



A DNA-based nanosensor for in situ precise imaging of lysosomal potassium ions via pH activation

Guangming Li, Ying An, Jiahui Sun, Mengxu Sun, Chaoyi Yang, Yi Ren, Shijiong Wei, Huangxian Ju ^{*} 

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry, Nanjing University, Nanjing, 210023, PR China

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ABSTRACT

For subcellular imaging, activating probes by specific species in targeted organelles is an attractive strategy to eliminate nonspecific signals during transport. The characteristic pH (~4.5–5.0) of lysosomes enables selective probe activation within lysosomes. In this work, a pH-activated K⁺ nanosensor for lysosomal K⁺ imaging was designed by capturing Y-typed DNA probe on linker modified gold nanoparticle. The DNA probe could be conveniently prepared by assembling dual fluorophores-labelled G-quadruplex (G4) strand, Sgc8c modified i-motif structure and capturing DNA sequence. The folding capability of G4 in the presence of K⁺ was inhibited at neutral pH, which prevented fluorescence resonance energy transfer (FRET) between two fluorophores labelled at both ends of the G4. Upon targeted delivery into lysosomes, the conformational change of the i-motif structure was activated by acidic pH, leading to G4 folding and thus K⁺-dependent FRET signal. Sgc8c modification enhanced target-cell-specific internalization for nanosensor delivery. Both in vitro experiments and in situ lysosomal K⁺ imaging along with external pharmacological stimuli demonstrated the excellent performance of the proposed K⁺ nanosensor, which expanded the application of DNA-based probes in subcellular imaging field.

1. Introduction

As primary degradative compartments of eukaryotic cells, lysosomes play vital significance in maintaining cellular homeostasis. They coordinate key functions including sensing, utilizing and recovering nutrients, which rely on synchronous interactions among hydrolytic enzymes, nutrient transporters and signaling factors (Settembre and Perera, 2024). Ion homeostasis and transmembrane ion fluxes are critical for regulating key lysosomal functions. Lysosomal dysfunction is linked to lysosomal storage diseases and neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, as well as cancers (Carmona-Gutierrez et al., 2016; Mondal et al., 2022; Wu et al., 2022). As the most abundant intracellular cation, potassium ion (K⁺) in biological environment is related to muscle contraction, heartbeat, nerve transmissions, and kidney functions (Zhou et al., 2011a). In lysosomes, K⁺ participate in maintaining stable lysosomal pH and membrane voltage (Lee et al., 2017; Steinberg et al., 2009). Therefore, developing reliable approaches for in situ monitoring of lysosomal K⁺ is essential for elucidating its regulatory role in lysosomal homeostasis.

Compared with cellular-level imaging, organelle-level imaging

requires to suppress the activity of probes toward specific targets prior to their localization in targeted organelles, enabling the imaging of biological species within organelles (Emmert et al., 2023). A common strategy is to utilize organelle-specific species to activate the probes within target organelles (Peng et al., 2019; Wu et al., 2025; Yi et al., 2024). In such a design, the probes remain inactive during transport and are specifically activated only upon their arrival at targeted organelles. For lysosomal imaging, low pH (~4.5–5.0) (Casey et al., 2010; Settembre and Perera, 2024) is an obvious feature compared with other organelles, and has been applied to activate the probes for in situ ATP detection (Du et al., 2019; L. L. Li et al., 2021; Peng et al., 2019), which are inert at neutral pH but are rendered active by H⁺-induced conformational change. However, such strategies have not been extended for the detection of other species in lysosomes, even if the DNA nanostructures logic-gated by both H⁺ and K⁺ have been reported (Dong et al., 2025; Li et al., 2011; Shao et al., 2020). The requirement of these nanostructures for exogenously supplied fluorogenic ligands also limits their practical applications in subcellular imaging field. Besides, some DNA nanostructures designed for simultaneous imaging of lysosomal pH and K⁺ lack effective blocking and activating mechanisms

^{*} Corresponding author.

E-mail address: hxju@nju.edu.cn (H. Ju).

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(Chen et al., 2021; X. Q. Li et al., 2021), which may thereby introduce nonspecific K^+ signals generated during transport into the lysosomes. Thus, it is necessary to develop a K^+ sensor with the pH-activated strategy for in situ precise imaging of K^+ in lysosomes.

In this work, we developed a pH-activated K^+ nanosensor by assembling dual fluorophores-labelled G-quadruplex (G4) strand (Cy5-G4-Cy3), Sgc8c modified i-motif structure (Gb1) and capturing DNA sequence (Gb2) to form a Y-typed DNA probe, and then binding the probe on linker modified gold nanoparticle (AuNP@linker) (Scheme 1a). As a class of G-rich DNA strands, G4 has been extensively used as a recognition element for K^+ sensing (Chen et al., 2021; Cui et al., 2020; Deng et al., 2024; Zhang et al., 2024) by its folding in the presence of K^+ (Mergny and Sen, 2019). To exclude the nonspecific K^+ signals, here pH-dependent conformation change of i-motif structure (Cheng et al., 2021; Du et al., 2019; Peng et al., 2019), which has been applied in pH sensing (Chen et al., 2020; Zhao et al., 2020), was utilized as an actuator to achieve the pH activation, and Sgc8c, an aptamer targeting to protein tyrosine kinase 7 (PTK7) on cell surface, was introduced to DNA probe to enhance cellular internalization and lysosomal localization (Chen et al., 2025; He et al., 2024; Su et al., 2025). Owing the prevented G4 folding in Y-typed DNA probe, the fluorescence resonance energy transfer (FRET) from Cy3 to Cy5 labelled at two ends of G4 strand was in “signal-off” state at neutral pH. After the nanosensor was specifically uptaken and delivered into lysosome, the low lysosomal pH activated the conformation change of i-motif structure, which induced the release of Cy5-G4-Cy3 and then K^+ -dependent G4 folding, resulting in “FRET-on” signal for K^+ response (Scheme 1b). The excellent performance of the proposed K^+ nanosensor for in situ imaging of lysosomal K^+ upon external pharmacological stimuli demonstrated its promising application in subcellular imaging field.

2. Experimental section

2.1. Materials, reagents and apparatus

The materials, reagents, and apparatus are presented in the Supplementary information.

