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Electronic Supplementary Information (ESI)

A Self-Assembled DNA Nanostructure as FRET Nanoflare for Intracellular ATP Imaging

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Experimental Section

Chemicals and Reagents.

Adenosine 5'-triphosphate (ATP) was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Oligomycin was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). Hoechst 33342, MitoLite ™ Blue FX490 and Lyso-tracker ™ blue DND-22 were obtained from Invitrogen Life Technologies Corporation. Loading buffer was purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (U.S.A.). All the chemicals were of analytical grade and used without further purification. MCF-7 cell lines were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Sartorius ultrapure water (18.2 MΩ cm, Milli-Q, Millipore) was used throughout the experiments. The sequences of the oligonucleotides are described in Table 1.

Apparatus. All fluorescence spectra were carried out on a QM40-NIR (Switzerland). Gel imaging was obtained from multifunctional molecular imaging analysis system Azure C600 (America). Dynamic Light Scattering (DLS) assay was performed on Zetasizer Nano ZS (Malvern). Cells were incubated in a humidified HF90 CO_2 incubator (Shanghai Lishen Scientific Equipment Co.Ltd), and were visualized under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The Atomic Force Microscope (AFM) were performed using Bioscope system (Bruker, USA). MTT assay were recorded on a RT 6000 microplate reader.

Preparation of DNA Nanostructures. the DNA nanostructures was assembled through a "one-pot" process. In brief, six customized single-stranded oligonucleotide strands (A1, A2, A3, A4, A5 and A6) were mixed in equal molar ratio in PBS buffer. The mixtures solutions were heated at 95 °C for 5 min and then cooled to room temperature and stored in a buffer at 4 °C for future use. The formation of DNA nanostructures was confirmed by 12% Polyacrylamide gel electrophoresis. DLS was employed to measure the hydrodynamic size of the nanostructure.

Polyacrylamide Gel Electrophoresis (PAGE) Analysis. To verify the successful assembly of DNA nanostructures, the 10 μL DNA solution was mixed with 2 μL 6 × loading buffer and 2 μL SYBR Gold. Afterwards, 1 μM nanostructures were incubated with 20 mM ATP in PBS buffer reacted at 37 °C, and then run on a 12% native polyacrylamide gel. The electrophoresis was conducted in 1 × TBE buffer at constant voltage of 90 V for 1.5 h.

AFM Characterization of DNA Nanostructure. Tore the transparent tape softly from the mica to eliminate one layer of the mica and obtained a freshly mica surface. Firstly, we need to make a layer of Ni^{2+} adsorbed on the mica surface with 100 mM $NiCl_2$ to let the mica surface positively charged. Therefore, we added 40 μL 100 mM $NiCl_2$ buffer to the middle of mica surface for 10 minutes, soon afterwards added 100 μL ultrapure water to surface immediately and blow off the water to clean the surface of mica, then washed away the solution with an ear wash. Subsequently, 20 μL DNA samples were added to the middle of mica surface for 10 minutes. After deposition,

wash away the solution with an ear wash. Finally, add 3 mL ultrapure water to the middle of mica surface immediately and blow it off to get a clean surface. Then we imaged the samples by a Multimode 8 Atomic Force Microscope with scanasyst-air Mode.

***In Vitro* Fluorescence Assay.** We confirmed outstanding efficiency of FRET by incubating 10 mM ATP with 200 nM nanostructures in PBS buffer at 37°C for 3h. The fluorescence emission spectrum of the mixture was collected from 550 nm to 750 nm, using the maximal excitation wavelength at 530 nm. The slit width of the excitation and the emission light path was set to 10 nm. To achieve the quantitation of ATP, a series of ATP at different concentrations were incubated with nanostructures (200 nM) in PBS buffer at 37 °C for 3h. For ATP selectivity test, UTP, GTP, CTP were mixed with the nanostructures at the same concentration as ATP. The following steps were the same as mentioned above. All experiments were repeated at least three times.

Biostability Analysis of DNA Nanostructures. To access the biostability of DNA nanostructures, 1 μM nanostructures were incubated with 10% (v/v) FBS at 37 °C for designated time, compared with only 1 μM nanostructure-free probes (A5+A6) incubated with FBS in the same condition. Afterwards, 10μL of each sample was mixed with 2 μL 6 × loading buffer and 2 μL SYBR Gold for subsequent electrophoresis analysis.

