DNA Photonic Nanowire with Tunable FRET Signals on the basis of Toehold-mediated DNA Strand Displacement Reaction

Supplementary Information

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S1 Materials and Methods

Materials

All DNA oligonucleotides used in this study, including the fluorophore-labeled strands, were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these oligonucleotides are shown in Table S1. Ultrapure water with 18.2 MΩ·cm (Millipore simplicity, USA) was used in all experiments. In this study, FAM was placed in an appropriate position around the central area of L (L') as donor. Cy3 and Texas Red were selected as acceptors and were respectively modified at assigned ends of A1 (A'-21) and A2 (A'-122). All dry powders of the purchased DNA were dissolved in 10 mM Tris-HCl buffer solution (300 mM NaCl, 5 mM $MgCl_2$, pH = 7.4). Transfection was performed by X-treme GENE HP DNA transfection Reagent (ROCHE). The cells were purchased from the Cell Bank, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China.

Annealing and Self-assembly of the DNA photonic wires

Linker (L) was mixed with 1.2×P1 and P2, heated to 95°C, and then allowed to cool slowly to room temperature. This complex was maintained at 4°C for further use. And the purity of the complex was tested by native-page gel (Figure S1).

Fluorescence measurements

The complex was diluted to 100 nM in Tris-HCl buffer with twice amount of A1 and A2. This solution was treated with target DNAs (T1, T2, or both, 100 nM) for 2 h at 25°C. The fluorescence spectra were recorded on an F-7000 fluorometer (Hitachi) with 430 nm light excitation. All the spectra became clearer after correction of acceptor fluorescence and buffer background. Acceptor fluorescence was normalized using the donor fluorescence.

Concentration gradient experiment

The complex was diluted to 100 nM in Tris-HCl buffer with twice the amount of A1 and A2. This solution was then treated with different concentration of target DNA (T1, T2, or both) for 2 h at 25°C. Accordingly, 0 nM, 0.5 nM, 2 nM, 5 nM, 10 nM, and 20 nM target DNAs were added to the solution. The fluorescence spectra were recorded on the fluorometer.

Direct annealing of A1 or A2 with L

L was mixed with A1, A2, or both at gradient molar ratios up to 100 nM, heated to 95°C, allowed to cool slowly to room temperature, and then scanned on a fluorometer (data are not shown). The value of $1\text{-}D/D_0$ is used to compare our design with direct annealing system at 2 nM target (Figure S2).

Time dependence experiment

The complex was diluted to 100 nM in Tris-HCl buffer with twice amount of A1, A2, and 2 nM target DNAs (T1, T2, or both). The fluorescence spectra were recorded by time (Figure S3).

Target concentration and Reaction time investigation when one kind of target existing

The complex was diluted to 100 nM in Tris-HCl buffer with twice amount of A1, A2, and 20 nM of one type target. The complex was then treated with another one at gradient concentration up to 20 nM for 2 h at 25°C (Figure S4a-c).

L was mixed with 50 nM A1 or A2 to replace P1 or P2 respectively, heated to 95°C, and then allowed to cool slowly to room temperature. It was then treated with 2 nM T1 or T2 and the fluorescence spectra were recorded by time. (Figure S4d-f). The spectra was normalized by the maximum intensity and cleared by the correction of acceptor fluorescence and buffer background.

Color photo experiment

The DNA reaction solution prepared in the 'Fluorescence measurements' was tested by a fluorescence microscope (Olympus IX71) with blue-light excitation.

Functional examination of Modified DNA photonic nanowire system

New complex and dx-Fuel were annealed similar to before (L':P'=1:1.2; LF:A'=1.2:1). The complex was diluted to 100 nM in Tris-HCl buffer with twice amount of dx-Fuel. This solution was treated with different concentration of target DNAs (miR-21-DNA, miR-122-DNA, or both) for 12 h at 25°C. The fluorescence spectra were recorded on an F-7000 fluorometer (Hitachi) with 430 nm light excitation at different time points. All the spectra became clearer after correction of acceptor fluorescence and buffer background. And the ratio of acceptor fluorescence to donor fluorescence was used to show reaction efficiency (Figure S6).

