorescence detection of DNA based on G-quadruplex hairpin DNA

Yi Liu, Rong Liao, Huan Wang, Hang Gong, Chunyan Chen, Xiaoming Chen*, Changqun Cai*

Key Laboratory of Environmentally Friendly Chemistry and Applications of Ministry of Education, College of Chemistry, Xiangtan University, Xiangtan, Hunan 411105, China

cxm@xtu.edu.cn

cai_mao3@hotmail.com

*Corresponding author. Tel.: +86 13973218593. Fax: +86-731-5829-2251.

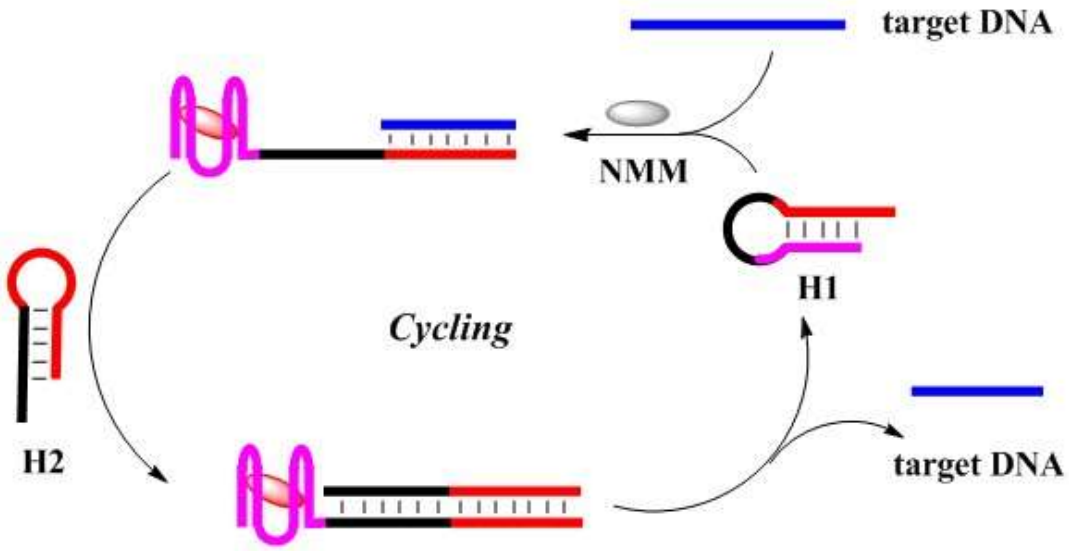
*Corresponding author. Tel.: +86 15273219560. Fax: +86-731-5829-2251.

Abstract

A facile and cost-effective fluorescence biosensor is constructed for the accurate and sensitive determination of DNA. The G-quadruplex-forming sequence in hairpin DNA sequence (H1) is originally locked. When target DNA is present, the hairpin structure of H1 can be unfolded and part of H1 hybridized with the target DNA to form a double-stranded DNA and a G-quadruplex. The hairpin DNA sequence (H2) hybridizes with the unfolded H1 and displaces the target DNA. Finally, the displaced target DNA again hybridizes with the H1 and initiates cycle amplification. Thus, the numerous G-quadruplexs at the H1 ends are formed, resulting in the fluorescent enhancement after binding with N-methyl mesoporphyrin IX (NMM). Lower background signal improves the accuracy of assay. The enzyme-free method can detect down to 40 pM and a linear range of 3 orders of magnitude. Moreover, this strategy has an ability to discriminate the target DNA from even single-base

mismatched or other sequence. The proposed method has potential applications in DNA-based molecular diagnostics.

Graphical Abstract



Accurate and sensitive label-free fluorescence detection of DNA based on G-quadruplex hairpin DNA.

Keywords: G-quadruplex; hairpin; low background; DNAs

1. Introduction

The development of DNA sensors for the sensitive, selective and cost-effective detection of trace amount of DNA is of great importance in various fields such as clinical diagnosis, genetic profiling and food safety [1-3]. In recent years, several strategies have been channeled into fabricating DNA sensors for DNA assay, including fluorescent [4,5], electrochemistry [6-8], colorimetric methods [9,10], and

surface-enhanced Raman spectroscopy (SERs) [11,12]. Among these biosensing methods, owing to the inherent advantages of easy operation, high sensitivity and homogeneity [13,14], the fluorescent methods for DNA detection is one of the most widely used methods [15,16].

Fluorescent probes are necessary for the construction of DNA fluorescent sensors. Several reports show that some probes require site-specfice labeling with fluorescent dye [16,17]. However, the fluorescence labeling and purification bring about high cost, complexity and time-consuming, which may limit its practical application [18,19]. Hence, it is interesting to develop a facile fluorescence sensor for the DNA detection. Recently, several label-free fluorescence strategy have been reported using G-quadruplex [20,21]. Three or four adjacent guanines (G-tracts) spontaneously formed four-stranded DNA structures called G-quadruplexes [22-24]. Guo et al. employed G-quadruplexes as the versatile signaling reporter to develop a new label-free and enzyme-free sensitive fluorescent detection of DNA [20]. A long DNA nanowire incorporated with numerous G-quadruplexes is formed by self-assembled DNA to amplify fluorescence signal. And the detection limit of 0.5 nM is obtained. Qu et al. developed a sensitive and selective DNA detection based on enzyme amplification and ligand-responsive quadruplex formation [21]. The simplicity of these sensing approaches without the modification of any fluorescence labeling still can generate the fluorescent signal output. However, these G-rich probe is compeletely unlocked and the intermolecular formation of G-quadruplex structure from two G-rich strands containing two G-tracts, resulting in a higher background signal [25,26]. This is an unfavorable factor in hindering accurate determination.

