Supporting Information

Programmable Engineered FRET-Nanoflare for Ratiometric Live-Cell ATP Imaging with Anti-Interference Capability

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Table of contents:

Experimental Section

Chemicals and Materials.

All DNA strands were synthesized and purified by Sangon Biotechnology Co., Ltd (Shanghai, China), and all the sequences of oligonucleotides are shown in Table S1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), oligomycin, bovine insulin, ATP, cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP), Mercaptoethanol (ME) were obtained from Sigma Aldrich (the U.S.A.). DNase I was purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium chloride (NaCl), tris aminomethane (Tris), calcium chloride and trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). AuNPs of 15 nm were purchased from Ted Pella (Redding, CA). Human cervical cancer cell (HeLa) was purchased from KeyGEN Biotechnology (Nanjing, China). All chemicals were used directly without further purification. All solutions were prepared with Milli-Q water from Milli-pore system.

Apparatus.

The UV-Vis absorption spectrum was recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). The transmission electron microscopic (TEM) images were performed on a JEM-2100F transmission electron microscope (JEOL, Japan). The fluorescence spectra were measured on a FluoLog-3 (Horiba, Japan). The pH analysis of buffers was monitored on a digital pH meter (PB-10, Sartorius, Beijing, China). The MTT assay were performed on an EL × 808 microplate reader (Bio Tek, the U.S.A.). The confocal fluorescence imaging was obtained on an FV1000-IX81 confocal laser scanning microscopy (Olympus, Japan) with a 40× objective. Flow cytometric assay was performed on FlowSight multi-dimensional panoramic cell flow counter (Amnis, Germany).

Preparation of the PolyA-mediated FRET-nanoflare.

The polyA_n-ATP aptamer-AuNPs or thiol-ATP aptamer-AuNPs were prepared with the previous reported salt aging method. The AuNPs (15 nm) were first mixed with

polyA$_n$-ATP aptamer or thiol-ATP aptamer in a 1:200 ratio and shaken at 25 ^oC overnight. Then, 1 M of sodium phosphate buffer (PBS, 1 M of NaCl, 100 mM of Na_2HPO_4 and NaH_2PO_4, pH 7.4) was added to the mixture for 5 times to reach a final concentration of 0.1 M PBS (0.1 M of NaCl, 10 mM of Na_2HPO_4 and NaH_2PO_4, pH 7.4) and incubated for another 40 h under room temperature. Then, the mixture was centrifuged at 12000 rpm for 20 min was washed 3 times with 0.1 M PBS. The prepared polyA-mediated DNA-AuNPs or thiol-ATP aptamer-AuNPs were resuspended in the reaction buffer (20 mM of Tris, 300 mM of NaCl，pH 8.0). Subsequently, the FRET-flare strand (R1, 1 μM) was added to the prepared DNA-AuNPs conjugates (5 nM) for 3-h incubation (37 ^oC, 300 rpm). The conjugates was purified via centrifugation at 12000 rpm for 20 min and washed with 0.1 M PBS to remove excess R1 strand. The concentration of prepared polyA-mediated FRET-nanoflare or thiol-mediated FRET-flares was determined by UV-Vis spectroscopy according to the Beer's law ($A=\varepsilon bc$, $\varepsilon_{(15\ nm\ AuNPs)} = 2.70\times10^8\ L\cdot mol^{-1}cm^{-1}$).

Fluorescence Analysis *in vitro*.

Different concentrations of ATP (0, 0.05, 0.075, 0.1, 0.25, 0.5, 1, 2 and 3 mM) were added into polyA$_n$-mediated FRET-nanoflare (6 nM) in the reaction buffer (20 mM of Tris, 300 mM of NaCl, pH 8.0) and incubated at 37 ^oC for 1.5 h. Then, the fluorescence emission spectra of solution were recorded from 540 nm to 800 nm at an excitation wavelength of 530 nm. For the specificity test, 6 nM polyA-mediated FRET-nanoflare were incubated with 0.25 mM CTP, GTP and UTP and ATP, respectively. The measuring steps were the same as mentioned above. All experiments were repeated at least 3 times.