Live Cell Imaging

HEK-293T, Hela and Huh-7 cells were seeded into 12-well plates (Corning Costar) and incubated for 24 h in DMEM/High glucose growth media (10% FBS, 37°C, 5% CO_2). The complex was transfected at 100 nM, as well as 200 nM dx-Fuel, by 1μL X-tremeGENE HP transfect reagent in 500 μL of medium into cells at 37°C for 2 h. After incubation, the transfection medium was removed and replaced with pure DMEM/High for imaging. Other experiments like only complex, single-stranded Fuel or dx-Fuel uptaken were performed similarly in Hela cells. It should be noted that for the experiments on transfection efficiency with only complex, single-stranded Fuel and dx-Fuel, 200 nM DNAs were used and the reaction time was 4 h.

The cellular images for uptaken examination were taken in bright-field and blue-light excitation by Fluorescence microscope (Olympus IX71) (Figure S7, S8) and the images for MicroRNA detection were taken in bright-field as well as 3 channels with corresponding emission wavelength range on Confocal Laser Scanning Microscopy (Leica TCS SP5). The signals were normalized with a coincident setting for each channels for uniformity, while different cells could have different settings since they were compared with colors rather than intensity and different cells had different uptaken efficiency. The fluorescence images were merged with bright-field images for final display.

Flow Cytometry examination for single detection in live cells

Dx-Fuel was annealed with only A'-21 or A'-122 and another complementary strand without fluorophore. After uptaken by Hela and Huh-7 cells using the same method above, the samples were analyzed with Flow Cytometry (Guava easyCyte 6-2L) in FITC excitation and 1 channel with corresponding emission wavelength range. To compare the differences of these colors, the data were normalized by using blank cells to adjust the autofluorescence of cells and only-donor system to adjust cross-color and keep acceptor dark (donor system only have complex strand and a double-stranded fuel without fluorophore). Compared to this control setting, other reaction groups were analyzed with same parameters. (Figure S9).

S2 DNA Sequences used in this design

All sequences were designed and analyzed by NUPACK online server. And the sequences were listed in Table S1.

Table S1. DNA sequences used in the experiments

Name	Sequence	Modification
L	5'-CACTCCTACCTCCACATCCTCCCATTC ACTCACACTCCCACTACTATCCACCTC-3'	Starting from the 5' end 26th base on T modified 6-FAM
P1	5'-GGTGGATAGTAGTGGGAGTG-3'	
P2	5'-GGAGGATGTGGAGGTAGGAG-3'	
T1	5'-GATAGTAGTGGGAGTGTGAGT-3'	
T2	5'-GAATGGGAGGATGTGGAGGTA-3'	
A1	5'-GAGGTGGATAGTAGTGGGAGTG-3'	3'-Cy3
A2	5'-GGAGGATGTGGAGGTAGGAGTG-3'	5'-Texas Red
L'	5'-CAAACACCATTGTCACACTCCACACCTCTTTT TTTCACTCCTCAACATCAGTCTGATAAGCTA-3'	Starting from the 5' end 31st base on T modified 6-FAM
LF	5'-CATACAACATCTACACAATATCCATAACCA-3'	
P'21	5'-TATCAGACTGATGTTGAGGAG-3'	
P'122	5'-GGTGTGGAGTGTGACAATGGT-3	
miR-21-DNA	5'-TAGCTTATCAGACTGATGTTGA-3'	
miR-122-DNA	5'-TGGAGTGTGACAATGGTGTTTG-3'	
A'-21	5'-TGTAGATGTTGTATGTATCAGACTGATGTT GAGGAGTG-3'	3'-Cy3
A'-122	5'-GAGGTGTGGAGTGTGACAATGGTTGGTT ATGGATATTG-3'	5'-Texas Red

S3 Supplementary Figures

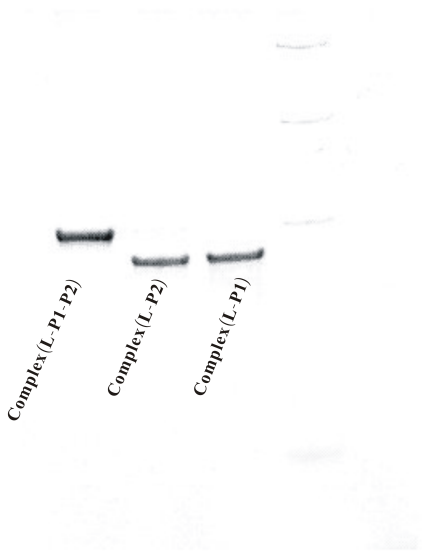

Fig. S1. Purity test of the complex (L-P1-P2). Native polyacrylamide gel was used to test the purity of complex. L-P1-P2 was annealed at a concentration of 3 μM, as well as incomplete complex, L-P1 and L-P2. They were loaded onto a 15% native PAGE gel and run at 120 V for 120 min.