Herein, a simple, new universal and enzyme-free fluorescent sensing platform was developed for the sensitive and accurate detection of DNA by the strand

displacement signal amplification strategy. In this sensor, N-methyl mesoporphyrin IX (NMM) was selected as detection signal sources. Two DNA hairpin probes (H1 and H2) were ingeniously designed. The G-quadruplex-forming sequences were initially locked in the hairpin structures of H1. In the absence of target DNA, the H1 and H2 basically remained locked in the hairpin structures. In the presence of the target DNA, H1 was unfolded via hybridization with target DNA. Then the H2 hybridized with the unfolded H1 and displaced the target DNA. The displaced target DNA again hybridized with H1 and initiated cycle amplification. Finally, numerous G-quadruplexs at H1 ends were formed. These G-quadruplex-forming sequences associated with N-methyl mesoporphyrin IX (NMM), and exhibited significantly enhanced fluorescent signal for sensitive monitoring of the target DNA. A lower background signal is obtained through locking stably G-rich sequence. The enzyme-free and cost-effective fluorescent sensor based on the strand displacement signal amplification strategy for sensitive and accurate DNA assay was realized. This detection platform might provide a versatile sensing platform for DNA-based molecular diagnostics.

2. Materials and methods

2.1. Materials

The oligonucleotides, 1× TE (pH 8.0, 10 mM Tris and 1 mM EDTA) buffer, diethylpyrocarbonate (DEPC) treated water, and 10× TM (pH 7.5, 500 mM Tris and 80 mM $MgSO_4$) buffer were obtained from Shanghai Sangon Co., Ltd. (Shanghai, China). NMM was purchased from J&K Scientific Ltd. (Beijing, China), Potassium chloride (KCl) was obtained from Comio Chemical Reagent Co., Ltd. (Tianjin, China). The sequences of the oligonucleotides were listed here (Table 1).

Table 1. Sequences of DNA$^{\alpha}$

note	Sequence
Target DNA	5'-ATC CGG GAT GGG CTG TCA TGC AA-3'
H1	5'-***TTGCA TGACA GCCCA TCCCG GAT***CT GGCAT GCAAT TGCAC TGGGC CGGGA TGGGC TGGG -3'
H2	5'-GTGCA ATTGC ATGCC AGATC CGGG AT GGGCT GTCAT GCAA-3'
Single-base mismatch target (SM)	5'-ATCCA GGATG GGCTG TCATG CAA-3'
double-base mismatch target (DM)	5'-ATC CA GGATG AGCTG TCATG CAA-3'
Three-base mismatch target (TM)	5'-ATCCA GGATG AGCTG TCATC CAA-3'
Four-base mismatch target (FM)	5'-ATCCA GGATG AGCTA TCATC CAA-3'
Non-complementary target (NC)	5'-TAGCA TCGCT ACGGA TCTAC CAT-3'

[α]The bold italic bases of H1 are the complementary sequence of the target DNA. The underlined bases of DNA are the mismatch base of the bold italic bases of H1.

2.2 Fluorescent DNA assay

The H1, H2 and DNAs were dissolved in 1× TE buffer. The oligonucleotide H1 (500 nM, pH 7.5, 125 mM Tris and 20 mM $MgSO_4$) and oligonucleotide H2 (500 nM, pH 7.5, 125 mM Tris and 20 mM $MgSO_4$)) were respectively heated to 95 ^oC for 15 min, and slowly cooled down to room temperature for 1 h before use. Then 100 μL H1, 100 μL H2, 10μL KCl solution (2 M), 10μL NMM (75 μM), a certain amount of ultra pure water and 10 μL target DNA of different concentrations were added to 2 mL centrifuge tube, volume of reaction solution was controlled to 250 μL. After the mixture was incubated at 40 ^oC for 80 min. Fluorescent emission spectra from 550 nm to 700 nm were recorded on a spectrofluorophotometer (RF-5301pc, SHIMAPZU,

Japan) at room temperature. An excitation wavelength of 392 nm was used for the fluorescence measurement. The fluorescence signal was observed at 610 nm. The excitation and emission slits were set to 5.0 and 10.0 nm, respectively.

2.3 Circular dichroism (CD) analysis

CD spectra were measured on a CD chiroptical spectrometer (Chirascan, Applied Photophysics, UK) at room temperature. The H1 (1 μM) and H2 (1 μM) was prepared in a 10× TM buffer solution (pH 7.5, 50 mM Tris, 8 mM $MgSO_4$). The target DNA was added to the reaction solution (100 nM). CD spectra were recorded between 220 and 320 nm, respectively, in 1 mm path length cuvettes. Spectra were recorded at 100 nm/min with a response time of 0.5 s and a bandwidth of 1.0 nm. Each tube was measured three times and spectra were averaged from 3 scans.