2.2. Preparation and characterization of DNA probe

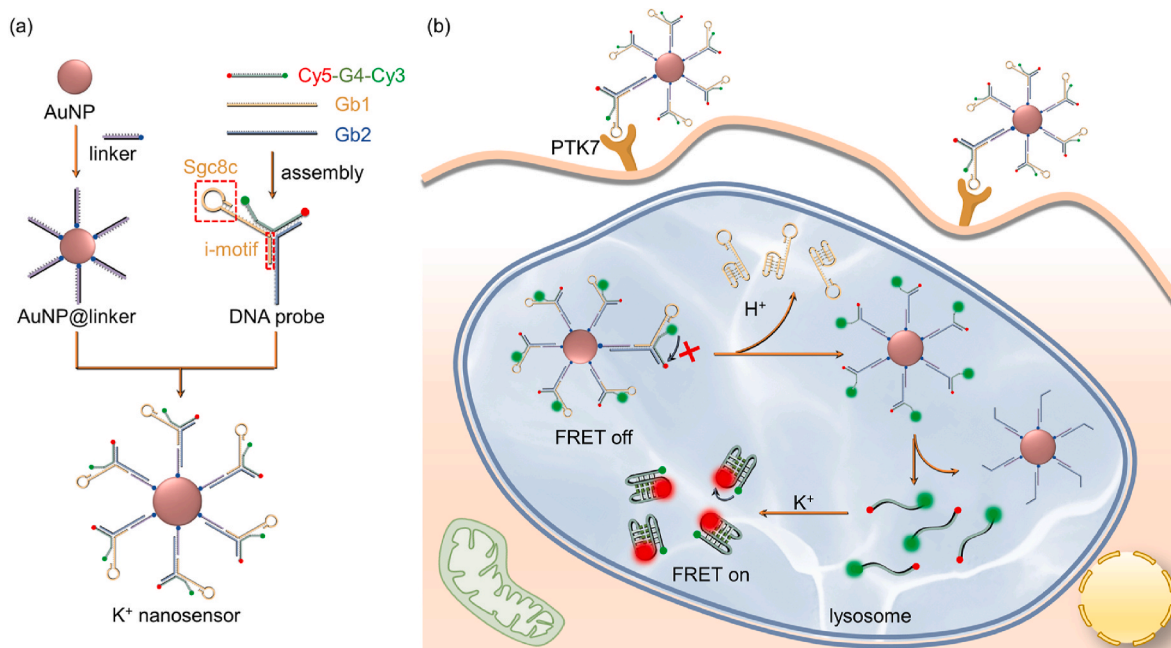
The assembly of DNA probe was conducted in PBS (10 mM, pH 7.5) containing 150 mM NaCl and 5 mM $MgCl_2$. After being quantified by UV-Vis spectra with Lambert-Beer's law, dual fluorophores-labelled G-quadruplex (G4) strand (Cy5-G4-Cy3), Sgc8c modified i-motif structure (Gb1) and capturing DNA (Gb2) were mixed at the same stoichiometry and incubated at 37 °C for 30 min. The prepared Y-typed DNA probe was stored at 4 °C in dark. Similarly, three control Y-typed DNA complexes were prepared by replacing Gb1 with Gb1 without Sgc8c or replacing Gb1 with a sequence without the i-motif portion (mGb1), Gb2 with mGb2, and Cy5-G4-Cy3 with another dual fluorophores-labelled DNA strand (Cy5-mG4-Cy3).

12% PAGE was used to confirm the formation of Y-typed DNA complexes, including the DNA probe. Specifically, 5 μ L of 1 μ M prepared DNA samples were mixed with 1 μ L of loading buffer, and then loaded on gels to perform the electrophoresis analysis at 110 V for 1 h at 4 °C in $1 \times$ TBE buffer, which was stained with Gel Red to obtain the image.

2.3. Preparation of K^+ nanosensor

AuNPs were firstly prepared by sodium citrate reduction according to previous report (Grabar et al., 1995). The concentration of prepared AuNPs was calculated according to previous report (Haiss et al., 2007). After the DNA linker was mixed with TECP (1:250, molar ratio) to reduce disulfide bonds at 37 °C for 2 h, 40 μ L of 50 μ M linker was added to 2 mL of 4 nM AuNPs and kept at −20 °C for 3 h to form AuNP@linker (Liu and Liu, 2017). The produced AuNPs@linker were centrifuged at 12000 rpm for 10 min, washed with PBS containing 0.1% Tween-20 for three times, resuspended in 200 μ L PBS and stored at 4 °C.

K^+ nanosensor was prepared by mixing above DNA probe and AuNP@linker at the ratio of 125:1 to incubate at 37 °C for 1 h. Unbonded DNA probe was removed by centrifugation and washed with PBS for three times. Similarly, three controls were prepared with the same procedure by replacing the DNA probe with three control DNA complexes. For quantification of the DNA probe loaded on K^+ nanosensor, the supernatant was diluted to measure the UV-Vis absorbance. The average number of DNA probes in each K^+ nanosensor was calculated with following equation:



Scheme 1. Illustration of (a) the stepwise assembly of K^+ nanosensor and (b) the mechanism for in situ imaging of K^+ in lysosome.

$$N = \frac{C_{\text{added}} \times V_{\text{added}} - C_{\text{supernatant}} \times V_{\text{supernatant}}}{C_{\text{AuNPs}} \times V_{\text{AuNPs}}} \quad (1)$$

where N is the average number of DNA probes in each K^+ nanosensor, C_{added} and V_{added} are the concentration and volume of DNA probe solution added to AuNP@linker, $C_{\text{supernatant}}$ and $V_{\text{supernatant}}$ represent the concentration of unbonded DNA probes in supernatant and the volume of supernatant, and C_{AuNPs} and V_{AuNPs} are the concentration and volume of the initial AuNPs solution. The loss of AuNPs during centrifugation and washing steps was considered negligible.

The resistance of K^+ nanosensor to degradation by FBS was electrophoretically analyzed on a 1% agarose gel at 90 V for 30 min.

2.4. Fluorescent measurements

The feasibility of K^+ detection with the nanosensor was confirmed in the presence or absence of 50 mM K^+ at pH 7.4 or 5.0. Briefly, 2 nM nanosensor (DNA probe loaded AuNPs) was incubated with 50 mM K^+ at pH 7.4 or 5.0 for different times to measure the fluorescence changes, which also led to an optimal incubating time of 5 min. The response curve was obtained by incubating 2 nM nanosensor with different K^+ concentrations (0, 1, 2.5, 5, 10, 20, 30 or 50 mM) at pH 5.0 at 37 °C for 5 min. The selectivity was examined by adding other metal ions in pH 5.0 PBS containing 2 nM nanosensor to incubate at 37 °C for 5 min. All fluorescent spectra from 550 to 750 nm were obtained at λ_{ex} of 530 nm.

2.5. Cell culture

HepG2 and MCF-7 cells were cultured in DMEM complemented with 10% FBS, 100 mg/mL streptomycin, and 100 mg/mL penicillin at 37 °C in a humidified atmosphere containing 5% CO_2 . The cell numbers were counted using a Countess II automated cell counter (Invitrogen, USA). The cells were digested by trypsin and conducted for subculture or latter assays when incubator flask coverage reached 80-90%. MCF-10A cells were cultured in RPMI-1640 medium under identical conditions.

2.6. Cytotoxicity assay

Cytotoxicity assay was conducted by CCK-8 method. Briefly, ~5000 MCF-7 cells were dispersed on 96-well microtiter plates and incubated for 24 h. Then, the cells were treated with nanosensor at different concentrations (0, 0.5, 1.0, 2.5 or 5.0 nM) in fresh medium. After incubation for 24 h, the cells were further incubated with 10% CCK-8 in fresh medium for another 2 h to measure the absorbance at 450 nm for evaluating the cell viability.

2.7. Confocal Laser Scanning Microscopic (CLSM) imaging

For CLSM imaging, $\sim 1 \times 10^4$ cells were seeded on 35 mm four-well confocal dishes and cultured for 24 h. After the medium was removed, the cells were incubated with 2 nM nanosensor in fresh medium at 37 °C for a series of times to collect the CLSM images. For colocalization experiments, the cells were incubated with 2 nM nanosensor in fresh medium for 3 h, washed three times with PBS, and stained with 200 nM Lyso Green for 10 min to collect the CLSM images. Cy3 channel was collected from 545 to 600 nm at an excitation wavelength of 530 nm, Cy5 channel was collected from 645 to 700 nm at an excitation wavelength of 630 nm, FRET signal was collected from 645 to 700 nm at an excitation wavelength of 530 nm, and Lyso Green was measured by collecting the image from 500 to 540 nm at an excitation wavelength of 488 nm.

For stimulus responsive experiments, corresponding stimulus were conducted by incubating the cells with stimulant drug for 1 h, and then the medium added with 2 nM nanosensor for 3 h. After the cells were washed with PBS for three times, they were subjected to CLSM imaging. Specifically, the concentrations of stimulant drugs such as chloroquine,

ATP, amphotericin B and digoxin were 100 μM , 100 μM , 3 μM and 50 nM, respectively.

