Quantify Na^+/K^+ ratio based on the different response of a new identified G-quadruplex to Na^+ and K^+

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Experimental Section

Sample preparation

MTC was prepared according to the method suggested by Hamer and Ficken,[1] and the purity has been proved by nuclear magnetic resonance (NMR) and mass spectroscopy (MS). The oligonucleotide, p25, was purchased from Invitrogen (Beijing, China), purified by PAGE. Methanol, NaCl, KCl, Tris, HCl, and EDTA were all in analytical grade. Ultrapure water was prepared by Milli-Q gradient ultrapure water system. All the samples were measured in 10 mM Tris-HCl buffer solution (pH 7.2).

Spectroscopy measurement

NMR spectra were recorded on a Bruker Avance 600 spectrometer equipped with a 5-mm BBI probe capable of delivering z-field gradient strength up to 50 G cm^{-1}. Samples were prepared in Tris-HCl buffer (10 mM Tris-HCl, 90% H_2O/10% D_2O, pH 7.2). A standard Bruker pulse program p3919gp that applies 3-9-19 pulses with gradients was used for water suppression. 256 scans were acquired for each spectrum with a relaxation delay of 2 s.

CD spectra were collected from 200 to 350 nm on a Jasco-810 automatic recording spectropolarimeter with a 1-cm pathlength quartz cell at 25℃. Spectra were collected with scan speed of 1000 nm/min. Each spectrum was the average of three scans.

Ultraviolet (UV) spectra were measured on a Agilent 8453 UV-visible spectrophotometer at the wavelength range 200 ～1000 nm using a 1 cm path cell at room temperature. Ultrapure water was used as reference.

The polyacrylamide gel electrophoresis (PAGE) was conducted in 1×TBE (Tris base-boric acid-EDTA) buffer solution with 15% native gels. The gels were run at 120V for 50 min at room temperature. Then the gels were incubated in SYBR Gold solution for 5 min, rinsed with ultrapure water, and then photographed by a CCD camera.

Fluorescence spectra were recorded on a Hitachi F4500 spectrofluorometer (Japan) in a 1-cm path-length quartz cell at room temperature. Xenon arc lamp was used as the excitation light source. The excitation and emission slits were both 10 nm. Excitation was set at 570 nm, and emission was collected from 580 to 720 nm. The scan speed was 240 nm/min.

Application.

Urine was used to confirm the feasibility of this aptasensor for analysis of real-world sample. Urine samples were harvested from six persons in a hospital, whose [Na^+] and [K^+] have been measured by an automatic chemistry analyzer (Beckman LX-20).The Na^+/K^+ ratios are calculated according to the equation [Na^+]/ [K^+]. Colorimetric measurement: Previously adding 500 μL Tris-HCl buffer solution with 10 μM p25 and 40 μL methanol solution with 200 μM MTC into the six urine samples (500 μL), then take photos using a camera. Quantitative measurement: the standard and urine samples are 10-fold diluted by using Tris-HCl buffer solution with 2.2 μM p25 and 4.4 μM MTC, then measure their fluorescence intensity at 598 nm after reacting 2 hours. Prepare a standard curve in which find the Na^+/K^+ ratios of the urine samples.

[1] a) F. M. Hamer, in *The Chemistry of Hererocyclic Compounds, Interscience*, New York, **1964**, pp.148; b) G. E. Ficken, in *The Chemistry of Synthetic Dyes*, Academic Press, New York, **1971**, pp.228.

Table 1. The sequences of different G-quadruplexes.

Name	Sequence 5'→3'
TBA	GGTTGGTGTGGTTGG
H24A	TTGGGTTAGGGTTAGGGTTAGGGA
Bcl2-2345	GGGCGCGGGAGGAATTGGGCGGG
c-myc	GGGTGGGTAGGGTGGG
c-kit	AGGGAGGGCGCTGGGAGGAGGG
VEGF	GGGCGGGCCGGGGGCGGG

