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Specific detection of potassium ion in serum by a modified G-quadruplex method

Shan Zhang\textsuperscript{1,+}, Ruibin Zhang\textsuperscript{2,+}, Baojin Ma\textsuperscript{1}, Jichuan Qiu\textsuperscript{1}, Jianhua Li\textsuperscript{1}, Yuanhua Sang\textsuperscript{1}, Wei Liu\textsuperscript{1,*}, Hong Liu\textsuperscript{1,*}

\textsuperscript{1} State Key Laboratory of Crystal Materials, Shandong University, Jinan, 250100, China

\textsuperscript{2} Blood Purification Center, Jinan Central Hospital, Jinan, 250013, China

* Corresponding Author. Hong Liu, hongliu@sdu.edu.cn;

Wei Liu, weiliu@sdu.edu.cn

\textsuperscript{+} These authors contributed equally to this work and they should be regarded as co-first author.
ABSTRACT

Potassium ion (K⁺) plays a central role in several fundamental physiological processes. Detection of the K⁺ concentration is an essential diagnostic tool for various medical diseases. However, most commercial detection methods are complex and expensive, which are not easily implemented in community hospitals or at home, in this study, we present a simple fluorescent K⁺ detection system based on the formation of G-quadruplex between K⁺ and dual-labelled thrombin aptamer oligonucleotide derivative (5’-FAM-TTTTTTAGGGTTGTTGTTTGG-TAMRA-3’). Furthermore, based on this method, highly sensitive and selective detection of K⁺ in actual serum was realized by using EDTA as chelating agent to avoid the interference of Ca²⁺ and Mg²⁺ at physiological concentrations. Thus, this study paves the road toward the design and manufacture of portable potassium ions sensors based on fluorescence.
1. Introduction

Potassium ion, K\(^+\), plays a pivotal role in many essential functions of human body, such as cellular transport, regulation of the membrane potential, maintenance of the blood pressure and muscle contraction.\(^{1-3}\) Several diseases related to the cardiovascular system, central nervous system and urinary tract are directly related to the incursion of the K\(^+\) concentration outside its normal range.\(^{4,5}\) For uremia patients, the concentration of K\(^+\) must be measured at regular intervals in order to determine hemodialysis time and duration. Thus, concentration of K\(^+\) in serum is one of the major markers in medical diagnostics.

Several commercial methods can be used to measure the concentration of K\(^+\), including flame luminosity, ion chromatography, use of ion-selective electrodes and dry chemistry methods.\(^{6-8}\) However, the existing potassium ion detection instruments are tedious, pricy and non-portable. Therefore, they are not suitable for community hospitals and home care.

To overcome these problems, the development of inexpensive, rapid, sensitive and selective detection methods has attracted considerable attention. Among those, methods based on the formation of G-quadruplex between K\(^+\) and a Thrombin Binding Aptamer (TBA) constitute a promising approach.\(^{9-12}\) In these methods, sensitive and effective detection of the G-quadruplex formation is essential for precise detection of K\(^+\) concentration. Although several strategies were explored to detect the formation of the G-quadruplex, including strategies of conjugating with a cationic polymer\(^{13,14}\) and strategies that monitor the fluorescence of a probe that either being
anchored to the oligonucleotide\textsuperscript{15-17} or added externally\textsuperscript{18-19}, how to avoid the interference of other metal ions such as Ca\textsuperscript{2+}, Mg\textsuperscript{2+} present in physiological serum is still a challenge.

In this work, as shown in Figure 1, a dual-labelled TBA oligonucleotide derivative (5'-FAM-T TTT TTA GGT TGG TGT GGT TGGTAMRA-3') was selected to detect the K\textsuperscript{+} based on the occurrence of fluorescent resonance energy transfer (FRET)\textsuperscript{20} between FAM and TAMRA after the formation of G-quadruplex between TBA oligonucleotide and K\textsuperscript{+}.\textsuperscript{21,22} Considering of that Ca\textsuperscript{2+} and Mg\textsuperscript{2+} could also induce the formation of G-quadruplex of TBA, a metal ion blocking agent was used to avoid their interference. Thus, K\textsuperscript{+} detection was achieved with high selectivity and high sensitivity. Furthermore, K\textsuperscript{+} in newborn calf serum (NBS), was successfully measured using this method, demonstrating the possibility of rapid K\textsuperscript{+} detection in serum based on formation of G-quadruplexes induced FRET.
Fig. 1. Schematic description of K⁺ detection based on the formation of G-quadruplex induced FRET. (a) G-quadruplex formation of dual-labelled TBA oligonucleotide derivative in the presence of K⁺ formation induced occurrence of FRET between FAM and TAMRA; (b) Eliminate the interference of Ca²⁺ and Mg²⁺ ions using EDTA as chelating agent to realize sensitive and selective K⁺ detection.

