**Electronic Supplementary Information (ESI)**

# Aptamer-Tethered Self-Assembled FRET-Flares for MicroRNA Imaging in Living Cancer Cells

Anmin Wang[1], Qing Lin[1], Shiyuan Liu[1], Jing Li[1], Jiaoli Wang[1], Ke Quan[2], Xiaohai Yang[1], Jin Huang[1,*] and Kemin Wang[1]

[1]State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha, China.

*E-mail: jinhuang@hnu.edu.cn

[2]School of Chemistry and Food Engineering, Changsha University of Science and Technology, Changsha, China

**Experimental Section**

**Chemicals and Reagents.** All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The oligonucleotides were purified by high-performance liquid chromatography (HPLC). All aqueous solutions were prepared using DEPC-treated ultrapure water (≥18 MΩ, Milli-Q, Millipore). The lipofectamine 3000 was obtained from Invitrogen (Thermo Fisher Scientific, USA). SYBR Gold was obtained from Invitrogen (USA). All cell lines were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell medium Dulbecco's modified Eagle's medium (DMEM) were purchased from Clontech (Mountain View, CA). The sequences of the oligonucleotides are described in Table S1.

**Apparatus.** The fluorescent spectra were measured using a Hitachi F-7000 fluorescence spectrometer (Japan). Confocal laser scanning microscopy (CLSM) studies were performed using an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. The flow cytometry analysis was gained from a Gallios machine (Beckman Coulter, USA).

**In Vitro DNA Assembly.** DNA probe consists of four single-stranded oligonucleotide strands (F, Apt-T, H1, and H2). H1 and H2-F complex were heated to 95 °C for 5 min and then allowed to cool to room temperature for at least 2 hours before use. They were mixed at the appropriate molar ratio (Apt-T:H1:H2-F=1:5:5) in HCR reacting buffer (10mM PBS containing 5 mM $MgCl_2$) and incubated for 3 h at 37 °C.

**Gel Electrophoresis Experiments.** For agarose gel electrophoresis, a sample containing 10 μL of each reaction product, 2 μL 6× loading buffer, and 2 μL of SYBR Gold was subjected to the 3% agarose gel electrophoresis for 1 h at 100 V in TBE buffer. For native polyacrylamide gel electrophoresis (PAGE), the DNA solution was mixed with 2 μL 6× loading buffer, and 2 μL of SYBR Gold, then run on a 12% native polyacrylamide gel. The electrophoresis was conducted in 1 × TBE buffer at constant voltage of 100 V for 2 h.

**Serum Assay of DNA nanoprobe.** Two groups of the DNA nanoprobe were spiked with or without fetal bovine serum (FBS), respectively, to a final concentration of 100 nM in 10% FBS. Both solutions were incubated at 37 ℃, and then the fluorescence of two samples was monitored for 6 h with an appropriate excitation wavelength.

**In Vitro Fluorescence Experiments.** All the assays were prepared in 10 mM PBS containing 5 mM $MgCl_2$, pH 7.4 at 37 ℃. For HCR amplification signal, four DNA probes (Apt-T, FAM-H1-BHQ1, H2-F) were mixed at the ratio of 1:5:5 at 37 ℃ for 3 h. The resultant solution was then excited at 488 nm, and the fluorescence emission was measured from 500 to 650 nm. For verified the FRET flare probe responses to miR-21, 100 Nm miR-21 was employed. For the detection of miR-21 in FRET-based HCR reaction, different concentrations of the target miR-21 were added into the DNA nanoprobe solutions, respectively. After reaction at 37 ℃ for 30 min, and then the solution was excited at 530 nm and the emission spectra were recorded from 550 to 750 nm, and the FRET spectra were normalized to the maximum Cy3 donor peak around 566 nm. For selectivity test, a certain concentration of miR-21, miR-429, miR-200b,

and let-7d stock solution were added into the solutions with a final concentration of 150 nM. The measuring processing was as the same as above.

**Cell Culture and transfection.** MCF-7 cells, HeLa cells, and L02 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL of 1% penicillin and streptomycin solution, and cultured at 37 ℃ in a humidified incubator containing 5% $CO_2$ and 95% air. Transfection assays were performed according to the manufacturer's protocol. Briefly, when using 35 mm glass dishes, transfection was carried out using 3 μL lipofectamine 3000 and certain concentrations of probes in 300 μL of Opti-Mem, then diluted to 1000 μL with DMEM and incubated at 37 °C for 3 h.

**Cell Viability Assay.** For the viability assay, MCF-7 cells were seeded in a 96-well plates at a density of $1 \times 10^6$ cells per well and a total volume of 200 μL/well. The plates were maintained in a humidified atmosphere with 5% $CO_2$ at 37 °C. After overnight incubation, the original incubating medium was discarded, and the MCF-7 cells were washed twice with PBS and incubated with serial concentrations of the probe for 24 h. Cells incubated with only the medium served as control. Subsequently, 20 μL of MTS and 100 μL of fresh medium (without FBS) were added to each well with incubation at 37 ℃ for 2 h after removal of the old medium. Finally, the absorption of the solution for each well was measured at 490 nm using a microplate reader (BioTek Instrument, Inc.) to determine cell viability after another vibration for 10 min.

**Flow Cytometry Assay.** MCF-7, HeLa and L02 cells were incubated with 100 nM Apt-T probes in reaction buffer for 30 min at 37 ℃, then the cells were collected by centrifugation at 2000 rpm for 4 min, washed three times with reacting buffer. Probes at the appropriate molar ratio was added and incubated with the cells for 2 h. After washing twice to remove nonbinding probes, the cells were resuspended in PBS for flow cytometric analysis on Beckman Coulter Gallios machine under 488 nm excitation by counting 10000 events. The data were analyzed with FlowJo software.