Cell Culture. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified 5% CO_2-containing atmosphere. The cells were grown to 80% confluence for 24 h before transfection with probes. The cell density was determined using a hemocytometer.

Cell Viability Assay. Cytotoxicity of the DNA nanostructures was measured by a standard MTT assay. MCF-7 cells were cultured in a 96-well plate at a density of 1×10^5 cells per well in a total volume of 200 μL and then incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 h. After discarding the original medium, different concentrations of DNA nanostructures (100, 200, 300 and 400 nM) were mixed with fresh medias and further incubated with MCF-7 cells for 12h. Still, discard the old mediums and add 10 μL MTS solution and 90 μL fresh medium to each well and incubate at 37°C for 4 h. Respectively, 150 μL of DMSO was added to dissolve the precipitated formazan violet crystals. The absorbance was recorded at 490 nM by a M1000 microplate reader. And the experiment was repeated for at least 3 times.

Confocal Fluorescence Imaging. MCF-7 cells were seeded in DMEM medium supplemented for 24 h. After washing 3 times with PBS, cells were incubated with 200 nM nanostructures dispersed in medium for 1 to 7 hours respectively in order to optimize the time. To detect the dynamic changes of ATP in cells, two groups of MCF-7 cells were first treated with oligomycin (10 μg mL^{-1}) and Ca^{2+} (5 mM) for 30min. Then 1 × PBS buffer was employed to wash the cells 3 times. Subsequently, the cells were mixed with nanostructures (200 nM) and incubated for 5h, One group of cells without treatment served as the control. As for endocytosis efficiency analysis, 200 nM nanostructures and nanostructure-free probes (A5 and A6) were incubated with two groups of MCF-7 cells for 5h, respectively, and other steps are the same as mentioned

above. The image of all cells was observed under an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. Cy3 fluorescence was excited by 561 nm and the emission was recorded in green channel from 570 to 620 nm; while the FRET (Cy5) fluorescence image was received in red channel with 561 nm excitation and emission recorded from 663 to 738 nm. All cells were observed under TI-E+A1 SI confocal microscope (Nikon, Japan). The fluorescence images were presented after handling by Image Proplus 6.0 software.

Cell Co-localization Assays. Three groups of MCF-7 cells were cultured in a 35-mm confocal dish for 24 h at 37 ℃, and then incubated with nanostructures for 5 h. After an incubation period of 5 h, 1 × PBS buffer was employed to wash the cells 3 times to remove the nanostructures that were not taken up by cells. Subsequently, the cells were treated with 5μL /mL hoechst-33342, MitoLite ™ Blue FX490 and Lyso-tracker ™ blue DND-22 respectively for 20 min. Afterwards, the cells were washed 3 times with 1mL PBS and subjected to confocal microscope imaging. The fluorescence of hoechst was carried out at the excitation of 405 nm and emissions were received in the blue channel at the same wave. While the excitation of MitoLite ™ Blue FX490 and Lyso-tracker ™ blue DND-22 were all λ = 405 nm and were collected in blue and purple channels, respectively.

Supporting Tables:

Oligo	Sequences(5'-3')
A1	ATTGTGACCCACCAGCAGTGTATGACCCGTTCGGA
A2	TCCGAACGGGTCATAGTGTCACTCTTGACATCCAAAAAAAAAAAAAAAAAAAAAAACC
A3	GGATGTCAAGAGTGAGTGGTCACGACGTCATTA
A4	TAATGACGTCGTGACGTGCTGGTGGGTCACAAT
A5	TCACCTTCCTCCGCAACCCAGGTGACGTAGGTTTTTTTTTTTTTTTTTTTTT
A6	Cy3-TACGTCACCTGGGGGAGTATTGCGGAGGAAGGTGACGTA-Cy5

Table S1. Oligonucleotide sequences used in this work.