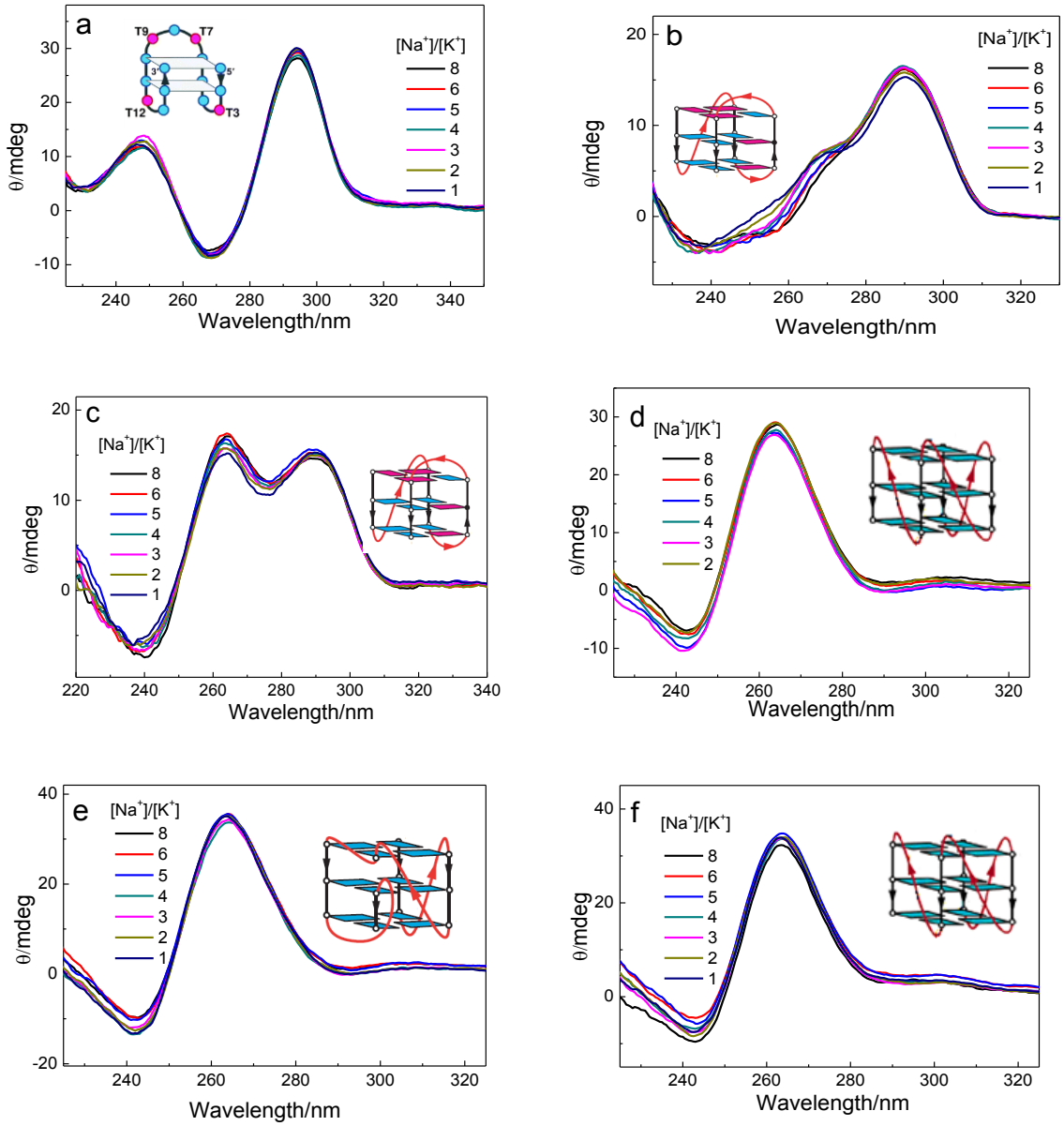

Fig. S1 The CD spectra of a) TBA, b) H24A, c) Bcl2-2345, d) c-myc, e) c-kit, and f) VEGF quadruplexes (3μM) with various Na^+/K^+ ratios in Tris-HCl buffer solution (pH 7.0) with 150 mM NaCl. The inserted is the G-quadruplex conformations according to the references [L.C. Bock, L. C. Griffin, J. A. Latham, et al. *Nature* **1992**, *355*, 564–566; D. J. Patal, A. T. Phan, V. Kuryavyi, *Nucleic Acid. Res.* **2007**, *35*, 7429-7455.]