For examining K^+ depletion triggered lysosomal acidification, cells were incubated in K^+ -free medium with or without 10 μM valinomycin for 1 h, and then the medium added with 2 nM nanosensor for 3 h, which was used for the monitoring of K^+ depletion in lysosome with the FRET signal. Afterward, 1 μM LysoSensor Green DND-189 was added in the medium to incubate for 30 min and collect the CLSM images for pH measurement. The LysoSensor Green DND-189 was excited at 470 nm to collect the image from 490 to 550 nm.

3. Results and discussion

3.1. Design and characterization of nanosensor

To obtain excellent responsive performance, a G4 sequence named PS2.M (Table S1) that exhibited high signal-to-background ratio and good specificity (Deng et al., 2024) was chosen for K^+ response. The assembly of Y-typed DNA probe was verified by polyacrylamide gel electrophoresis (PAGE) (Fig. 1a). Owing to the limited complementary base sequences, G4 strand hardly hybridized with either Gb1 or Gb2 (lanes 4 and 5), but could hybridize with Gb1-Gb2 complex (lanes 6 and 7) to form a Y-typed DNA probe (Scheme 1a), which could thus further hybridize with the DNA linker (lane 8) for probe capture on AuNPs.

The transmission electron microscopic (TEM) image of AuNPs exhibited uniform spherical morphology with an average diameter of 15.48 ± 1.17 nm (Fig. 1b). A two-step conjugation procedure was performed for the preparation of K^+ nanosensor. After DNA linker was assembled on AuNPs via S-Au bond, the characteristic absorption peak of DNA occurred at 260 nm, while the typical absorption peak of AuNPs red-shifted from 519 nm to 523 nm (Fig. 1c), demonstrating the successful preparation of AuNP@linker (Hu et al., 2015; Qian et al., 2014). Compared to AuNP@linker, the obtained K^+ nanosensor showed further.

Increase of DNA characteristic absorption and red-shift of AuNP absorption peak to 525 nm, indicating the successful capture of the DNA probe by AuNP@linker. The Zeta potentials of AuNPs, AuNPs@linker and K^+ nanosensor were tested to be -19.2 mV, -26.1 mV and -28.8 mV, while their hydrodynamic diameters were tested to be 23.2 nm, 34.9 nm and 53.9 nm, respectively (Fig. 1d), further verifying the coupling processes. From the concentration of unbound DNA probe

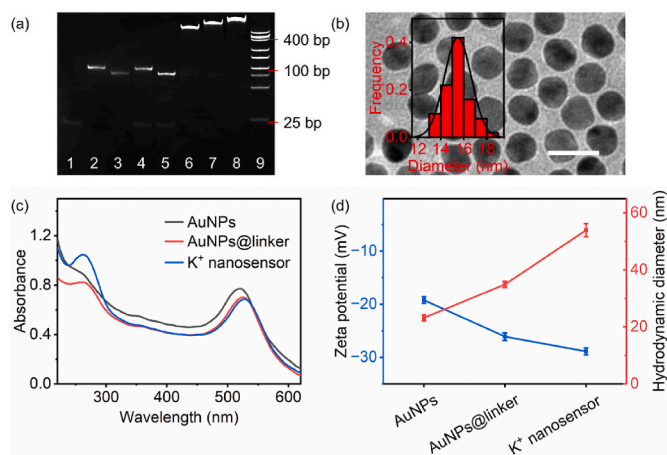


Fig. 1. (a) PAGE analysis for the assembly of DNA probe. Lane 1: G4, lane 2: Gb1, lane 3: Gb2, lane 4: G4 + Gb1, lane 5: G4 + Gb2, lane 6: Gb1 + Gb2, lane 7: G4 + Gb1 + Gb2, lane 8: G4 + Gb1 + Gb2 + linker, lane 9: DNA marker. (b) Representative TEM image of AuNPs. Scale bar: 25 nm. Inset: Size distribution histogram derived from 50 particles. (c) UV-Vis adsorption spectra of AuNPs, AuNPs@linker and K^+ nanosensor. (d) Zeta potentials and hydrodynamic diameters of AuNPs, AuNPs@linker and K^+ nanosensor ($n = 3$).

in the supernatant, which was quantified using a standard curve based on its characteristic absorbance at 260 nm (Fig. S1), the average number of DNA probe captured on single AuNP was measured to be 70 ± 2 .

3.2. Fluorescent response of K^+ nanosensor to K^+

To evaluate the feasibility of pH-activated K^+ sensing, the fluorescent responses of the nanosensor to different solutions were examined at lysosomal pH (5.0) and physiological pH (7.4). The fluorescence spectrum of the nanosensor in pH 7.4 PBS with an excitation wavelength of 530 nm showed a strong emission peak of Cy3 at 565 nm, while the emission of Cy5 was very weak, and the presence of 50 mM K^+ did not change the fluorescence spectrum (Fig. 2a). Moreover, the change of fluorescence spectrum was negligible upon the increasing incubation time in pH 7.4 PBS containing 50 mM K^+ (Fig. S2a), indicating the inhibited folding capability of G4 strand in the DNA probe. Thus, the nanosensor could not respond to K^+ at pH 7.4. After incubating the nanosensor in pH 5.0 PBS for 5 min, the fluorescence spectrum showed both the emission peak of Cy3 at 565 nm and an emission peak at 665 nm (Fig. 2a). The latter could be attributed to the emission of Cy5 due to the acidic-pH activated conformation change of i-motif structure, which led to the release of flexible Cy5-G4-Cy3 from the nanosensor to produce FRET from Cy3 to Cy5. Besides, the presence of Na^+ in the buffer might also induce a minor FRET (Deng et al., 2024). The FRET made the Cy3 fluorescence weaker than that in pH 7.4. After 50 mM K^+ was added in pH 5.0 PBS to incubate the nanosensor, the FRET signal increased, while the Cy3 fluorescence further weakened, indicating the formation of K^+ induced G-quadruplex structure, which further shortened the distance between Cy3 and Cy5 and thus resulted in significant increase of the ratio of FRET signal at 665 nm to Cy3 fluorescence at 565 nm (FRET/Cy3 ratio) from 0.138 ± 0.009 to 0.857 ± 0.011 (Fig. 2b). The K^+ -dependent ratio change provided the feasibility of the nanosensor for pH-activated K^+ detection.

With the increasing incubation time of the nanosensor in pH 5.0 PBS containing 50 mM K^+ , the fluorescence spectrum became more stable (Fig. S2b) and the FRET/Cy3 ratio trended to the maximum value at 5 min (Fig. 2c), which could be used as an optimal incubation time for in vitro detection of K^+ with a linear range of 0 to 10 mM and detection

limit of 0.45 mM at 3σ (Fig. 2d and e). Interestingly, the fluorescent response of K^+ nanosensor was pH sensitive (Fig. S3a), and the FRET/Cy3 ratio showed cliff-like reduction at pH 5.0–5.5 (Fig. S3b), which was just the pH range of lysosomes, providing the sufficient specificity to distinguish lysosomes from other organelles and thus assuring the feasibility of pH-activated in situ imaging of in lysosomes. Moreover, the K^+ nanosensor possessed excellent selectivity. Other metal ions did not interfere with the detection of K^+ (Fig. 2f).

3.3. Cellular uptake and lysosomal localization of K^+ nanosensor

To perform in situ fluorescence imaging of lysosomal K^+ in living cells, the stability of the nanosensor in fetal bovine serum (FBS) and its cell cytotoxicity were firstly evaluated. The PAGE images of DNA probe showed gradual downward shift of the DNA band upon the increasing incubation time with 10% FBS (Fig. 3a), indicating its degradation and instability against FBS. However, the electrophoretic band of the nanosensor remained the same position and intensity upon FBS incubation (Fig. 3b), exhibiting its exceptional stability against FBS. CCK-8 assays showed that over 95% of the MCF-7 cells remained their viability after treated with the nanosensor ranging from 0.5 to 5 nM for 24 h (Fig. S4), indicating the negligible cytotoxicity of the nanosensor.