2. Experimental Section

2.1 Materials

TBA oligonucleotide derivative (5’-FAM-T TTT TTA GGT TGG TGT GGT TGG-TAMRA-3’) was synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. Other chemicals were all of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. Newborn calf serum (NBS) of New Zealand origin was bought from Gibco (product line of Thermo Fisher Scientific) and were stored frozen before use. The use of NBS was approved by the ethics committee of Shandong University.

2.2 Measurements and methods

Relationship between fluorescence and concentration of K⁺. 40 ul 5 µM Dual-labelled thrombin binding aptamer (TBA) oligonucleotide was added into 3 ml KCl Tris-HCl (20 mM, pH=7.4) solution at a series of concentration of 0, 2, 4, 6, 8, 10, 15, 20, 25, 30 mM. Then the fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation of 490 nm.

Interference of Na⁺ to the detection system. 40 ul 5 µM Dual-labelled thrombin
binding aptamer (TBA) oligonucleotide was added into 3 ml NaCl Tris-HCl solution at a series of concentration of 130, 133, 136, 139, 142, 145, 148, 151 mM. Then the fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation of 490 nm.

**Interference of Ca²⁺ to the detection system.** 40 ul 5 µM Dual-labelled thrombin binding aptamer (TBA) oligonucleotide was added into 3 ml CaCl₂ Tris-HCl solution at a series of concentration of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 mM. Then the fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation of 490 nm.

**Interference of Mg²⁺ to the detection system.** 40 ul 5 µM Dual-labelled thrombin binding aptamer (TBA) oligonucleotide was added into 3 ml MgCl₂ Tris-HCl solution at a series of concentration of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mM. Then the fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation of 490 nm.

**Eliminate the interference of Ca²⁺ and Mg²⁺ through metal ion blocking by Ethylene Diamine Tetraacetic acid, Disodium Salt (EDTA-2Na).** 40 ul 5 µM dual-labelled thrombin binding aptamer (TBA) oligonucleotide was added into 3 ml KCl Tris-HCl solution at 5 mM. Then different amount of CaCl₂ were added and got the solution containing 2.25, 2.375, 2.5, 2.625, 2.75 mM Ca²⁺ respectively and finally 10 mM EDTA-2Na were added. The fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation of 490 nm.

40 ul 5 µM dual-labelled thrombin binding aptamer (TBA) oligonucleotide was
added into 3 ml KCl Tris-HCl solution at 5mM. Then different amount of MgCl₂ were
added and got the solution containing 0.7, 0.8, 0.9, 1.0, 1.1 mM Mg²⁺ respectively and
finally 10 mM EDTA-2Na were added. The fluorescence spectra of TBA and K⁺
mixture were measured at 25 °C under excitation of 490 nm.

**Detection of potassium ions in simulated serum.** A certain amount of NaCl,
CaCl₂ and MgCl₂ were added into 0.2 µM dual-labelled thrombin binding aptamer
(TBA) oligonucleotide solution and got the solution containing 145 mM Na⁺, 2.5 mM
Ca²⁺ and 0.9 mM Mg²⁺. Then different amount of KCl were added into the mixture
and got solutions containing 0, 2, 4, 6, 8, 10, 15, 20, 25, 30 mM K⁺ respectively. The
fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation
of 490 nm.

**Detection of potassium ions in NBS.** The concentration of K⁺ ions in serum was
measured by a cobas® 8000 automatic biochemistry analyzer beforehand, and the test
result was 5.9 mM. Different amount of KCl were added into 1 mL NBS containing
0.2 µM dual-labelled thrombin binding aptamer (TBA) oligonucleotide and got the
solution containing 5.9, 9.9, 13.9, 17.9, 21.9, 25.9, 29.9 mM K⁺ respectively. The
fluorescence spectra of TBA and K⁺ mixture were measured at 25 °C under excitation
of 490 nm.