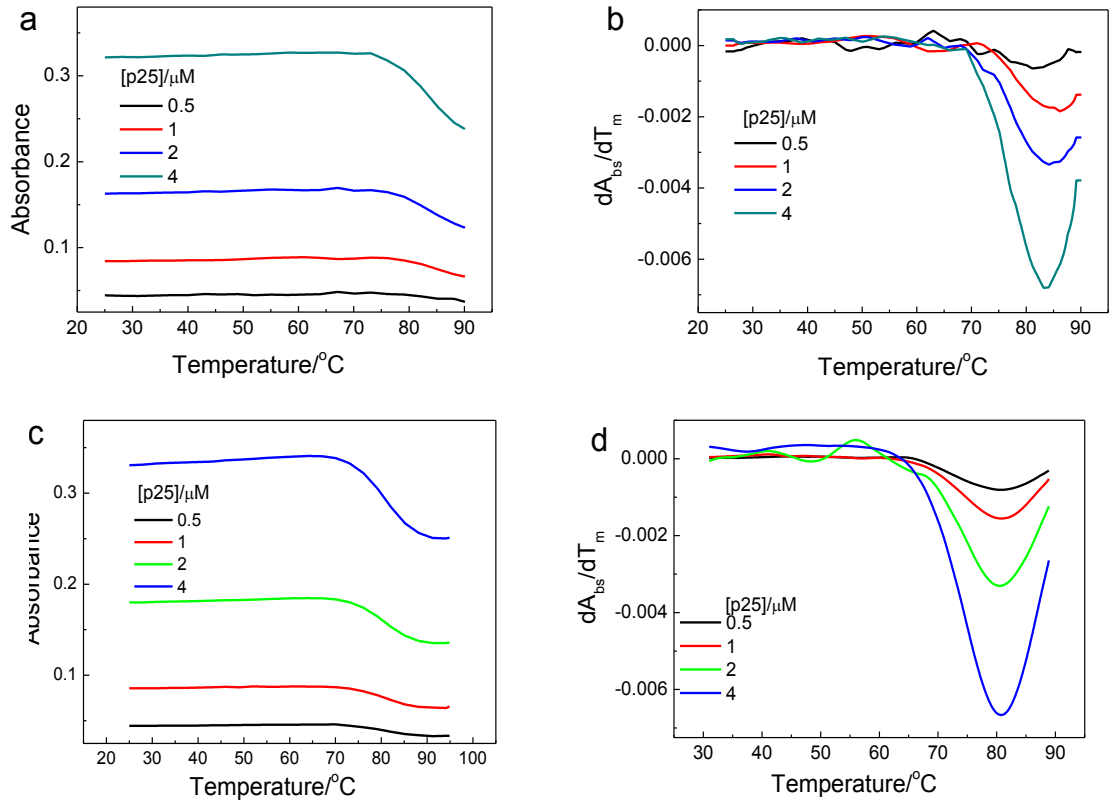

Fig. S2 a) and c) The curves of the absorbance at 295nm versus temperature for different amounts of p25 respectively with 150mM NaCl and 150 mM KCl. b) and d) The differential plots respectively are derivative from the data of Figure S1a and S1c.

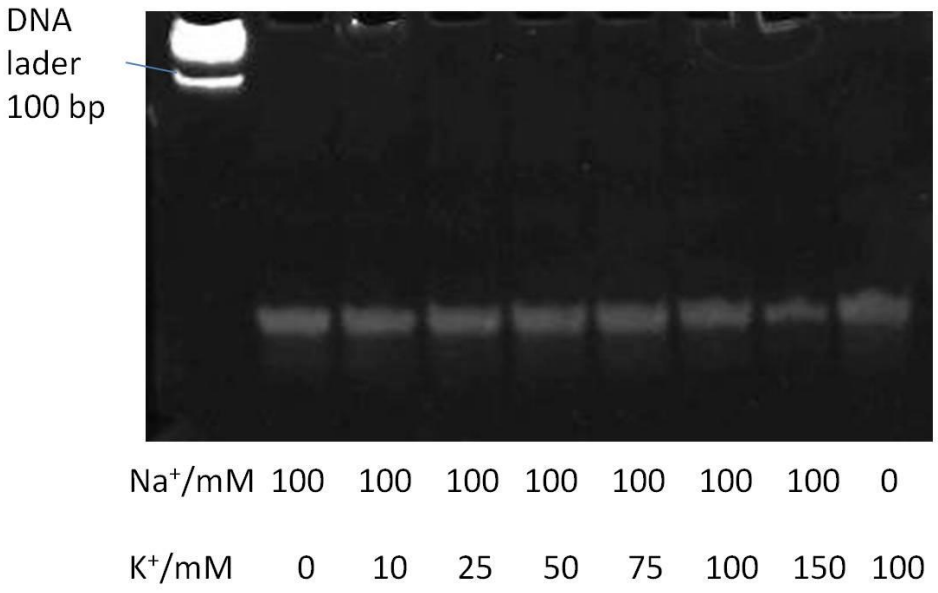

Fig. S3 Nondenaturing PAGE analysis of 2 μM p25 under different Na^+ and K^+ concentrations in 10 mM Tris-HCl buffer (pH 7.2).

