Electronic Supplementary Information (ESI)

I-Motif-Based Nano-Flares for Sensing pH Changes in Live Cells

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Experimental Section

1. Buffer solution

Phosphate Buffer (**PB**): 100 mM Na_4HPO_4, 100 mM NaH_2PO_4. The pH depends on the ratio.

Phosphate Buffered Saline (**PBS**): 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4.

Washing Buffer (**WB**): 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 5 mM $MgCl_2$, 250 mM glucose, pH 7.4.

Binding Buffer (**BB**):10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 5 mM $MgCl_2$, 250 mM glucose, 0.1 mg/mL tRNA, 1 mg/mL BSA, pH 7.4.

2. DNA sequences

All the DNA sequences used in this work were synthesized by Sangon Biotechnology Co.Ltd. (Shanghai, China). All the sequences are listed as below (from 5' to 3'):

(1) I-motif: SH-T_{10}CCCTAACCCTAACCCTAACCC

(2) Flare (a): GTTAGGGTTAGGGTTAGTT-RhG (match with i-motif)

(3) Flare (b): GTTAGTGTTAGGGTTAGTT- RhG (1-base mismatch)

(4) Flare (c): GTTAGTGTTAGTGTTAGTT- RhG (2-base mismatch)

(5) Helper: SH- TTTTTTTTTT

(6) Control: BHQ1-CCCTAACCCTAACCCTAACCC

3. Instrumentation

The steady-state UV-vis absorption, fluorescence, circular dichroism (CD) spectra, and

confocal microscope were measured using a Biospec-nano UV-vis spectrophotometer (Japan), a Hitachi F-7000 fluorescence spectrometer (Japan), a Biologic MOS-500 CD spectrophotometer (France), and an Olympus laser-scanning confocal microscope (Japan).

4. Preparation of I-Motif-Based Nano-Flares

Citrate-stabilized AuNPs (~13 nm) were prepared using published procedures.[1] Thiolated i-motif sequences and flares (1:1.2) were mixed in PBS, heated to 95 ℃, slowly cooled to room temperature, and stored in the dark for at least 12 hours to allow hybridization. These pre-formed DNA duplexes and "helper" DNA were then added to AuNPs and shake overnight. After 16 hours, sodium dodecylsulfate (SDS) solution (10%) was added to the mixture to achieve a 0.1% SDS concentration, PB (0.1 M; pH=7.4) was added to the mixture to achieve a 0.01 M phosphate concentration, and aliquots of sodium chloride 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. The solution containing the functionalized particles was centrifuged (13000 rpm, 30 min) and resuspended in PBS three times to produce the purified AuNPs used in all subsequent experiments (Data seen in **Figure S3**). The concentration of the particles was determined by measuring their extinction at 524 nm ($\varepsilon=2.7\times10^8$ L $mol^{-1}cm^{-1}$).

5. Determination of DNA amount on AuNP

DNA sequences loaded on AuNPs were quantitated according to the published protocol.[2] Mercaptoethanol (20 mM) was added to the nano-flares solution, which was incubated overnight with shaking at room temperature. 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 (**Figure S4**) that was prepared with known concentrations of RhG labeled flares with same conditions.

6. Fluorescence experiments

Nano-flare probes were diluted to a concentration of 5 nM in PB and treated with different pH buffer (pH: 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5). The fluorescence spectra were recorded on an F-7000 fluorescence spectrometer exciting at 505 nm and measuring emission from 515 to 650 nm in 1 nm increments. Both excitation and emission slits were set at 5 nm. The time-scanning fluorescence spectra were excited at 505 nm and recorded at 535 nm.

7. Nuclease assay

Nano-flare probes were diluted to a concentration of 2.5 nM in PBS (pH 7.0), 0.25 mM $MgCl_2$ and 50 mg/L Bovine Serum Albumin (Sigma Aldrich). DNase I (TaKaRa) was added immediately before reading (concentration, 0.38 mg/L). Then, time-scanning fluorescence spectra were recorded on F-7000 fluorescence spectrometer with excitation at 505 nm and emission at 535 nm. Control probes (BHQ1 instead of AuNP) were tested in a same manner at a concentration of 150 nM. The approximate rates of degradation under these experimental conditions were determined from the slop of the linear region of the degradation curves (**Figure S6**).