3. Results and Discussion

3.1 Relationship between fluorescence intensity and concentration of potassium
ions.
In this work, a dual-labelled TBA oligonucleotide derivative (5’-FAM-T TTT TTA GGT TGG TGT GGT TGG-TAMRA-3’) was designed for K⁺ detection. The ketonic oxygen in TBA oligonucleotide will combine with potassium ions and therefore the oligonucleotides fold into the form of G-quadruplex. When G-quadruplex forms between K⁺ and TBA oligonucleotide, two fluorophores FAM and TAMRA approach, and cause FRET occurrence. Fluorescence changes can be recorded by fluorescence spectrophotometer, which reflects the K⁺ concentration.¹⁵, ¹⁶, ²¹

Fig. 2. (a) Fluorescence spectra of the dual labelled TBA in the presence of various K⁺ concentrations; (b) I₅₇₈nm/I₅₁₆nm as a representative of fluorescence changes of dual labelled TBA in the presence of various K⁺ concentrations. Error bars show standard deviation of triplicate measurements.

As shown in Figure 2a, when no K⁺ in solution, there is fluorescence at 516 nm, but almost no fluorescence at 578 nm can be detected. However, when K⁺ exists, as the concentration of K⁺ in the dual labelled TBA increases, the fluorescent intensity at 516 nm representative of FAM decreases while the fluorescent intensity at 578 nm
representative of TAMRA increases, indicating that the presence of K\(^+\) causes the formation of G-quadrurlex between K\(^+\) and TBA, which induces occurrence of FRET from FAM to TAMRA. The variation of the fluorescence intensity at 516 nm and 578 nm reflects the occurrence of FRET between FAM and TARAM, which is induced by G-quadrurlex formation after the addition of K\(^+\). Then the ration of fluorescence intensity at 516 nm and 578 nm was used to quantitatively evaluate the relationship between the between K\(^+\) concentration. As shown in in Figure 2b, the ration of fluorescence intensity, R (defined as R=I\(_{578\text{nm}}\)/I\(_{516\text{nm}}\)), increases linearly with the concentration of K\(^+\) in the 0-30mM range, and the value of slope is 0.0040. As well know, normal K\(^+\) concentration in human serum is in the region of 3.5-5.5 mM, which is covered in the range of 0-30 mM designed in this method. These results indicates that dual-labelled TBA oligonucleotide based method is effective for K\(^+\) detection.

3.2 Interference of other metal ions on the detection of potassium ions.

However, considering the existence of large amount of Na\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) in real serum, these metal ions may have potential interference to K\(^+\) detection in real serum. So, the influence of Na\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) on the detection of K\(^+\) was further evaluated.

The effect of sodium ions on the fluorescent properties of the system. Sodium ions are the most abundant ions in human blood. To check ether Na\(^+\) has an interference of K\(^+\) detection in this system, various amounts of Na\(^+\) were added to the dual-labelled oligonucleotide solution (Figure 3). In this measurement, the Na\(^+\)
concentration was in the range of 130-150 mM, which corresponds to the human serum concentration (135-145 mM).

![Fluorescence spectra of the dual labeled TBA in the presence of various Na⁺ concentrations](image)

Fig. 3. (a) Fluorescence spectra of the dual labeled TBA in the presence of various Na⁺ concentrations; (b) \( I_{578\text{nm}}/I_{516\text{nm}} \) as a representative of fluorescence changes of dual labelled TBA in the presence of various Na⁺ concentrations. Error bars show standard deviation of triplicate measurements.

As shown in Figure 3a, with the addition of Na⁺, the fluorescence intensity of dual-labelled TBA oligonucleotide at 516 nm decreases and the peak at 578 nm increases, which has a similar phenomenon to K⁺. What is different from K⁺ is that the variation of the fluorescence intensity is much lower with Na⁺ than with K⁺. As shown in Figure 3b, the slope of the linear fit of \( R=I_{578\text{nm}}/I_{516\text{nm}} \) with Na⁺ concentration is only 0.00037, which is over 10 times smaller than for K⁺ ions. Therefore, the interference of Na⁺ ions is negligible.