Quantification of PolyA-mediated ATP aptamer loading on AuNPs.

The concentration of AuNPs and the Cy3-labeled polyA$_n$-ATP aptamer (polyA$_n$-ATP aptamer-F) in each sample were measured to quantify the number of DNA attached to each AuNP. The concentrations of AuNPs were determined by UV-visible spectroscopy measurements and the absorbance values were related to the concentration of nanoparticles via Beer's law (For 15 nm AuNPs, $\varepsilon = 2.4 \times 10^8\ M^{-1}cm^{-1}$). To determine the concentration of Cy5-labeled DNA in each probe, the DNA

was chemically displaced by adding ME (with a final concentration of 20 mM in 0.3 M NaCl, 10 mM phosphate buffer solution (pH 7.4)) into the $polyA_n$-ATP aptamer-F modified AuNPs solution. The mixture was then incubated for 18 h with shaking at room temperature. The released DNA probes were then separated via centrifugation and the fluorescence was recorded by a fluorescence spectrometer. The fluorescence was converted to molar concentrations of probes by comparing to a standard linear calibration curve which was prepared with known concentrations of oligonucleotides with identical buffer pH, ionic strength and ME concentration. The number of oligonuleotides per AuNP was calculated by dividing the concentration of $polyA_n$-ATP aptamer-F by the concentration of AuNPs. All experiments were repeated three times using fresh samples to obtain error bars.

Enzyme-Resistance Stability Assay.

As a comparison, $polyA_{30}$-mediated single-dye-based nanoflare was prepared by hybridizing single dye labeled reporter (R2, 1 μM) with 5 nM $polyA_{30}$-mediated DNA-AuNPs for 3-h incubation (37 ^oC, 300 rpm). The conjugates was purified via centrifugation at 12000 rpm for 20 min and washed with 0.1 M PBS to remove excess R2 strand. For stability test, DNase I with different concentrations (0.25 U/mL and 2.5 U/mL) was added to the 6 nM $polyA_{30}$-mediated FRET-nanoflare and $polyA_{30}$-mediated single-dye-based nanoflare for 12-h incubation at 37 ^oC, respectively. As a control, the system without DNase I treatment was measured under the same conditions. The fluorescence was measured on an RF-5301PC fluorescence spectrophotometer with the excited wavelength at 530 nm by recording emission from 540 nm to 800 nm.

Cell culture.

HeLa cells were cultured in RPMI-1640 medium containing bovine insulin (0.01 mg/mL), 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 g/mL), which were incubated in a cell culture incubator at 37 ^oC with 5% CO_2.

Cytotoxicity.

The cytotoxicity of the $polyA_{30}$-mediated FRET-nanoflare was determined by a standard MTT assay. The Hela cells (200 μL, 1×10^4 cells/well) were cultured in 96-well microtiter plates at 37 ^oC for overnight. Then, the original medium was removed

and HeLa cells were respectively incubated with bare AuNPs (1 nM), polyA$_{30}$-mediated FRET-nanoflare (1 nM and 5 nM) for 6 h, 12 h, 24 h and 48 h, respectively. After removing the medium, 100 μL MTT solution (0.5 mg/mL) were added to each well and incubated for 4 h. Then, MTT solution was removed and 150 μL DMSO were added to each well for solubilize the precipitates. The absorption was measured at 490 nm with a microplate reader (EL × 808, BioTek) in order to evaluate cell viability.

Confocal Fluorescence Microscopy Imaging.