3. Results and discussion

3.1 Principle of the sensor

As illustrated in **Scheme 1**, the sensor included a fluorescent dye, NMM, an initiator strand (target DNA), and two hairpin structures (H1, H2). H1 includes the complementary strands of target DNA and G-quadruplex-forming sequence. NMM is weakly fluorescent by itself, but exhibit a dramatic fluorescencent in the presence of G-quadruplexs [27,28]. The G-quadruplex-forming sequences were initially locked in the hairpin structures of the H1. In the absence of the target DNA, the H1 and H2 basically remained locked in the hairpin structures, which avoided the association of the fluorescent dye, NMM. In this case, low fluorescent emission can be expected. When the target DNA was present, the red part of H1 completely hybridized with the target DNA, and unfolded the hairpin structure of H1. Then the H2 hybridized with the unfolded H1 and displaced the target DNA through the strand displacement mechanism. The displaced target DNA again hybridized with the hairpin sequence of

H1 and initiated the strand displacement cycles, resulting in the generation of massive active G-quadruplex at the H1 ends. This active G-quadruplex associated with NMM, and exhibited significantly enhanced fluorescent signal for sensitive monitoring of the target DNA.

3.2 Feasibility of the sensor for DNA detection

To confirm the feasibility of the sensor for DNA detection, fluorescence spectra were measured (**Fig. 1A**). A small fluorescent response (black line) was obtained from the NMM and K^+ because of the weak fluorescent emission of NMM at 610 nm. The H1 (200 nM) also obtained a weak fluorescent emission (red line) due to the fact that G-quadruplex-forming sequences was locked stably. The mixture of H1 and H2 (200 nM) showed slightly higher fluorescencent response than that of the H1 solution (blue line vs. red line) after incubation with NMM. This result attributed the weak interaction between the H1 and H2. Upon incubation of the target DNA (30 nM) with this H1 and H2 solution for 90 min, an obviously increased fluorescent signal at 610 nm (green line) was observed. The results indicated that numerous G-quadruplex structures was formed, and selectively bind to NMM. Then**,** the feasibility of the sensor for DNA detection by signal amplification was confirmed. 5 nM, 20 nM and 50 nM the target DNA was adde to the H1 (200 nM), the mixture of H1 and H2 (200 nM), respectively. As shown in **Fig. 1B**, a slightly change was observed when the target DNA bounded to the H1 and the H1 was folds into G-quadruplex structures. However, upon incubation of the target DNA with the mixture of H1 and H2 solution, an obviously change was shown, which indicated that signal amplification was successfully implemented.

3.3 CD assay

CD spectroscopy was measured to investigate relationship between the fluorescence signal and the G-quadruplex structure (**Fig. 2**). A weak positive peak at 265 nm and a negative peak at 240 nm were obtained by the H1 reaction solution. This is charateristic peaks (265 nm and 240 nm) in parallel G-quadruplex [29,30]. The result showed that a G-quadruplex-forming sequence was locked stably (black line). When the target DNA (100 nM) and the H1 (1 μM) were mixed, the spectrum intensity increased and exhibited a positive peak at 265 nm (red line). This reslut indicated that part of the H1 was unfolded, and a positive parallel G-quadruplex structure was formed. The peak in the same position was presented at the mixture H1 and H2 solution (1 μM) (blue line). Upon incubation of the target DNA (100 nM) with the H1 and H2 solution, an obviously increased signal at 265 nm (green line) was observed. The different reaction solution presented different the spectrum intensity, it was also shown that the CD assay produced consistent results with the fluorescence experiment.

3.4 Optimization of detection conditions

Detection conditions were optimized, which included the concentration of H2, K^+, NMM, work temperature and reaction time, to achieve high sensitivity. The influence of different concentrations of H2 (100, 150, 200, 300, 400 nM) was investigated firstly. 200 nM of H1 and different concentrations of H2 were fixed after the formation of hairpin structure. 50 nM target DNA, 80 mM K^+ and 12 μM NMM were added to the mixture of H1 and H2 solution. After the mixture were incubated at 40 ^oC for 90 min. As shown in **Fig. 3A**, the biggest fluorescence change ($\Delta F=F-F_0$, F_0 is the fluorescence signal in the absence of target DNA; F is the fluorescence signal in presence of target DNA) was observed when the concentrations of H2 was 200 nM.

K^+ is coordination cation to stabilize G-quadruplexs. 50 nM target DNA, different concentration of K^+ and 12μM NMM were added to the mixture of H1 and H2 solution (200 nM). After the mixture were incubated at 40 ^oC for 90 min. The effect of K+ concentration on the fluorescence intensity changes was shown in **Fig. 3B**. The weak fluorescence intensity change ($\Delta F=F-F_0$, F_0 is the fluorescence signal in the absence of target DNA; F is the fluorescence signal in the presence of target DNA) was observed. The optimal concentration of K^+ was chosen at 80 mM.

NMM is weakly fluorescent by itself, but exhibit a dramatic fluorescencent in the presence of G-quadruplexs [24,25]. 50 nM target DNA, different concentration of NMM and 80 mM K^+ were added to the mixture of H1 and H2 solution (200 nM). After the mixture were incubated at 40 ^oC for 90 min. As shown in **Fig. 3C**, 3 μM was chosen as the optimum concentration of NMM. Therefore, the concentration of H2, K^+, NMM were chosen at 200 nM, 80 mM, and 3 μM, respectively.