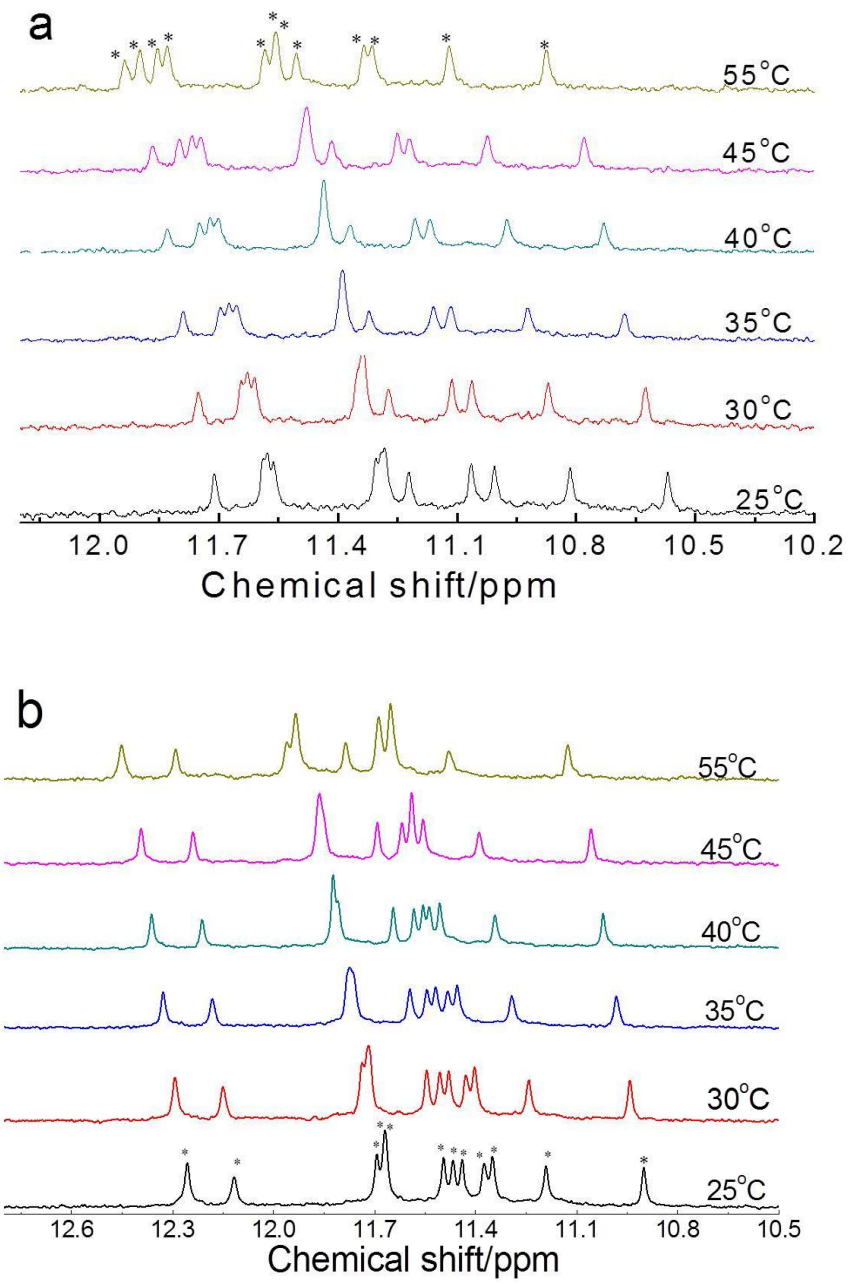

Fig. S4 Part of the ^{1}H-NMR spectra of imino protons changes with increasing temperature for p25 with a) 150mM KCl and b) 150mM NaCl present.

Table 2. The P32 and its modified sequence.

Name	DNA sequences
p25	GGGCCAGGGAGCGGGGCGGAGGGGG
p25-1	GGGCCAGGGAGCGGGGCGGAGGGG**A**
p25-2	GGGCCAGGGAGCGGGGCGGAGGG**A**G
p25-3	GGGCCAGGGAGCGGGGCGGAG**A**GGG
p25-4	GGGCCAGGGAGCGGGGCGGA**A**GGGG
p25-5	GGGCCAGGGAGC**A**GGGCGGAGGGGG
p25-6	GGGCCAGGGAGCGGG**A**CGGAGGGGG
p25-7	GGGCCAGGGAGCG**A**GGCGGAGGGGG
p25-8	GGGCCAG**A**GAGCGGGGCGGAGGGGG
p25-9	G**A**GCCAGGGAGCGGGGCGGAGGGGG