The effect of calcium and magnesium ions on the fluorescent properties of the system. As well known, Ca²⁺ and Mg²⁺ are main divalent cations found in human
serum. Although, several researchers have shown that the formation of the G-quadruplex is specific for potassium\textsuperscript{27,28}, other researchers have demonstrated that Ca\textsuperscript{2+} and Mg\textsuperscript{2+} can also react with these oligonucleotides and form G-quadruplexes.\textsuperscript{29,30} To evaluate the influence of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} on the detection of K\textsuperscript{+}, the fluorescence changes of the dual-labelled oligonucleotide solution was also measured in the presence of various concentrations of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. As shown in Figure 4, the fluorescence intensity at 516 nm decreases and the fluorescence intensity at 578 nm increases with Ca\textsuperscript{2+} concentration, which indicates that Ca\textsuperscript{2+} has an interference to K\textsuperscript{+} detection. In this measurement, the Ca\textsuperscript{2+} concentration was in the range of 0-3.5 mM, which corresponds to the human serum concentration (2.25-2.75 mM). The slope of R vs Ca\textsuperscript{2+} (Figure 4b) is 0.1027, which is 24 times larger than with the K\textsuperscript{+} case, indicating that the binding affinity of Ca\textsuperscript{2+} with the oligonucleotide is much greater than that of K\textsuperscript{+}.\textsuperscript{,}
Fig. 4. (a) Fluorescence spectra of the dual labeled TBA in the presence of various \( \text{Ca}^{2+} \) concentration; (b) \( I_{578\text{nm}} / I_{516\text{nm}} \) as a representative of fluorescence changes of dual labeled TBA in the presence of various \( \text{Ca}^{2+} \) concentrations; (c) Fluorescence spectra of the dual labeled TBA in the presence of various \( \text{Mg}^{2+} \) concentration; (d) \( I_{578\text{nm}} / I_{516\text{nm}} \) as a representative of fluorescence changes of dual labelled TBA in the presence of various \( \text{Mg}^{2+} \) concentrations. Error bars show standard deviation of triplicate measurements.

As for \( \text{Mg}^{2+} \) in the range of 0-1.4 mM (the \( \text{Mg}^{2+} \) concentration in human serum is 0.7-1.1 mM)\(^{31,32}\), the results are similar to those obtained with \( \text{Ca}^{2+} \) (Figure 4c and 4d). The slope for \( \text{Mg}^{2+} \) is 0.1060, which is also about 24 times larger than for \( \text{K}^{+} \), thus indicating a strong interference of \( \text{Mg}^{2+} \) on the detection of \( \text{K}^{+} \) by the
3.3 Eliminating the interference of other metal ions for the detection of K⁺.

To eliminate the interference of Mg²⁺ and Ca²⁺, the most widely used chelating agent EDTAT₂Na was used to sequester Ca²⁺ and Mg²⁺ (Figure 5b). An EDTAT₂Na concentration of 10 mM was selected in order to always be in excess compared to Ca²⁺ (2.25-2.75 mM) and Mg²⁺ (0.7-1.1 mM) in serum. Figure 5c shows the fluorescence spectrum of the oligonucleotide, EDTAT₂Na, and the oligonucleotide in the presence of EDTAT₂Na. EDTAT₂Na does not have fluorescence and the fluorescence of the oligonucleotide is only slightly weakened by the presence of EDTAT₂Na.

Fig. 5. (a) Structure of EDTAT₂Na; (b) structure of the complex formed by Ca²⁺ and EDTAT₂Na; (c) Fluorescence spectrum of EDTAT₂Na and the dual-labelled oligonucleotide, in the absence or in the presence of EDTAT₂Na. (n=3, n-number of measurements)

In order to check that whether EDTAT₂Na can prevent the interference of Ca²⁺ and
Mg\(^{2+}\) on the fluorescent K\(^+\) detection, the fluorescence of the dual-labelled oligonucleotide was measured in the presence of 10 mM EDTA-2Na, 5 mM K\(^+\) and various amounts of Ca\(^{2+}\) and Mg\(^{2+}\) (Figure 6). When the Ca\(^{2+}\) concentration changes from 2.25 to 2.75 mM (Figure 6a), there is no obviously changes of fluorescent intensity (Figure 6a). The value of R is almost unchanged (Figure 6b), and the slope of the linear equation is about 0, which indicates that, under these conditions, Ca\(^{2+}\) does not interfere with the fluorescence of the dual-labelled oligonucleotide. A similar situation occurs when the Mg\(^{2+}\) concentration increases from 0.7 to 1.1 mM (Figure 6c and d).

Fig. 6. (a) Fluorescence spectra of the dual labeled TBA in the presence of EDTA-2Na for Ca\(^{2+}\) concentrations ranging from 2.25 to 2.75 mM; (b) \(I_{578\text{nm}}/I_{516\text{nm}}\) as a representative of fluorescence changes of dual labelled TBA in the presence of
various Ca$^{2+}$ concentrations with EDTA-2Na existing; (c) Fluorescence spectra of the dual labeled TBA in the presence of EDTA-2Na for Mg$^{2+}$ concentrations ranging from 0.7 to 1.1 mM; (d) $I_{578\text{nm}}/I_{516\text{nm}}$ as a representative of fluorescence changes of dual labelled TBA in the presence of various Ca$^{2+}$ concentrations with EDTA-2Na existing. Error bars show standard deviation of triplicate measurements.