**Confocal Fluorescence Imaging.** For imaging of MCF-7 and L02 cells, the cells were seeded into a confocal dish for 24 h at 37 ℃, then incubated with 100 nM Apt-T probes in reaction buffer for 30 min at 37 ℃, washed three times with PBS. Probes at the appropriate molar ratio was added and incubated with the cells for 2 h, cells were washed three times with 1 mL of PBS and subjected to confocal microscope imaging. The FAM fluorescence emission channels were collected using an excitation laser at 488 nm. For imaging of miR-21 in MCF-7, HeLa and L02 cells, the cells were seeded into a confocal dish for 24 h at 37 ℃, and then incubated with DNA probes for 4 h or various time (for time optimization). To detect the dynamic changes of miR-21 in cells, two groups of MCF-7 cells were transfected with anti-miR-21 (300 nM) and miR-21 mimics (300 nM) for 3 h, respectively. One group of cells without treatment served as the control. Other steps were performed the same as described above. Cy3 fluorescence was excited by 561 nm and emission recorded from 570 to 620 nm; FRET-induced fluorescence of Cy5 was excited by 561 nm 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 processing by Image Proplus 6.0 software and ImageJ version 1.38× software.

**Cell co-localization assays.** The MCF-7 cells were seeded in a 35-mm confocal dish and incubated for 24 h at 37 ℃, and then incubated with DNA probes for 4 h. After incubation, cells were washed three times with 1 mL of PBS, and then the cells were treated with 5 μg/mL hoechst-33342 for 10 min. After incubation, cells were washed twice with 1 mL of PBS and subjected to confocal microscope imaging. Excitation of hoechst was carried out at $\lambda = 405$ nm and emissions were collected in the blue channel. For excitation of Cy3 was carried out at $\lambda = 561$ nm, and the emissions of Cy3 and Cy5 were recorded in green and red channels, respectively.

**qRT-PCR.** Total cellular RNAs was extracted from HeLa cells, MCF-7 cells, and L02 cells respectively, using Trizol reagent (Sangon Co. Ltd., Shanghai, China) according to the manufacturer's instructions. The cDNA samples were prepared by using the reverse transcription (RT) reaction with AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). qPCR analysis of miRNA was performed with SG Fast qPCR Master Mix (2×; BBI), according to the indicated protocol on an LightCycler480 Software Setup (Roche). The relative expression of miR-21 was calculated using the $2^{-\Delta\Delta Ct}$ method.

**Supporting Tables:**

**Table S1 Oligonucleotide sequences used in this work.**

| Oligo | Sequences(5'-3') |
|---|---|
| F | (Cy3)TTCAGTCTGTAGCTTATCAGACTGAA (Cy5) |
| H1 | ATGAAGGACGAT(BHQ)GTATGCTTAGGGTCGACTTCCATAG ACCCTAAGCATACAT(FAM) |
| H1 | ATGAAGGACGATGTATGCTTAGGGTCGACTTCCATAGACCC TAAGCATACAT |
| H2 | TCAACATCAGTCTGATAAGCTACAGTTTTGACCCTAAGCAT ACATCGTCCTTCATATGTATGCTTAGGGTCTATGGAAGTC |
| Apt-trigger | GACCCTAAGCATACATCGTCCTTCATTTTTGGTGGTGGTGG TTGTGGTGGTGGTGG |
| miR-21 | UAGCUUAUCAGACUGAUGUUGA |
| Anti-miR-21 | U*C*A*ACAUCAGUCUGAUAAGC*U*A* |
| miR-21 mimics | U*A*G*CUUAUCAGACUGAUGUU*G*A* |
| miR-200b | UAAUACUGCCUGGUAAUGAUGA |
| miR-429 | UAAUACUGUCUGGUAAAACCGU |
| let-7d | AGAGGUAGUAGGUUGCAUAGUU |

The * represents phosphorothioate modification.