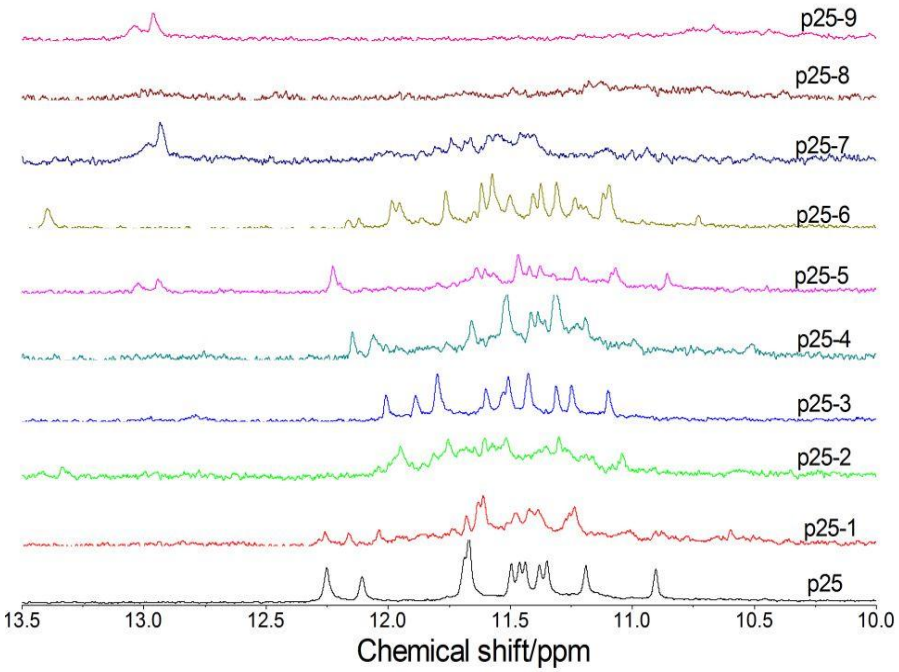

Fig. S5 Part of the ^{1}H-NMR spectra of imino protons for the mutated oligomers of p25 with 150mM KCl.

Oligomers containing mutations within each of the four G-tracts were designed and shown in Table 2. The mutation of G-to-A base was made based on the assumption that the mutation would denature the G-quadruplex structure if this guanine had participated to form G-tetrads. The mutation in the central position of the first, second and third G-tracts (p25-9, p25-8, and p25-7) obviously inhibited G-quadruplex formation. The mutated oligomers including p25-2, p25-3, and p25-6 exhibit different spectral feature compared with p25. The result indicates the guanines located in these above mutated positions had participated to form G-tetrads. Contrarily, p25-1, p25-4, and p25-5 show similar spectra as that p25, meaning the guanines in these mutated positions did not contribute to G-quadruplex formation.

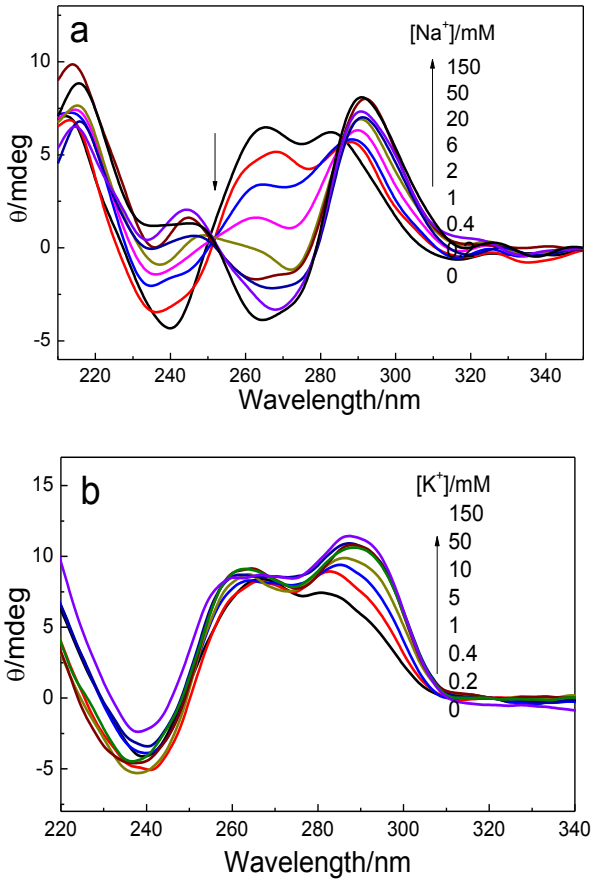

Fig. S6 The CD spectra of 3 μM p25 with increasing amount of a) NaCl, and b) KCl in 10 mM Tris-HCl buffer solution (pH 7.2).

