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RAPID COMMUNICATION

Fluorescence-Enhanced Potassium Ions Detection Based on Inherent Quenching Ability of Deoxyguanosines and K^+-Induced Conformational Transition of G-Rich ssDNA from Duplex to G-Quadruplex Structures

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Abstract Herein, we develop a novel single fluorophore-labeled double-stranded oligonucleotide (OND) probe for rapid fluorescence-enhanced K^+ detection, based on an inherent quenching ability of guanine bases and G-rich OND conformation transition from duplex to G-quadruplex. This probe presents high sensitivity and good selectivity for the detection of K^+, and the assay process is simple and fast.

Keywords Fluorescence · Potassium ion · Quenching · Deoxyguanosine · Conformational transition · G-quadruplex structure

Introduction

Potassium ions play a very important role in biological systems, such as the maintenance of cell membrane potential and the regulation of the concentration of other ions in the living cell [1, 2]. Besides, an unbalance of K^+ ions is associated with several disease [3, 4]. Therefore, it is of great importance to develop a simple and sensitive detection method for K^+. However, owing to the presence of sodium and other cations in physiological conditions, it is still a challenge to selectively assay the extracellular K^+ level. Recently, there have been increasing interests on the construction sensor for K^+ detection based on G-quadruplex DNAs as sensing elements [5–12]. G-quadruplexes are four-stranded DNA structures consisting of consecutive G-tetrads, generally stabilized by alkali metal cations [13]. Among all alkali cations, K^+ presents the highest efficiency at stabilizing G-quadruplexes due to its appropriate size, can be located in the cavity between two adjacent G-tetrads of a G-quadruplex binding to eight carbonyl oxygen atoms from the G-tetrads [14]. Hence, a number of K^+ sensors have been developed utilizing G-quadruplex DNAs labeled with fluorescent or electrochemical indicators [5–12, 15]. However, the development of a simple, fast, and cost-effective sensor with high sensitivity and good selectivity is still the focus of research efforts.

DNA molecules have been extensively utilized to construct various biosensors for target detection. The introduction of simple methods for fluorescent labeling of nucleic acids has opened the door that enables nucleic acid hybridization probes to be used for research and development [16]. Indeed, in recent years, fluorescent probes have multiplied at a high rate and the homogeneous fluorescence assays based on FRET (fluorescence resonance energy transfer) or non-FRET quenching mechanisms have been widely developed [17]. However, most of probes are labeled with both a fluorescent reporter and a quencher dye and the fluorescence is only released from the reporter when the two dyes are physically separated after hybridization occurs. Although extensively used in a broad spectrum of applications [16, 18], they still have some drawbacks in that they require labeling at both ends of the OND probe with specific dyes that suffer in overall yield and high cost [19]. To solve these problems, single fluorophore-labeled probe with only one fluorophore tag has been

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developed. To signal target detection event, however, a nanostructure such as gold nanoparticle, carbon nanotube, or graphene is also used as a "nanoquencher" at the same time [17, 20]. It is further demonstrated that the fluorescence of some fluorescent dyes can be efficiently quenched by an interaction between the dye and a nucleotide base and very effective quenching often occurs between dye and a guanine base via potoinduced electron transfer [21]. On the other hand, a dual-labeled double-stranded DNA (dsDNA) probe based on competitive strand-displacement reaction has also been developed and successfully used in homogeneous detection of target without any separation step [22]. Based on the above background research about inherent quenching ability of guanine bases and competitive strand-displacement reaction, we are inspired to introduce G-rich OND which has rich guanine bases and K^+-induced stable G-quadruplex structure into a single fluorophore-labeled double-stranded OND probe system for the detection of K^+.

Herein, a rapid, nanostructure-free, fluorescence-enhanced K^+ detection method has been successfully developed using a novel single fluorophore-labeled double-stranded OND probe. The design takes advantage of an inherent quenching ability of guanine bases and thus the nonfluorescent quencher is eliminated from the probe. The short strand of the probe is designed with an end-labeled fluorophore that is placed adjacent to three guanines located on the long opposite strand, leading to great fluorescence quenching. In the presence of K^+, which could induce and stabilize the G-quadruplex conformation formed from the long strand of the probe, a conformational transition from duplex to G-quadruplex occurs and the two strands of the probe are separated from each other, leading to fluorescence increase. We further demonstrate that such probe is sensitive and selective against other common interfering cations, and is very promising for K^+ detection in practical application.