**Supporting Figures:**

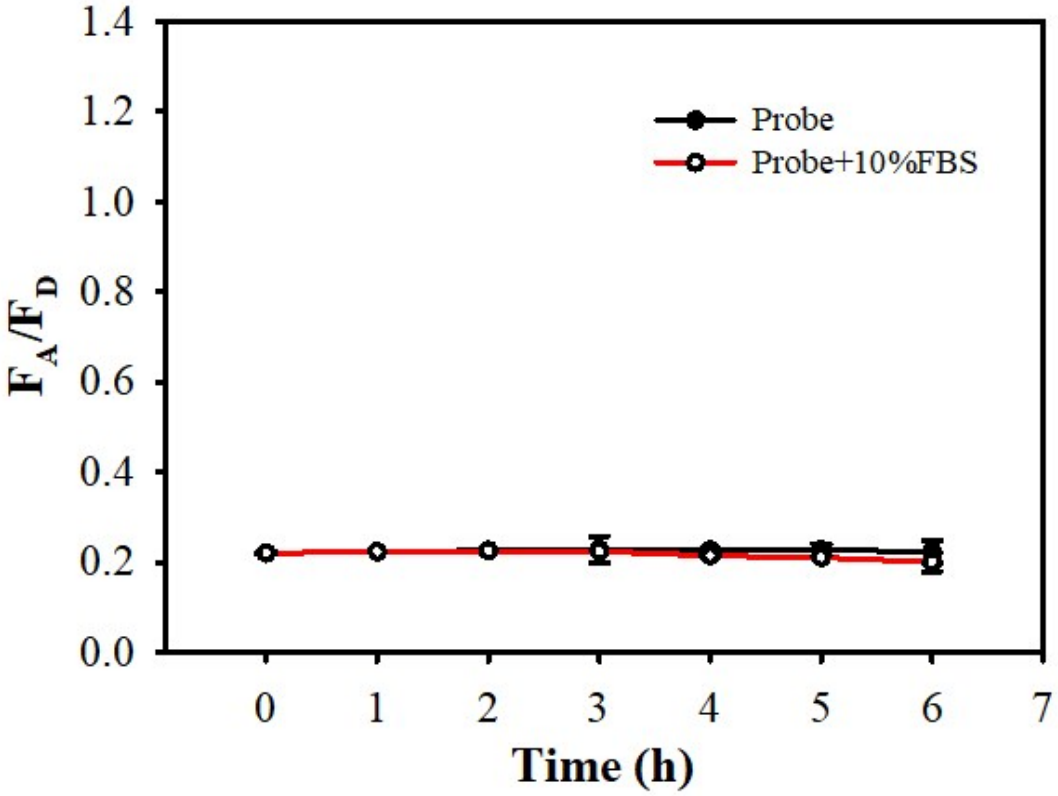


**Figure S1.** Fluorescence curves of the nanoprobe in buffer with or without 10%FBS, as a function of time. The result showed that the introduction of 10%FBS did not lead to the obvious changes of FRET signal of the nanoprobe. The data error bars indicate means ± SD (n=3).