The influence of work temperature and reaction time of amplification process were also studied under the optimized assay conditions. 20 nM target DNA, 80 mM K^+ and 3 μM NMM were added to the mixture of H1 and H2 solution (200 nM). First, the optimizations of reaction temperatures were achieved through target DNA detection experiments at 25, 35, 40, 45 and 50 °C. After the reaction solution was incubated at 90 min, the flourescence emission spectra were recorded. **Fig. 3D** showed that the highest fluorescence intensity change ($\Delta F=F-F_0$, F_0 is the fluorescence signal in the absence of target DNA; F is the fluorescence signal in presence of target DNA) was observed when the temperature was 40 °C. Reaction time of the amplification process was optimized. **Fig. 3E** showed that the fluorescent intensity change increased with the increase of reaction time and were approaching stable at 80 min. Thus, 40 °C was

chosen as the optimum temperature and 80 min was selected for the reaction time for further studies.

3.5. Sensitivity and selectivity

Under optimum experimental conditions, the proposed strategy was evaluated by quantitative analysis the target DNA. H1 (200 nM), H2 (200 nM), 80 mM K^+ and 3 μM NMM were mixed upon incubation for 80 min at 40 °C. As shown in **Fig. 4A**, the fluorescence intensity was increased gradually with the increase of the target DNA (0-50 nM, a-k). **Fig. 4B** shown the fluorescence intensity changes ($\Delta F=F-F_0$) in response to the different target DNA concentrations. The linear relationship between ΔF value and the concentration of target DNA (0-25 nM) can be expressed as $\Delta F=3.066C + 2.982$, where $R^2=0.9938$ **(Fig. 4C)**. The linear relationship between ΔF value and the concentration of target DNA (25-50 nM) can be expressed as $\Delta F=9.458C - 163.4$, where $R^2=0.9923$ **(Fig. 4D)** (C is the concentration of the target DNA). The proposed method had a broad linear range, the liner range from 0.05 nM to 25 nM and 25 nM to 50 nM. The limit of detection of target DNA was 0.04 nM (3σ/k). The two DNA concentration regions were obtained due to the amplification effect of fluorescence signal is different at the high concentration of target and the low concentration of target. As shown in Fig. 1B, The fluorescence signal amplification effect of high concentration target is better than that of the low concentration target.

The selectivity of the assay was further assessed. the complementary, single-base mismatched, double-base mismatched, three-base mismatched, four-base mismatched and Non-complementary mismatched target DNA were used as initiators, respectively. As shown in **Fig. 5**, the fluorescence intensity changes of the 20 nM mismatched and non-complementary sequence are lower than that of the complementary target DNA. The single-base mismatched, double-base mismatched, three-base mismatched, four-

base mismatched and Non-complementary DNA could recover 34.1%, 20.4%, 17%, 12.2% and 11.6% of the fluorescence signal, respectively. The results indicated the high selectivity for the target DNA. Then, the detection limit and linear range of the reported methods for detection of DNA were compared, and the corresponding results were listed in Table 2.

Table. 2 Comparison of Different Methods for DNA Detection

Detection methods	Linear range	Detection limit	Ref.
Fluorescence	50 pM-10 nM	20 pM	[4]
Fluorescence	50 pM-20 nM	50 pM	[5]
electrochemial	0.1 pM-0.1 nM	0.1 pM	[6]
colorimetric	10 μM-100 nM	10 nM	[9]
SERs	——	10-100 pM	[12]
Fluorescence	25 nM-2 μM	25 nM	[15]
Fluorescence	0.5 nM-10 nM	0.29 nM	[16]
Fluorescence	3 orders	45.6\17.4 nM	[17]
Fluorescence	3 nM-60 nM	0.5 nM	[20]
Fluorescence	0.6 nM-3.0 nM	36 pM	[21]
This work	50pM-25nM 25nM-50nM	40 pM	——

3.6 Detection of DNA in serum samples

Experiments were carried out in human serum samples. The human serum was obtained from the Hospital of Xiangtan University. Various concentrations of the target DNA were added in 100-fold dilution of human serum samples. As shown in Table 3, the data exhibited good recovery, thereby indicating that the strategy offered great potential for specific detection of DNA in biological fluids.

Table. 3 Results for the Determination of the target DNA in 100-fold Dilution of human serum.[β]

samples	the concentration of target DNA ($nmol \cdot L^{-1}$)			
	Spiked	Measured	Recovery (%)	RSD (%)
Human serum	5.00	4.73	94.6	2.2
	15.00	14.36	95.73	3.9
	30.00	31.95	106.5	1.7

[β] Condition: H1 and H2 dosages: 200 nM, temperature: 40 ^{o}C

4. Conclusion

A facile and enzyme-free fluorescence sensor has been developed for the detection of DNA based on the strand displacement signal amplification strategy. The sensor is designed with the hairpin structure of H1. The H1 contains the G-quadruplex-forming sequuences in structure. The detection limits is 40 pM, and the wide linear ranges from 50 pM to 25 nM and 25 nM to 50 nM is achieved by utilizing the assay. The method is simple and cost-efficient in design, rapid in operation. The sensitive and selective strategy has potential applications in DNA-based molecular diagnostics.

