Dual-Stimuli Responsive I-motif/Nanoflares for Molecule Imaging in Lysosome†

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Experimental Section

Chemicals and Apparatus. All oligonucleotides were prepared by Sangon Biotechnology Co. (Shanghai, China). They were dissolved in sterilized Milli-Q ultrapure water (18.2 MΩ) as stock solutions and were kept at -4°C. Cytosine-rich oligonucleotide hybrided assembly ATP-aptamer particles were diluted to a concentration of 30 nM in PBS containing 2 mM $MgCl_2$ (Sigma). Hela cells were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). All of the fluorescence spectra were recorded on a PTI-QM4 Fluorescence System (Photo Technology International, Birmingham, NJ) exciting at 560 nm and measuring emission from 570 to 700 nm. UV-Vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Hitachi U-4100 UV/Vis spectrophotometer (Kyoto, Japan).

Synthesis of AuNPs. AuNPs were prepared by citrate reduction of $HAuCl_4$.[1] A 250 mL aqueous solution consisting of 1 mM $HAuCl_4$ was brought to a vigorous boil and stirred in a round-bottom flask fitted with a reflux condenser, and then 38.8 mM trisodium citrate (25 mL) was added rapidly to the solution. The solution was boiled for another 15 min, during which time its color changed from pale yellow to deep red. The solution was cooled to room temperature with continuous stirring. The sizes of the nanoparticles were verified by TEM (H7100, Hitachi High Technologies Corp., Tokyo, Japan); they appeared to be nearly monodispersed, with an average size of 13 nm. A Hitachi U-4100 UV/Vis spectrophotometer (Kyoto, Japan) was used to measure absorption of the AuNPs solution. The particle concentration of the AuNPs (13 nm) was determined according to Beer's law using an extinction coefficient of 10^8 M^{-1} cm^{-1} at 520 nm for AuNPs of 13-nm diameter[1] (Fig. S1).

Preparation of Apt-AuNPs. The thiol-modified 41-mer DNA oligonucleotides were attached to the AuNPs according to modified literature procedures.[2] Thiolated aptamer was mixed with reporter strands (1:3) in phosphate buffered saline (PBS; 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4), heated to 70℃, slowly cooled to room temperature, and stored in the dark for at least 12 hours to allow hybridization. The mixtures were centrifuged for 25 min at 16 000 rpm to remove the excess thiol-DNA. Following removal of the supernatants, the precipitates were washed with buffer. The mixture was stirred at room temperature for 12 h to obtain aptamer-Au nanoparticles. The nanoparticles were then centrifuged (10000 r/min, 10 min), washed four times with buffer. The aqueous phase was combined. The sequence of anti-ATP aptamer (P1) 5′-SH-TTTTGGTTAGACGGACCTGGGGGAGTATTGCGGAGGAAGGT-3′, and cytosine-rich single-stranded DNA (P2) is 5′-TCCGCATCGTCCCTAACCCTAACCCTAACCC-TMR-3′. ATP-AuNPs hydried with cytosine-rich single-stranded DNA, incubulated for 30 min at room temperature. According to the method reported in literature,[3] we got the binding affinities K_d of the anti-ATP aptamer is about 10μM, which was comparable with the reported K_d values.[4] The K_d demonstrated little increment, which means several bases added had little effect on the affinity of the aptamer.

The solution containing the functionalized particles was centrifuged and resuspended in phosphate buffered saline three times to produce the purified Au NPs used in all subsequent experiments. The concentration of the particles was determined by measuring their extinction at 524 nm (ε = 2.7 x 10^8L $mol^{-1}cm^{-1}$). The concentration of the DNA of modified gold particles was determined by monitoring the UV signal of DNA at 254 nm, using total DNA minus DNA of resuspended.The number(s) of DNA on a single gold particle is 50.

Co-Incubation of Nanoparticles with Cells: First, Hela cells were seeded into 6-well plates with glass bottoms for confocal imaging at a seeding density of 3.0 $\times 10^5$ cells per well. After the cells reached 80% confluence, our aptasensor were incubated with cell at 150 nM concentration for each well. After 2 h of incubation, cells were washed with PBS 3 times and stained with LysoTracker Green (Invitrogen, CA) for cell membranes for next 0.5 h. After removing the medium and then washing with PBS (1 mL), cell samples were examined on a Nikon TE2000-E confocal laser scanning microscope (Tokyo, Japan). Fluorescence emission was collected at 505 – 525 nm and 580 – 620nm, LysoTracker Green and TAMRA were excited at 488 and 560 nm, respectively. In all experiments, the pinhole and gain settings of each individual collection channel were determined using control nanoparticle treated cells. The settings were held constant throughout the experiment.

