Electronic Supplementary Information (ESI)

A Label-free fluorescent assay for potassium ions using riboflavin as a G-quadruplex ligand

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Materials and Methods

Reagents and Apparatus

Riboflavin and FAD were purchased from Sigma-Aldrich (St. Louis, MO, USA). FMN was bought from J&K Scientific Ltd. (Beijing, China). FMN and FAD were prepared in water and stored at -20℃. Purified DNA sequences were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). DNA stock solutions were prepared by dissolving DNA in water and stored at -20℃. The concentrations of DNA in single-stranded state were determined by measuring the absorbance at 260 nm. Molar extinction coefficients were determined using a nearest neighbor approximation (http://www.idtdna.com/calc/analyzer). Other chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Milli-Q ultrapure water was used throughout the experiments. Fluorescence measurements were carried out on a F-4600 spectrofluorometer (Hitachi, Tokyo, Japan). The emission spectra were recorded in the wavelength of 480-700 nm upon excitation at 448 nm at ambient temperature. The highest emission intensity for flavins was observed at ~530 nm. UV-Vis absorption spectra were recorded on a Lambda 25 Spectrometer (PerkinElmer, Singapore) from 390 to 550 nm.

Fluorescence measurements

Aliquots of KCl were added to Tris-HCl (20 mM pH 7.0) solutions containing EDTA, Na^+, and DNA, incubated at room temperature for 15 min. Then riboflavin in water solution was added into each DNA solution and fluorescence spectra were measured. As to the procedure for selectivity, Na^+, Li^+, NH_4^+, Pb^{2+}, Hg^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, and Cu^{2+} were used instead of K^+. The assay procedures for FAD and FMN were the same as those for riboflavin, except that FAD and FMN were used instead of riboflavin. The final concentrations of the components were provided

in each part of this paper.

Absorbance measurements

Aliquots of PW17 (0, 2, 5, 10, 15, 20, 40 μM) were added to Tris-HCl (20 mM pH 7.0) solutions containing EDTA and potassium ions, incubated at room temperature for 15 min. Then riboflavin in water solution was added into each G-quadruplex solution and absorption spectra were measured. The final concentrations of EDTA, K^+, and riboflavin were 1 mM, 50 mM and 10 μM, respectively.

Analysis of real samples

Urine was determined to estimate the performance of this assay for analysis of real samples. Urine samples, excreted from healthy members of our group, were filtered through 0.22 μm membrane before using. After adding aliquots of K^+ (0 or 30 mM), these samples were diluted with Tris-HCl buffer, and then mixed with EDTA (1 mM), PW17 (4 μM) and Rb (1 μM) successively. After incubated at ambient temperature for 15 min, fluorescence spectra were recorded. The urine samples were diluted 300-fold finally. All the samples in quintuplicate were detected.

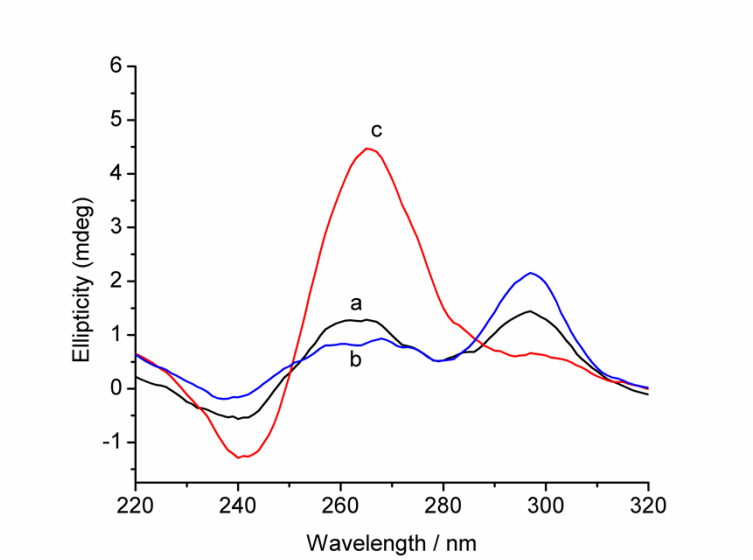

Figure S1. Circular dichroism (CD) spectra of PW17 (4 μM) in the absence (a) and the presence of Na^+ (50 mM) (b) and Na^+ (50 mM) and K^+ (50 mM) (c). Solutions were prepared in 20 mM Tris–HCl (pH 7.0) buffer containing 1 mM EDTA.