Notes

The authors declare no competing financial interest.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 21305118, 21402168, and 21505112), Scientific Research Foundation of Hunan Provincial Education Department (No. 15B232, 16A204), and Hunan 2011 Collaborative Innovation Center of Chemical Engineering & Technology with Environmental Benignity and Effective Resource Utilization.

References

[1] H. Li, Y. Zhang, L. Wang, J. Tian, X. Sun, Nucleic acid detection using carbon nanoparticles as a fluorescent sensing platform, Chem. Commun. 47 (2011) 961-963.

[2] W. Qiang, W. Li, X. Li, X. Chen, D. Xu, Bioinspired polydopamine nanospheres: a superquencher for fluorescence sensing of biomolecules, Chem. Sci. 5 (2014) 3018-3024.

[3] D. Gresham, D. M. Ruderfer, S. C. Pratt, J. Schacherer, M. J. Dunham, D. Botstein, L. Kruglyak, Genome-wide detection of polymorphisms at nucleotide resolution with a single DNA microarray, Science 311 (2006) 1932-1936.

[4] R. Hu, T. Liu, X.-B. Zhang, S.-Y. Huan, C. Wu, T. Fu, W. Tan, Multicolor fluorescent biosensor for multiplexed detection of DNA, Anal. Chem. 86 (2014) 5009–5016.

[5] F. Li, J. Chao, Z. Li, S. Xing, S. Su, X. Li, S. Song, X. Zuo, C. Fan, B. Liu, W. Huang, L. Wang, L. Wang, Graphene oxide-assisted nucleic acids assays using conjugated polyelectrolytes-based fluorescent signal transduction, Anal. Chem. 8 (2015) 3877–3883.

[6] S. Liu, Y. Lin, L. Wang, T. Liu, C. Cheng, W. Wei, B. Tang, Exonuclease III-aided autocatalytic DNA biosensing platform for immobilization-free and ultrasensitive electrochemical detection of nucleic acid and protein, Anal. Chem. 86 (2014) 4008–4015.

[7] K. Hsieh , R. J. White, B. S. Ferguson, K. W. Plaxco, Y. Xiao, H. T. Soh, Polarity-switching electrochemical sensor for specific detection of single-nucleotide mismatches, Angew. Chem. Int. Ed. 50 (2011) 11176–11180.

[8] Q. Shen, M. Fan, Y. Yang, H. Zhang, Electrochemical DNA sensor-based strategy for sensitive detection of DNA demethylation and DNA demethylase activity, Anal. Chem. Acta 934 (2016) 66-71.

[9] X. Xiang, M. Luo, L. Shi, X. Ji, Z. He, Droplet-based microscale colorimetric biosensor for multiplexed DNA analysis via a graphene nanoprobe, Anal. Chim. Acta 751 (2012) 155-160.

[10] S. Wu, P. Liang, H. Yu, X. Xu, Y. Liu, X. Lou, Y. Xiao, Amplified single base-pair mismatch detection via aggregation of exonuclease-sheared gold nanoparticles, Anal. Chem. 86 (2014) 3461–3467.

[11] G. Pelossof, R. Tel-Vered, X.-Q. Liu, I. Willner, Amplified surface plasmon resonance based DNA biosensors, aptasensors, and $Hg2^{+}$ sensors using hemin/G-quadruplexes and Au nanoparticles, Chem. Eur. J. 17 (2011) 8904–8912.

[12] H. J. Lee, Y. Li, A. W. Wark, R. M. Corn, Enzymatically amplified surface plasmon resonance imaging detection of DNA by exonuclease III digestion of DNA microarrays, Anal. Chem. 77 (2005) 5096–5100.

[13] L. Yuan, W. Y. Lin, K. B. Zheng, L. W. He, W. M. Huang, FarRed to near infrared analyte-responsive fluorescent probes based on organic fluorophore platforms for fluorescence imaging, Chem. Soc. Rev. 42 (2013) 622-661.

[14] Y. M. Yang, Q. Zhao, W. Feng, F. Y. Li, Luminescent chemodosimeters for bioimaging, Chem. Rev. 113 (2013) 192-270.

[15] Y. Zhang, C. Zhu, L. Zhang, C. Tan, J. Yang, B. Chen, L. Wang, H. Zhang, DNA-templated silver nanoclusters for multiplexed fluorescent DNA detection, Small 11 (2015) 1385–1389.

[16] Y. Guo, Q. Chen, Y. Qi, Y. Xie, H. Qian, W. Yao, Label-free ratiometric DNA detection using two kinds of interactionresponsive emission dyes. Biosens. Bioelectron. 87 (2017) 320-324.

[17] A. H. Loo, Z. Sofer, D. Bouša, P. Ulbrich, A. Bonanni, Carboxylic carbon quantum dots as a fluorescent sensing platform for DNA detection. Appl. Mater. Interfaces 8 (2016) 1951-1957.

[18] G. Zhu, L. Liang, C.-y. Zhang, Quencher-free fluorescent method for homogeneously sensitive detection of microRNAs in human lung tissues, Anal. Chem. 86 (2014) 11410-11416.

[19] R. Liao, K. He, C. Chen, X. Chen, C. Cai, Double-strand displacement biosensor and quencher-Free fluorescence strategy for rapid detection of microRNA, Anal. Chem. 88 (2016) 4254-4258.