The HeLa cells were first seeded into 15 mm confocal dishes for 24 h at 37 ^{o}C with 5% CO_2. Then the medium was removed and washed three times with 0.1 M PBS. Subsequently, 1 mL of fresh medium supplemented containing 1 nM polyA$_{30}$-mediated FRET-nanoflare was added to the dishes and incubated for 3 h. After that, the cells were washed three times with 0.1 M PBS to remove the excess polyA$_{30}$-mediated FRET-nanoflare. Confocal fluorescence imaging was performed on the Olympus FV1000 confocal laser scanning system with a 40× objective. To test the ability of the polyA$_{30}$-mediated FRET-nanoflare for distinguish different level of intracellular ATP, oligomycin and Ca^{2+} was used as the ATP inhibitor and inducer to down-regulate and up-regulate the level of intracellular ATP. One group of HeLa cells was treated with 5 mM Ca^{2+} for 30 min. Another group of HeLa cells was treated with 5 mM 2-deoxy-D-glucose and 10 μg/mL oligomycin for 30 min. Then cells were washed with 0.1 M PBS and incubated with 1 nM polyA$_{30}$-mediated FRET-nanoflare for 3 h. Fluorescence images were recorded under the same above.

Flow Cytometry Analysis.

HeLa cells were seeded in a culture dish and cultured in a cell culture incubator at 37 ^{o}C with 5% CO_2 for 24 h. After treated with 1 nM nanoprobes at 37 ^{o}C for another 3 h, the cells were detached with trypsin and neutralized with RPMI-1640 medium. After that, the mixture was centrifuged under 750 rpm for 3 min and washed with 0.1 M PBS for three times and suspended in 200 μL 0.1 M PBS. Finally, HeLa cells were analyzed with flow cytometry. To detect the different level of ATP in cells, two groups of HeLa cells were pretreated with 5 mM Ca^{2+} or 5 mM 2-deoxy-D-glucose and 10 μg/mL oligomycin for 30 min as above, respectively. Then 0.1 M PBS buffer was employed

to wash the cells for three times. One group of HeLa cells without treatment served as the control. Subsequently, the cells were incubated with 1 nM polyA_{30}-mediated FRET-nanoflare for 3 h and subjected for flow cytometry assay.

Table S1. Oligonucleotide sequences used in this study.

Name	Sequence (5' to 3')
polyA$_{10}$-ATP aptamer	AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA
polyA$_{20}$-ATP aptamer	AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA
polyA$_{30}$-ATP aptamer	AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA
polyA$_{40}$-ATP aptamer	AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA
thiol-ATP aptamer	AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT-SH
polyA$_{10}$-ATP aptamer-F	Cy3-AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA
polyA$_{20}$-ATP aptamer-F	Cy3-AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA
polyA$_{30}$-ATP aptamer-F	Cy3-AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA
polyA$_{40}$-ATP aptamer-F	Cy3-AC CTGGG GGAGT ATTGC GGAGG AAGGT TTTTT AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA
R1	Cy5-AGTAT TGCAC CTTCC TCCGC AATAC T-Cy3
R2	CCTTC CTCCG CAATA CT-Cy3