Supporting Figures:

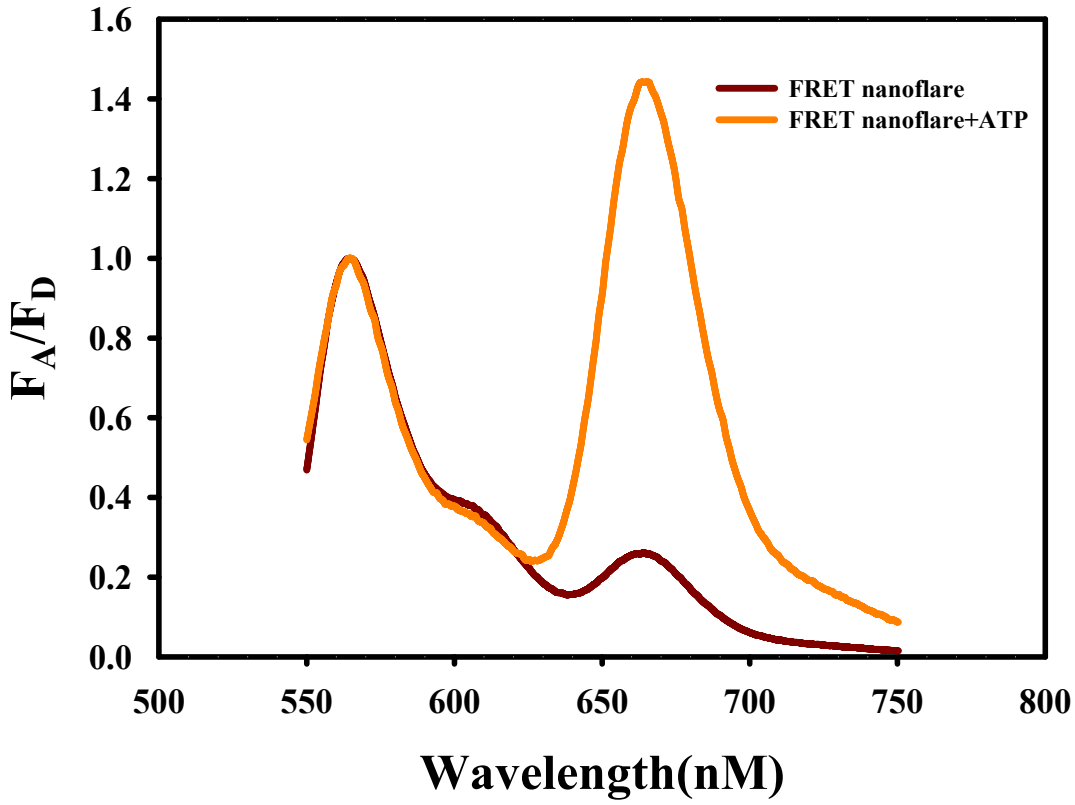

Figure S1. Fluorescence analysis for the feasibility of the self-assembled FRET nanoflare.