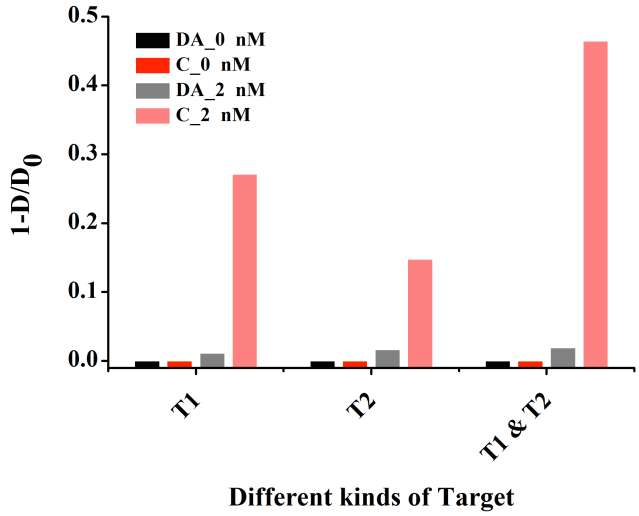

Fig. S2. Comparison of direct annealing with our design. The reaction efficiency value compares of 1-D/D_0 at 2 nM Targets. DA represents Direct Annealing and C is Catalysis which is our design. D_0 is the initial fluorescence intensity of donor and D is the intensity in the presence of targets.

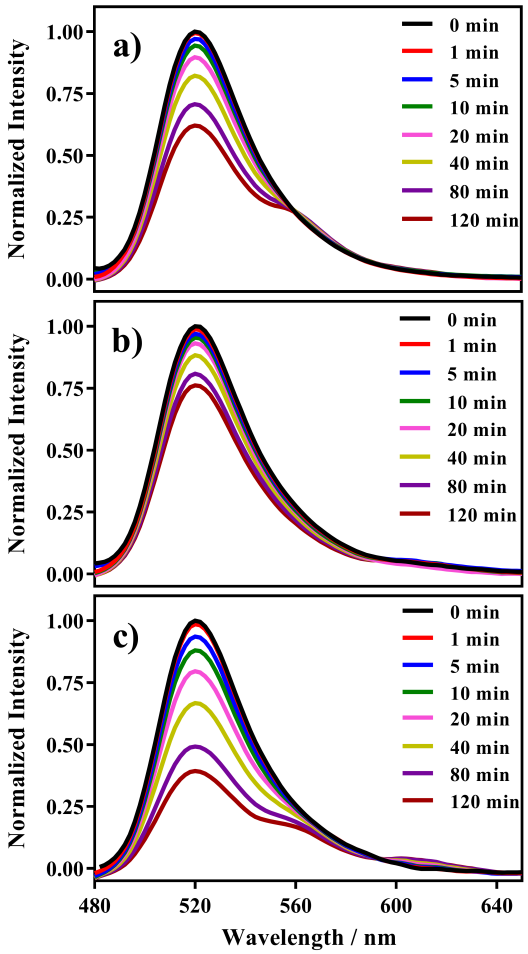

Fig. S3. The fluorescence spectra got from time dependent experiments. **a**, [T1]=2 nM, and the fluorescence signals were obtained at different time points until 120 min. **b**, [T2]=2 nM. **c**, [T1]=2 nM and [T2]=2 nM.

