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# A label-free biosensor for DNA detection based on ligand-responsive G-quadruplex formation



Yahui Guo, Pei Xu, Hui Hu, Xiaodong Zhou\*, Jiming Hu\*

Key Laboratory of Analytical Chemistry for Biology and Medicine, Ministry of Education, College of Chemistry & Molecular Sciences, Wuhan University, Wuhan 430072, PR China

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## ABSTRACT

A facile and label-free assay with label-free molecular beacons (MBs) and fluorescent dye N-methyl mesoporphyrin IX (NMM) was developed for the detection of specific single-stranded DNA sequences. It was demonstrated by a reverse transcription oligonucleotide sequence (target DNA, 20 bases) of RNA fragment of human immunodeficiency virus (HIV) as model systems. In the absence of target DNA, the MBs were in the stem-closed form, the G-quadruplex structure could not form and the fluorescence signal of NMM was very low. In the presence of target DNA the MBs turned "Off" to "On", thus promoting the formation of G-quadruplex which could greatly enhance the fluorescence of NMM. This biosensor was simple in design, fast in operation, and more convenient and promising than other methods. It took less than 30 min to finish and its detection limit was 1.4 nM. No sophisticated experimental techniques or chemical modification for DNA sequences were required. This new approach could be widely applied to sensitive and selective nucleic acids detection.

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## 1. Introduction

Sensitive and selective single-stranded DNA (ssDNA) sequences detection is of great significance in drug screening, gene profiling, environmental analysis, clinical diagnostics, food safety and a variety of biomedical studies [1–3]. Motivated by this demand, a number of techniques for specific ssDNA sequences have been developed, such as colorimetric [4], electrochemical, fluorescent [5–7] and chemiluminescent [8] methods. As a powerful and important analytical technique, fluorescence has been proved to be of particular utility in this regard. Due to its inherent properties, fluorescence has been used as an alternative to the conventional DNA detection methods [9–11].

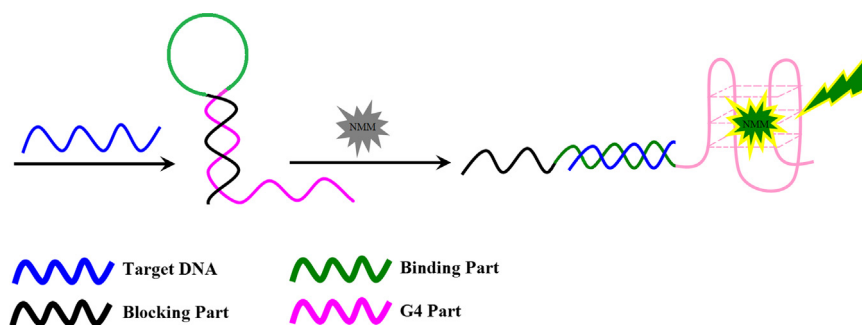
Molecular beacons (MBs), proposed by Tyagi and Kramer in 1996 [12] have rapidly become a mainstay in molecular biology and biotechnology because they rapidly and specifically report the presence of a given nucleic acid sequence in homogeneous solution [13–17]. While MBs-based detection systems are one of the most successful separation-free probes [18,19], there remain some challenging problems. First, MBs need to be labeled with two non-native moieties (i.e., a donor fluorophore and a quencher), thus suffering from problems associated with double labeling such as high cost, low yield, and singly labeled impurities [20]. Second, fluorescence

enhancement of MBs is generally limited by background fluorescence, which comes from imperfect quenching of donors and conformational fluctuations of the hairpin structure [21]. Although some molecular beacon-type approaches based on nanomaterials can provide highly sensitive detection methods, their practical use has been limited due to complex processes, high-cost, essential labeling, hydrolyzation, and/or the use of masking agents [22–25]. Consequently, the fabrication of a label-free, sensitive simple, and low cost detection of nucleic acids is important for gene therapy, mutation analysis and clinical diagnosis.

N-methyl mesoporphyrin IX (NMM) is a commercially available unsymmetrical anionic porphyrin characterized by a pronounced structural selectivity for G-quadruplexes but not for duplexes (DNA, RNA, and RNA–DNA hybrids), triplexes, or single-stranded forms [26,27]. The fluorescence of NMM is very weak, but exhibits a > 20-fold enhancement upon binding to G-quadruplex DNA (with excitation and emission wavelengths centered at 399 and 614 nm, respectively). However, similar properties were not at all evident in the presence of duplex, triplex or ssDNA structures. Owing to its unique features, the NMM has been successfully utilized in the applications for the DNA detection [28–31].

Herein, a facile, label-free DNA detection system was described based on ligand-responsive quadruplex formation. In comparison with conventional MBs-based methods based on FRET, this super simple method consists of only an oligomer and a quadruplex-specific dye NMM that, avoiding the complex and expensive labeling procedure, also saves the trouble of nanomaterial fabrication. So this biosensor has the advantages of easier to design,

\* Corresponding authors. Tel.: +86 27 68752439x8701; fax: +86 27 68754067.  
E-mail addresses: [zhouxd@whu.edu.cn](mailto:zhouxd@whu.edu.cn) (X. Zhou), [jmhu@whu.edu.cn](mailto:jmhu@whu.edu.cn) (J. Hu).