Experimental Section

All chemically synthesized ONDs were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA concentration was estimated by measuring the absorbance at 260 nm. All the other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. Millipore MilliQ water was used in all the experiments. Fluorescent emission spectra were recorded on a RF-5301PC spectrofluorometer (Shimadzu, Japan). The CD spectra were collected by a JASCOJ-810 spectropolarimeter (Tokyo, Japan), and the measurements were performed in the TE buffer (pH: 7.4, containing 10 mM Tris, 0.1 mM EDTA, 5 mM Mg^{2+}).

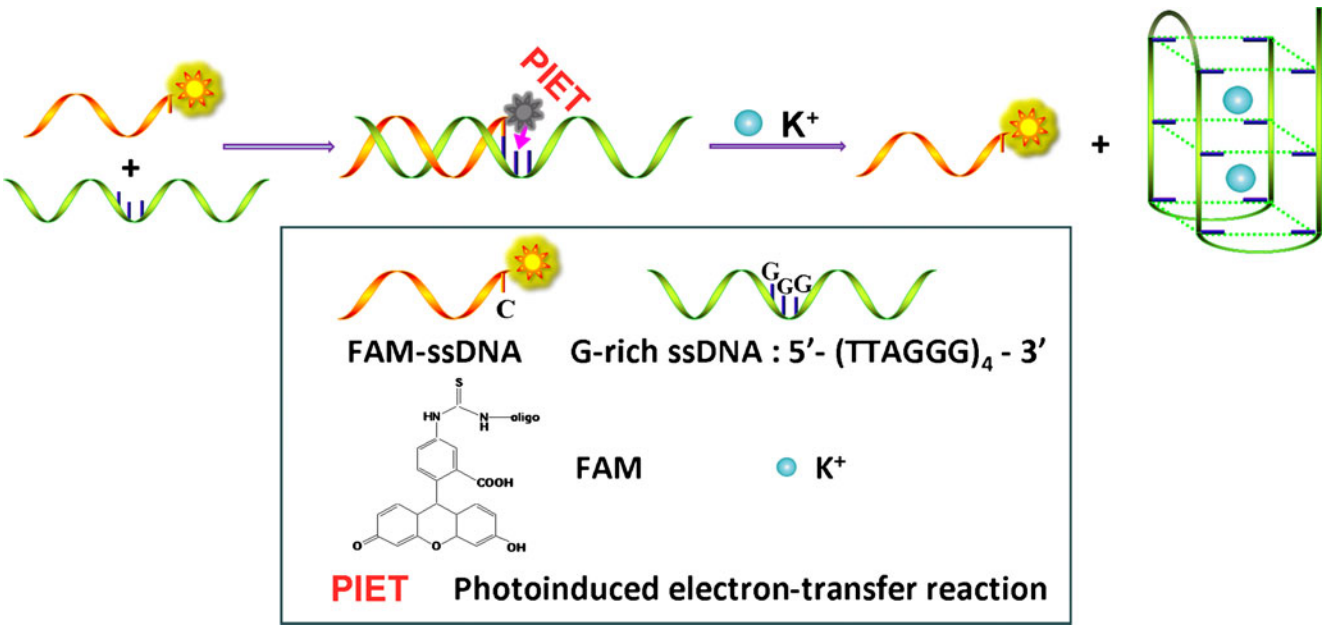
OND sequences used are listed below:

(1) The FAM dye-labeled short strand of the probe (FAM-OND):

5′-FAM-CTAACCCTAA-3′ (FAM=fluorescein-based dye)

(2) The G-rich long strand of the probe (G-OND):

5′-TTAGGGTTAGGGTTAGGGTTAGGG-3′



Scheme 1 A schematic diagram (not to scale) to illustrate the fluorescence-enhanced potassium ions detection mechanism using single fluorophore-labeled double-stranded OND as a probe based on the inherent quenching ability of guanine bases and conformational transition from duplex and G-quadruplex structures