Table S1. DNA sequences used in this study.

name	DNA sequences (5′-3′)
PW17	GGGTAGGGCGGGTTGGG
G3T2	G3(T2G3)3
AGRO 100	GGTGGTGGTGGTTGTGGTGGTGGTGG
G3T4TT4	G3T4G3TG3T4G3
PS2.M	GTGGGTAGGGCGGGTTGG
Rs-4-ds	GGAACGAGGGGATGGGAGGGAGGGAGTCGTTCC
VEGF	GGGCGGGCCGGGGGCGGG
G3T3	G3(T3G3)3
c-myc	TGAGGGTGGGGAGGGTGGGGAA
G3T4	G3(T4G3)3
Oxy28	G4(T4G4)3
TBA	GGTTGGTGTGGTTGG
Hum 21	GGG(TTAGGG)3
G3T	G3(TG3)3
G3T4TT	G3T4G3TG3TG3
23A	TATAATACACGTAGCATCTGACA
23B	TGTCAGATGCTACGTGTATTATA

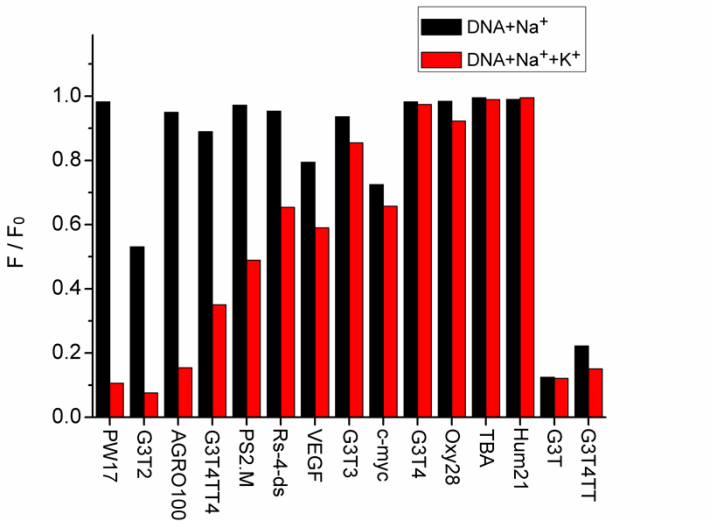

Figure S2. Fluorescence ratio of Rb with different G-rich sequences in the absence or presence of K^+ (10 mM). Experimental conditions: 20 mM pH 7.0 Tris-HCl buffer containing 1 mM EDTA and 50 mM NaCl. [Rb] = 1 μM, [G-rich sequence] = 10 μM. F_0 and F are the fluorescence intensities of Rb in the absence or presence of G-rich sequences, respectively.