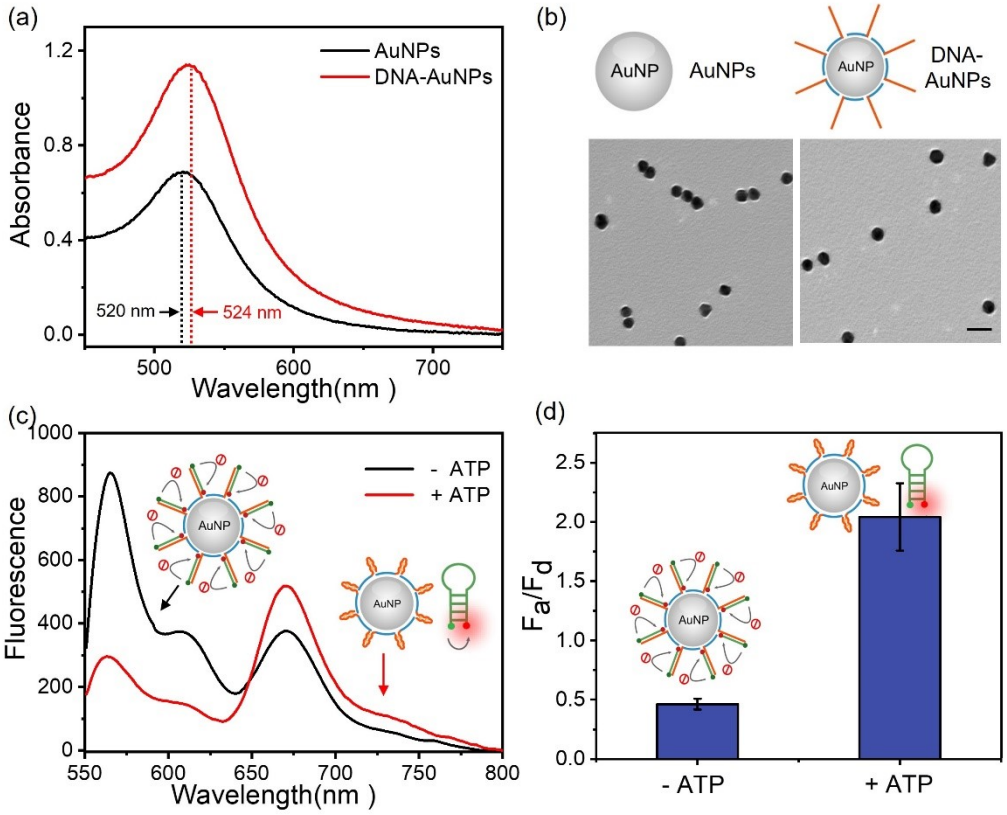

Fig. S1 Characterization of the PolyA-mediated FRET-nanoflare and the feasibility for ATP Detection. (a) UV-Vis absorption spectra of AuNPs (black, λ_{max} = 520 nm) and DNA-AuNPs (red, λ_{max} = 524 nm). (b) TEM images of bare AuNPs (left) and DNA-AuNPs (right). (c) Fluorescence emission spectra of the polyA-mediated FRET-nanoflare in the presence (red) or absence (black) of ATP (λ_{ex} = 530 nm). The polyA length was A_{30}. [ATP] = 1 mM; [FRET-nanoflare] = 6 nM. (d) Corresponding fluorescence emission ratio of acceptor and donor (F_a/F_d) in (c).

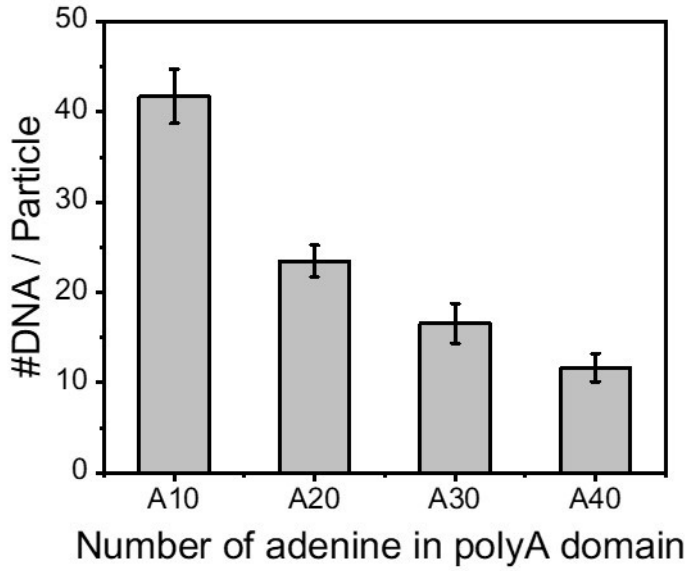

Fig. S2 The ATP aptamer loading on AuNPs with different polyA$_n$ domain.