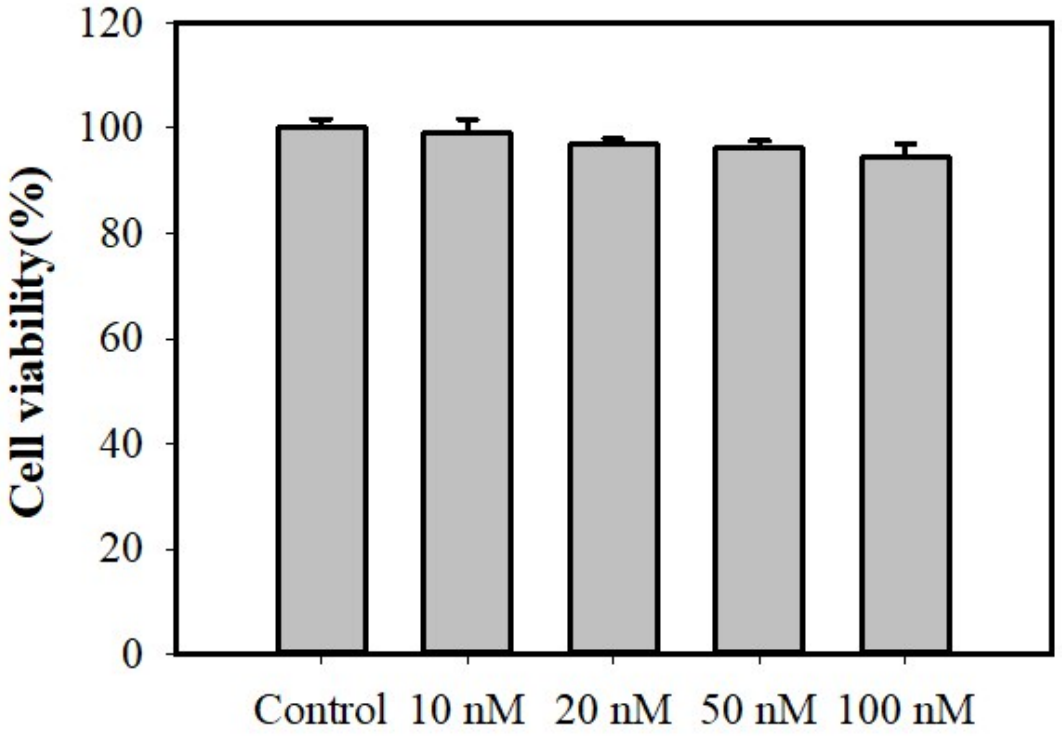


**Figure S2.** Cytotoxicity of the probe incubated with MCF-7 cells at different concentrations of probes.

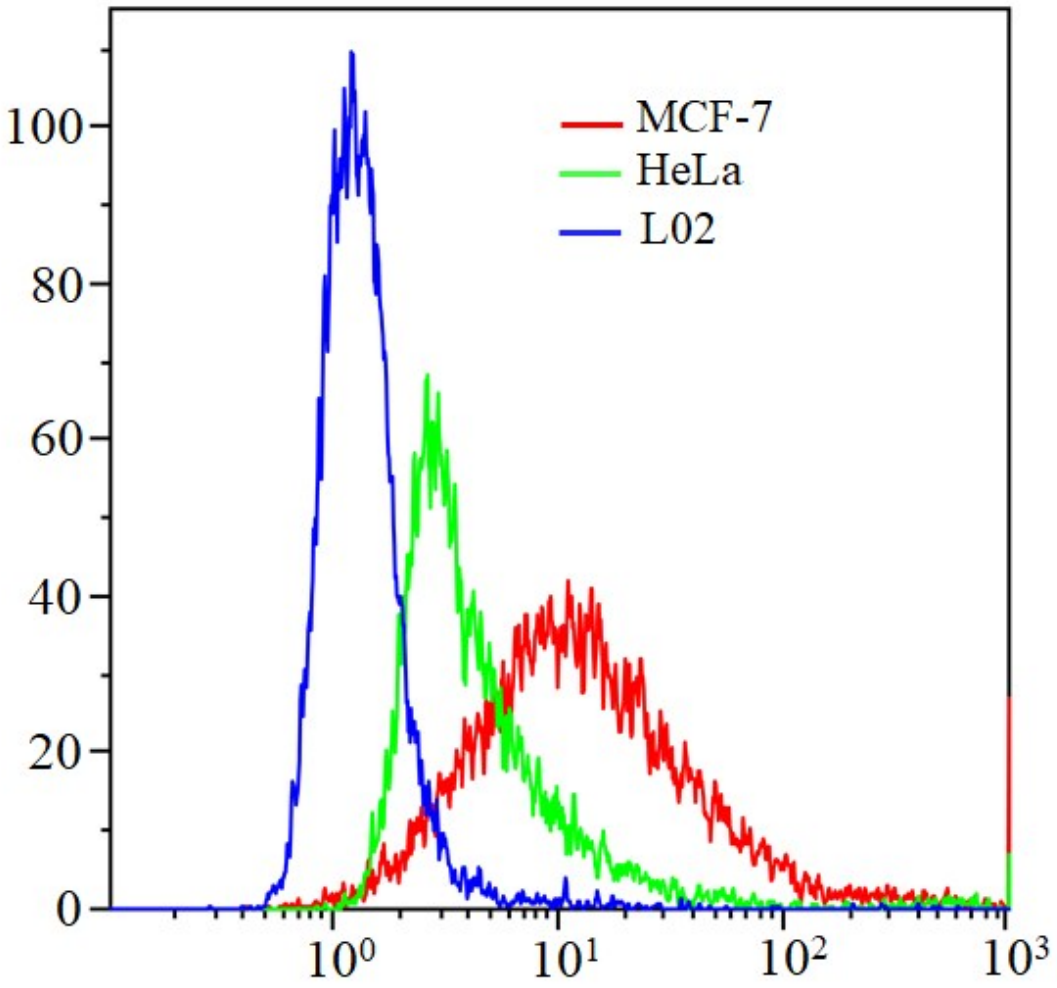


**Figure S3.** Flow-cytometric analysis the selective recognition of probe to MCF-7 cells, HeLa cells and L02 cells, respectively.H1 in probe were labeled with FAM.

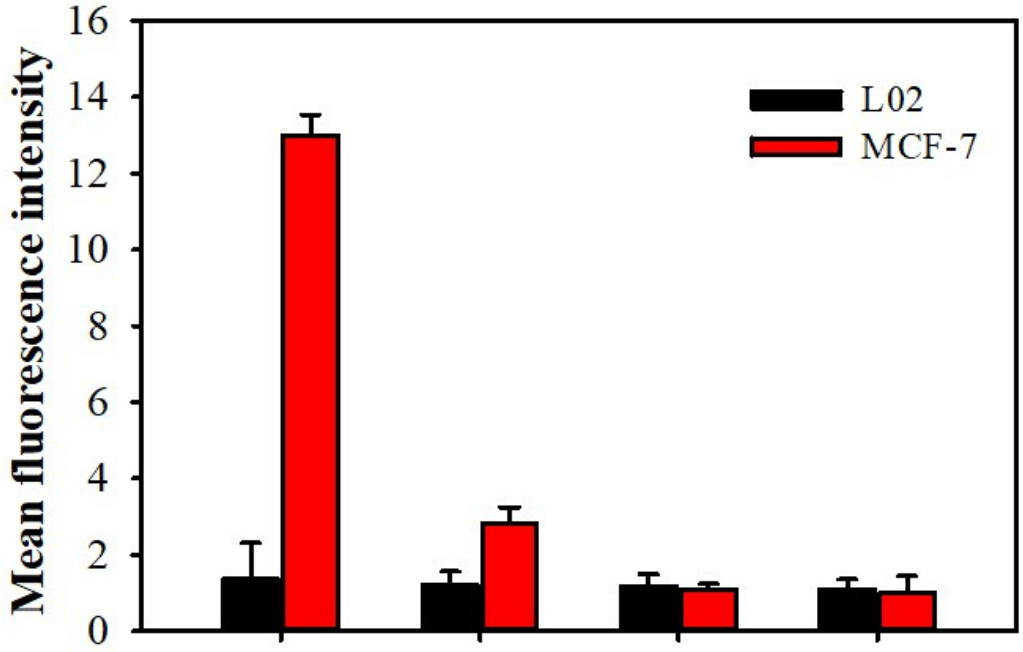


**Figure S4.** Flow cytometry quantification of cell surface aptamer-tethered HCR in different conditions between MCF-7 cell line and L02 cell line;