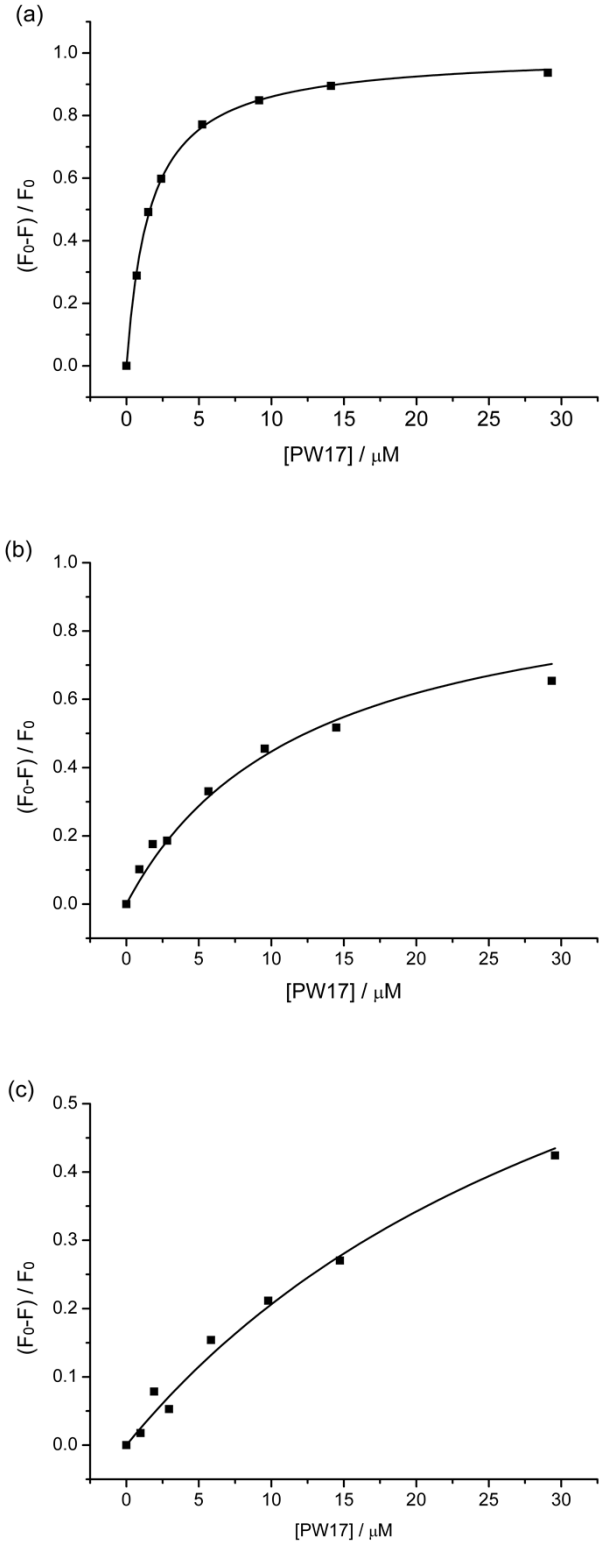

Figure S3. Nonlinear regression analysis of fluorescence binding curve of (a) Rb, (b) FMN and (c) FAD with PW17. Experimental conditions: 20 mM pH 7.0 Tris-HCl (1 mM EDTA, 50 mM KCl). The concentration of flavins was 1 μM. F_0, F denotes the fluorescence intensity of flavins in the absence and presence of PW17, respectively. Horizontal axis represents the concentration of unbound PW17. F_∞ is fluorescence intensity of the saturated binding. As many aromatic ligands

do, flavins possibly bind to G-quadruplex with the end-stacking mode. When the concentration of PW17 was 100 μM, the fluorescence of FMN was quenched 91 %. For the concentration of PW17 at 350 μM, the fluorescence of FAD was quenched 90%. So we assumed that the value of F_∞ is 0 for flavins in this study. [L_t], [L] denotes the concentrations of total and unbound PW17, respectively. [S_t] is the total concentration of flavins. Kd is the dissociation constant, obtained from the fluorescence titration experiments. The dissociation constants are 1.6 μM, 12.4 μM and 38.5 μM for Rb, FMN and FAD, respectively, based on the equation of (1) and (2) with 1:1 binding.[1]

$$F_0 - F = (F_0 - F_\infty)[L] / (Kd + [L]) \quad (1)$$

$$[L] = [L_t] - [S_t] (F_0 - F) / (F_0 - F_\infty) \quad (2)$$

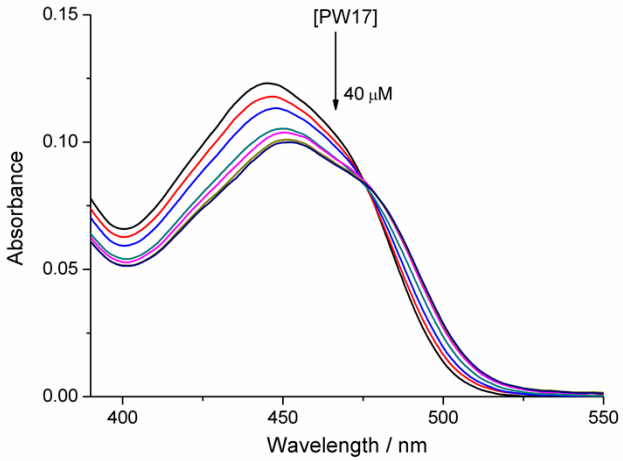

Figure S4. Absorption spectra of Rb (10 μM) with PW17 (0, 2, 5, 10, 15, 20, 40 μM) in 20 mM pH 7.0 Tris-HCl buffer containing 1 mM EDTA and 50 mM KCl.