The cellular uptake of the nanosensor was evaluated with CLSM imaging of different cells, including MCF-7 and HepG2 cells with high expression of PTK7 on the cytomembrane, and PTK7-negative MCF-10A cells as control (Albright et al., 2023; Chen et al., 2025) (Fig. 3c). After incubated with the nanosensor, MCF-10A cells showed.

Negligible fluorescence in all channels, while both HepG2 and MCF-7 cells displayed strong fluorescence in both Cy3 and Cy5 channels, indicating the uptake of the nanosensor into the PTK7-positive cells. As expected, HepG2 and MCF-7 cells showed the fluorescence signal in FRET channel, demonstrating that the uptaken nanosensor entered lysosomes, which activated the conformation change of i-motif structure to release Cy5-G4-Cy3. In the absence of Sgc8c the treatment with control nanoparticles did not produce the fluorescence signals of both Cy3 and Cy5 in MCF-7 cells, indicating the specific recognition of Sgc8c to targeting cells and PTK7-mediated endocytosis, which could be further verified by pretreating MCF-7 cells with free Gb1 to occupy PTK7

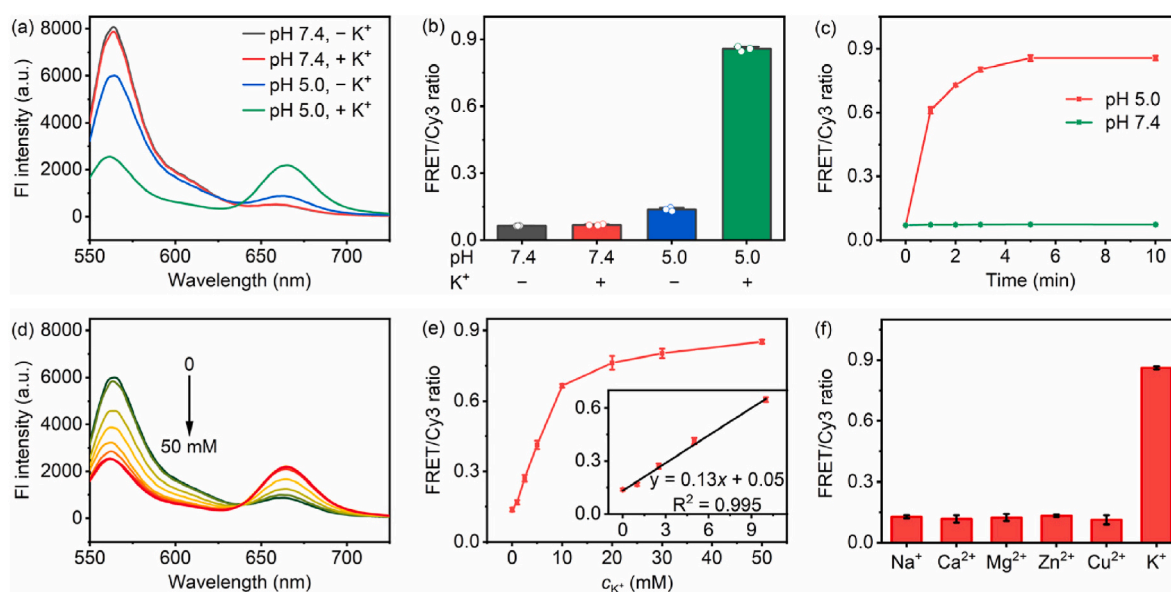


Fig. 2. (a) Fluorescence spectra of 2 nM K^+ nanosensor after incubated for 5 min in pH 5.0 or 7.4 PBS in presence or absence of 50 mM K^+ . λ_{ex} = 530 nm. (b) Corresponding FRET/Cy3 ratios. (c) Plot of FRET/Cy3 ratio of 2 nM nanosensor in pH 7.4 or 5.0 PBS containing 50 mM K^+ vs incubation time (n = 3). (d) Fluorescence spectra of 2 nM nanosensor incubated for 5 min in pH 5.0 PBS containing 0, 1, 2.5, 5, 10, 20, 30 and 50 mM K^+ . λ_{ex} = 530 nm. (e) Plot of FRET/Cy3 ratio vs K^+ concentration. Inset: linear plot in the range of 0–10 mM (n = 3). (f) Selectivity of K^+ nanosensor for 50 mM K^+ at pH 5.0 against 150 mM Na^+ , 5 mM Ca^{2+} , 5 mM Mg^{2+} , 100 μ M Zn^{2+} , and 100 μ M Cu^{2+} (n = 3).

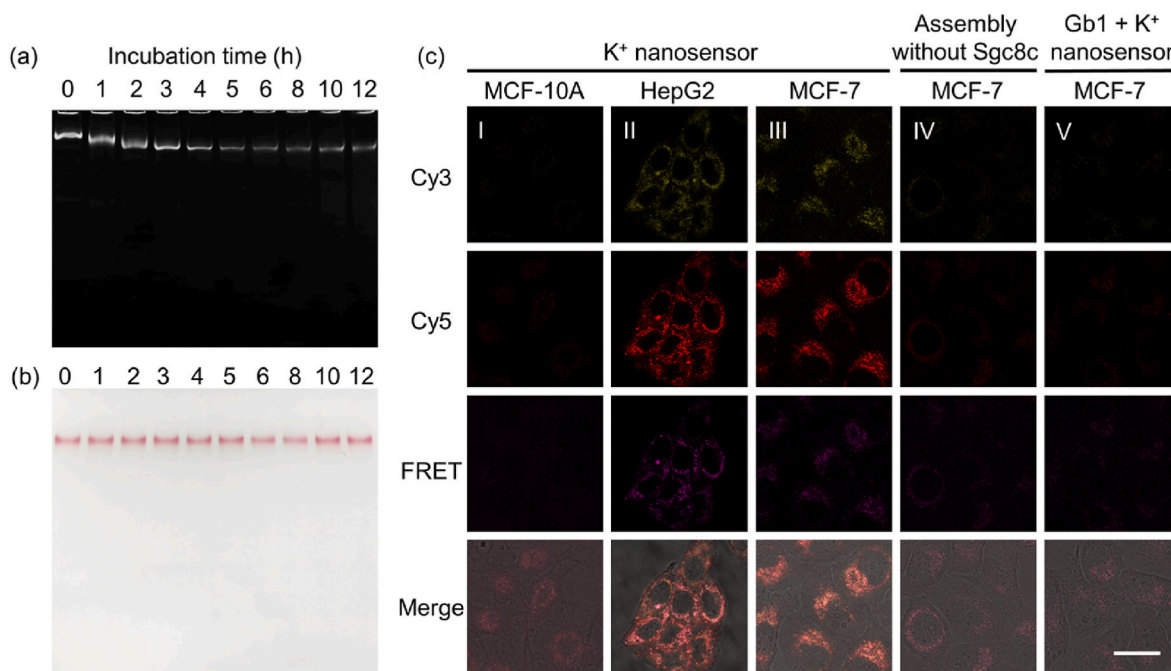


Fig. 3. (a) PAGE image of DNA probe and (b) agarose gel electrophoresis image of nanosensor after incubation with 10% FBS for different times. (c) Representative CLSM images of (I) MCF-10A cells, (II) HepG2 cells and (III) MCF-7 cells after treated with K⁺ nanosensor for 3 h, (IV) MCF-7 cells after treated with the assembly without Sgc8c, and (V) MCF-7 cells after treated with 200 nM Gb1 for 1 h and then K⁺ nanosensor for 3 h. Scale bar: 25 μm . Cy3 channel was collected in 545-600 nm with λ_{ex} of 530 nm. Cy5 channel was collected in 645-700 nm with λ_{ex} of 630 nm. FRET channel was collected in 645-700 nm with λ_{ex} of 530 nm.

binding sites and then incubating the cells with the nanosensor, which resulted in the disappearance of fluorescence signals in all channels.

