Electronic Supporting Information

A Membrane-anchored Fluorescent Probe for Detecting K^+ in the Cell Microenvironment

Mengyi Xiong, Huijie Zhu, Qiming Rong, Chan Yang, Liping Qiu, Xiao-bing Zhang*
and Weihong Tan

Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha 410082. P. R. China.
* To whom correspondence should be addressed.
E-mail: xbzhang@hnu.edu.cn

Experimental Section

Materials

The DNA synthesis reagents were purchased from Glen Research (Sterling, VA, USA). All oligonucleotides sequences were synthesized on a PolyGen synthesizer and purified by HPLC (Agilent Model 1260 HPLC system). A solution of 0.1 M triethylamine acetate (pH 6.5) was used as HPLC buffer A, and HPLC-grade acetonitrile (Sigma Aldrich) was used as HPLC buffer B. 10 × Tris-HCl buffer (100 mM, pH = 7.4); 2 × HEPES buffer (100 mM, NaCl 270 mM, pH = 7.4); 2 M KCl and 2M NaCl solution were prepared and used.

Fluorescence measurements

The fluorescence measurements were conducted on a Fluoromax-4 spectrofluorometer (HORIBA JobinYvon, Edison, NJ) at room temperature. For all fluorescence detection, excitation wavelength was set at 495 nm (excitation wavelength of FAM) with a 2 nm slit width.

Cell culture

The CCRF-CEM (T-cell line, human acute lymphoblastic leukemia) were cultured in RPMI 1640 medium with the addition of 10% FBS (fetal bovine serum, Invitrogen, Carlsbad, CA, USA) and 0.5 mg/mL penicillin - streptomycin (KeyGEN Biotech, Nanjing, China) in a 5% CO_2 environment at 37 °C.

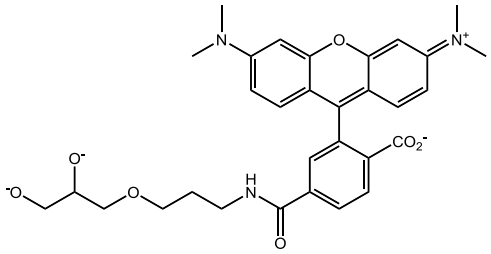
Flow cytometry and CLSM analysis

The cells were washed 3 times by centrifugation (900 rpm, 5 minutes) with 1 × HEPES buffer and incubated with lipid - FT5-1 probe for 40 min. Then the cells were washed for another 3 times to remove the excess probes and dispersed in 1 × HEPES buffer for use. In flow cytometry, the samples were analyzed on a BD FACSVerse™ flow cytometer. The concentration of K^+ was changed through removing the primary solution by centrifugation and adding solution contained different K^+ concentration repeatedly. After flow cytometric analysis, the samples were analyzed using a FV1000-X81 confocal microscope (Olympus) with the continuous addition of KCl.

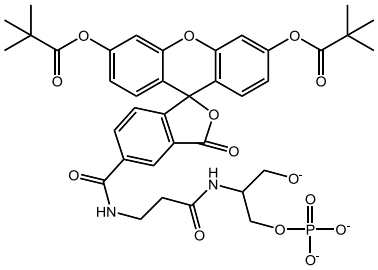
Table S1 . The oligonucleotides sequences for optimization and sensors.

Name	Sequence
FT1-1	**5' - FAM - T GGT TGG TGT GGT TGG T - TAMRA - 3'**
FT5-0	**5' - FAM - TT TTT GGT TGG TGT GGT TGG - TAMRA -3'**
FT5-1	**5' - FAM - TT TTT GGT TGG TGT GGT TGG T - TAMRA - 3'**
FT6-2	**5' - FAM - TTT TTT GGT TGG TGT GGT TGG TT - TAMRA - 3'**
FT7-2	**5' - FAM - T TTT TTT GGT TGG TGT GGT TGG TT - TAMRA - 3'**
FT5-5	**5' - FAM - TT TTT GGT TGG TGT GGT TGG TTT TT - TAMRA - 3'**
FT9-2	**5' - FAM - TTT TTT TTT GGT TGG TGT GGT TGG TT - TAMRA - 3'**
FT2-9	**5' - FAM - TT GGT TGG TGT GGT TGG TTT TTT TTT - TAMRA - 3'**
Lipid-FT5-1	**5' - Lipid - PEG - PEG - FAM - TT TTT GGT TGG TGT GGT TGG T - TAMRA - 3'**
Lipid-FB5-1	**5' - Lipid - PEG - PEG - FAM - TT TTT GGT TGG TGT GGT TGG T – BHQ_1 - 3'**