For the colorimetric MTT assay MTT (5 mg/mL, 25 μL) was added into each well containing treated cells; after 4 h, the supernatant was discarded, and DMSO (100 μL) was added to each well; the mixture was shaken and measured at 570 nm using an ELISA reader scanning multiwell spectrophotometer (PerkinElmer VICTOR 2, GMI, Minnesota, USA). For all samples, cell incubation was performed for 24 and 48 h, and then cell viability and toxicity were determined using MTT assays. The results, summarized in Figure S10, are presented as mean viability(standard deviation from three independent experiments, each of which were performed in triplicate). Figure S8 shows that both the reference AuNPs and nanoflares exhibited survival rates close to 100% after incubation for 24 h or 48 h (MTT assay), suggesting no cytotoxic effects of our aptasensor.

Spectroscopic studies For ATP detection, a certain concentration of ATP was added to the 500 μL PBS buffer solution containing 30 nM aptamer. After incubation at room temperature for 30 min, the mixtures were centrifuged for 10 min at 10 000 rpm, the fluorescence emission spectra of supernatants were recorded.

UV-visible spectroscopic measurements were performed to monitor the change of the nanoparticle solution (Fig. S2). The maximum absoption wavelength red-shift from 518 nm to 522 nm when conjucated with ATP aptamer.

1. R. C. Mucic, J. J. Storhoff, C. A. Mirkin, and R. L. Letsinger, *J. Am. Chem. Soc.*1998, **120**, 12674–12675.

2. L. R. Hirsch, J. B. Jackson, A. Lee, N. J. Halas, and J. L. West, *Anal. Chem.* 2003, **75**, 2377–2381.

3. (a) R. Nutiu and Y. F. Li, *J. Am. Chem. Soc.* 2003, **125**, 4771–4778. (b) J. Zheng, J. Li, Y. Jiang, J. Jin, K. Wang, R. Yang and W. Tan, *Anal. Chem.* 2011, **83**, 6586–6592.

4. D. E. Huizenga and J. W. Szostak, *Biochemistry* 1995, **34**, 656–665.

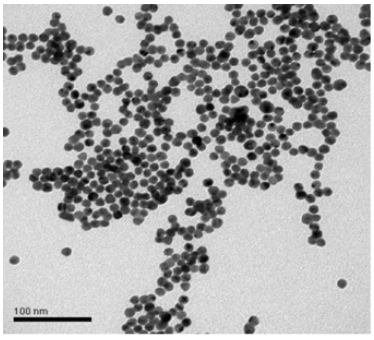

Fig. S1 Transmission electron microscopy (TEM) of the attained AuNPs.

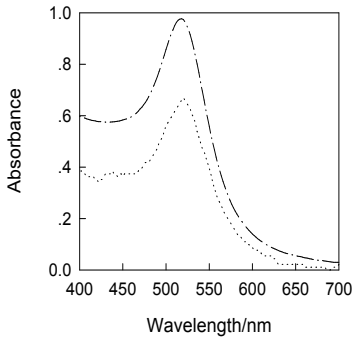

Fig. S2 Absorption spectra of AuNPs (-·-·-) and P1-modified AuNPs (·····).

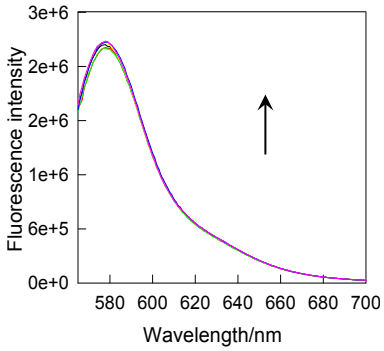

Fig. S3 Fluorescence emission spectra of ATP-aptamer (P1) strand in the PBS buffer solution upon addition of different concentrations of ATP. The arrows indicate the fluorescence signal changes as increases in the ATP concentration (0, 0.05, 0.1, 0.5, 1, 2 and 3 mM). pH= 5.2 and the concentration of ATP-aptamer strand (P1) is 30 nM. λex= 560 nm.

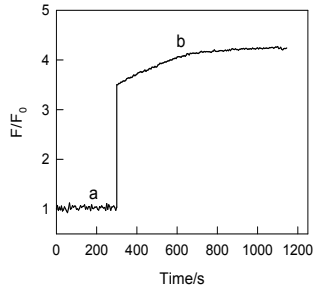

Fig. S4 Real-time fluorescence records of nanoflares (a) upon addition of 3 mM ATP (b). The concentration of nanoflares is 30 nM. λex= 560 nm.

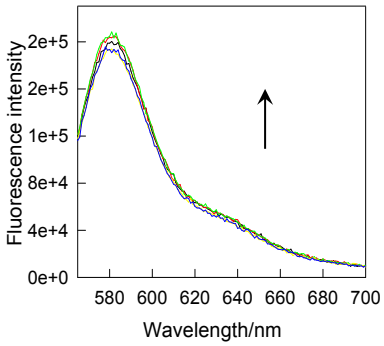

Fig. S5 Fluorescence emission spectra of non-ATP binding aptamer/i-Motif on gold nanoparticle in different pH. The arrows indicate the fluorescence signal changes as pH (7.4, 7, 6.5, 6, and 5.2). The concentration of non-ATP binding aptamer/i-Motif strand (P1) is 30 nM. λex= 560 nm.