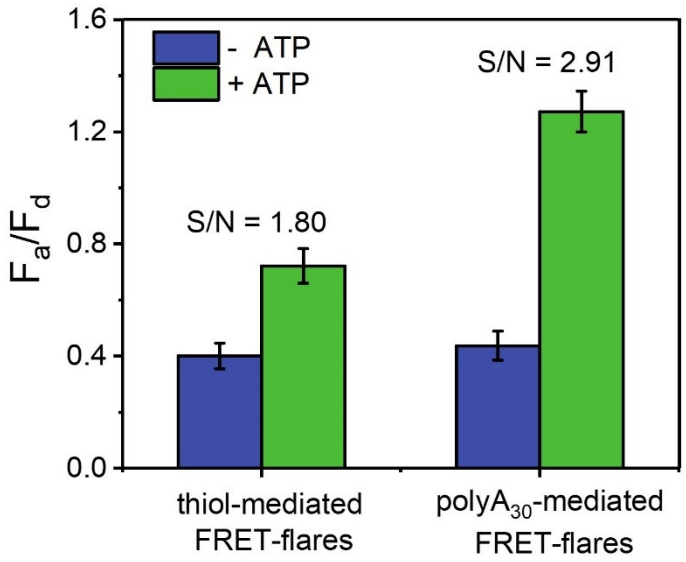

Fig. S3 Fluorescence signal response of polyA30-mediated FRET-flares and thiol-mediated FRET-flares. [$polyA_{30}$-mediated FRET-flares] = [thiol-mediated FRET-flares] = 6 nM; [ATP] = 0.25 mM. All experiments were repeated for three times.

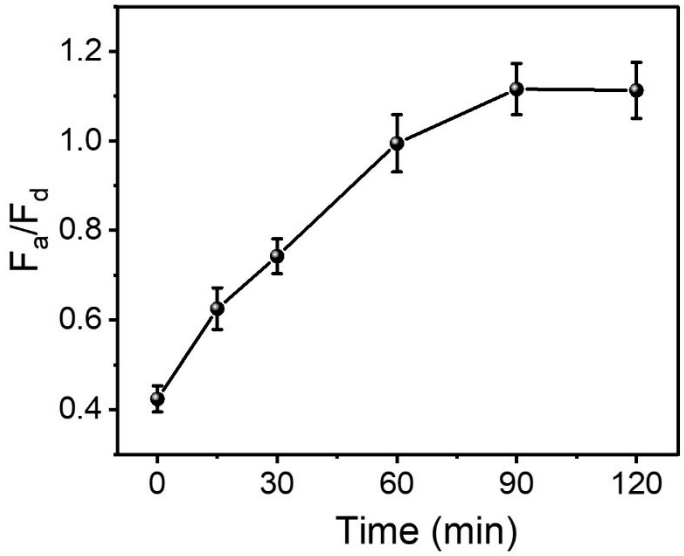

Fig. S4 The kinetics study of polyA$_{30}$-mediated FRET-flares for ATP detection *in vitro*. [polyA$_{30}$-mediated FRET-flares] = 6 nM; [ATP] = 0.25 mM. All experiments were repeated for three times.

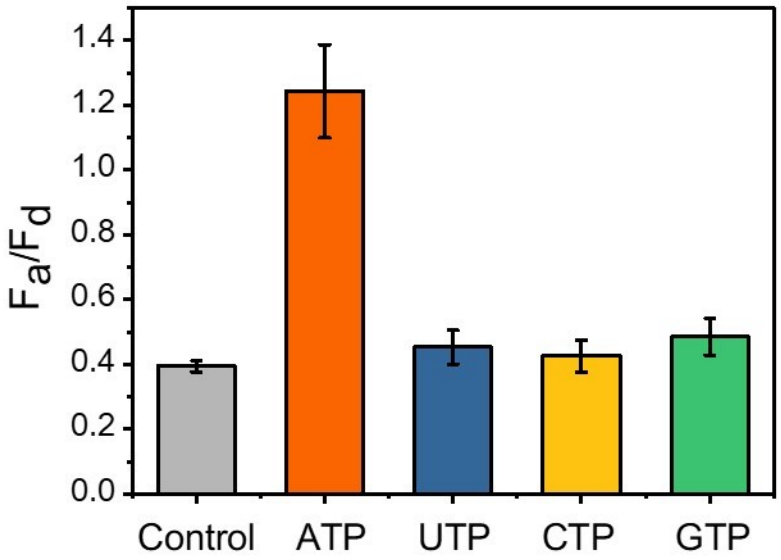

Fig. S5 Selectivity studies for target ATP and other analogs (UTP, CTP, and GTP). [FRET-nanoflare] = 6 nM. [ATP] = [UTP] = [CTP] = [GTP] = 0.25 mM. λ_{ex} = 530 nm; λ_{em} for F_a and F_d was 565 nm and 670 nm, respectively.