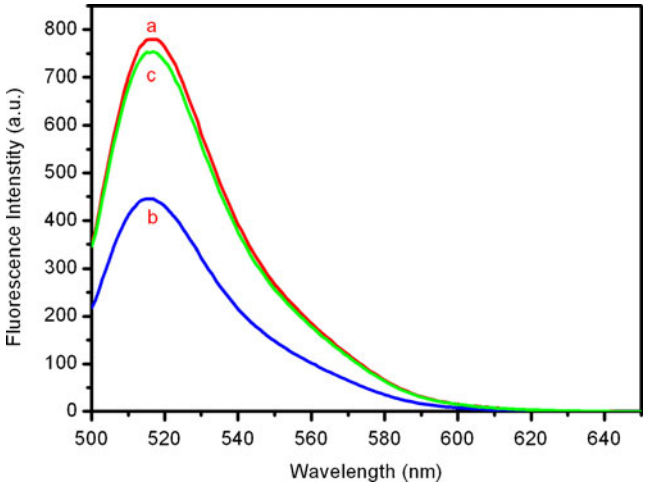


Fig. 1 Fluorescence emission spectra of the FAM-OND (100 nM) at different conditions: **a** FAM-OND; **b** FAM-OND + 100 nM G-OND; **c** FAM-OND + 100 nM G-OND + 10 mM K^+. Excitation was at 480 nm, and the intensity of emission peak at 517 nm was monitored. All measurements were performed in TE buffer (pH: 7.4, 10 mM Tris, 0.1 mM EDTA, 5 mM Mg^{2+})

Results and Discussion

Scheme 1 presents a schematic diagram to illustrate the fluorescence-enhanced potassium ions detection mechanism using single fluorophore-labeled double-stranded OND as a probe based on the inherent quenching ability of guanine bases and conformational transition from duplex and G-quadruplex structures. In the absence of K^+, the fluorescent dye-labeled short strand forms a duplex with the long opposite strand, bringing the dye very close to the three guanines on the long strand. Subsequently, a great fluorescence quenching occurs owing to the photoinduced electron transfer between the dye and the guanines. In contrast, in the presence of K^+, a conformational transition from duplex to G-quadruplex occurs and the long strand forms a K^+-stabilized G-quadruplex conformation. As a result, the two strands of the probe are separated from each other and the dye and the guanines are no longer in close proximity, resulting in a significant fluorescence increase. Therefore, based on the above strategy, potassium ions detection could be realized by monitoring the fluorescence intensity changes of the double-stranded OND probe.

To demonstrate a proof of concept that our probe can be used for fluorescence-enhanced potassium ions detection, we chose an OND sequence (G-OND) associated with human telomere sequence as a model system. Figure 1

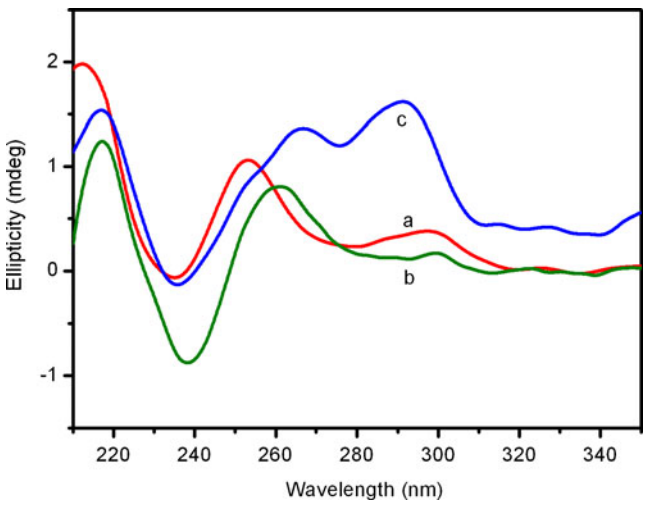


Fig. 2 CD spectra of G-OND with different conformation: **a** G-OND random coil, **b** the duplex formed by FAM-OND and G-OND, **c** G-quadruplex conformation formed in the presence of 10 mM K^+. All measurements were performed in TE buffer (pH: 7.4, 10 mM Tris, 0.1 mM EDTA, 5 mM Mg^{2+})