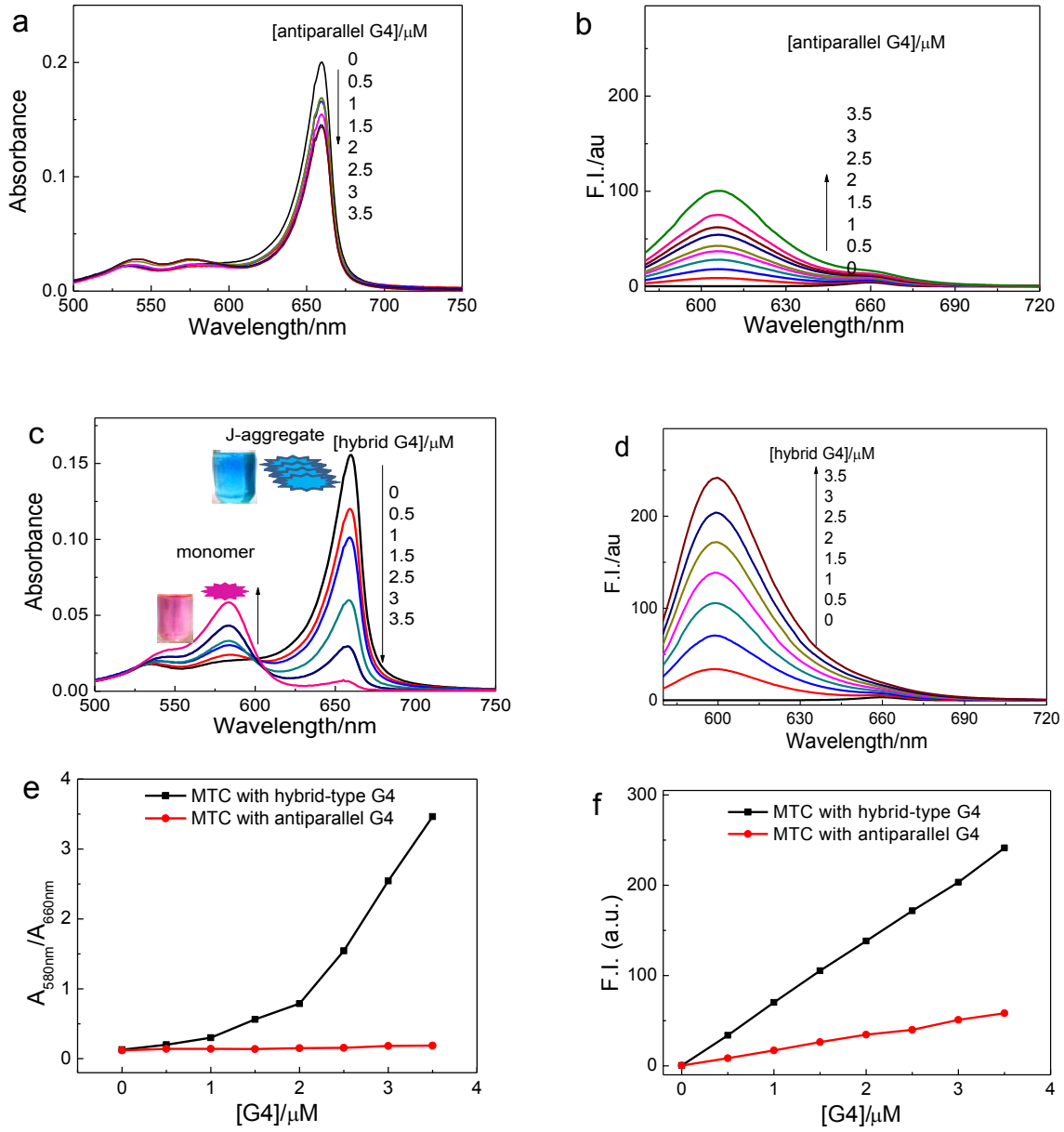

Fig. S7 The (a) absorption and (b) fluorescence spectra of 3.3μM MTC with increasing amount of antiparallel p25 G-quadruplexes in Tris-HCl buffer solution containing 20mM NaCl. The (c) absorption and (b) fluorescence spectra of 3.3 μM MTC with increasing amounts of hybrid-type p25 G-quadruplexes in 10 mM Tris-HCl buffer solution (pH 7.2) containing 20 mM K^+. The insert in (c) shows the solution colour corresponding to the MTC J-aggregate and monomer. (e) The curves of the ratio A_{580nm}/A_{660nm} and (f) the fluorescent intensity at 598 nm versus [G4] with hybrid-type (black line) and antiparallel (red line) motif. The inserted in the absorption spectra is the sample solution images.

The increased absorbance ratio at 580 nm and 660 nm as well as the enhancement of fluorescence intensity at 600 nm reflects the switch from MTC J-aggregates to monomers.

Compared with the hybrid-type G-quadruplexes, the antiparallel G-quadruplexes aroused the switch of MTC more weakly, meaning the weaker interaction between the antiparallel G-quadruplexes and MTC.