[20] Q. Guo, Y. Chen, Z. Song, L. Guo, F. Fu, G. Chen, Label-free and enzyme-free sensitive fluorescent detection of human immunodeficiency virus deoxyribonucleic acid based on hybridization chain reaction, Anal. Chim. Acta 852 (2014) 244–249.

[21] C. Zhao, L. Wu, J. Ren, X. Qu, A label-free fluorescent turn-on enzymatic amplification assay for DNA detection using ligand-responsive G-quadruplex formation, Chem. Commun. 47 (2011) 5461–5463.

[22] D. Sen, W. Gilbert, Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis, Nature 334 (1988) 364–366.

[23] W. I. Sundquist, A. Klug, Telomeric DNA dimerizes by formation of guanine tetrads between hairpin loops, Nature 342 (1989) 825-829.

[24] J. R. Williamson, M. K. Raghuraman, T. R. Cech, Monovalent cation-induced structure of telomeric DNA: the G-quartet model, Cell 59 (1989) 871–880.

[25] D. Rhodes, R. Giraldo, Telomere structure and function, Curr. Opin. Struct. Biol. 5 (1995) 311–322.

[26] H. Zhou, Z.-S. Wu, G.-L. Shen, R.-Q. Yu, Intermolecular G-quadruplex structure-based fluorescent DNA detection system, Biosens. Bioelectron. 41 (2013) 262–267.

[27] H. Arthanari, S. Basu, T. L. Kawano, P. H. Bolton, Fluorescent dyes specific for quadruplex DNA, Nucleic Acids Research 26 (1998) 3724–3728.

[28] L. Q. Guo, D. D. Nie, C. Y. Qiu, Q. S. Zheng, H. Y. Wu, P. R. Ye, Y. L. Hao, F. F. Fu, G. N. Chen, A G-quadruplex based label-free fluorescent biosensor for lead ion, Biosens. Bioelectron. 35 (2012) 123–127.

[29] J. Kypr, I. Kejnovska, D. Renciuk, M. Vorlícková, Circular dichroism and conformational polymorphism of DNA. Nucleic Acids Res. 37 (6) (2009) 1713-1725.

[30] D. M. Gray, J.-D. Wen, C. W. Gray, R. Repges, C. Repges, G. Raabe, J. Fleischhauer, Measured and calculated CD spectra of G-quartets stacked with the same or opposite polarities. Chirality 20 (3-4) (2008) 431-440.

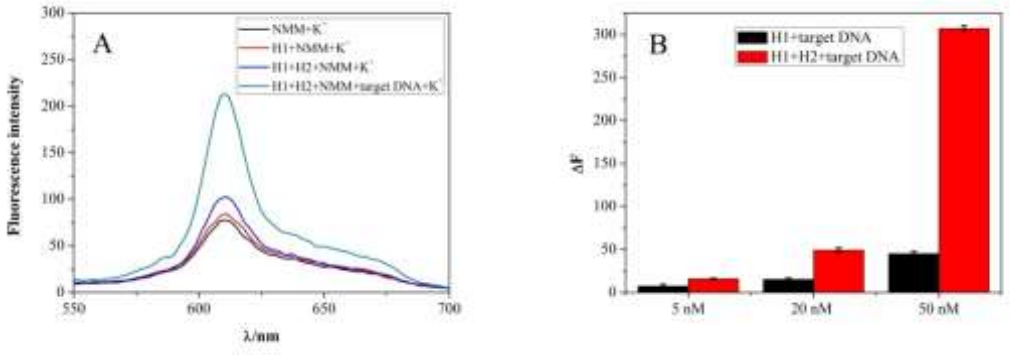


Fig 1. The fluorescent spectra of the sensor (A) and fluorescence intensity change of the sensor in the absence of H2 and the presence of H2 (B).

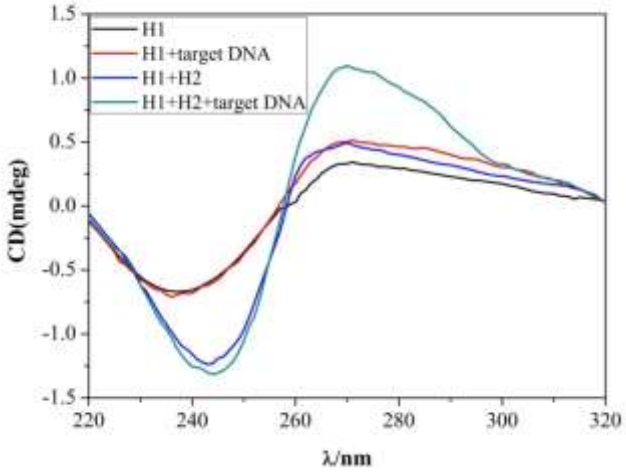


Fig 2. CD spectra of 1 μM H1 and 1 μM H2 upon addition of target DNA in 10× TM buffer solution (50 mM Tris, 8 mM $MgSO_4$, pH 7.5): 0 nM target DNA; 100 nM target DNA