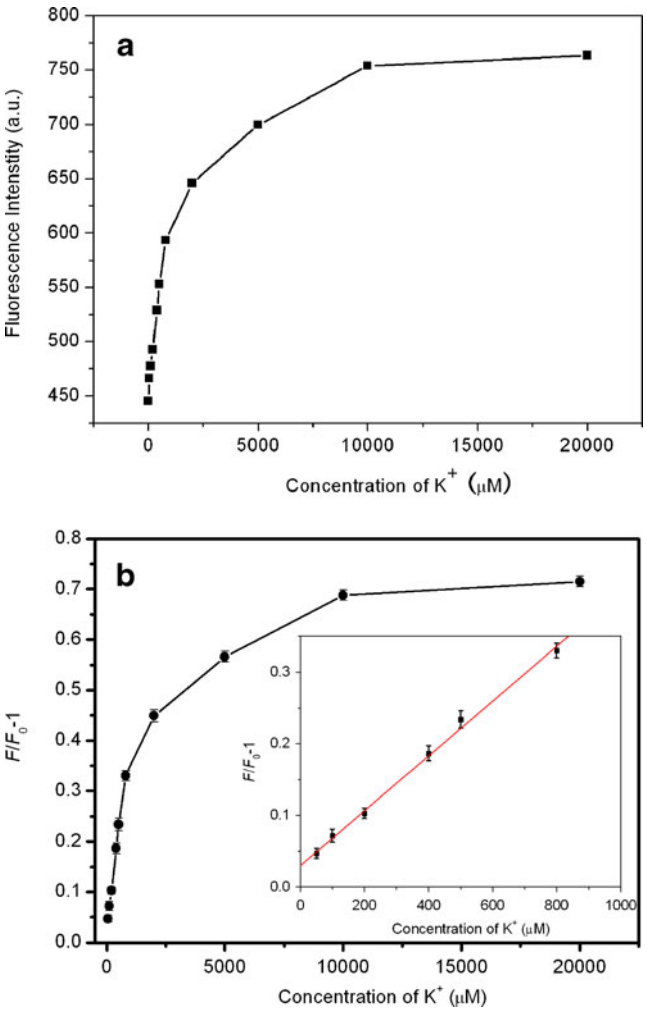


Fig. 3 **a** Fluorescence intensity of the probe (100 nM FAM-OND +100 nM G-OND) in the presence of different concentration of K^+. **b** Fluorescence intensity changes of the probe (F/F_0-1, where F and F_0 are the fluorescence intensity of the probe with and without the presence of K^+, respectively) plotted against the concentration of K^+ with error bars. The inset shows a linear range of 50–800 μM. All measurements were performed in TE buffer (pH: 7.4, 10 mM Tris, 0.1 mM EDTA, 5 mM Mg^{2+})

shows the fluorescence emission spectra of FAM-labeled short strand OND (FAM-OND) of the probe at different conditions. In TE buffer (pH: 7.4, containing 10 mM Tris-HCl, 0.1 mM EDTA, 5 mM Mg^{2+}), FAM-OND exhibits a strong fluorescence emission at 517 nm which can be attributed to the presence of the fluorescein-based dye (curve a). In the presence of long opposite strand G-OND, about 42.8% quenching of the fluorescence emission is observed (curve b), indicating that a duplex is formed and the three guanine bases in the middle of G-OND can effectively quench the dye fluorescence of FAM-OND due to their very close proximity to each other and the subsequent occurrence of photoinduced electron transfer from dye to the guanine bases [21]. It should be pointed out that our measurement was performed right after the addition of G-OND into the FAM-OND solution and the involvement of longer incubation time does not lead to an observable decrease of the fluorescence emission, indicating that the quenching process is very rapid. Next, a significant fluorescence enhancement is observed immediately after the introduction of K^+ (10 mM) into the double-stranded probe solution, leading to a 96.7% fluorescence recovery (curve c). It is also worthwhile mentioning that the fluorescence of the free FAM-OND was, however, scarcely influenced by the addition of K^+ in the absence of G-OND. These observations indicate that this K^+ detection method is very fast and convenient. In addition, the conformational transition and the formation of G-quadruplex induced by K^+ can be confirmed from CD spectra of Fig. 2. As shown in Fig. 2c, the characteristic positive ellipticity peak near

