Electronic Supplementary Information (ESI)

Aptamer-Based FRET Nanoflares for Imaging Potassium Ions in Living Cells

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Experimental Section

Chemicals and Materials. Trisodium citrate was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Chloroauric acid (HAuCl₄·H₂O) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Oligomycin, ATP, cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). DNA loading buffer was purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (USA). SMMC-7721 cells were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Other reagents from commercial suppliers were analytical grade and used without further purification. All aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore). All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the involved oligonucleotides are listed as below (From 5' to 3'):

G-quadruplex:

FAM- TCTACGGGTTAGGTTAGGGTTAGGTT- TAMRA
three-bases mismatched G-quadruplex :

FAM- TCTACGGGTTAGCGTTAGAGTTAGCGT- TAMRA

cDNA: CTAACCGGTAGAT₁₀⁻ SH

Apparatus. The transmission electron microscopic (TEM) images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The fluorescence spectra were obtained on a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan). The excitation wavelength was set at 488 nm and the emission spectra from 510 to 650 nm were collected with a quartz cuvette containing 100 μL of solution. The UV-vis absorption spectra were obtained with a Biospec-nano UV-vis spectrophotometer (Japan). The cells were visualized under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The laser excitation was 488 nm, the bright-field image was recorded simultaneously by transmission PMT, and the fluorescent images were collected in the range of 505-525 nm (first column, FAM) and >560 nm (second column, TAMRA), respectively. The intracellular concentrations of Au were determined by ICP-MS (Thermo Fisher, X Series II). Cellular TEM imaging was performed with a FEI Tecnai G2 Spirit at an accelerating voltage of 120 kV.

Preparation of Aptamer-based FRET Nanoflares. Citrate-stabilized AuNPs (~13 nm) were synthesized by the classical citrate reduction route.¹ Thiolated cDNA strands were reduced by Tris (2-carboxyethyl) phosphine hydrochloride (TCEP-HCl). After 1 h, the cDNA strands were mixed with G-Quadruplex strands in phosphate buffered saline (PBS: 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4). Then the mixture was heated to 90 °C and maintained 10 min, then slowly cooled down to room temperature, and stored in the dark for overnight to allow complete hybridization. On the second day, the solution was added to the 13 nm AuNPs at a concentration of 3 μmol of oligonucleotide per 1 mL of 10mM AuNPs and shaken overnight. After 16 h, Phosphate buffer (0.2 M; pH = 7.4) was added to the mixture to achieve a 0.01 M phosphate concentration. In the subsequent salt aging process, aliquots of NaCl solution (2.0 M) were added to the mixture over an eight-
hour period to achieve a final sodium chloride concentration of 0.15 M. Finally, the solution was centrifuged (13,000g, 30min) and washed three times with 0.01 M phosphate buffer saline (0.3 M NaCl, pH 7.4). Concentrations of functionalized AuNPs were determined by measuring their extinction at 524 nm (ε=2.7×10^4 L mol⁻¹ cm⁻¹).

**Determination of DNA Amounts on AuNP.** DNA sequences loaded on AuNPs were quantitated according to the published protocol.² Briefly, mercaptoethanol (ME) was added (final concentration 20 mM) to the aptamer-based FRET nanoflares solution (2 nM). After it was incubated overnight with shaking at room temperature, the DNA strands were released. Released DNA probes were then separated via centrifugation and the fluorescence was measured with a fluorescence spectrometer. The fluorescence was converted to molar concentrations of DNAs by interpolation from a standard linear calibration curve that was prepared with known concentrations of TAMRA-labeled G-quadruplex with identical buffer pH, ionic strength and ME concentrations.

**Fluorescence Experiments.** Prepared aptamer-based FRET nanoflares were diluted to a concentration of 2 nM in 20 mM Tris-HCl (pH 7.4). 95 μL aliquots of the above sample were transferred into the fluorometer thermostated at 37°C and 5 μL aliquots of concentrated K⁺ stock solution were added. Then the mixtures were incubated and measured. For kinetic study, the A/D signal change was registered upon the subsequent variation of K⁺ concentration. For selectivity test, a certain concentration of Na⁺, NH₄⁺, K⁺, and Li⁺ stock solution were added into the aptamer-based FRET nanoflares with a final concentration of 150 mM.

**Nuclease Assay.** Two groups of the aptamer-based FRET nanoflares were diluted to a concentration of 2 nM in 20 mM Tris-HCl (pH 7.4). After allowing the samples to equilibrate (10 min), 1.3 μL of DNase I in assay buffer (2 U/L) was added to one of them, the other untreated group was served as control. The fluorescence of two samples was monitored for 1 h with an appropriate excitation wavelength.