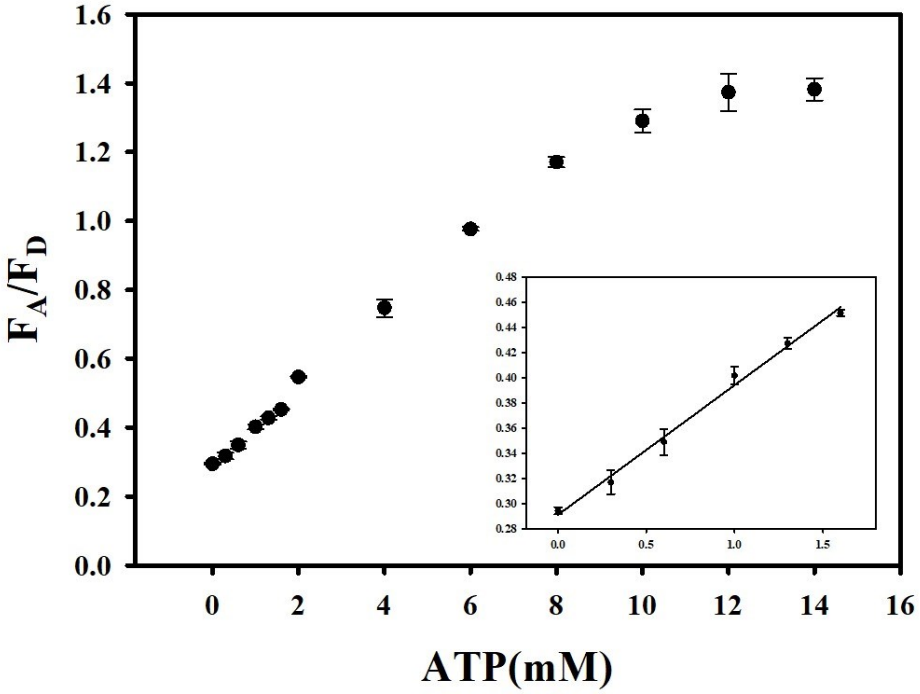

Figure S2. A plot of the F_A/F_D ratio as a function of ATP concentrations. Inset plot is a linear calibration curve of F_A/F_D ratio as a function of ATP in the lower concentration range.

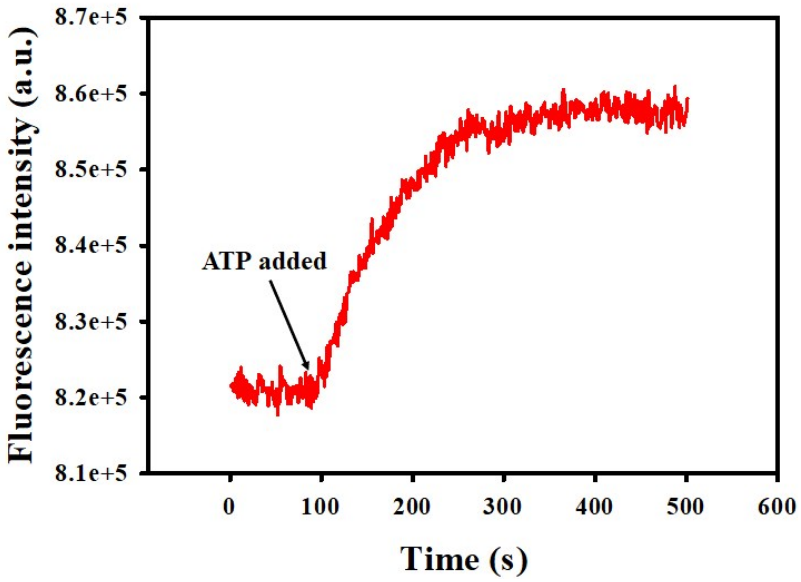

Figure S3. Dynamics Study of the FRET nanoflare response to ATP (Ex=530 nm, Em=665 nm).

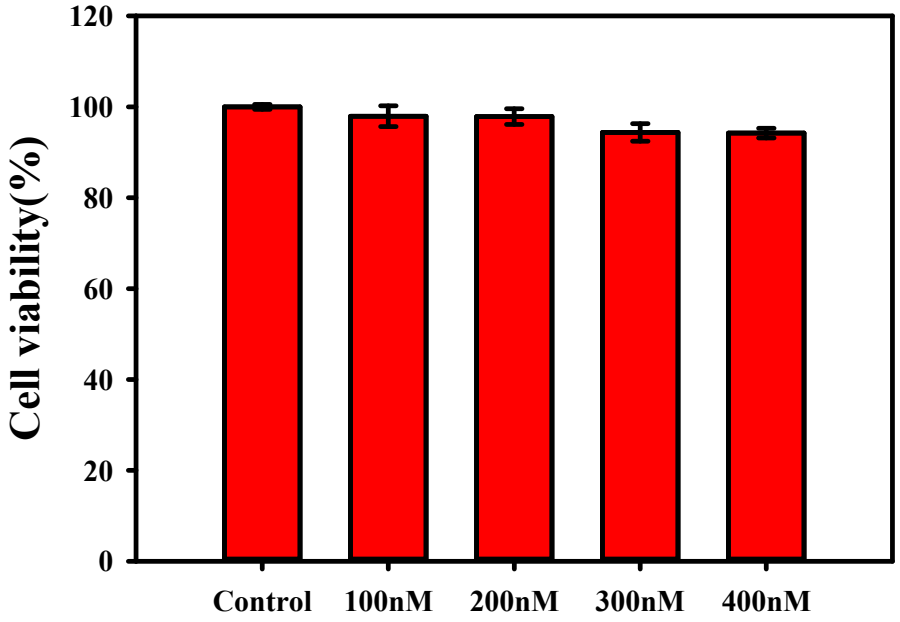

Figure S4. Cell viability assay (MTT) of the MCF-7 cells incubated with different concentrations of the DNA nanostructure-based FRET nanoflare for 24 h.