**Scheme 1.** Schematic illustration of the label-free molecular beacon for DNA detection. Target DNA hybridized with the beacon leading to the quadruplex-forming oligomer to form a quadruplex structure. The strong interaction between the folded quadruplex and NMM brought about great fluorescence enhancement.

synthesize, purify and thus is much cheaper and more applicable. More importantly, it takes less than 30 min to finish and its detection limit is 1.4 nM. No sophisticated experimental techniques or chemical modification for DNA sequences are required, the assay can be accomplished by using a common spectrophotometer. It is simple in design, fast in operation and possesses high sensitivity and selectivity. Therefore, this platform has the prospect of becoming a practical and powerful tool for HIV diagnosis and DNA detection in clinic field.

## 2. Experimental

### 2.1. Chemicals and materials

NMM was purchased from J&K Scientific Ltd. (Beijing, China), and its concentration was measured by using absorbance spectroscopy and was found to be  $\lambda = 379$  nm, assuming an extinction coefficient of  $1.45 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ . The stock solution of NMM (2 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in darkness at  $-20$  °C. All the other chemicals were of analytical reagent grade and were used as received without further purification. Solutions were prepared with ultrapure water (18.2 M $\Omega$  resistivity). All measurements were performed in Na–K–Mg buffer (20 mM Tris–HCl, 0.1 M NaCl, 10 mM KCl, 10 mM MgCl<sub>2</sub>, and pH 8.0), unless stated otherwise. All oligonucleotides with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China) and had the following sequences:

HIV probe (HP): 5/-GGG TTT TGG GTT TTG GGT TTT GGG TGC ATC CAG GTC ATG TTA TTC CCA AAA-3/

Reverse transcription oligonucleotide sequence of RNA fragment of HIV (HT): 5/-AAT AAC ATG ACC TGG ATG CA-3/

Mismatched target sequences: MT1: 5/-AAT AAC ATG ACC TGC ATG CA-3/; MT3: 5/-AAT AAC ATG ACC ACC ATG CA-3/; MTR: 5/-AAA TGT TGG AGA ACA GTA TC-3/

### 2.2. Apparatus

The concentration of NMM was measured on a UV-2550 spectrophotometer (SHIMADZU, Japan). Fluorescence spectra data were collected with an F-4600 fluorescence spectrophotometer (Hitachi Co. Ltd., Japan) equipped with a Xenon lamp excitation source, slit widths for the excitation and emission were set at 5 and 10 nm respectively. Circular dichroism (CD) spectra were collected using a Jasco J-810 spectropolarimeter (Tokyo, Japan) at 20 °C. CD measurements from 220 to 320 nm were taken, the scan speed was 50 nm min<sup>-1</sup> and bandwidth was 1 nm. Ultrapure water was produced by a Millipore-Q Academic purification set (Millipore, Bedford, MA, USA). A pH-10 potentiometer (Sartorius) was used to measure pH of solutions.

### 2.3. Fluorescence measurements

Different concentrations of target DNA were added to 300  $\mu$ L Tris–HCl working buffer which contained 200 nM probe DNA and 2  $\mu$ M NMM, followed by incubating at 25 °C for 30 min. Later the fluorescence emission spectra of G-quadruplex–NMM complexes were collected from 580 to 650 nm by using synchronous scanning fluorescence spectrometry.

### 2.4. Single nucleotide polymorphism (SNP) analysis

100 nM target DNA and the same concentration of other mismatched strands were used to perform the label-free reaction according to the procedure of detection of target DNA mentioned above, and the fluorescence intensities of NMM were measured by using time-scan fluorescence spectrometry.

## 3. Results and discussion

### 3.1. Principle of operation

As shown in Scheme 1, the DNA detection system consisted of a probe DNA and N-methyl mesoporphyrin IX (NMM). The probe DNA was composed of three parts: a quadruplex-forming oligomer part, the probe part that could complementarily bind with target DNA and the block part which complemented the quadruplex-forming part. NMM is an anionic porphyrin characterized by a pronounced structural selectivity for G-quadruplexes but not for duplexes, triplexes, or single-stranded forms. In the absence of target DNA, the probe was in stem-loop structure and NMM was weakly fluorescent owing to the block of the quadruplex-forming part. When the probe was challenged with target DNA, the hairpin-probe hybridized with target DNA to form double-stranded structure, then induced the G-quadruplex formation in the presence of monovalent ions. The strong interaction between the “activated” quadruplex and NMM, a specific quadruplex binder, brought about a great fluorescence enhancement.

To validate the formation of the G-quadruplex, the CD spectra of the probe were recorded. As shown in Fig. 1A, when the probe was challenged with target DNA, the peak in 290 nm indicated the formation of the G-quadruplex [32–34]. Fig. 1B shows the fluorescence spectra of NMM in different conditions. The probe and NMM were weakly fluorescent by itself, but exhibited a dramatic fluorescence enhancement upon binding to target DNA, which suggested NMM was enhanced by the G-quadruplex owing to the conformational change.

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