8. Cell culture, particle incubation and imaging

Hela cell lines were established in our lab and were grown in RPMI 1640 cell medium with 10% inactivated fetal bovine serum (Hyclone, USA) at 37 ℃ in 5% CO_2. Cells were seeded on 35-mm glass bottom dishes (MatTek, MA) and grown for 1-2 days prior to treatment. The cell density was determined using a hemocytometer, and this was performed prior to any experiment. On the day of treatment, the cells were approximately 50% confluent.

For lysosome localization experiment, cells were incubated with fresh media containing nano-flares (particle concentration, 1 nM) for 2 hours. And then, the cells were washed to remove excess nano-flares by WB and incubated in 2 mL BB containing 1 μL Lyso Tracker blue (Invitrogen, USA) for 15 min. Subsequently, the cells were washed three times and resuspensed in 2 mL BB and imaged on a laser scanning confocal microscope.

For the chloroquine (CQ) experiment, cells were treated with CQ for 40 min and then washed three times by WB. The media was replaced with fresh media containing nano-flares (particle concentration, 1 nM) for 2 hours incubation. Subsequently, the cells were washed to remove excess nano-flares and resuspensed in 2 mL BB and imaged on a laser scanning confocal microscope.

References

[1] D. S. Seferos, D. A. Giljohann, H. D. Hill, A. E. Prigodich and C. A. Mirkin, J. Am. Chem. Soc., 2007, 129, 15477.

[2] L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian, G. Viswanadham, Anal. Chem., 2000, 72, 5535.

Supporting Figures

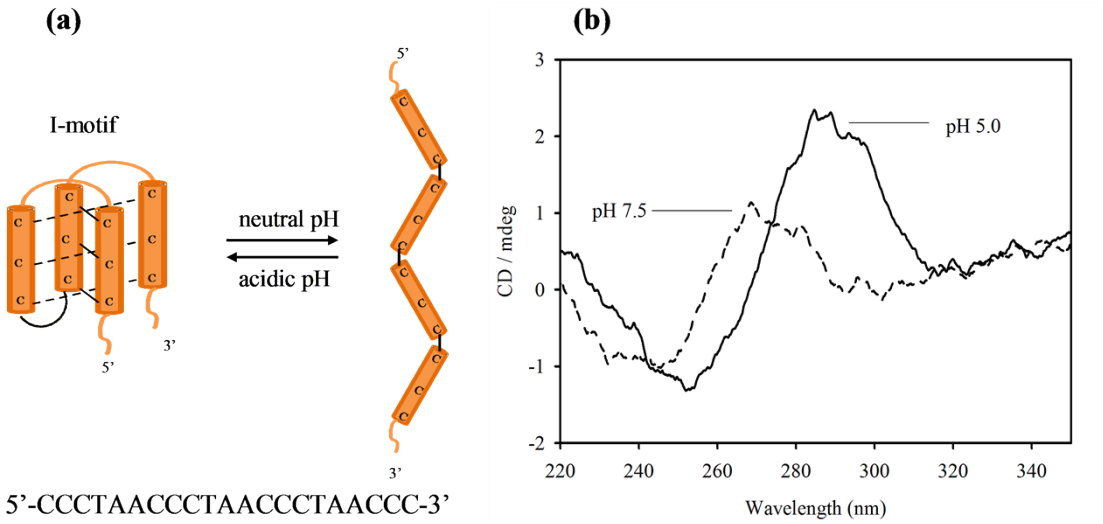

Figure S1. (a) Schematic illustration of the reversible pH-induced conformational change of i-motif and its sequence used in this study; (b) CD spectra of the i-motif sequence in pH 5 and pH 7.5 solutions.