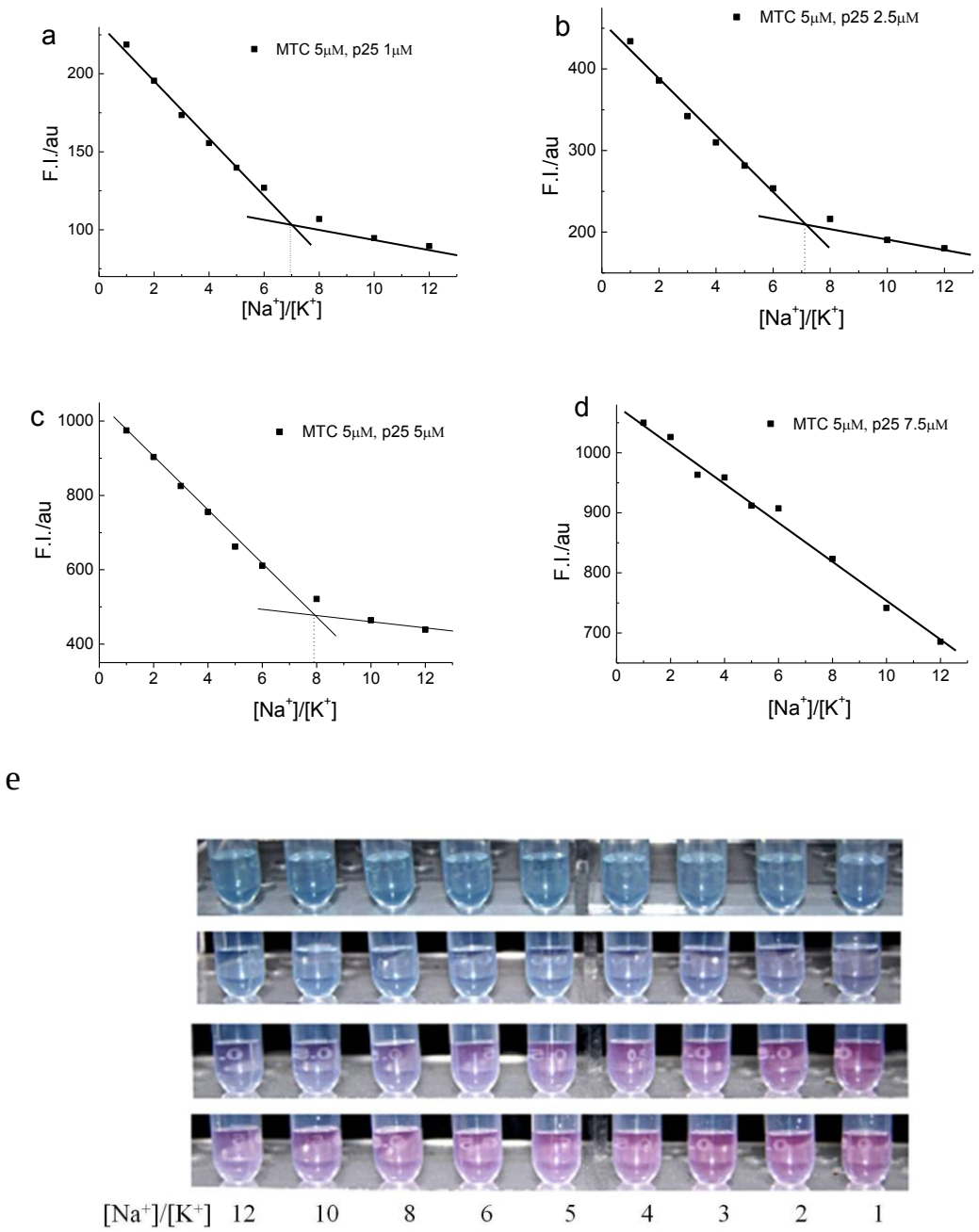

Fig. S8 (a), (b), (c), and (d) The plots of the fluorescence intensity of 5 μM MTC at 598 nm versus the ratios of $[Na^+]/[K^+]$ with various concentrations of p25 in Tris-HCl buffer solution (pH 7.4). (e) The photos of the solutions correspond to the (a), (b), (c), and (d) samples.

The result indicates a [p25]-dependent response of MTC to the Na^+/K^+ ratios: a higher concentration of p25 provides a larger linear range for quantitative measurement; but p25 with about a half-concentration of MTC can make the probe solution exhibit various colours corresponding to different Na^+/K^+ ratios. The ratio of [p25]/[MTC] close to 1/2 will be appropriate for realizing both of colorimetric and quantitative measurements.

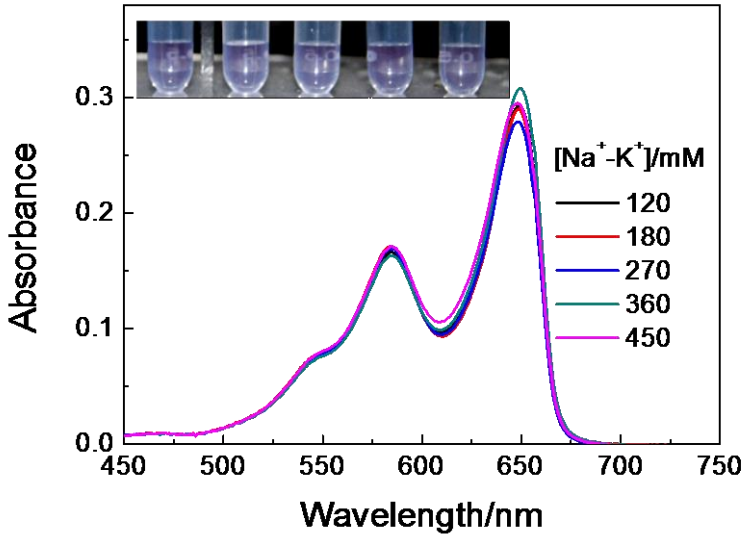

Fig. S9 The absorption spectra of 5 μM MTC together with 3 μM p25 in 10 mM Tris-HCl buffer solution (pH 7.2) when the ratio of $[Na^+]/[K^+]$ is 4 but different total concentrations of Na^+ and K^+. The inserted is the images of the solution colour.