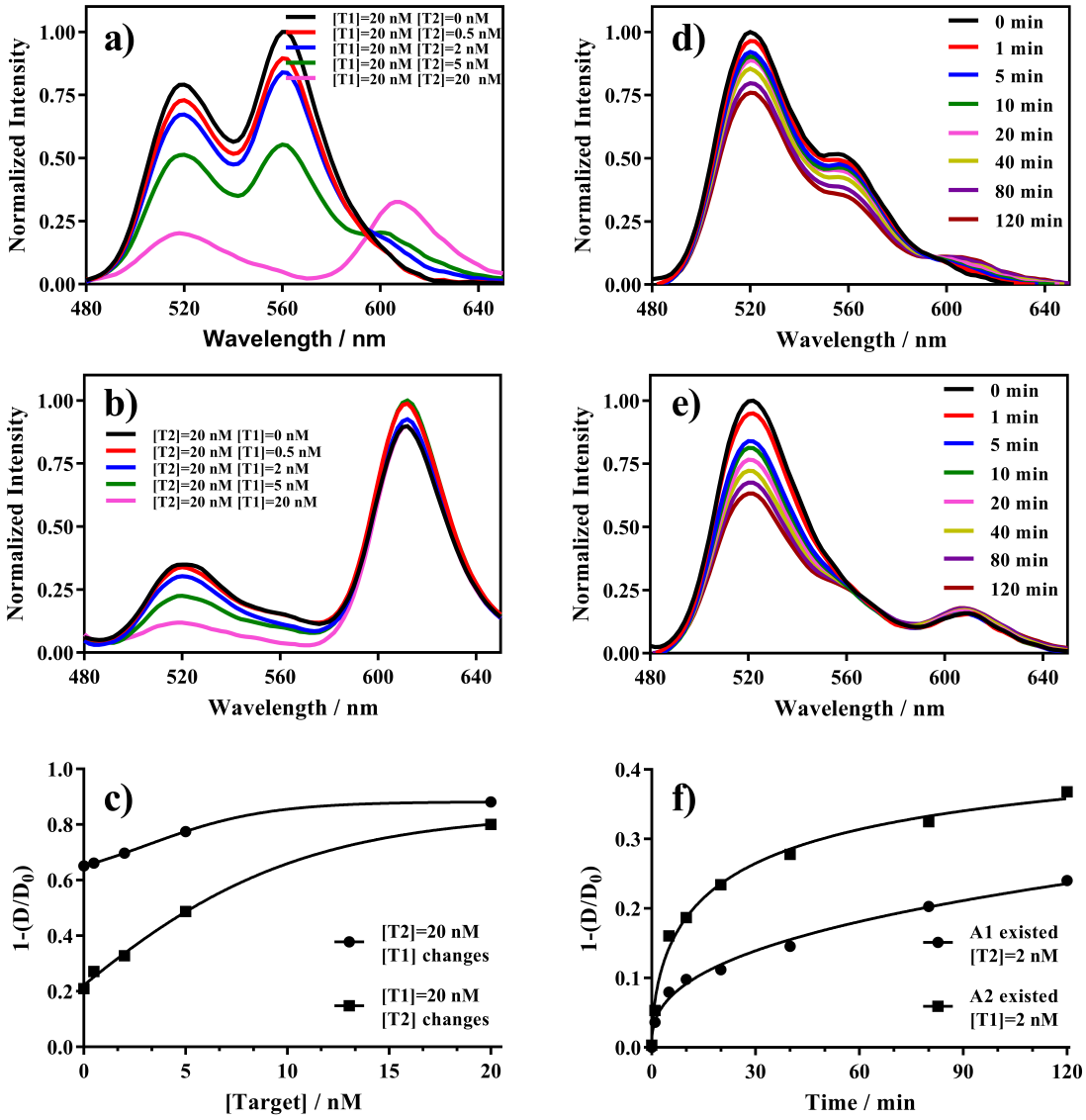

Fig. S4. Target concentration and Reaction time investigation when one kind of target existing. a-b and d-e, The fluorescence spectra of one kind of target input with the maximum effect from another target which has already existed. **a**, [T1]=20 nM, T2 was added with different concentration changing up to 20 nM. **b**, [T2]=20 nM, T1 was added with different concentration changing up to 20 nM. **c**, The reaction efficiency value changes of 1-D/D_0 with different concentration of targets. **d**, A1 already existed, 2 nM T2 was added and the FRET spectra were recorded by time. **e**, A2 already existed, 2 nM T1 was added and the FRET spectra were recorded by time. **f**, The reaction efficiency value changes of 1-D/D_0 by time at 2 nM target with another target already existing.