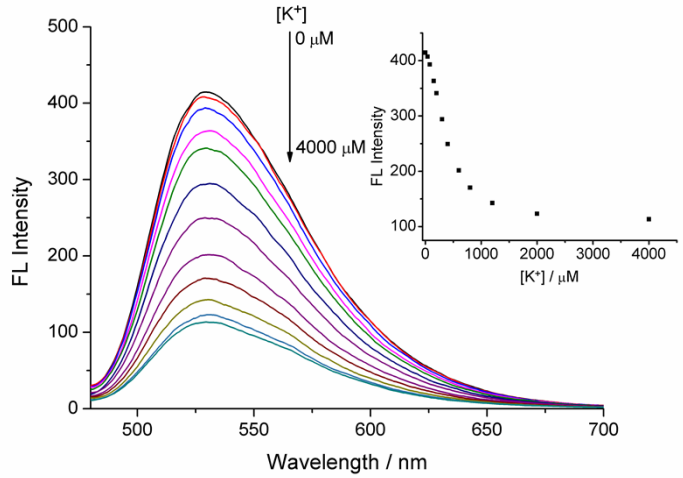

Figure S5. Fluorescence emission spectra of Rb in the presence of PW17 and K^+(0-4000 μM). The concentrations of Rb and PW17 were 1 μM and 4 μM, respectively. Experimental conditions: 20 mM pH 7.0 Tris-HCl buffer containing 1 mM EDTA and 50 mM NaCl. Inset was the

fluorescence intensities of this assay against the concentration of K^+.

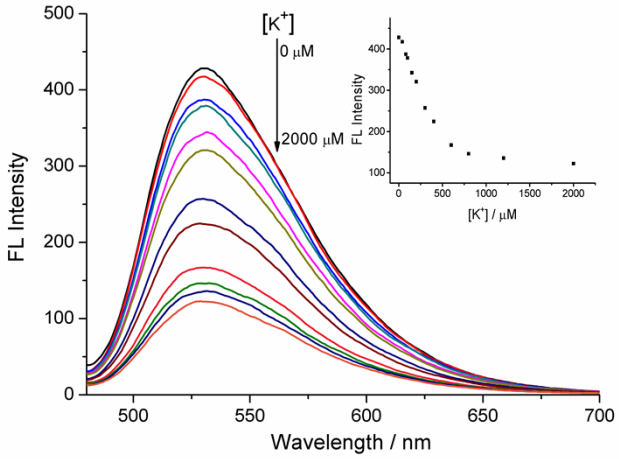

Figure S6. Fluorescence emission spectra for analyzing mimic physiological samples. The mimic physiological solutions contained Na^+(145 mM), Mg^{2+}(1.5 mM), Ca^{2+}(2.5 mM) and K^+ (0-20 mM) and diluted with 20 mM Tris-HCl buffer (pH 7.0, 1 mM EDTA) before measuring. The concentrations of Rb and PW17 were 1 μM and 4 μM, respectively. Inset was the fluorescence intensities of this assay against the concentration of K^+ in mimic physiological samples diluted 10-fold.

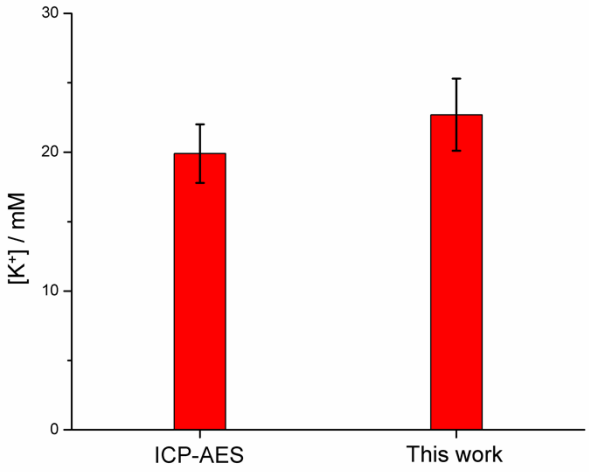

Figure S7. Concentration of K^+ in a urine sample measured using ICP-AES and the proposed assay.

References

1. Connors K A (1987) Binding Constants, John Wiley & Sons Inc, New York, pp 339-343.