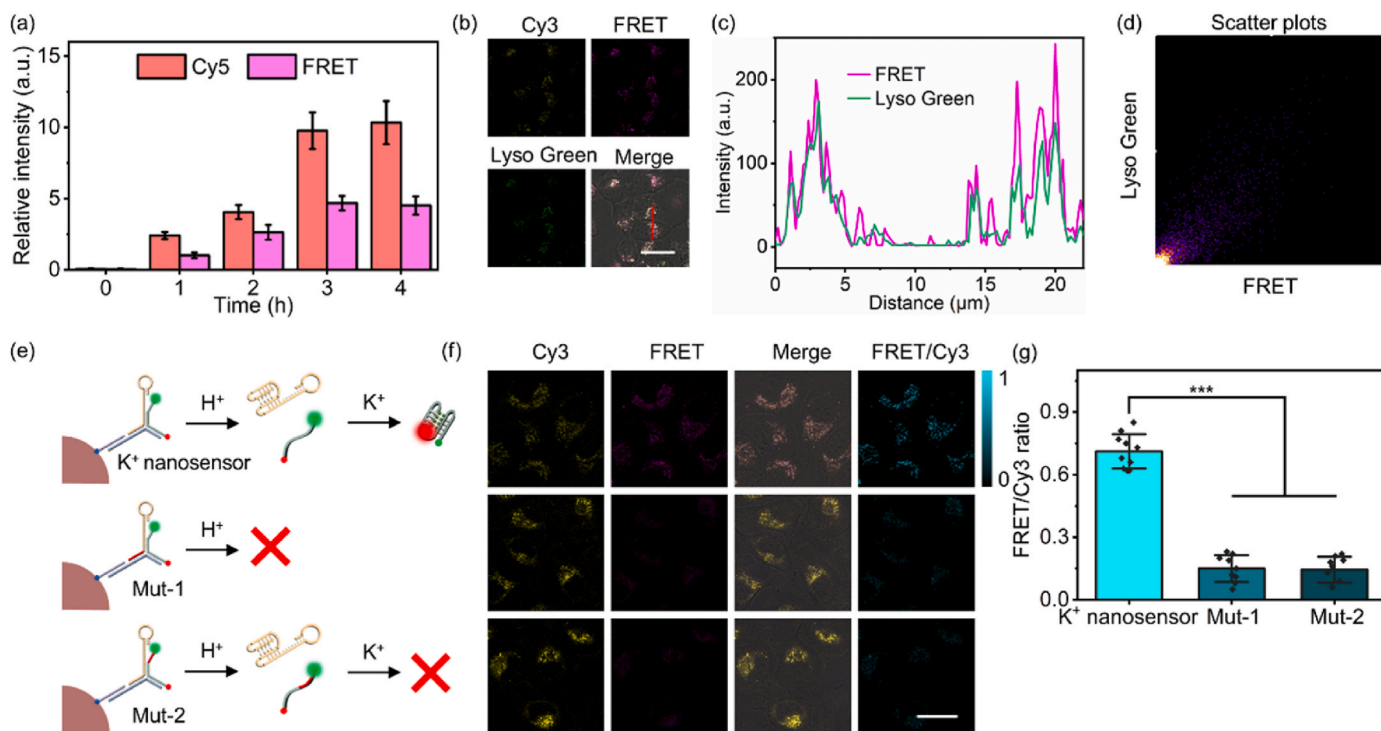


Fig. 4. (a) Histogram of fluorescence intensity of Cy5 and FRET channels from CLSM images of MCF-7 cells incubated with 2 nM nanosensor for different times. ($n = 10$). (b) Representative CLSM images of MCF-7 cells incubated with 2 nM nanosensor for 3 h and then stained with 200 nM Lyso Green for 10 min. Lyso Green channel was collected in 500-540 nm with λ_{ex} of 488 nm. The merged image displays the FRET and Lyso Green channels. Scale bar: 25 μm . (c) Fluorescence intensity profiles along the red straight line in (b). (d) Scatter plots for FRET and Lyso Green channels. (e) Schematic diagrams and fluorescence response mechanism of K⁺ nanosensor, Mut-1 or Mut-2. The red parts in Mut-1 and Mut-2 represent mutant bases. (f) Representative CLSM images of MCF-7 cells incubated with 2 nM nanosensor, Mut-1 or Mut-2 for 3 h. Scale bar: 25 μm . (g) Histogram of FRET/Cy3 ratio in (f) ($n = 10$). Two-tailed Student's *t*-test was used to assess statistical significance, *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The incubation time for cellular internalization of the nanosensor was optimized with MCF-7 cells. With the increasing incubation time, the fluorescent intensity in all channels gradually increased (Fig. S5), and the fluorescence intensity of Cy5 channel plateaued at 3 h (Fig. 4a), indicating saturated internalization of the nanosensor. Meanwhile, the nanosensor showed obvious FRET response to lysosomal K^+ (Fig. S5), and the FRET signal reached the maximum value at 3 h (Fig. 4a). Thus 3-h incubation was used in following experiments. After the nanosensor-treated cells were further stained with Lyso Green, a commercial lysosome-targeting fluorescent dye, for 10 min, colocalization experiment was performed to assess the location of nanosensor. The FRET signal occurred at the identical region to Lyso Green fluorescence (Fig. 4b and c), and the Pearson's correlation coefficient was 0.87 (Fig. 4d), indicating significant colocalization of nanosensor and lysosomes, and thus the potential application of the nanosensor for in situ imaging of K^+ in lysosomes.

3.4. pH-activated lysosomal K^+ sensing in living cells

To reveal the pH-activated K^+ -responsive mechanism, two control Y-typed DNA complexes were prepared by replacing Gb1 with a sequence without the i-motif portion (mGb1), Gb2 with mGb2, and Cy5-G4-Cy3

with another dual fluorophores-labelled DNA strand (Cy5-mG4-Cy3) for the fabrication of Mut-1 and Mut-2, respectively (Fig. 4e). The same structure of the formed DNA complexes as the DNA probe was demonstrated with PAGE analysis (Fig. S6). The Mut-1 or Mut-2 showed the similar fluorescence spectra as well as their negligible changes under different incubation conditions (Figs. S7a and S8a). Compared to the K^+ nanosensor (Fig. 2a and b), the change of FRET/Cy3 ratios were also negligible upon incubation in pH 7.4 or 5.0 in the presence or absence of 50 mM K^+ (Figs. S7b and S8b), indicating the necessity of both the i-motif structure for pH-activated release of Cy5-G4-Cy3 and the G4 sequence for K^+ -sensitive G4 folding to produce the pH-controllable K^+ response. Therefore, after MCF-7 cells were incubated with Mut-1 or Mut-2, the FRET signal was also markedly weaker compared to that treated with K^+ nanosensor (Fig. 4f and g), further demonstrating the K^+ sensing mechanism in lysosomes.