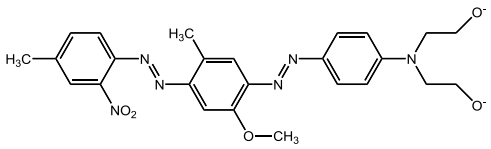
Structures of TAMRA, FAM, BHQ_1, C_{18} diacyllipid, PEG and Lipid-FT5-1



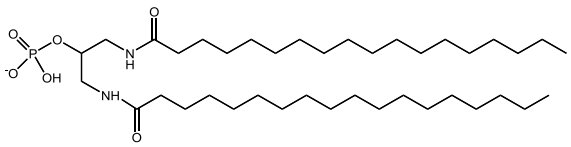
TAMRA



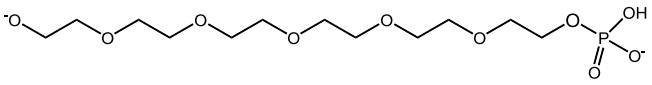
FAM



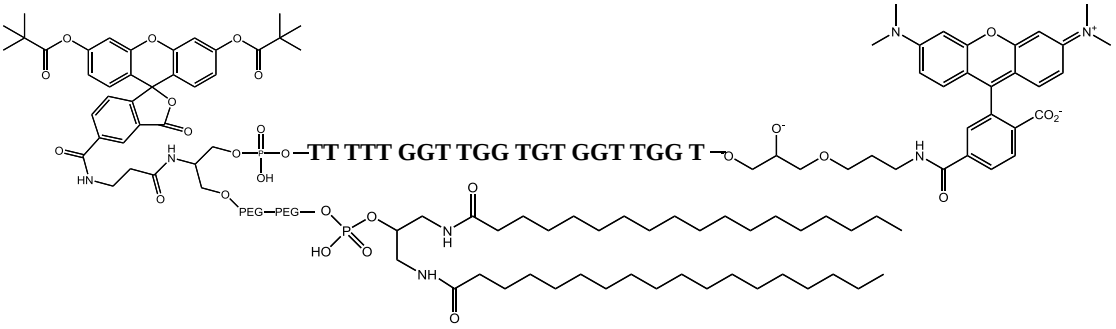
BHQ_1



C_{18} diacyllipid



Polyethylene glycol-PEG

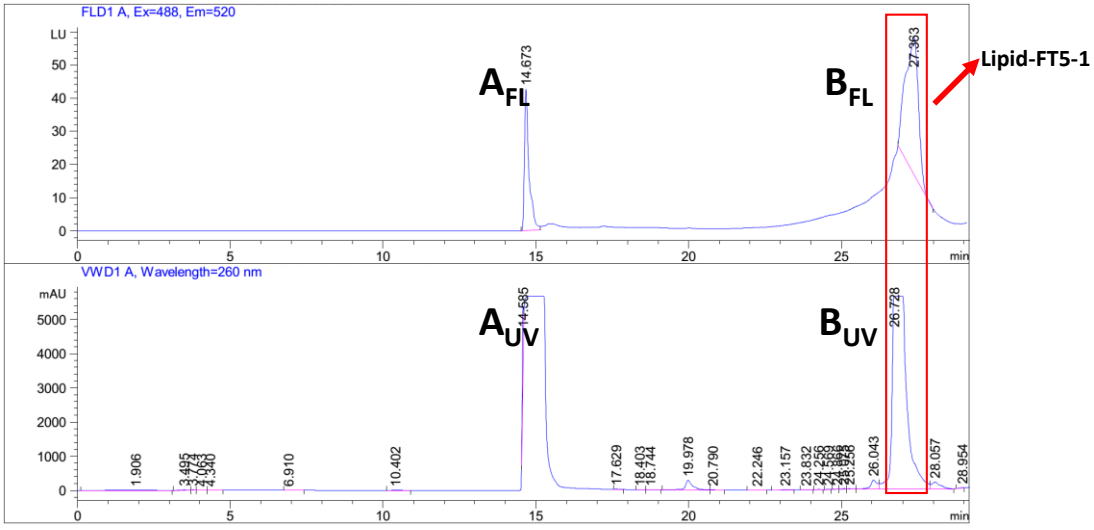


Lipid-FT5-1

Synthesis of probes

The C_{18} diacyllipid was synthesized according to previous published method.[1] The DNA synthesis reagents were purchased from Glen Research (Sterling, VA, USA). Oligonucleotides sequence were synthesized on a PolyGen synthesizer by soild state method following the protocol of previous report.[2] Briefly, oligonucleotide sequences were synthesized using a PolyGen synthesizer on a 1.0 micromole scale. Lipophilic phosphoramidites dissolved in dichloromethane were coupled onto the 5' ends of the oligonucleotides (This procedure was unnecessary for sequences without C_{18} diacyllipid). After synthesis, oligonucleotides were deprotected and cleaved from the corresponding controlled pore glass (CPG) according to reagent instructions. Then, all oligonucleotides were precipitated by adding 3 M NaCl (0.1-times volume) and cold ethanol (2.5-times volume). After having been frozen for 2h, the precipitated oligonucleotides were collected by centrifugation, dissolved in 0.1 M triethylammonium acetate (TEAA) , and then purified by HPLC (Agilent Model 1260 HPLC system) using 0.1 M TEAA buffer and acetonitrile (Sigma Aldrich) as the eluents.