**Cytotoxicity Assay.** SMMC-7721 cells (1×10⁵ cells/well) were plated in 96-well microtiter plates to a total volume of 200 μL well⁻¹. After overnight culture, the aptamer-based FRET nanoflares were added to each well. SMMC-7721 cells were incubated with aptamer-based FRET nanoflares (0, 2, and 5 nM) for 6, 12, 18, and 24 h. Subsequently, 0.1 mL of MTT solution (0.5 mg mL⁻¹ in PBS) was added to each well with incubation at 37°C for 4 h. Then the cells were washed with PBS. After the addition of DMSO (100 μL well⁻¹), the assay plate was shaken at room temperature for 10 min. The absorbance was measured at 490 nm with a microplate reader.

**Cell Culture.** SMMC-7721 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml 1% antibiotics penicillin/streptomycin and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air.

**AuNPs Uptake.** To estimate the amount of probes and control probes per cell, SMMC-7721 cells were seeded at 1×10⁶/mL in a 6-well plate. After 24 h, cells were incubated with the probes and control probes (2 nM) for different times. Then cells were washed with PBS five times, trypsinized to remove them from the bottom and collected by centrifugation. The trypsinized cell dispersion was further sonicated in a hot water bath (60 °C) to completely disrupt the cell membranes. Finally, the probes and control probes were
dissolved by successfully adding 0.3 mL hydrochloric acid and 0.1 mL nitric acid to the solution. Following the incubation overnight, the sample was diluted to 10 mL using ultrapure water. Based on the previous protocol, the concentration of Au, determined by ICP-MS (Thermo Fisher, X Series II), was converted to the number of AuNPs per cell. For each data point, we obtained the average and standard deviation by testing 6 samples (two separate trials, with 3 parallel experiments in each trial).

**Imaging Intracellular K⁺.** SMMS-7721 cells were plated on 35 mm glass-bottom dishes and grown to 80% confluence before imaging. For comparative experiment, two groups of cells were treated with 5nM probes or control probes respectively. After 4h, the cells were washed three times with PBS (pH 7.4) and then observed under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. TAMRA fluorescence image was recorded in red channel with 488 nm excitation and a 560 nm (±10 nm) bandpass filter. In the experiments for expression levels of K⁺, SMMS-7721 cells were treated with ATP, amphotericin, and the mixture of nigericin, bumetanide, and ouabain respectively.

**Flow Cytometric Assay.** SMMS-7721 cells were incubated with probes and control probes. After 4h, the cells were washed to remove the redundant particles. Cells were then detached from culture dishes using Trypsin-EDTA Solution. The solution containing treated cells was centrifuged (2000 rpm, 4 min) and resuspended in PBS three times. Flow cytometric assay was performed using Beckman Coulter Gallios machine.

**Supporting Reference:**


Supporting Figures

Figure S1. (A) TEM micrograph of AuNPs; (B) Absorption spectra of AuNPs (black line) and aptamer-based FRET nanoflares (red line). The maximum optical absorption was shifted from 519 nm to 524 nm after modification.
**Figure S2.** Standard linear calibration curve of fluorescence signal against the concentration of TAMRA labeled strands. The excitation wavelength was 543 nm and the emission wavelength was from 565 to 630 nm. The error bars represent the standard deviations.
Figure S3. The ultraviolet absorption of nanoprobe incubated with various biologically important metal cations at their physiological concentrations: K⁺ (150 mM), Na⁺ (150 mM), Ca²⁺ (5.0 mM), Mg²⁺ (5.0 mM), Cu²⁺ (50 μM), Zn²⁺ (50 μM), Fe³⁺ (50 μM), in 20 mM Tris-HCl buffer (pH 7.4).
Figure S4. Circular dichroism spectra of 2 mM G-Quadruplex in 20 mM Tris-HCl (pH 7.4) (red) with 0, 145 mM NaCl (green), or 145 mM KCl (blue).
Figure S5. Fluorescence changes of aptamer-based FRET nanoflares registered upon the subsequent increase of K⁺ concentration (from 11mM to 15mM) in 20 mM Tris-HCl buffer (pH 7.4).
Figure S6. SMMS-7721 cells were incubated with different concentrations (0 nM, 2 nM and 5 nM) of aptamer-based FRET nanoflares for 6 h, 12 h, 18 h and 24 h. Black bar stands for no aptazyme-AuNP; light grey bar stands for the aptamer-based FRET nanoflares (2nM); dark grey bar stands for higher concentration of the aptamer-based FRET nanoflares (5nM). Error bars represent variations between three measurements.
Figure S7. (a) Fluorescence curves of the nanoprobe (2 nM) in buffer with (○) or without (●) DNase I, as a function of time. (b) Fluorescence curves of the nanoprobe (2 nM) in buffer with (○) or without (●) Exo III, as a function of time. The results showed that the introduction of DNase I or Exo III did not lead to the obvious changes of FRET signal of the nanoprobe.
**Figure S8.** ICP-MS for cellular uptake amount of the nanoprobe and control nanoprobe. The error bars are the standard deviations by testing 3 samples.
Figure S9. Colocalization experiments involve the aptamer-based FRET nano-flares and DAPI (stained nuclei) in SMMS-7721 cells lines. Scale bar: 10μm.