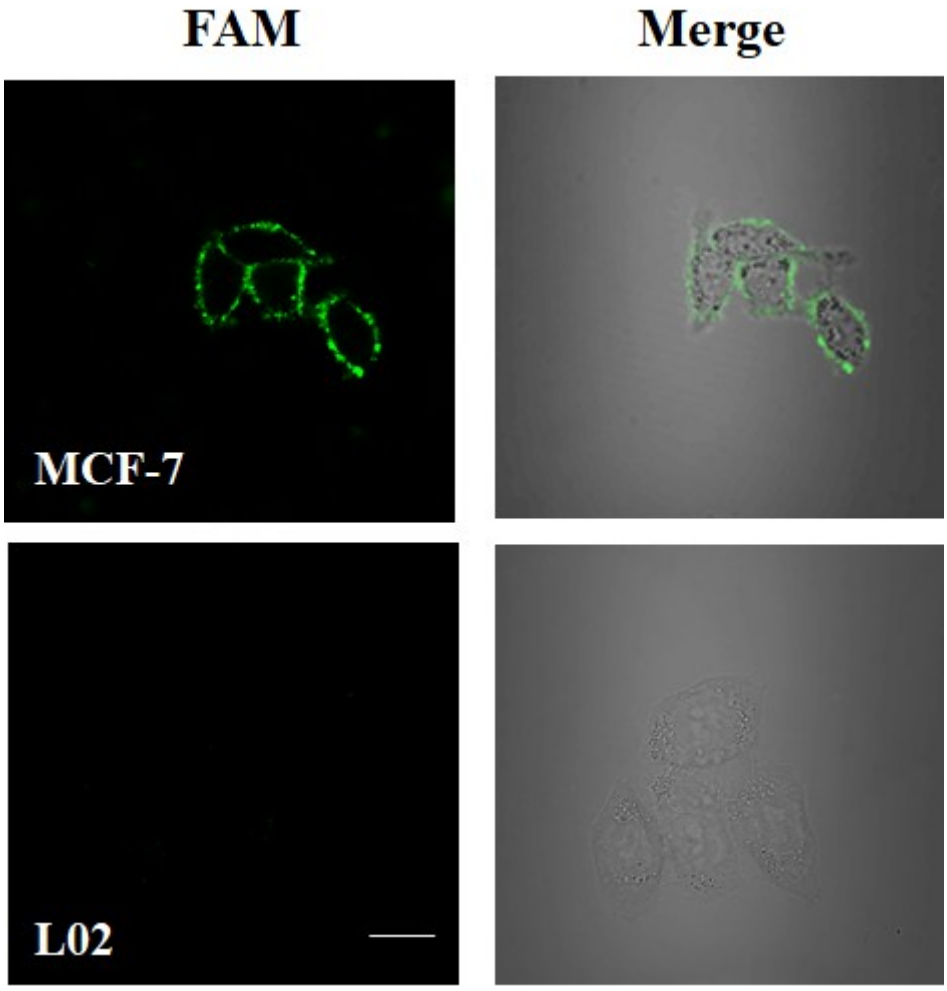


**Figure S5.** Fluorescence images of MCF-7 and L02 cells incubated with Apt + H1 + H2, The FAM fluorescence emission channels were collected using an excitation laser at 488 nm. Scale bar is 20 μm.

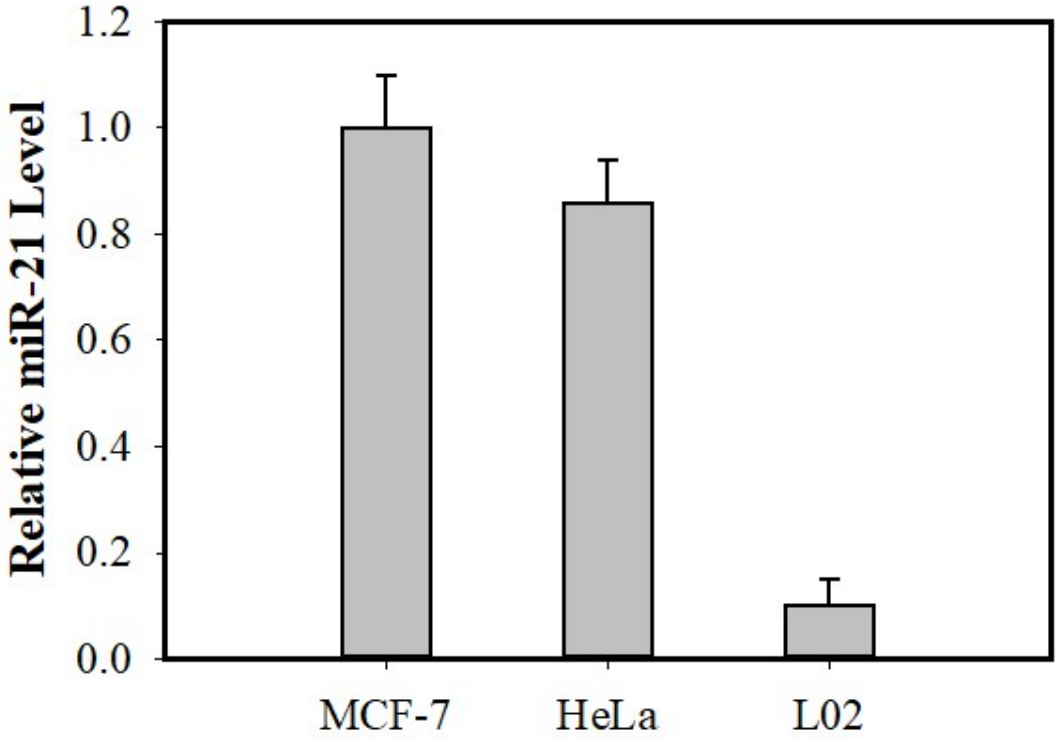


**Figure S6.** qRT-PCR analysis of relative expression levels of miR-21 in MCF-7 cells, HeLa cells and L02 cells, respectively.