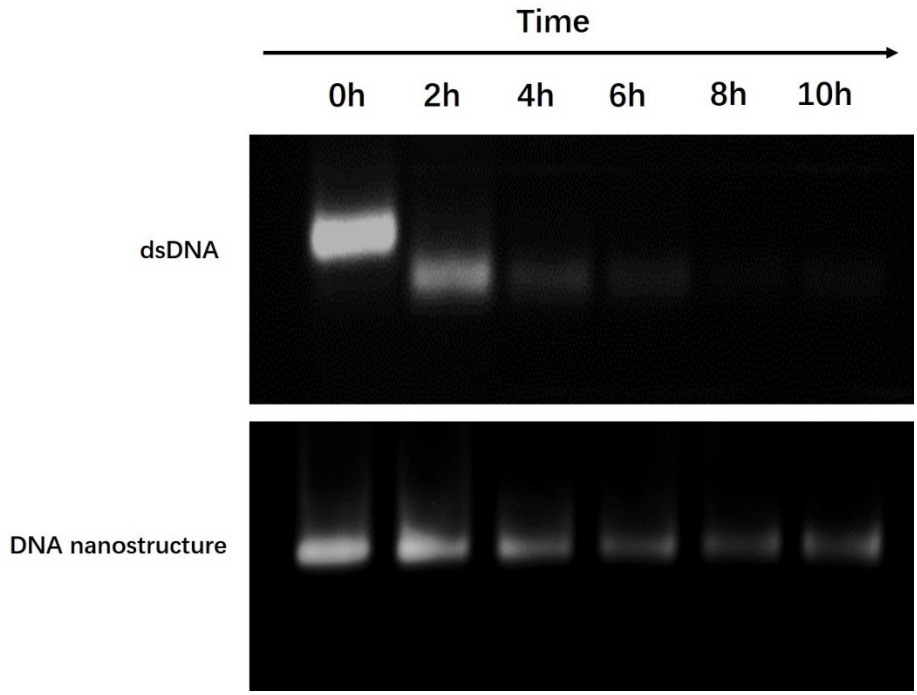

Figure S5. Native gel electrophoresis analysis of degradation between the DNA nanostructure and dsDNA in FBS.

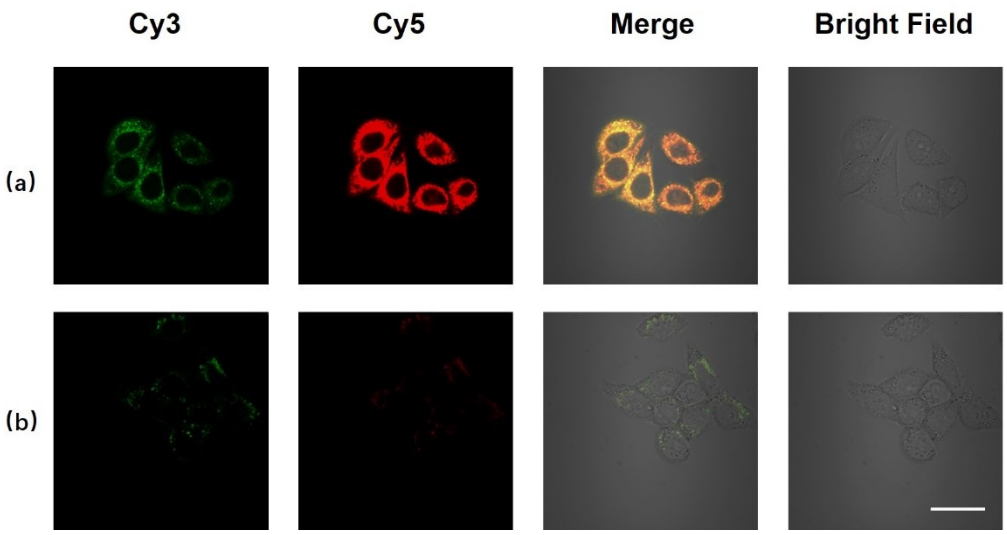

Figure S6. Confocal fluorescence images of MCF-7 cells incubated with (a) DNA nanostructure-based FRET nanoflares, and (b) nanostructure-free probes (A5+A6), respectively. The scale is 20 μm.