The result of purification from HPLC was shown as follows. The UV-vis absorption of oligonucleotides (at 260 nm) and fluorescence signal of FAM (Em=520 nm) were used to verify the structure of all sequences. The probes without C_{18} diacyllipid (FT1-1, FT5-0, FT5-1, FT6-2, FT7-2, FT5-5, FT9-2, FT2-9) showed a UV-vis absorption (~260 nm, absorption of oligonucleotides) at ~15 min, and simultaneously a fluorescence emission of FAM was observed. Since the synthesis of oligonucleotides by a automatic DNA synthesizer was consecutive, only when the FAM was coupled on the correct sequences, can both UV-vis absorption and fluorescence emission be observed at the same time. In the synthesis of lipid-FT5-1 and lipid-FB5-1, because of the hydrophobic of C_{18} diacyllipid, an absorption at ~27 min (peak B_{UV}) was observed, which was much different from the sequence without C_{18} diacyllipid at ~15 min (peak A_{UV}). Simultaneously the fluorescence signal of FAM appeared (B_{FL}), which demonstrated the successfully synthesized of lipid-FT5-1.



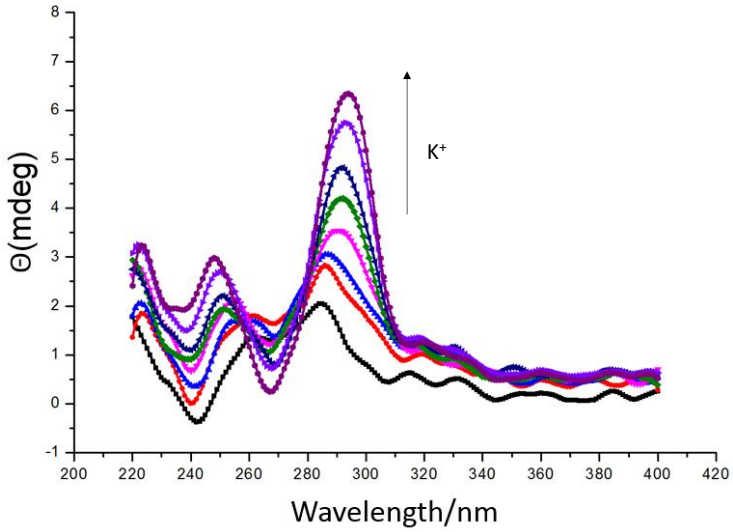


Figure S1. The changes of CD spectrum when the concentration of KCl range from 0 to 60 mM in 20 mM Tris-HCl and the FT5-1 was 5 μM.