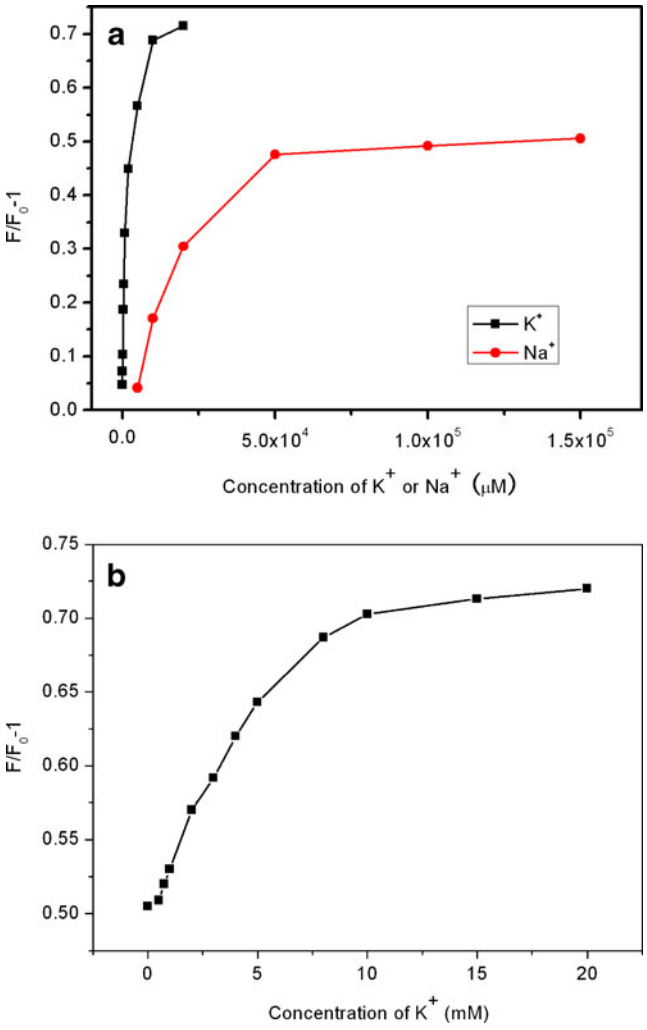


Fig. 4 **a** Fluorescence intensity enhancement of the probe (100 nM FAM-OND +100 nM G-OND) (F/F_0-1, where F and F_0 are the fluorescence intensity of the probe with and without the presence of K^+ or Na^+, respectively) plotted against the concentration of corresponding ions. **b** Fluorescence intensity enhancement (F/F_0-1) of the probe (100 nM FAM-OND +100 nM G-OND) plotted against the concentration of K^+ in coexistence with 150 mM Na^+

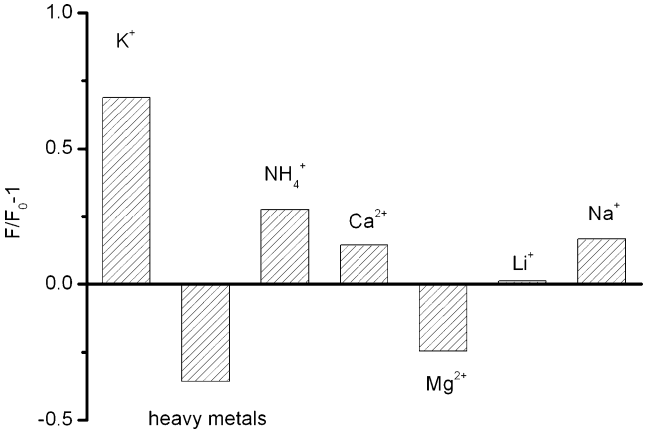


Fig. 5 Selectivity assay of this K^+ sensor against other common ions, including Na^+, Li^+, NH_4^+, Ca^{2+}, Mg^{2+} (every ion concentration is 10 mM), and 10 mM heavy metal ions mixture (Zn^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}). The histograms of F/F_0-1 value, where F_0 and F are the fluorescence intensity of the probe (100 nM FAM-OND +100 nM G-OND) in the absence and presence of different tested ions, respectively