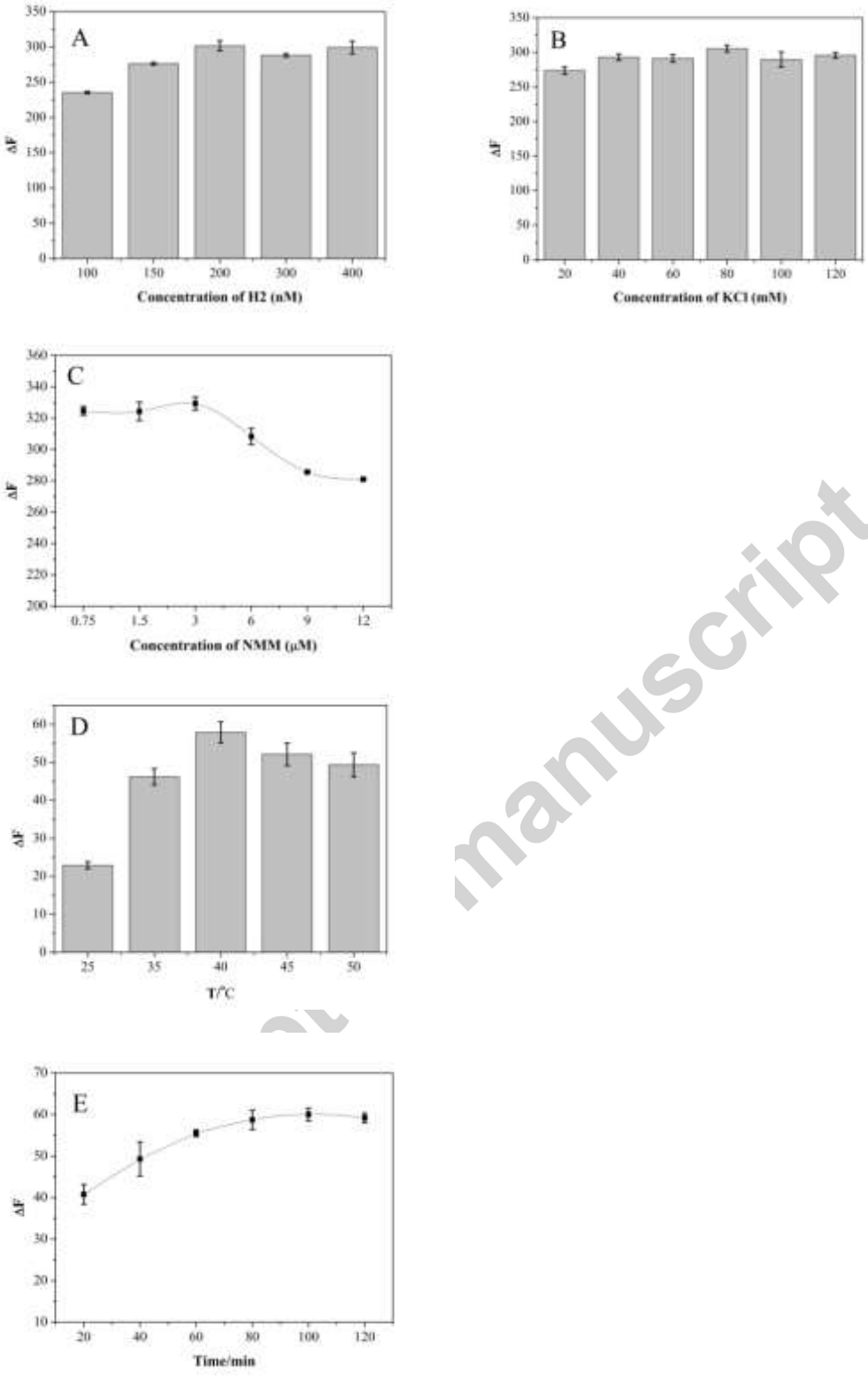


Fig 3. (A) Variance of fluorescence intensity change with the different concentration of H2. (B) Variance of fluorescence intensity change with the different concentration of K^+. (C) Variance of fluorescence intensity change with the amount of NMM. (D) Variance of fluorescence intensity change with the different temperature. (E) Variance of fluorescence intensity change with the reaction time.