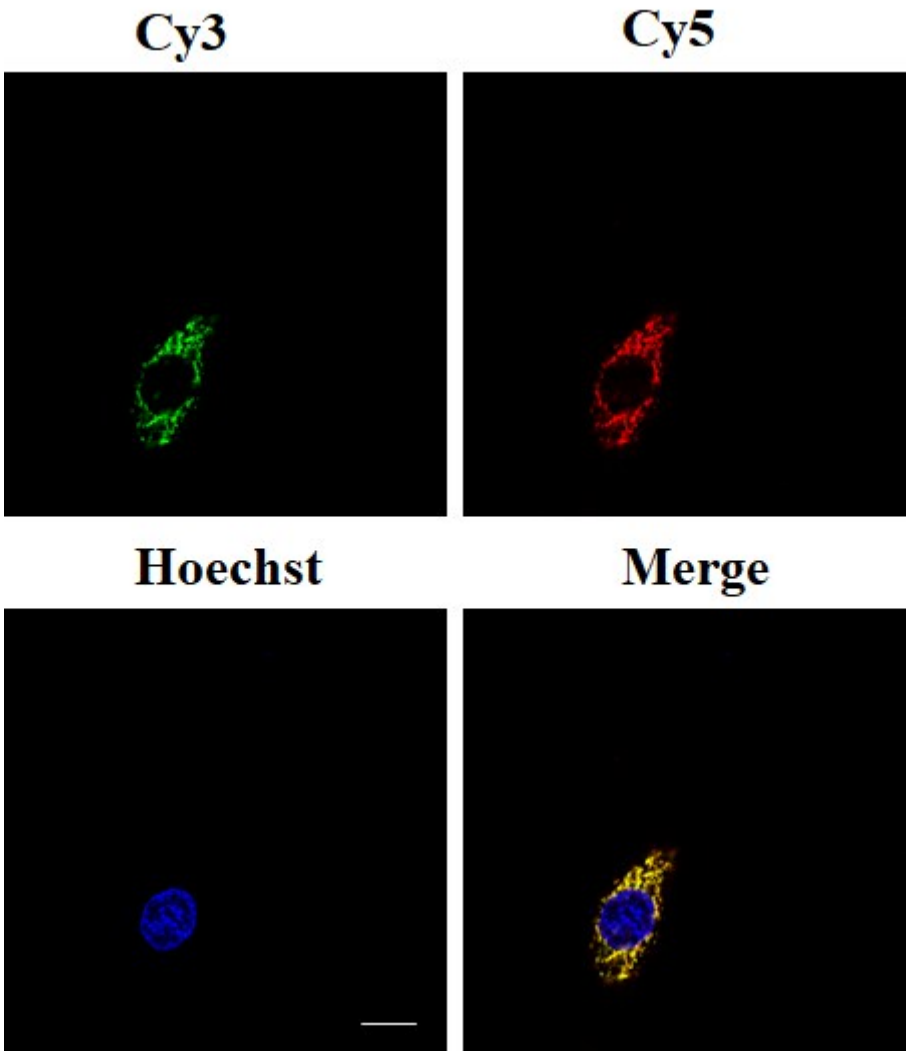


**Figure S7.** Colocalization experiments involve the probe and Hoechst (stained nuclei) in MCF-7 cell lines. The green fluorescence represents Cy3, the red fluorescence represents Cy5 and the blue fluorescence represents Hoechst stained cell nuclei . Scale bar is 20 μm.

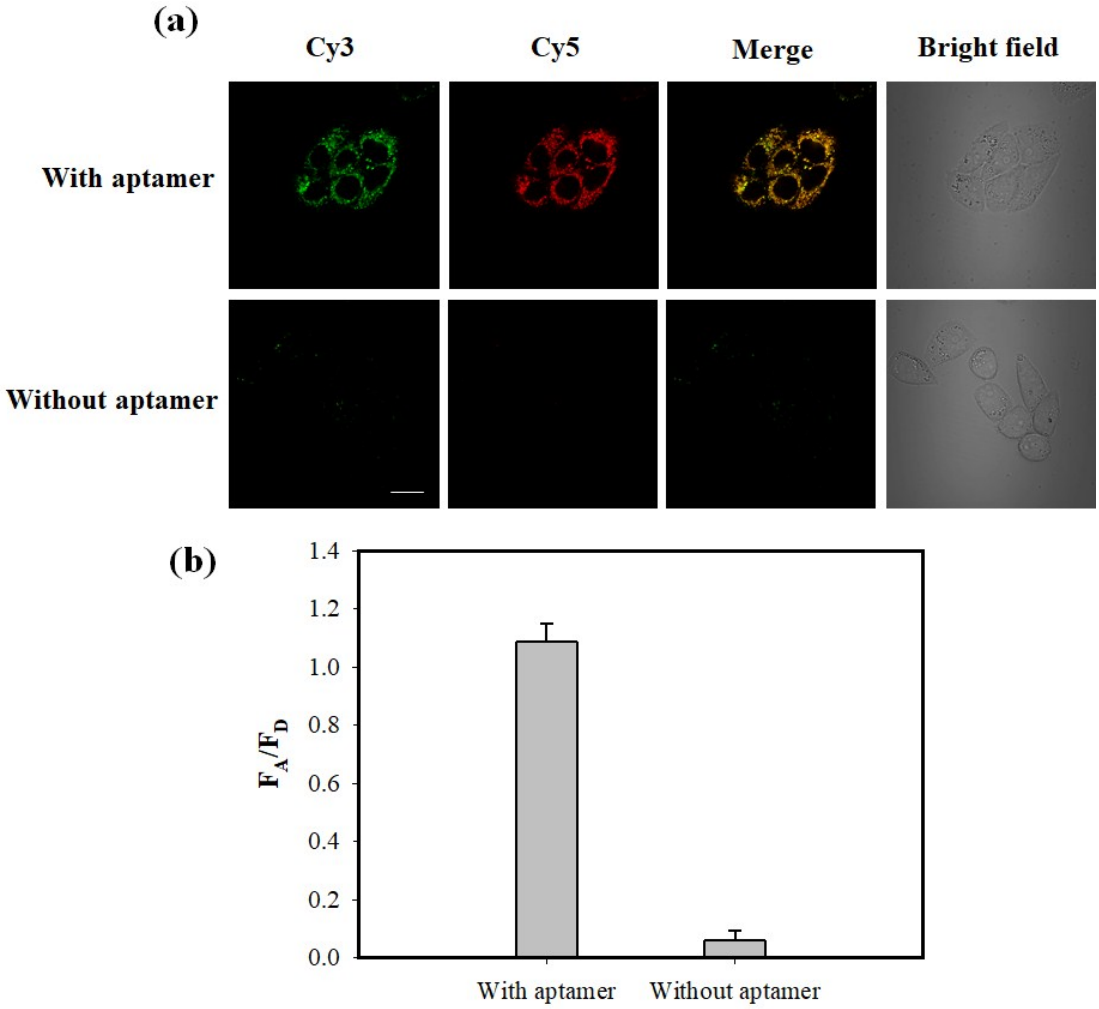


**Figure S8.** (a) Confocal fluorescence images of MCF-7 cells incubated with nanoprobe, and nanoprobe without aptamer. Scale bar is 20 μm; (b) Statistical histogram analysis of the relative fluorescence intensity ($F_A$/ $F_D$) of MCF-7 cells incubated with nanoprobe, and nanoprobe without aptamer.