The above results indicates that the addition of EDTA-2Na can successfully inhibit the combination of Ca$^{2+}$ or Mg$^{2+}$ with the oligonucleotide, without affecting the fluorescence of the G-quadruplex system.

3.4 Detection of potassium ions in simulated serum.

To investigate the reliability of the K$^+$ detection by the dual-labelled oligonucleotide, simulated serum solutions containing 145 mM Na$^+$, 2.5 mM Ca$^{2+}$, 0.9 mM Mg$^{2+}$, 10mM EDTA-2Na and K$^+$ in concentrations ranging from 2.5 to 25 mM were prepared. The fluorescence spectra of the dual-labelled oligonucleotide G-quadruplex were measured, as shown in Figure 7. The fluorescent intensities at 516 nm decreased and at 578 nm increased with increasing K$^+$ concentration (Figure 7a) and R is linearly correlated to the concentration of K$^+$ (Figure 7b). This test demonstrates the possibility of detecting K$^+$ in the presence of Na$^+$, Mg$^{2+}$ and Ca$^{2+}$. 
Fig. 7. (a) Fluorescence spectra of the dual labeled TBA in simulated serum containing various concentrations of $K^+$ and EDTA-2Na (10 mM); (b) Plot of $R$ vs $Ca^{2+}$ concentration and linear fit. Error bars show standard deviation of triplicate measurements.

3.5 Detection of potassium ions in NBS.

To demonstrate the possibility of dual-labeled TAB for $K^+$ detection in blood, we detected the concentration of potassium ions in serum extracted from the blood of a newborn calf. The concentration of $K^+$ ions in serum was measured by a cobas® 8000 automatic biochemistry analyzer beforehand, and the test result was 5.9 mM. The $K^+$ concentration was tuned by adding standard $K^+$ solution into the serum, thus getting samples with a gradient of $K^+$ concentrations. To realize the measurements, 0.2 μM of dual-labelled oligonucleotides and 10 mM of EDTA-2Na were added. Furthermore, in order to test the efficiency of EDTA-2Na, the fluorescence of the solutions was also measured in the absence of EDTA-2Na. When no EDTA-2Na is present (Figure 8a), the intensities of the fluorescent peaks at both 518 nm and 577 nm are unchanged.
when the K$^+$ concentration varies, and the values of R=$I_{577\text{nm}}/I_{518\text{nm}}$ are nearly identical (Figure 8b). However, when EDTA-2Na is added to serum (Figure 8c), the intensity of the fluorescent peak at 518 nm decreases with increasing K$^+$ concentration while the peak at 577 nm increases which is consistent with the measurements above. Remarkably, the R value is linearly correlated to K$^+$ concentration (Figure 8d).

![Figure 8](image)

**Fig. 8.** (a) Fluorescence spectra of the dual labeled TBA in NBS containing 0-30 mM K$^+$ in the absence of EDTA-2Na; (b) Corresponding plot of R vs Ca$^{2+}$ concentration and linear fit; (c) Fluorescence spectra of the dual labeled TBA in NBS containing 0-30 mM K$^+$ in the presence of EDTA-2Na (10 mM); (d) Corresponding plot of R vs Ca$^{2+}$ concentration and linear fit. Error bars show standard deviation of triplicate measurements.
The feasibility of developed sensor to detect K\(^+\) in serum using fluorescence spectrophotometer was investigated in spiked calf serum samples. Serum was diluted 3 times in 20 mM Tris-HCl buffer (pH=7.4) containing 10 mM EDTA-2Na and then various concentrations of K\(^+\) were added. We can see from Table 1 that the recoveries were in the range of 96–101%. Therefore, the developed fluorescence sensor based on G-quadruplex is applicable for the determination of K\(^+\) in real samples.

Table 1

Recovery of K\(^+\) in a calf serum sample using fluorescence method based on G-Quadruplex (n-number of measurements).

<table>
<thead>
<tr>
<th>Background content (mmolL(^{-1}))</th>
<th>Added concentration (mmolL(^{-1}))</th>
<th>Detected concentration (mmolL(^{-1})) (n=3)</th>
<th>Recovery ratio (%)</th>
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<td>5.9</td>
<td>16</td>
<td>22.08</td>
<td>1.01</td>
</tr>
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Fluorescence response was measured in a calf serum sample diluted 3 times in 20 mM Tris-HCl buffer (pH=7.4) containing 10 mM EDTA-2Na at 25 °C.