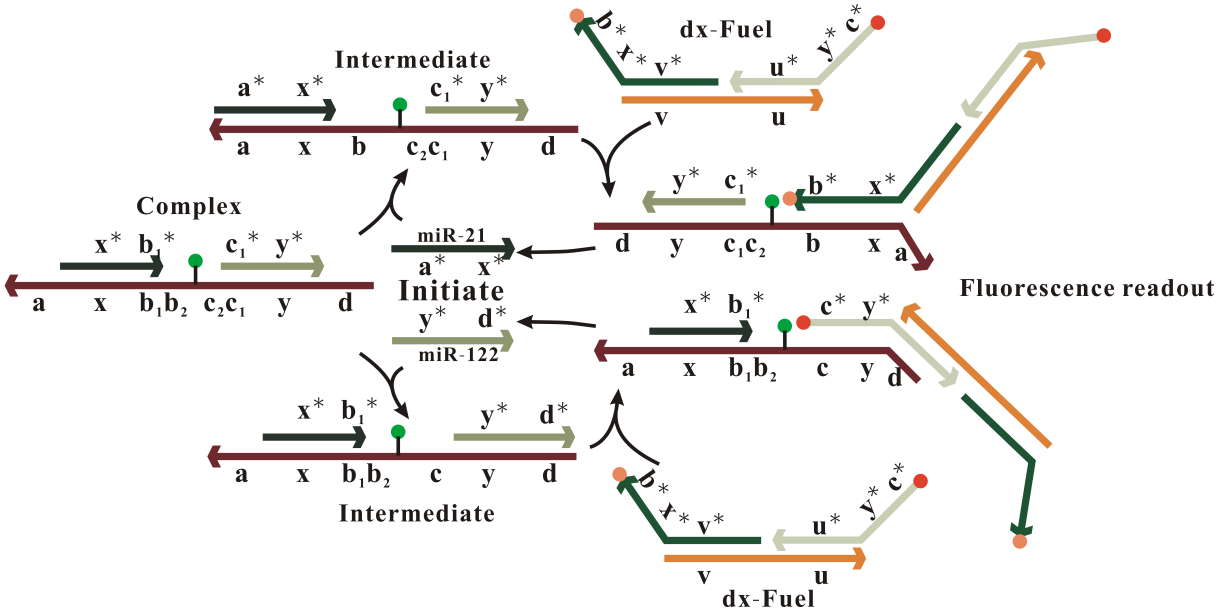

Fig. S5. Schematic representation of modified DNA photonic nanowire network based on the toehold-mediated DNA strand displacement reactions. Each letter-labelled segment is different part of domains. Starred domains are complementary to unstarred domains.(e.g. a* is complementary to a).

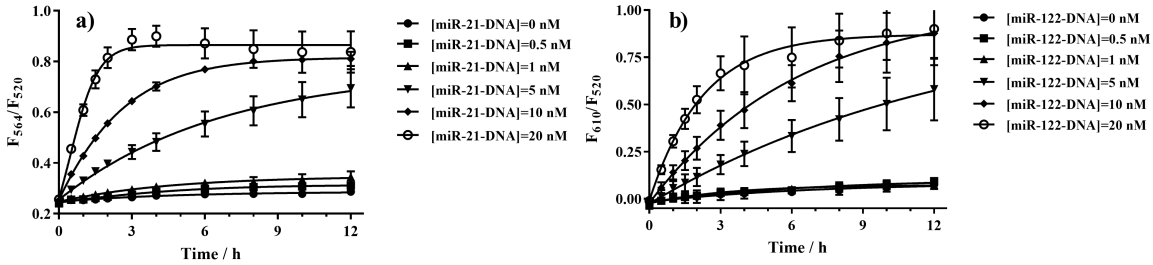

Fig. S6. Functional examination of Modified DNA photonic nanowire system. a, Kinetics of modified DNA photonic nanowire systems with different concentration of miR-21-DNA. **b**, Kinetics of modified DNA photonic nanowire systems with different concentration of miR-122-DNA. The reaction efficiency is shown by the fluorescence intensity ratio of acceptor to donor, (*e.g.* F_{564}/F_{520} represents the ratio of acceptor-Cy3 to donor-FAM).