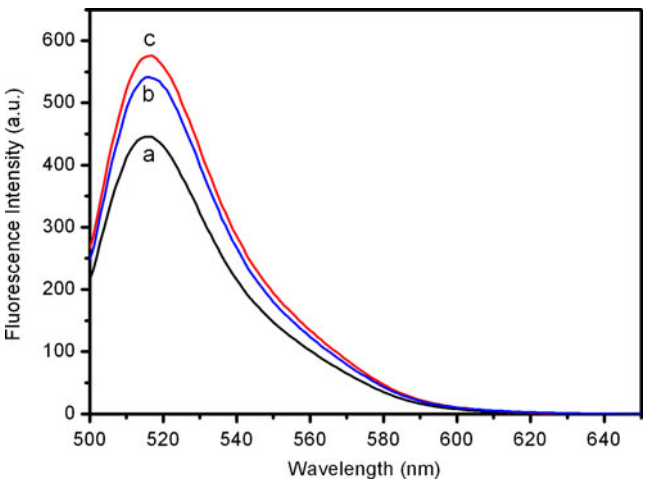


Fig. 6 Fluorescence spectra for analyzing K^+ in lake water: **a** blank; **b** lake water; **c** lake water + 200 μM K^+

295 nm of antiparallel G-quadruplex structure can be identified immediately upon the addition of K^+ [23].

We also collected fluorescence emission spectra of the probe in the presence of different concentrations of K^+, as shown in Fig. 3. The fluorescence intensity of the probe increases with the increasing concentration of K^+, and then near saturation when reach to 10 mM of K^+ concentration, as shown in Fig. 3a. It is clearly seen that an obvious fluorescence intensity enhancement can be observed upon the addition of 50 μM K^+, hence, a detection limit of as little as 50 μM in TE buffer is obtained. Figure 3b further illustrates the relationship between the fluorescence intensity changes (F/F_0-1) of the probe and K^+ concentration, where F_0 and F are the probe fluorescence intensities at 517 nm in the absence and the presence of K^+, respectively. The inset in Fig. 3b presents a good linear relationship (R^2=0.996) in the K^+ concentration range from 50 to 800 μM.

For the detection of K^+, it is essential to exclude or minimize the interference from Na^+. Figure 4a gives the comparison diagram of fluorescence intensity changes induced by K^+ and Na^+, respectively. It is clearly seen that the fluorescence enhancement induced by Na^+ is considerably smaller, even at higher concentration of 50–150 mM. For example, 150 mM of Na^+ induced fluorescence emission enhancement was about the same as that induced by 3 mM of K^+. Furthermore, we also examined the fluorescence intensity enhancement of the probe at different concentrations of K^+ in the presence of 150 mM Na^+, as shown in Fig. 4b. It is obvious that our sensor can still detect as little as 0.5 mM K^+, even in coexistence with high concentration Na^+. To test the specificity of our K^+ sensor, the possible interference of some common ions (e.g., Li^+, Ca^{2+}, Mg^{2+}, NH_4^+ and heavy metals) are evaluated, as shown in Fig. 5. Figure 5 shows that this sensor has a good selectivity for K^+ over other tested ions. Besides, Mg^{2+} and other heavy metal ions themselves may quench dye fluorescence. In order to demonstrate the practicality of our sensor for K^+ detection, we employed it to assay K^+ in real sample lake water. Firstly, the probe (100 nM FAM-OND +100 nM G-OND) in TE buffer (pH: 7.4, 10 mM Tris, 0.1 mM EDTA, 5 mM Mg^{2+}) without the addition of lake water or K^+ are used as blank. Next, lake water sample was diluted 2-fold with TE buffer for the detection of K^+. Then, the diluted lake water spiked with 200 μM K^+ was also examined using our K^+ sensor. As shown in Fig. 6, the response of the sensor toward K^+ in the lake water is obvious, and the K^+ level is estimated to be about 0.9 mM, via diluting lake water to half of the origin concentration within linear detection range for K^+. Besides, through addition of appropriate concentrations of K^+ (200 μM) into the diluted lake water sample, the K^+ level in the sample was confirmed once again. This result can be supported utilizing inductively coupled plasma mass spectrometry.

Conclusions

A novel single fluorophore-labeled double-stranded OND probe for rapid, fluorescence-enhanced K^+ detection is proposed for the first time. The detection is based on the inherent quenching ability of guanine bases and conformational transition from duplex to G-quadruplex structures. Our design is simple and cost effective and the detection is convenient, fast and high sensitivity, with a detection limit of 50 μM in TE buffer for the analysis of low concentration K^+. Such probe also presents good selectivity against other common interfering ions, and it is very promising for K^+ detection in higher concentration Na^+ (150 mM) interference.

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