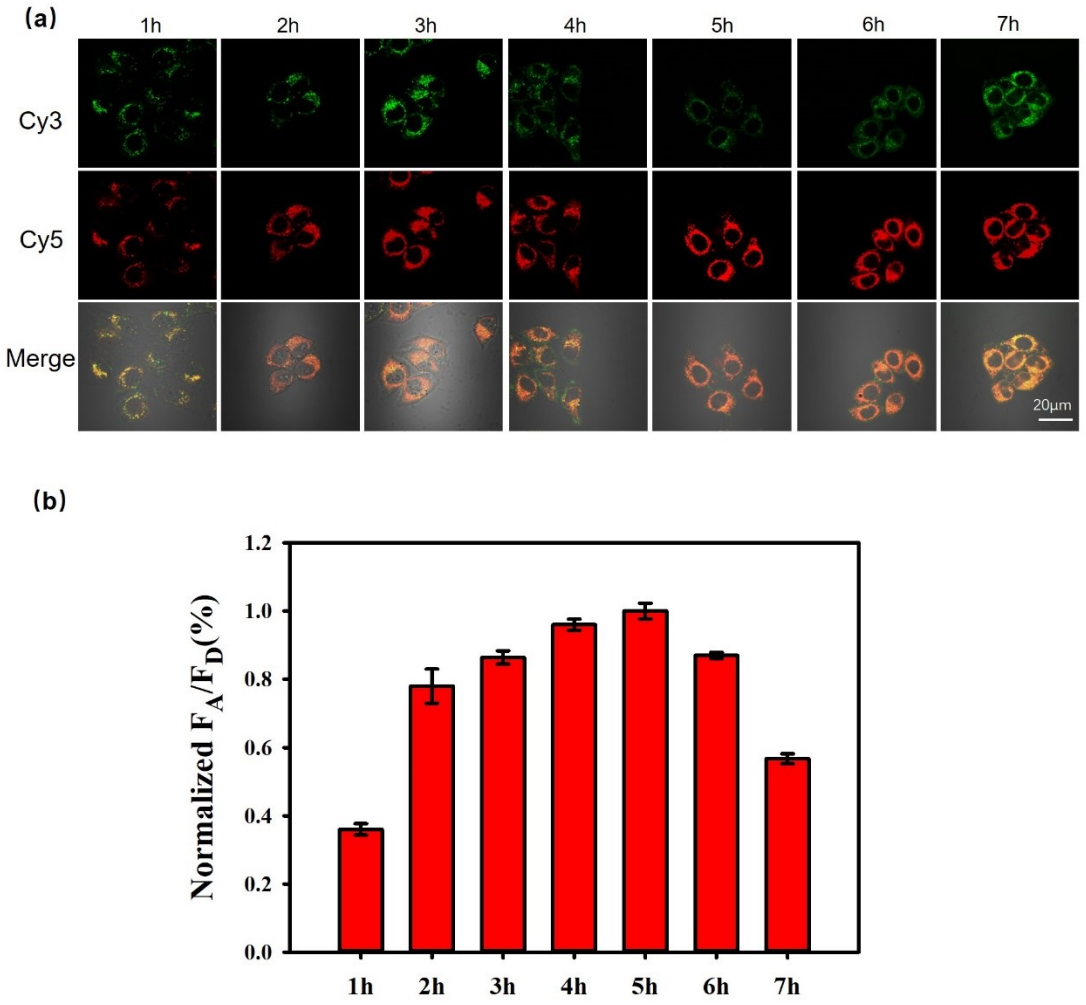

Figure S7. Studies of incubation time for the DNA nanostructure-based FRET nanoflares with MCF-7 cells. (a) Confocal fluorescence images of the cells with the nanoprobes for different incubation time. (b) Histogram of the FRET signals of the above cells for different incubation time.