To demonstrate the practical application of the K^+ nanosensor in living cells, external stimuli with chloroquine (CQ), ATP, amphotericin B or digoxin were applied to modulate lysosomal pH or intracellular K^+ concentration. CQ can raise lysosomal pH to above 6.0 in living cells (Yoshimori et al., 1991; Zhao et al., 2020). ATP acts as an agonist for ATP-sensitive K^+ channels (Chen et al., 2021), and amphotericin B and digoxin act as potent inducers of cellular K^+ efflux (Ren et al., 2021;

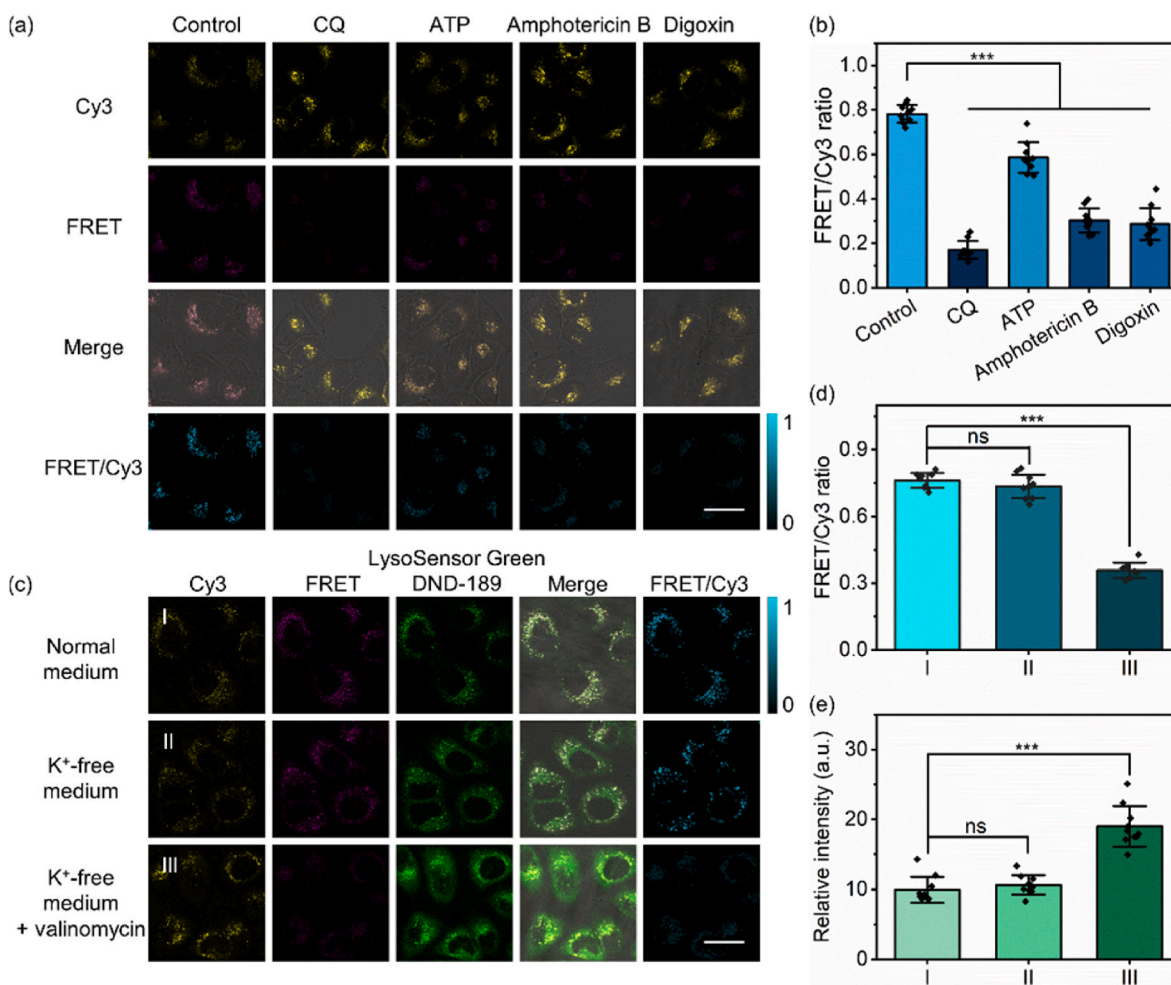


Fig. 5. (a) Representative CLSM images of MCF-7 cells treated with 100 μ M CQ, 100 μ M ATP, 3 μ M amphotericin B, or 50 nM Digoxin for 1 h, and then the mixture added with 2 nM nanosensor for 3 h. Scale bar: 25 μ m. (b) Histogram of FRET/Cy3 ratio in (a) ($n = 10$). (c) Representative CLSM images of MCF-7 cells incubated continuously with (I) normal medium, (II) K^+ -free medium or (III) K^+ -free medium containing 10 μ M valinomycin for 1 h, the mixture added with 2 nM nanosensor for 3 h, and then stained with the mixture further added with 1 μ M LysoSensor Green DND-189 for 30 min. LysoSensor Green DND-189 channel was collected in 490–550 nm with λ_{ex} of 470 nm. Scale bar: 25 μ m. (d) FRET/Cy3 ratios and (e) fluorescence intensity of LysoSensor Green DND-189 channel in (c) ($n = 10$). Two-tailed Student's t -test was used to assess statistical significance. ns, not significant; *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Yang et al., 2016; Zhou et al., 2011b; Zhou et al., 2011a), which reduce the K⁺ concentration in lysosomes. After MCF-7 cells were treated with CQ for 1 h and then its mixture with the nanosensor for 3 h, they showed very weak FRET signal along with a FRET/Cy3 ratio of 0.170 ± 0.040, which was obtained from Adobe Photoshop software and was much smaller than 0.782 ± 0.041 of untreated cells (Fig. 5a and b). The low FRET/Cy3 ratio could be attributed to the raised lysosomal pH, as observed in vitro experiment (Fig. S3). The treatments with ATP, amphotericin B or digoxin also resulted in the reduced FRET signals (Fig. 5a), and the FRET/Cy3 ratios were 0.585 ± 0.069, 0.302 ± 0.054 and 0.287 ± 0.072, respectively, demonstrating the reduction of K⁺ concentration to varying extents. Thus the proposed nanosensor could be effectively used for in situ precise monitoring of K⁺ in lysosomes of living cells.

Lysosomal ionic homeostasis is generally maintained by a sophisticated network, in which K⁺ serves as a critical counterion to preserve the acidity (Steinberg et al., 2009). To dissect the impact of K⁺ on lysosomal pH, MCF-7 cells were subjected to different culturing conditions for lysosomal K⁺ and pH measurements with the K⁺ nanosensor and pH indicator LysoSensor Green DND-189, respectively. The MCF-7 cells pre-cultured in K⁺-free or normal medium for 1 h showed similar FRET/Cy3 ratios (0.762 ± 0.033 and 0.736 ± 0.053) and fluorescence intensity of LysoSensor Green DND-189 (9.94 ± 1.86 and 10.64 ± 1.38) (Fig. 5c–e), indicating that the lysosomal K⁺ and pH were preserved despite the absence of extracellular K⁺, and the cells possessed tolerance and self-regulatory capacity during short-term K⁺ deprivation. When the cells were pre-treated in K⁺-free medium containing 10 μM valinomycin, a K⁺ ionophore to balance intracellular and extracellular K⁺ concentrations (Yang et al., 2025), they displayed dramatic changes in both K⁺ and pH signals (Fig. 5c). The FRET/Cy3 ratio decreased to 0.357 ± 0.035 (Fig. 5d), while the LysoSensor Green DND-189 fluorescence showed approximately 1.9-fold increase (Fig. 5e), indicating the decreasing lysosomal K⁺ concentration due to valinomycin-forced efflux of intracellular K⁺ and marked acidification of lysosomes via the induced influx of H⁺, which demonstrated the regulation of K⁺ on lysosomal pH.

To provide a comprehensive overview of the analytical performance and functional advantages, the pH-activated K⁺ nanosensor was compared with previously reported G4-based K⁺ fluorescent probes for cellular applications (Table 1), focusing on signal readout mode, subcellular targeting, and the ability to suppress nonspecific signals during transport. Obviously, the pH-activated K⁺ nanosensor effectively suppressed nonspecific signals during delivery, ensuring that the signals originate specifically from lysosomal K⁺.