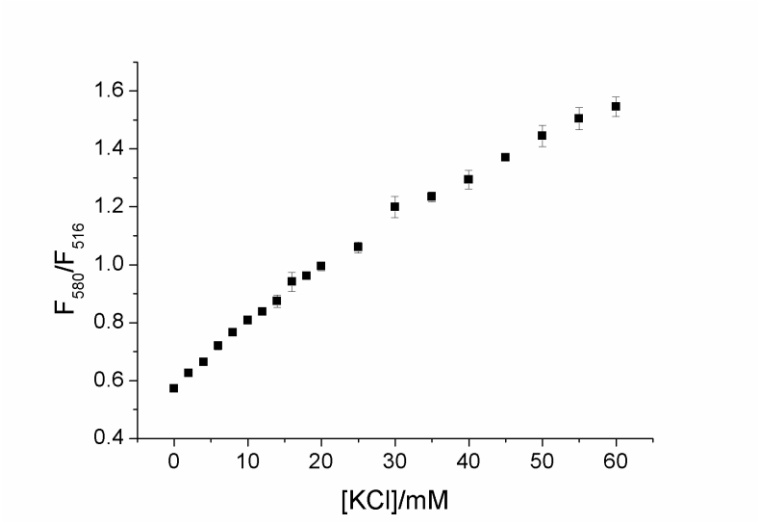


Figure S2. Fluorescence intensity ratio of acceptor (TAMRA, emission wavelength=580) to donor (FAM, emission wavelength = 516) in HEPES at room temperature.

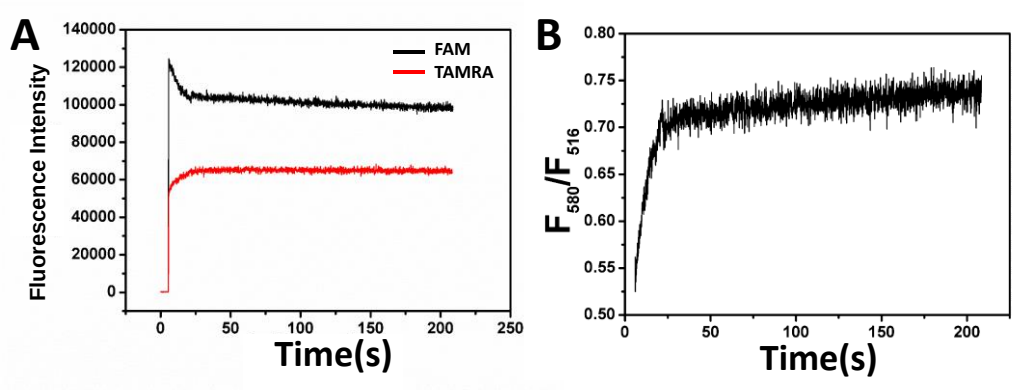


Figure S3. (A) Kinetics and (B) ratio value of fluorescence intensity from FT5-1 when the concentration of KCl was added to 10 mM in HEPES buffer.

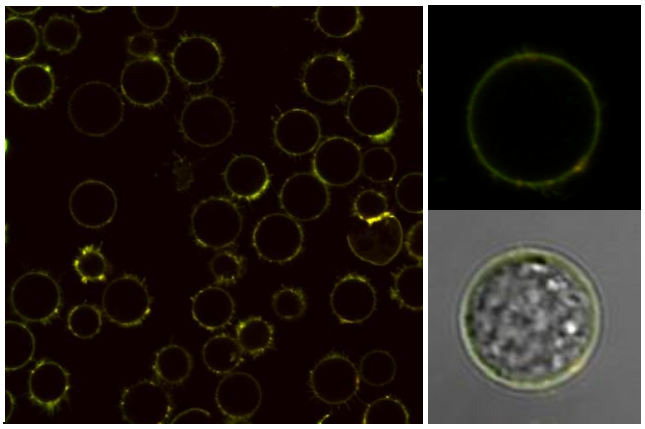


Figure S4. The outline of lipid - FT5-1 modified CEM cells by CLSM.