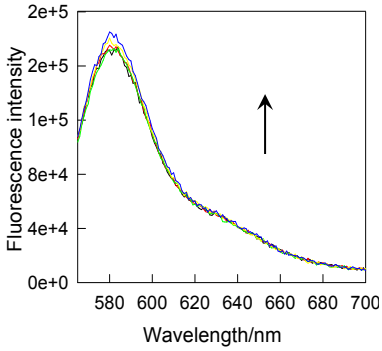

Fig. S6 Fluorescence emission spectra of non-ATP binding aptamer/i-Motif on gold nanoparticle in the PBS buffer solution upon addition of different concentrations of ATP. The arrows indicate the fluorescence signal changes as increases in the ATP concentration (0, 0.05, 0.1, 0.5, 1, 2 and 3 mM). pH= 5.2 and the concentration of non-ATP binding aptamer/i-Motif strand (P1) is 30 nM. λex= 560 nm.

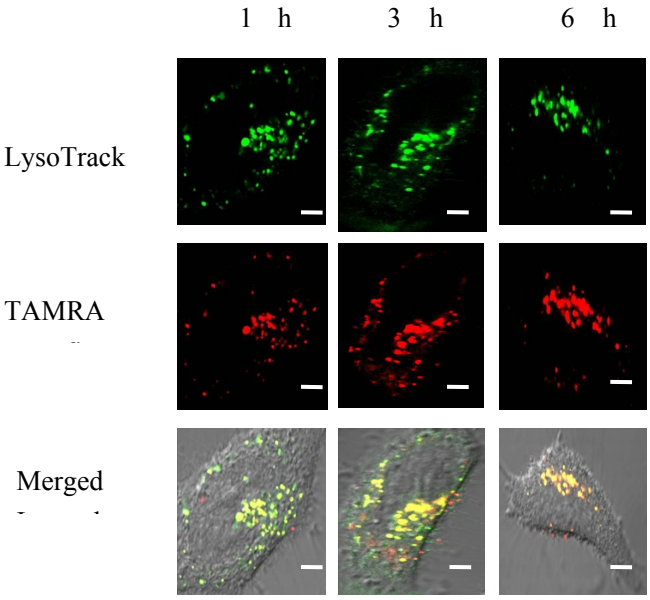

Fig. S7 Laser sanning confocal microscopy images of Hela cells that were incubated with 30 nM TAMRA-aptamer nanoflares for 1.0, 3.0, and 6.0 h, respectively. The scale bar is 5μm.

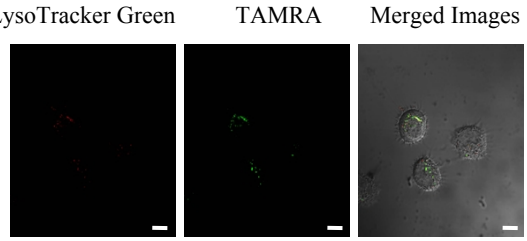

Fig. S8 Laser sanning confocal microscopy images of Hela cells that were incubated with 30 nM TAMRA-aptamer nanoflares overtime. The scale bar is 10 μm.

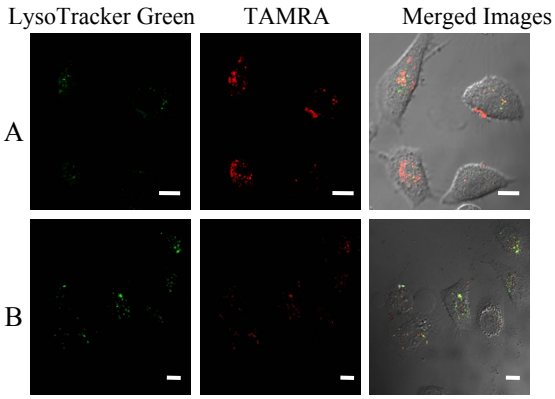

Fig. S9 Laser sanning confocal microscopy images of Hela cells that were incubated with 30 nM TAMRA-aptamer nanoflares and 100 μM etoposide (A) or 3 μg/mL oligomycin (B), respectively. The scale bar is 10 μm.

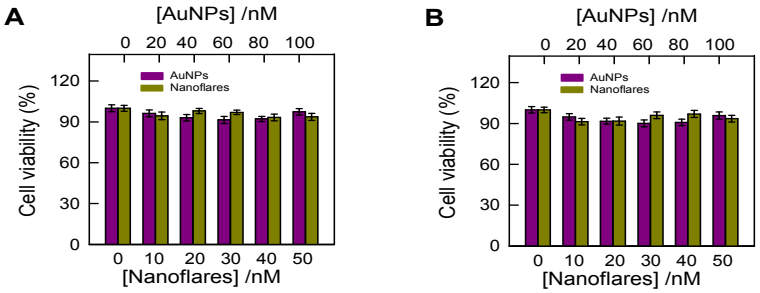

Fig. S10 MTT assays of the reference AuNPs and nanoflares for 24 (A) and 48 h (B).