4. Conclusion

In conclusion, a pH-activated K⁺ nanosensor for in situ precise imaging of lysosomal K⁺ in living cells has been developed by capturing a Y-typed DNA probe on DNA linker modified AuNP. The DNA probe contains a pH-sensitive i-motif structure for activating its conformational change at acidic lysosomal pH, a dual-fluorophores labelled G4 strand for K⁺ induced G4 folding, and a Sgc8c sequence for target-cell-specific internalization of the nanosensor. In vitro tests have confirmed the pH-activated conformational change of i-motif structure to release the G4 strand from the DNA probe and thus produce specific response to K⁺, which leads to a selective method for pH-activated K⁺ detection by using G4 folding-enhanced ratio of FRET to Cy3 fluorescence as the response signal. The nanosensor can be conveniently delivered into lysosomes via PTK7-mediated endocytosis without generation of nonspecific signals, which results in excellent performance for lysosomal pH-activated fluorescent imaging of K⁺. Its practical application is also demonstrated by monitoring external drug-modulated changes of lysosomal pH and intracellular K⁺ level and dissecting the impact of K⁺ on lysosomal pH. The proposed nanosensor provides a tool for a glimpse of lysosomal ionic homeostasis and can be expanded for subcellular imaging.

Table 1
Comparison of previously reported G4-based K⁺ fluorescent probes for cellular imaging.

Number	Signal readout mode	Subcellular targeting	Ability to suppress nonspecific signals	Reference
1	Signal off	Lysosome	No	Chen et al. (2021)
2	Signal off	Lysosome	No	Li et al. (2021)
3	Ratiometric fluorescence	None	Yes	Cui et al. (2020)
4	Ratiometric fluorescence	Mitochondrion	No	Zhang et al. (2024)
5	Signal on	Extracellular	No	Deng et al. (2024)
6	Ratiometric fluorescence	Lysosome	Yes	This work.

CRediT authorship contribution statement

Guangming Li: Writing – original draft, Methodology, Data curation, Conceptualization. **Ying An:** Writing – review & editing, Methodology. **Jiahui Sun:** Writing – review & editing, Methodology. **Mengxu Sun:** Validation, Software, Methodology, Conceptualization. **Chaoyi Yang:** Software, Methodology, Investigation. **Yi Ren:** Methodology, Conceptualization. **Shijiong Wei:** Methodology, Conceptualization. **Huangxian Ju:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. ASupplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2026.119145>.

Data availability

Data will be made available on request.

References

Albright, S., Cacace, M., Tivon, Y., Deiters, A., 2023. Cell surface labeling and detection of protein tyrosine kinase 7 via covalent aptamers. *J. Am. Chem. Soc.* 145, 16458–16463. <https://doi.org/10.1021/jacs.3c02752>.
Carmona-Gutierrez, D., Hughes, A.L., Madeo, F., Ruckenstein, C., 2016. The crucial impact of lysosomes in aging and longevity. *Ageing Res. Rev.* 32, 2–12. <https://doi.org/10.1016/j.arr.2016.04.009>.
Casey, J.R., Grinstein, S., Orlowski, J., 2010. Sensors and regulators of intracellular pH. *Nat. Rev. Mol. Cell Biol.* 11, 50–61. <https://doi.org/10.1038/nrm2820>.
Chen, B., Wang, Y.T., Ma, W.J., Cheng, H., Sun, H.H., Wang, H.Z., Huang, J., He, X.X., Wang, K.M., 2020. A mimosa-inspired cell-surface-anchored ratiometric DNA nanosensor for high-resolution and sensitive response of target tumor extracellular pH. *Anal. Chem.* 92, 15104–15111. <https://doi.org/10.1021/acs.analchem.0c03250>.
Chen, F., Lu, Q.J., Huang, L.N., Liu, B.W., Liu, M.L., Zhang, Y.Y., Liu, J.W., 2021. DNA triplex and quadruplex assembled nanosensors for correlating K⁺ and pH in lysosomes. *Angew. Chem. Int. Ed.* 60, 5453–5458. <https://doi.org/10.1002/anie.202013302>.
Chen, Z.P., Zeng, W.J., Lei, Y.M., Liang, W.B., Yang, X., Yuan, R., Yang, C.Y., Zhuo, Y., 2025. Engineering of a multi-modular DNA nanodevice for spatioselective imaging