Detecting the levels of K^+ on the cell-surface quantitatively

The CLSM imaging of CEM cells under different levels of K^+ (0 mM, 30 mM and 60mM) were utilized, then Olympus software was used to analyze the pixel signal fluorescence intensity of these images. The pixel signal intensity of green channel from the images of CEM cells under 0 mM KCl was defined as 1.0, and the relative pixel intensity of both green and red channel under different levels of K^+ were displayed in Fig. S5A. With the addition of K^+ from 0 mM to 60 mM, the relative pixel intensity of green channel reduced and the relative pixel intensity of red channel increased. The ratio value of the relative pixel intensity (red points) was compared with the ratio of fluorimetric titration of K^+ (black points), as shown in Fig. S5B. The result indicated that this probe was capable of measuring K^+ levels on cell-surface.

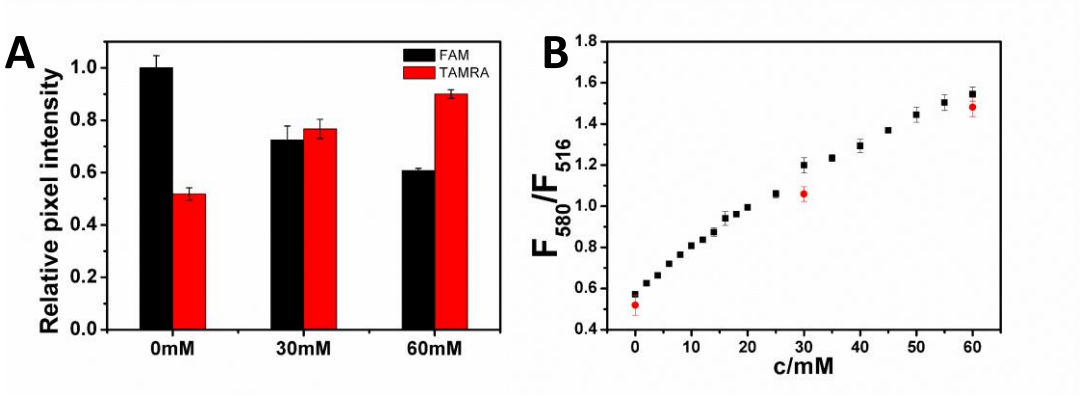


Figure S5. (A) The pixel fluorescence signal intensity of CEM cells imaging under different levels of K^+. (B) The ratio of the pixel fluorescence signal intensity from two channels (red points) and the in vitro fluorescence intensity ratio (black points).

The interference of the membrane lipids

The ratio of the relative pixel intensity was a little lower than the ratio of fluorimetric titration (Fig. S5B). This slight reduction of sensitivity might be caused by the interference of the membrane lipids which decreased the FRET efficiency between FAM and TAMRA. To investigate the interference of the membrane lipids, various counts of CEM cells were added to the solution of FT5-1 and Lipid-FT5-1 respectively. The solutions contained 2 μM FT5-1 or Lipid-FT5-1, 60 mM KCl and 1×HEPES. Then the fluorescence spectrum was measured and the ratio was dispalyed in Fig. S6. The result indicated that FT5-1 exhibited negligible changes while the ratio of Lipid-FT5-1 reduced slightly, which implied the sensitivity of this DNA-diacyllipid diblock probe is influenced by the nature of membrane lipids, such as steric hindrance.