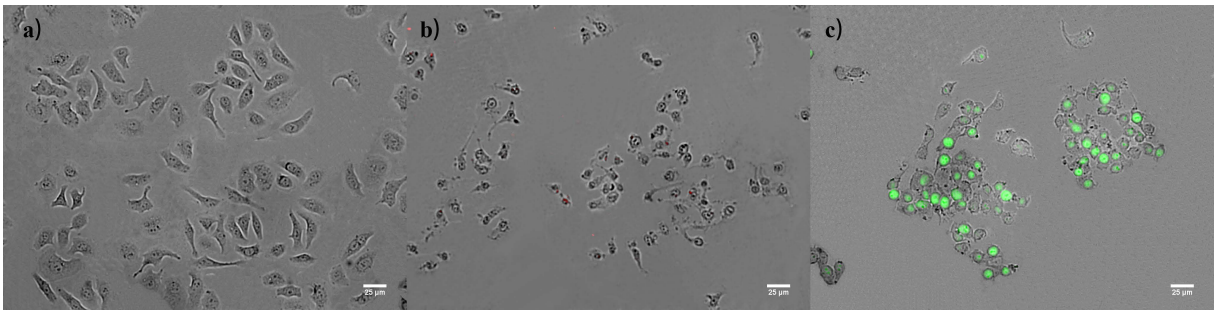

Fig. S7. Comparison of single-strand and duplex with certain amount of transfection reagent in Hela cells. a, Only transfection reagent. **b**, 200 nM single-strand modified by Texas Red (A'-122) was transfected into Hela cells and incubated for 4 h. The images were taken by fluorescence microscope with yellow-light excitation. **c**, 200 nM duplex modified by FAM (L'-P'21-P'122) was transfected into Hela cells and incubated for 4 h. The images were taken by fluorescence microscope with blue-light excitation.

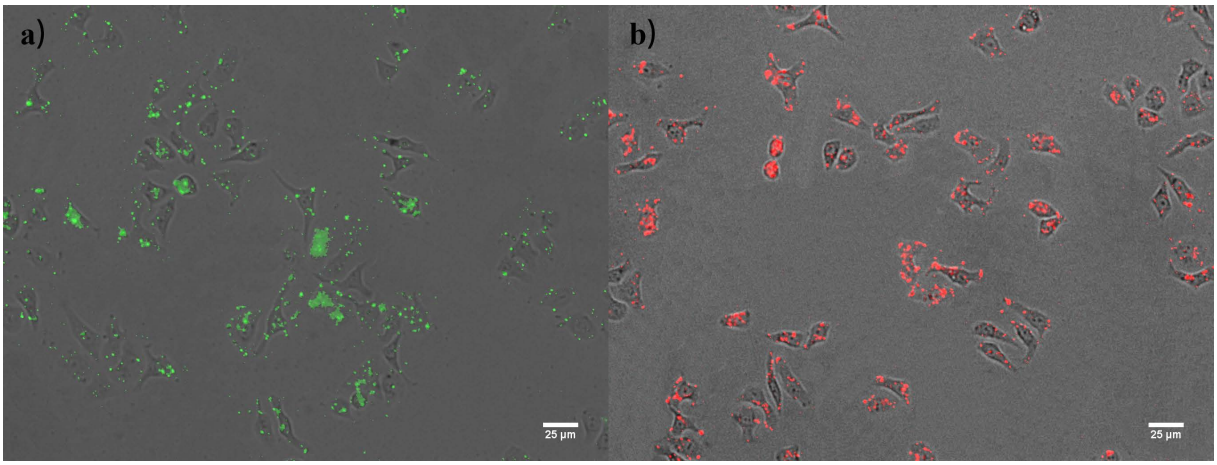

Fig. S8. Examination for the amount of transfection reagent. **a**, 200 nM complex was transfected into Hela cells with 1 uL transfection reagent. The images were taken by fluorescence microscope with blue-light excitation. **b**, 200 nM dx-Fuel was transfected into Hela cells with 1 uL transfection reagent. The images were taken by fluorescence microscope with yellow-light excitation.