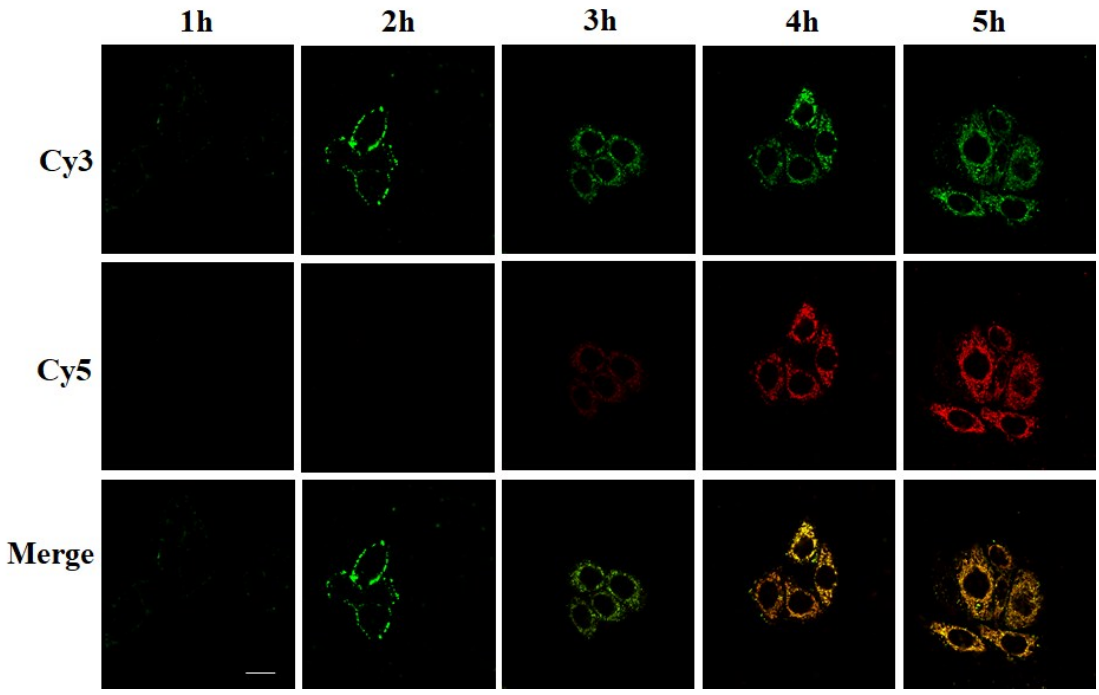


**Figure S9.** Real-time fluorescence imaging of the self-assembled FRET flares responded to miR-21 in MCF-7 cells. Cy3 fluorescence was excited by 560 nm and emission recorded from 570 to 620 nm; Cy5 fluorescence was excited by 560 nm and emission recorded from 663 to 738 nm. Scale bar: 20 μm.

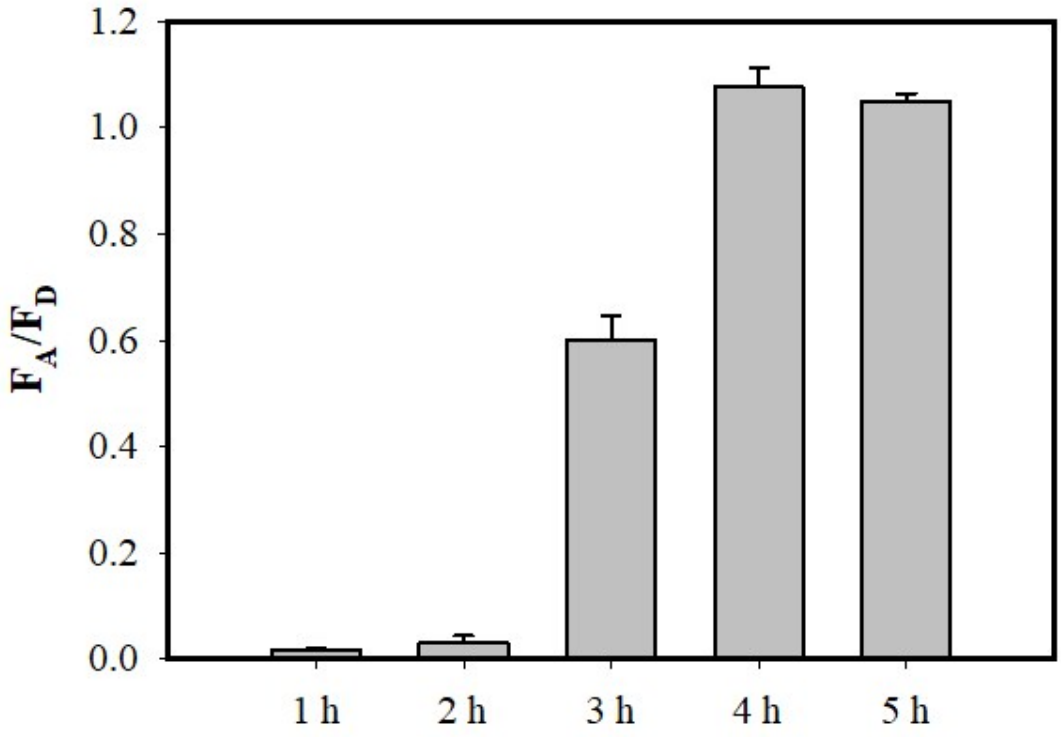


**Figure S10.** Statistical histogram analysis of the relative fluorescence intensity ($F_A/ F_D$) of the different incubation time for MCF-7 cells through self-assembled FRET flares system.