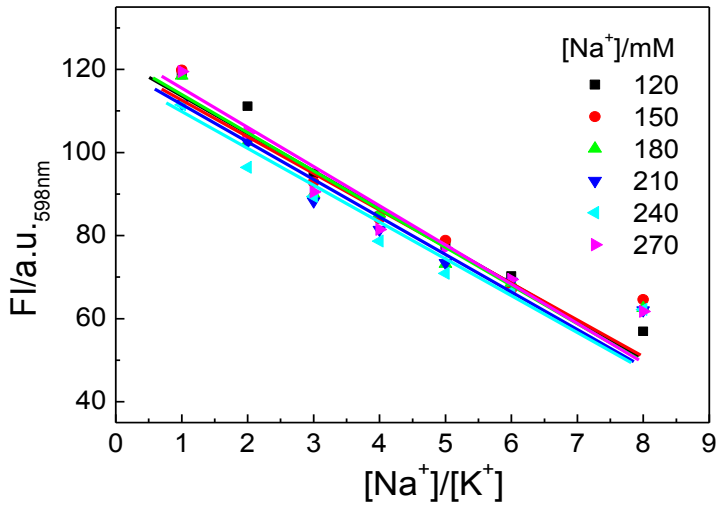

Fig. S10 The plots of the fluorescence intensity at 598 nm versus the ratio of $[Na^+]/[K^+]$ with various concentrations of Na^+ in Tris-HCl buffer solution (pH 7.4).

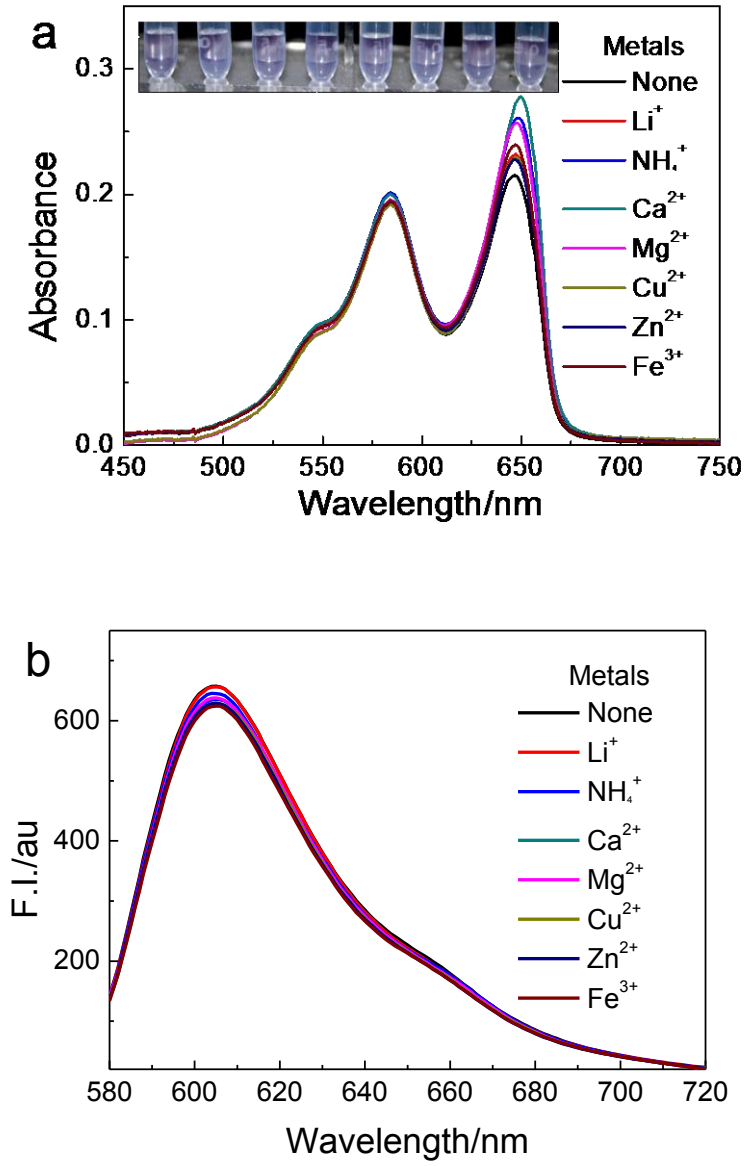

Fig. S11 The (a) absorption and (b) fluorescence spectra of 5 μM MTC without and with metal ions present in 10 mM Tris-HCl buffer solution (pH 7.2) containing 3 μM p25, 150 mM NaCl/75 mM KCl.