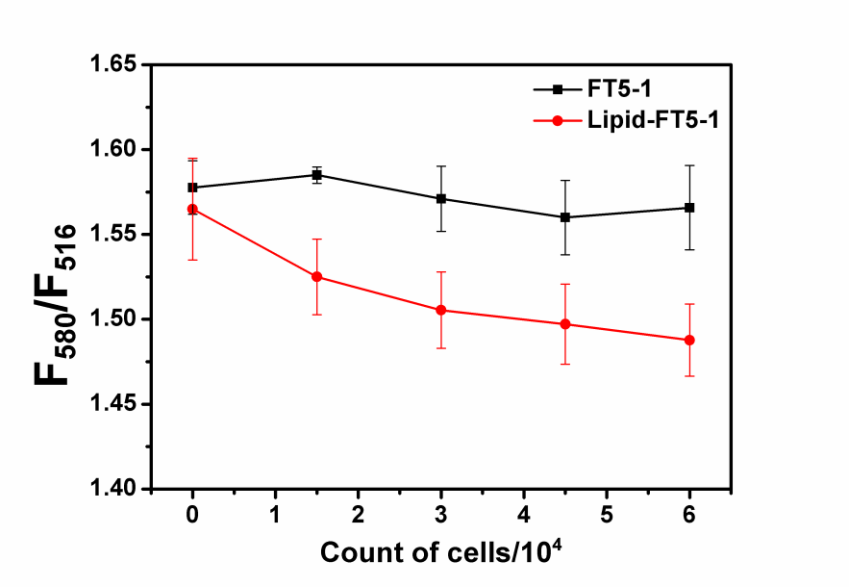


Figure S6. The fluorescence ratios of FT5-1 and Lipid-FT5-1 in which various counts of CEM cells were added.

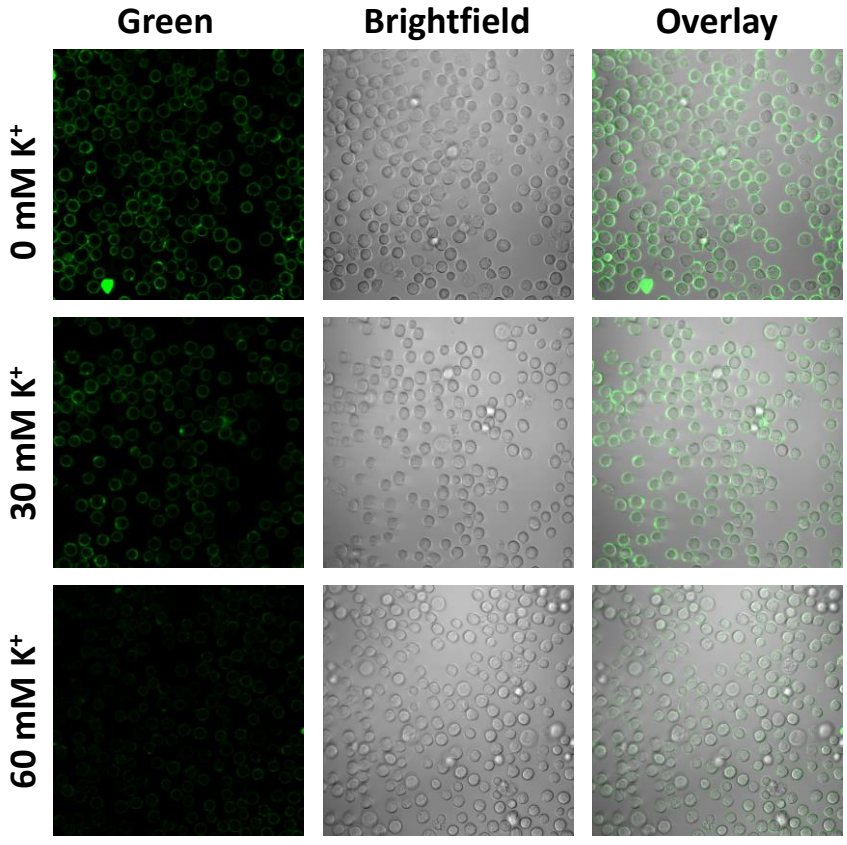


Figure S7. CLSM of Lip-FB5-1 on cell membrane under different concentrations of K^+.

References

1. H. Liu, Z. Zhu, H. Kang, Y. Wu, K. Sefan and W. Tan, *Chem. Eur. J.*, 2010, **16**, 3791-3797.
2. Y. R. Wu, K. Sefah, H. Liu, R. Wang and W. Tan, *Proc. Natl. Acad. Sci. USA.*, 2010, **107**, 5-10.