- and evaluation of NK cell-mediated cancer immunotherapy. *Angew. Chem. Int. Ed.* 64, e202414064. <https://doi.org/10.1002/anie.202414064>.
- Cheng, M.P., Qiu, D.H., Tamon, L., Istvankova, E., Viskova, P., Amrane, S., Guedin, A., Chen, J.L., Lacroix, L., Ju, H.X., Trantirek, L., Sahakyan, A.B., Zhou, J., Mergny, J.L., 2021. Thermal and pH stabilities of i-DNA: confronting in vitro experiments with models and in-cell NMR data. *Angew. Chem. Int. Ed.* 60, 10286–10294. <https://doi.org/10.1002/anie.202016801>.
- Cui, M.R., Chen, L.X., Li, X.L., Xu, J.J., Chen, H.Y., 2020. NIR remote-controlled "lock-unlock" nanosystem for imaging potassium ions in living cells. *Anal. Chem.* 92, 4558–4565. <https://doi.org/10.1021/acs.analchem.9b05820>.
- Deng, Z.W., Ding, J.C., Bu, J.Q., Li, J.C., Liu, H., Gao, P.R., Gong, Z., Qin, X.X., Yang, Y.J., Zhong, S.A., 2024. Fluorophore label-free light-up near infrared deoxyribonucleic acid nanosensor for monitoring extracellular potassium levels. *Anal. Chem.* 96, 4023–4030. <https://doi.org/10.1021/acs.analchem.3c03881>.
- Dong, P.T., Li, Z.J., Wang, Q.W., Shi, L.L., Li, T., 2025. Engineering logic-gated i-motif/G-quadruplex (iG4) hybrid structures for DNA nanotweezer-based aptasensing. *Anal. Sens.* 5, e202400064. <https://doi.org/10.1002/ans.202400064>.
- Du, Y., Peng, P., Li, T., 2019. DNA logic operations in living cells utilizing lysosome-recognizing framework nucleic acid nanodevices for subcellular imaging. *ACS Nano* 13, 5778–5784. <https://doi.org/10.1021/acsnano.9b01324>.
- Emmert, S., Quagnali, G., Thallmair, S., Rivera-Fuentes, P., 2023. A locally activatable sensor for robust quantification of organellar glutathione. *Nat. Chem.* 15, 1415–1421. <https://doi.org/10.1038/s41557-023-01249-3>.
- Grabar, K.C., Freeman, R.G., Hommer, M.B., Natan, M.J., 1995. Preparation and characterization of Au colloid monolayers. *Anal. Chem.* 67, 735–743. <https://doi.org/10.1021/ac00100a008>.
- Haiss, W., Thanh, N.T.K., Aveyard, J., Fernig, D.G., 2007. Determination of size and concentration of gold nanoparticles from UV-vis spectra. *Anal. Chem.* 79, 4215–4221. <https://doi.org/10.1021/ac0702084>.
- He, A., Wan, L.Q., Zhang, Y.C., Yan, Z.Z., Guo, P., Han, D., Tan, W.H., 2024. Structure-based investigation of a dna aptamer targeting PTK7 reveals an intricate 3D fold guiding functional optimization. *Proc. Natl. Acad. Sci. U. S. A.* 121, e2404060121. <https://doi.org/10.1073/pnas.2404060121>.
- Hu, Y.L., Zhang, L., Zhang, Y., Wang, B., Wang, Y.W., Fan, Q.L., Huang, W., Wang, L.H., 2015. Plasmonic nanobiosensor based on hairpin DNA for detection of trace oligonucleotides biomarker in cancers. *ACS Appl. Mater. Interfaces* 7, 2459–2466. <https://doi.org/10.1021/am507218g>.
- Lee, C., Guo, J., Zeng, W., Kim, S., She, J., Cang, C., Ren, D., Jiang, Y., 2017. The lysosomal potassium channel TMEM175 adopts a novel tetrameric architecture. *Nature* 547, 472–475. <https://doi.org/10.1038/nature23269>.
- Li, L.L., Lv, W.Y., Wang, Y., Li, Y.F., Li, C.M., Huang, C.Z., 2021. DNA logic nanodevices for real-time monitoring of ATP in lysosomes. *Anal. Chem.* 93, 15331–15339. <https://doi.org/10.1021/acs.analchem.1c02829>.
- Li, T., Zhang, L.B., Ai, J., Dong, S.J., Wang, E.K., 2011. Ion-tuned DNA/Ag Fluorescent nanoclusters as versatile logic device. *ACS Nano* 5, 6334–6338. <https://doi.org/10.1021/nn201407h>.
- Li, X.Q., Liu, X.N., Jia, Y.L., Luo, X.L., Chen, H.Y., Xu, J.J., 2021. Dual recognition DNA triangular prism nanoprobe: toward the relationship between K⁺ and pH in lysosomes. *Anal. Chem.* 93, 14892–14899. <https://doi.org/10.1021/acs.analchem.1c04056>.
- Liu, B.W., Liu, J.W., 2017. Freezing directed construction of bio/nano interfaces: reagentless conjugation, denser spherical nucleic acids, and better nanoflares. *J. Am. Chem. Soc.* 139, 9471–9474. <https://doi.org/10.1021/jacs.7b04885>.
- Mergny, J.L., Sen, D., 2019. DNA quadruple helices in nanotechnology. *Chem. Rev.* 119, 6290–6325. <https://doi.org/10.1021/acs.chemrev.8b00629>.
- Mondal, B., Dutta, T., Padhy, A., Das, S., Gupta, S.S., 2022. Lysosome-targeting strategy using polypeptides and chimeric molecules. *ACS Omega* 7, 5–16. <https://doi.org/10.1021/acsomega.1c04771>.
- Peng, P., Du, Y., Zheng, J., Wang, H.H., Li, T., 2019. Reconfigurable bioinspired framework nucleic acid nanoplatform dynamically manipulated in living cells for subcellular imaging. *Angew. Chem. Int. Ed.* 131, 1662–1667. <https://doi.org/10.1002/anie.201811117>.
- Qian, R.C., Ding, L., Yan, L.W., Lin, M.F., Ju, H.X., 2014. Smart vesicle kit for in situ monitoring of intracellular telomerase activity using a telomerase-responsive probe. *Anal. Chem.* 86, 8642–8648. <https://doi.org/10.1021/ac502538w>.
- Ren, Y., Wu, S.J., Burdette, J.E., Cheng, X., Kinghorn, A.D., 2021. Structural insights into the interactions of digoxin and Na⁺/K⁺-ATPase and other targets for the inhibition of cancer cell proliferation. *Molecules* 26, 3672. <https://doi.org/10.3390/molecules26123672>.
- Settembre, C., Perera, R.M., 2024. Lysosomes as coordinators of cellular catabolism, metabolic signalling and organ physiology. *Nat. Rev. Mol. Cell Biol.* 25, 223–245. <https://doi.org/10.1038/s41580-023-00676-x>.
- Shao, X.L., Zhu, L.J., Zhang, Y.Z., Du, Z.H., Sun, C.Y., Xu, W.T., 2020. Integration of ion-tuned oligonucleotide structural motifs and DNA-templated copper nanoclusters as a manipulable logic device. *Sens. Actuators, B* 325, 128769. <https://doi.org/10.1016/j.snb.2020.128769>.
- Steinberg, B.E., Huynh, K.K., Brodovitch, A., Jabs, S., Stauber, T., Jentsch, T.J., Grinstein, S., 2009. A cation counterflux supports lysosomal acidification. *J. Cell Biol.* 189, 1171–1186. <https://doi.org/10.1083/jcb.200911083>.
- Su, M.H., Li, Y., Li, H.X., Wang, X.X., Ying, D.X., Zhang, L.Z., Yang, C., Jiang, M.Y., Xu, L.J., Wang, X., Sun, Y., Xu, H.Y., Zhang, Z.W., Wang, X.J., Fu, T., Xie, S.T., He, J.X., Liu, X.S., Tan, W.H., 2025. An aptamer-drug conjugate for promising cancer therapy with comprehensive evaluation from rodents to non-human primates. *Signal Transduct. Target. Ther.* 10, 316. <https://doi.org/10.1038/s41392-025-02399-1>.
- Wu, B., Zhao, J., Yu, F.Z., Li, L.L., Zhao, Y.L., 2025. Spatiotemporal imaging of noncoding RNAs in mitochondria via an organelle-specific DNA assembly strategy. *Nano Lett.* 25, 1948–1955. <https://doi.org/10.1021/acs.nanolett.4c05559>.
- Wu, Y., Xu, M., Wang, P.P., Syeda, A.K.R., Huang, P., Dong, X.P., 2022. Lysosomal potassium channels. *Cell Calcium* 102, 102536. <https://doi.org/10.1016/j.ceca.2022.102536>.
- Yang, Y.J., Huang, J., Yang, X.H., Quan, K., Xie, N.L., Ou, M., Tang, J.L., Wang, K.M., 2016. Aptamer-based FRET nanoflares for imaging potassium ions in living cells. *Chem. Commun.* 52, 11386–11389. <https://doi.org/10.1039/c6cc05379c>.
- Yang, Z.L., Shao, X.L., Wu, Y.T., Roy, A., Garcia, E., Farrell, A., Pradhan, S., Guo, W.J., Gan, H., Korkmaz, Z., Adams, E., Lu, Y., 2025. Decoding potassium homeostasis in cancer metastasis and drug resistance: insights from a highly selective DNzyme-based intracellular K⁺ sensor. *J. Am. Chem. Soc.* 147, 18074–18087. <https://doi.org/10.1021/jacs.5c03781>.
- Yi, D.Y., Li, L.L., Li, M.Y., 2024. Subcellular compartment-specific amplified imaging of metal ions via ribosomal RNA-regulated DNzyme sensors. *Angew. Chem. Int. Ed.* 64, e202412387. <https://doi.org/10.1002/anie.202412387>.
- Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M., Tashiro, Y., 1991. Bafilomycin A1, a specific inhibitor of vacuolar-type H⁺-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J. Biol. Chem.* 266, 17707–17712. [https://doi.org/10.1016/S0021-9258\(19\)47429-2](https://doi.org/10.1016/S0021-9258(19)47429-2).
- Zhang, N., Cheng, H.J., Liu, Y.L., 2024. Mitochondria-targeted ratiometric KLA-tweezers for fluorescence imaging of potassium ions. *Anal. Chem.* 96, 15355–15363. <https://doi.org/10.1021/acs.analchem.4c03040>.
- Zhao, J., Li, Y.H., Yu, M.M., Gu, Z.J., Li, L.L., Zhao, Y.L., 2020. Time-resolved activation of pH sensing and imaging in vivo by a remotely controllable DNA nanomachine. *Nano Lett.* 20, 874–880. <https://doi.org/10.1021/acs.nanolett.9b03471>.
- Zhou, X.F., Su, F.Y., Gao, W.M., Tian, Y.Q., Youngbull, C., Johnson, R.H., Meldrum, D.R., 2011b. Triazacryptand-based fluorescent sensors for extracellular and intracellular K⁺ sensing. *Biomaterials* 32, 8574–8583. <https://doi.org/10.1016/j.biomaterials.2011.07.081>.
- Zhou, X.F., Su, F.Y., Tian, Y.Q., Youngbull, C., Johnson, R.H., Meldrum, D.R., 2011a. A new highly selective fluorescent K⁺ sensor. *J. Am. Chem. Soc.* 133, 18530–18533. <https://doi.org/10.1021/ja207345s>.