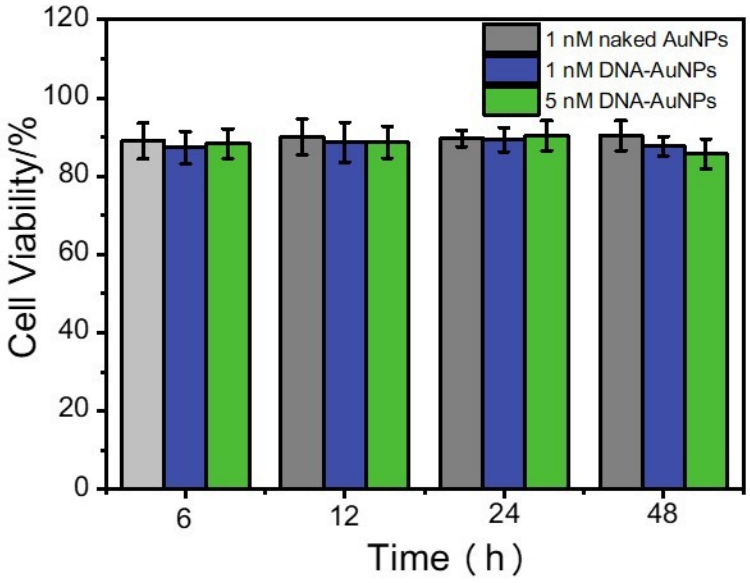

Fig. S6 Cell viability of HeLa cells after treating with naked AuNPs (1 nM) and the polyA$_{30}$-mediated FRET-nanoflare (1 and 5 nM) for 6 h, 12 h, 24 h, and 48 h at 37 °C, followed by MTT assay.

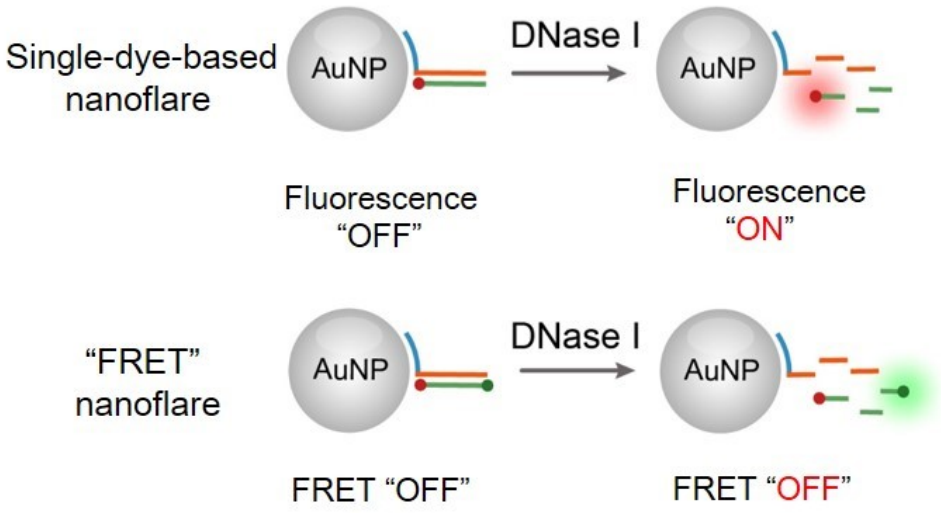

Fig. S7 Schematic of the traditional polyA$_{30}$-mediated nanoflare and FRET-nanoflare treated with high concentrations of DNase I.