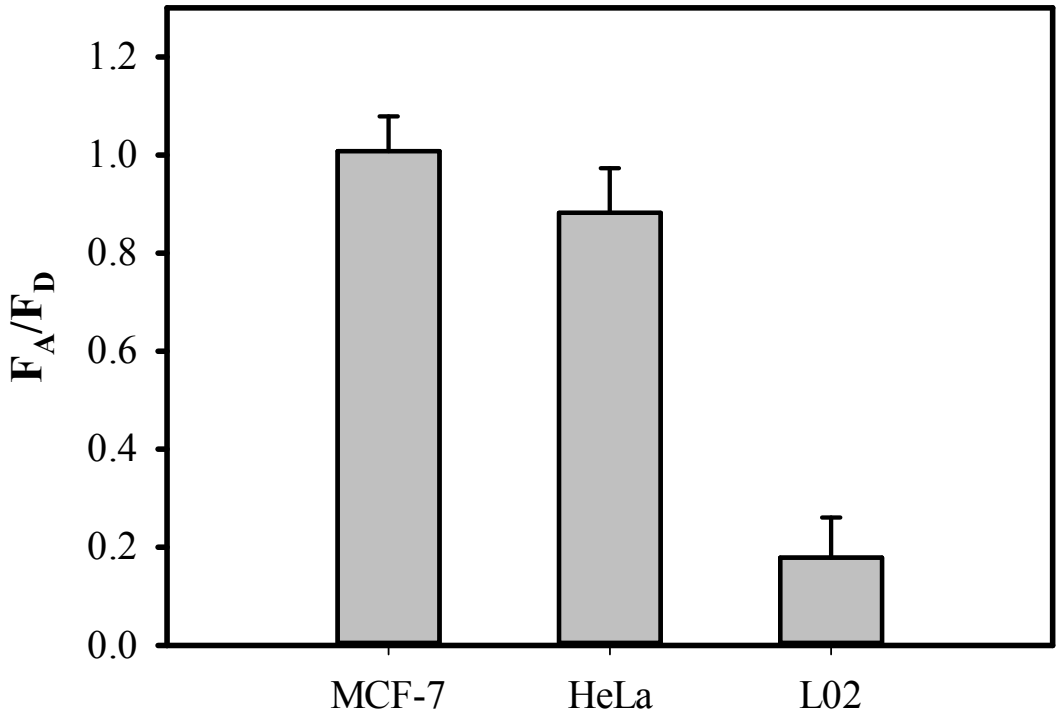


**Figure S11.** Statistical histogram analysis of the relative fluorescence intensity ($F_A/ F_D$) of MCF-7 cells, HeLa cells and L02 cells through self-assembled FRET flares system.

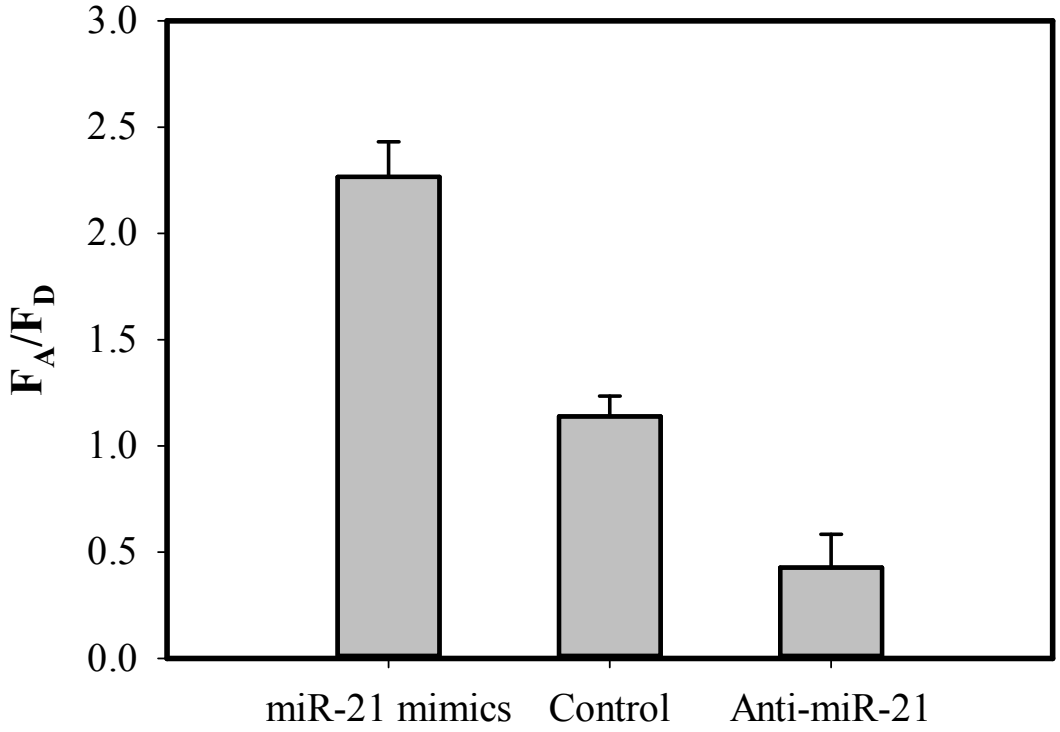


**Figure S12.** Statistical histogram analysis of the relative fluorescence intensity ($F_A/ F_D$) of MCF-7 cells treated with miR-21 mimics transfection and anti-miR-21 transfection and the untreated control.