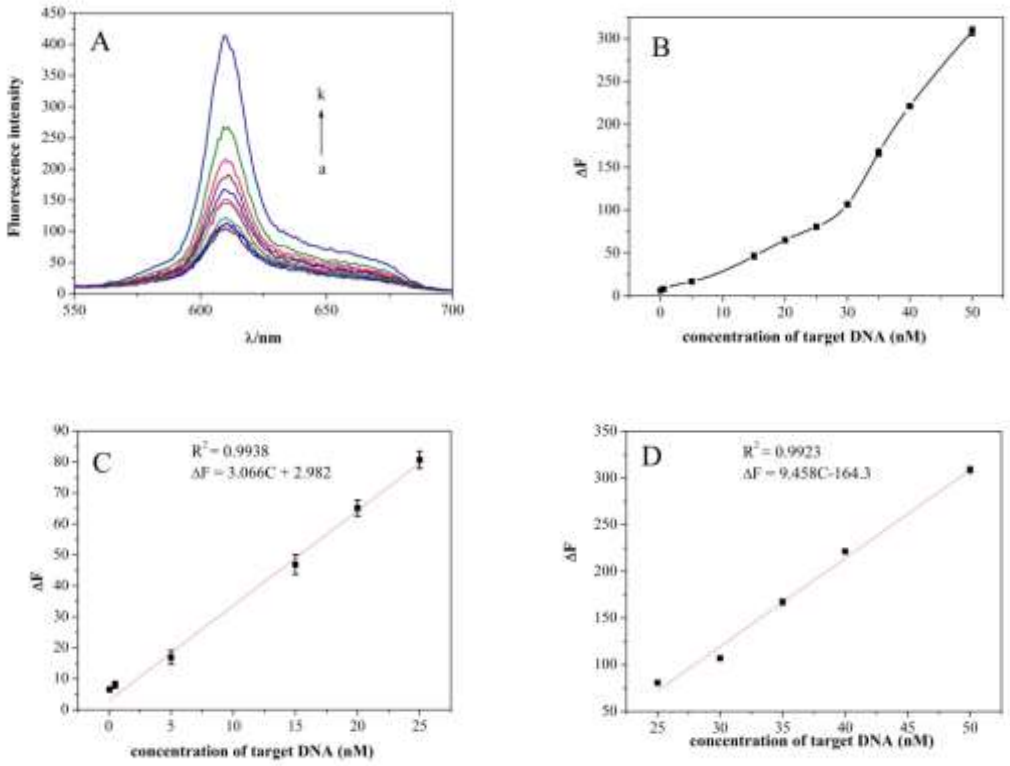


Fig 4. (A) Fluorescence emission spectra as a function of target DNA concentration. The concentration of target DNA is 0, 0.05, 0.5, 5, 15, 20, 25, 30, 35, 40, 50 nM from bottom to top, respectively. (B) Fluorescence intensity changes as a function of the concentration. (C) Liner relation between the fluorescence intensity changes at 610 nm and target DNA concentration (0.05 nM-25 nM). (D) Liner relation between the fluorescence intensity changes at 610 nm and target DNA concentration (25 nM-50 nM).

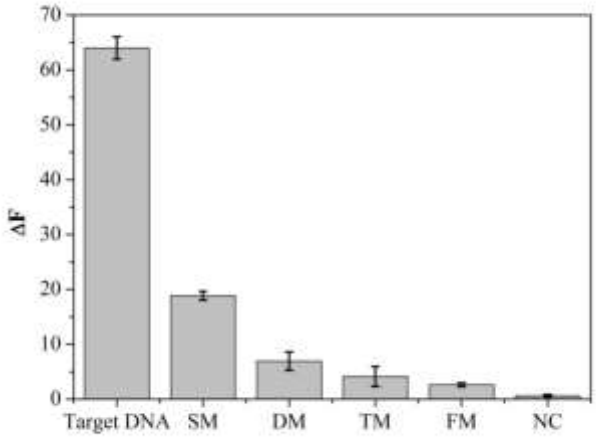
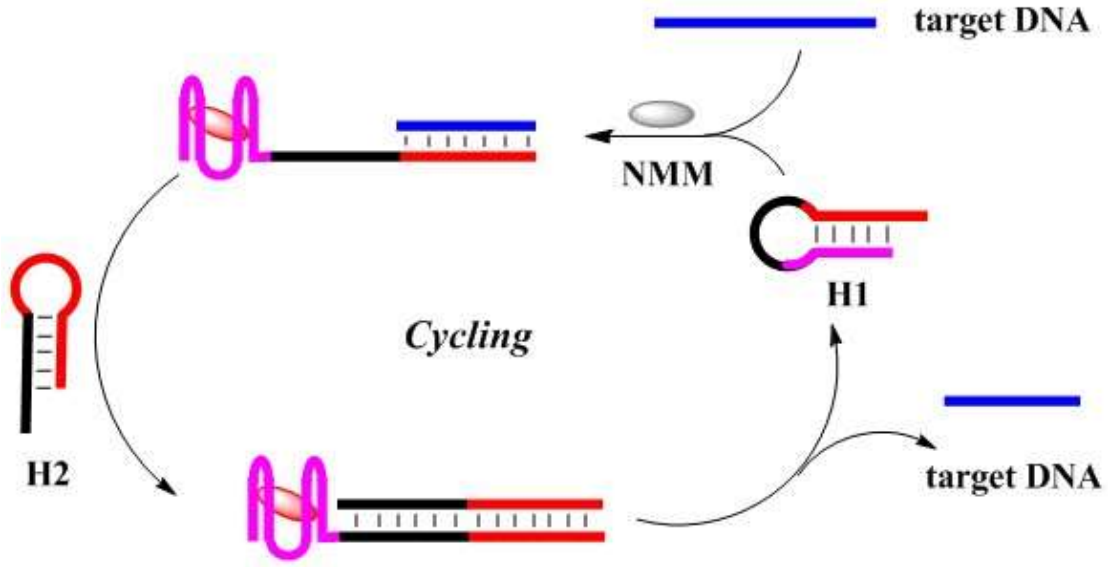


Fig 5. Specificity investigation for target DNA against single-base mismatch, double-base mismatch, three-base mismatch, four-base mismatch, non-complementary t DNA sequences.

Scheme 1



Highlights

1. A facile and enzyme-free sensing method was developed.
2. The method is simple and cost-efficient in design, rapid in operation.
3. The accurate, sensitive and selective detection of DNA was successfully achieved.