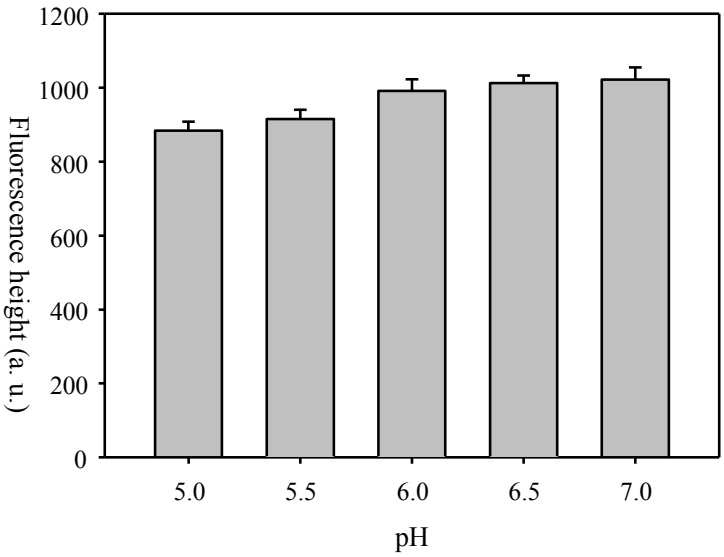

Figure S2. Study the stability of Rhodamine Green (RhG) at various pH solutions.

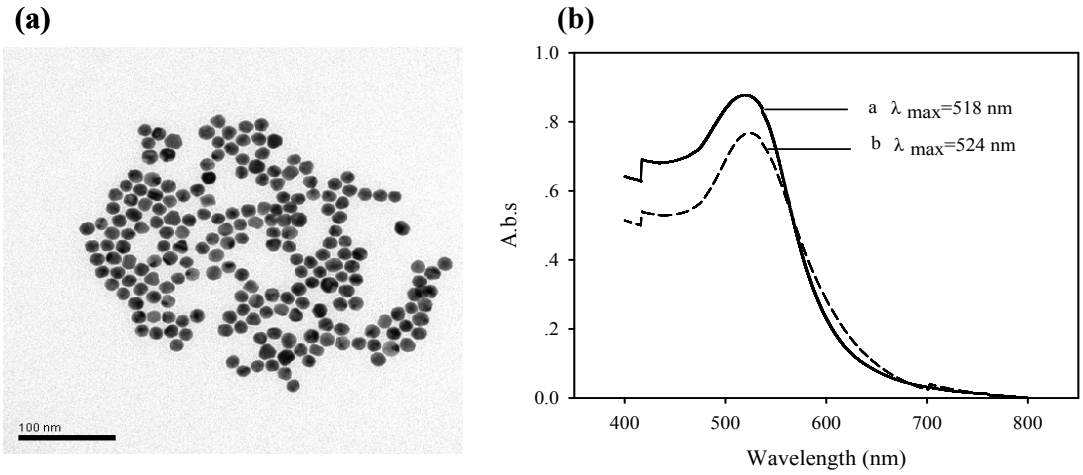

Figure S3. (a) TEM micrograph of AuNPs; (b) Absorption spectra of AuNPs (real line) and DNA functionalized AuNPs (dashed line).

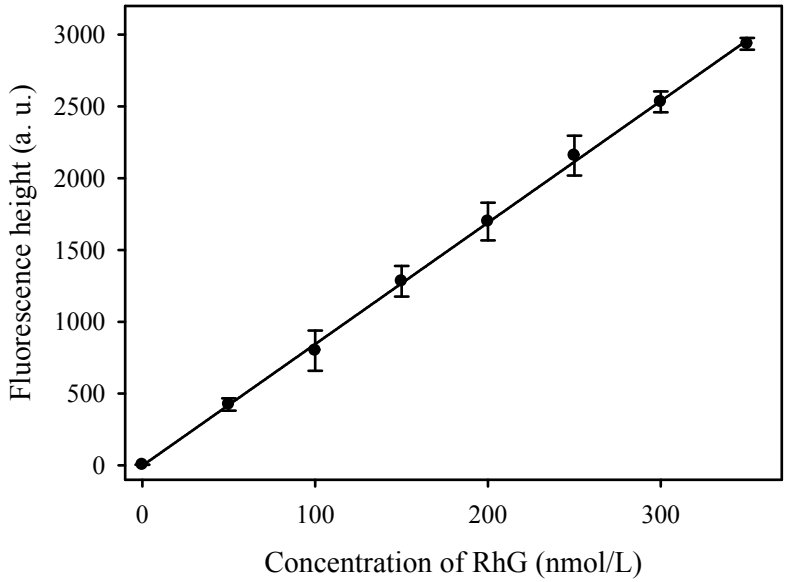

Figure S4. Standard linear calibration curve of fluorescence signal against the concentration of RhG labeled flares. The fluorescence of released DNA from AuNP was converted to molar concentrations of DNAs by interpolation from the above standard linear calibration curve. The result reveals each AuNP contains approximately 90 DNAs (60 “flares” and 30 “helper”).