The comparison of properties with other existing method is shown in Table S1 in supporting information. T\(_3\)TT\(_3\) sequences (5’-GGGTTTGGGTGGGTTTGGG) in method of crystal violet-G-quadruplex complexes have good selectivity to K\(^+\) when Na\(^+\) exists, but can not recognize K\(^+\) very well when Ca\(^{2+}\) and Mg\(^{2+}\) exist; Hum21
sequences (5'-GGGTTAGGGTTAGGGTTAGGG) in method of crystal violet-G-quadruplex complexes have good selectivity to K$^+$ when Ca$^{2+}$ and Mg$^{2+}$ exist, but can not recognize K$^+$ very well when Na$^+$ exists. The linearity between fluorescence and concentration of K$^+$ is good when high concentration of Na$^+$ exists and this method can sense K$^+$ in a range of 0-10 mM. The method of G-quadruplex with cationic conjugated polymer can recognize K$^+$ well in the presence of Na$^+$, but the interference of Ca$^{2+}$ and Mg$^{2+}$ can not be ignored. The linearity between fluorescence and concentration of K$^+$ is good when Na$^+$ exists and the limit of detection is 0-50mM. Method of dual-labeled oligonucleotide derivative has studied the effects of spacer bases to the efficiency of FRET, and found that with the spacer bases TTTTTTA, the efficiency of FRET can be greatly improved. However, no further study works on the selectivity, linearity, and applicability of this dual-labeled oligonucleotide derivative system. Our method can detect K$^+$ with Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ all existing and has perfect linearity even in real calf serum. The detection range is 0-30 mM which is large enough for K$^+$ detection in serum. Above all, method of modified dual-labeled G-quadruplex in our study is most promising to be used in medical diagnosis.

To further explore the availability of this detection system in different conditions, we also tested the stability in different pH and temperature. As shown in Figure S1, the EDTA-2Na modified dual-labeled oligonucleotide system is nearly not affected by the variation of pH, so it is available at different pH. For the effect of temperature, the results indicate that $R=I_{578\text{nm}}/I_{516\text{nm}}$ decreases with increase of measurement.
temperature, which indicates that the efficiency of FRET is lowered by the increasing temperature. It may be because of the equilibrium state of G-quadruplex is influenced by the increasing temperature and reduced the energy transfer. However, it would not influence the application of the detection system as we can optimize experimental parameters under different temperatures. Moreover, the temperature in human body is 36.5 to 37.5°C, and this range would not affect very much during the detection.

As discussed above, the $K^+$ detection method we developed using an EDTA-2Na standard solution to eliminate the interference of divalent ions only necessitates a fluorescence spectrophotometer, and can be adjusted to be available at different pH and temperatures. Therefore, this method is adapted to small-size portable devices for uses in hospitals or for family care.

4. Conclusion

Fluorescent-labeled TBA oligonucleotides offer a promising platform for the detection of $K^+$. However, the selectivity of such system is quite low, as Ca$^{2+}$ and Mg$^{2+}$ at physiological concentrations also induce a change of conformation of the aptamer, resulting in an even larger FRET. The interference of Ca$^{2+}$ and Mg$^{2+}$ can simply be eliminated by adding EDTA-2Na which acts as a scavenger for these divalent ions. Thus, this modified dual-labelled G-quadruplex method has been successfully used to detect $K^+$ in serum in the presence of Ca$^{2+}$, Mg$^{2+}$ and Na$^+$ at their physiological concentrations. Therefore, this work connects the theory and practice of potassium ions fluorescent detection, and lays the foundations for the design and
manufacture of a portable $K^+$ sensor which would have a wide application in community hospitals and family care.

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Author Contributions Statement

The experiments were designed by Hong Liu and Wei Liu, and carried out by Shan Zhang and Ruibin Zhang, the results were analysed by Shan Zhang, Ruibin Zhang, Baojin Ma and Jichuan Qiu, Jianhua Li and Yuanhua Sang compiled the supporting information. Shan Zhang and Ruibin Zhang wrote the main manuscript text, Baojin Ma, Jichuan Qiu, Hong Liu and Wei Liu made significant contribution to the revision.

Competing financial interests

The authors declare no competing financial interests.

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Graphical and textual abstract

A modified dual-labelled G-quadruplex method is successfully designed to rapidly detect K⁺ in complex matrix of real serum.