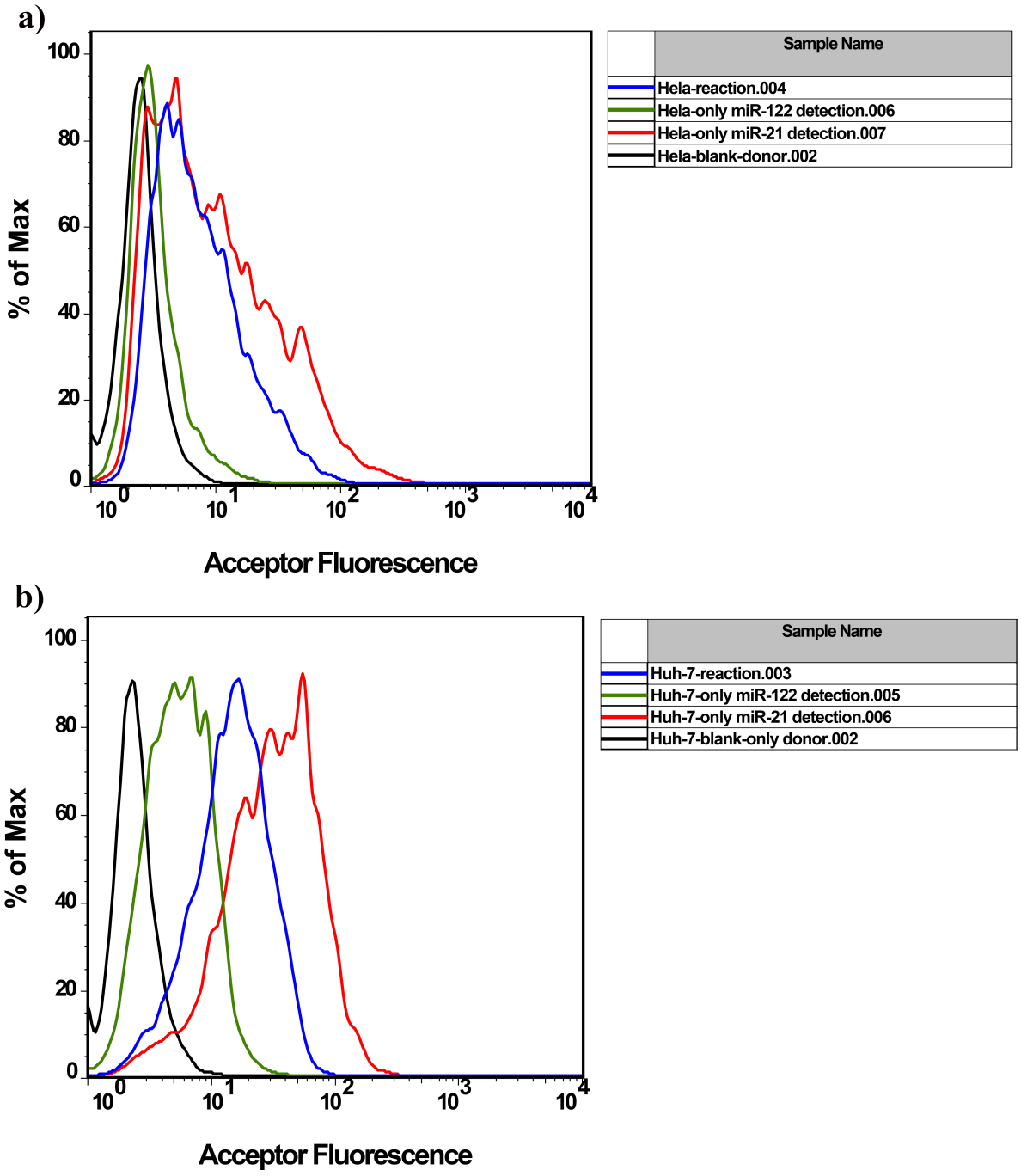

Fig. S9. Representative Flow Cytometry spectra for HEK-293T, Hela and Huh-7 cells with different treatment. a, Hela cells were incubated with 4 different kinds of system. Hela-only donor system only had complex and a double-stranded fuel without fluorophore incubating with Hela cells. Hela-only miR-21 detection was the system of dx-fuel modified with only A-21' and another complementary strand without fluorophore. And in Hela-only miR-122 detection, A-122' was existed and A-21' was replaced by a strand with same sequence but no fluorophore. By contrast, Hela-reaction was the system with complex and normal dx-Fuel. **b**, Similar to Hela cells, Huh-7 cells were also treated with only-donor, miR-21 detection and miR-122 detection systems, as well as normal reaction system.