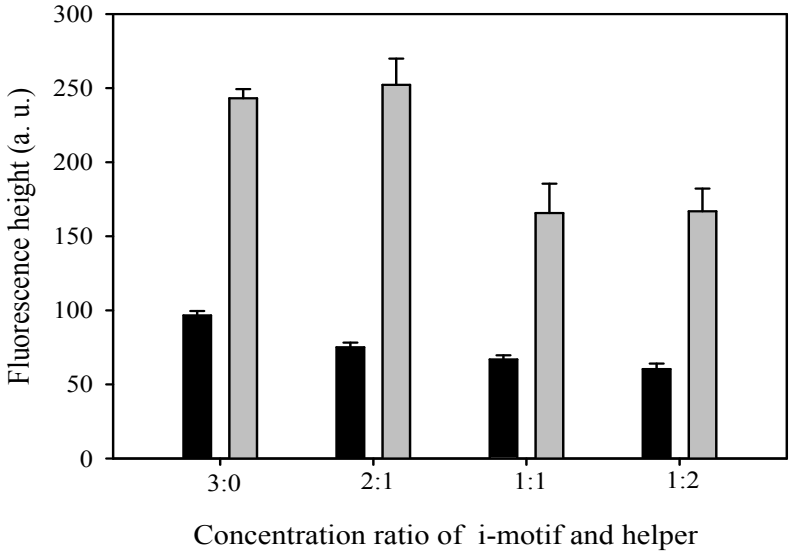

Figure S5. Optimization of i-motif to help ratio, the black pillar demotes pH 7.0 and the gray pillar means pH 5.0.

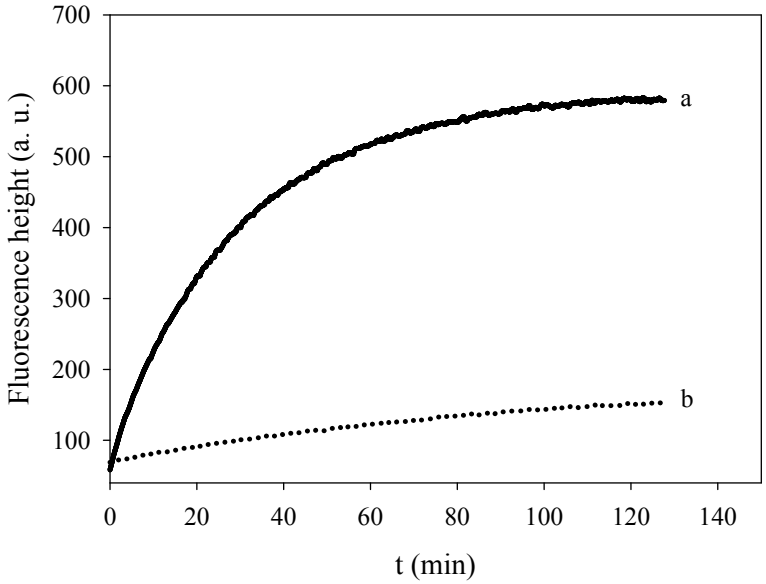

Figure S6. Enzymatic degradation of nano-flares and control probes (BHQ1 instead of AuNP). Nano-flares (2.5 nM) and control probes (150 nM) were treated with enzyme DNase I (0.38 mg/L). The fluorescence signal was recorded as a function of time (a: control probes; b: nano-flares). In these experiments, the concentrations of the probes was adjusted equalized the number of fluorescent oligonucleotides in solution. The concentration of control probes was 60 times compared to the nano-flares, since each nano-flare particle contains 30 helper DNAs and 60 fluorescent flares.