

Electronic Supporting Information

Hybridization Chain Reaction-Based Nanoprobe for Cancer Cell

Recognition and Amplified Photodynamic Therapy

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Experimental Section

Chemicals and Materials

All the DNA oligonucleotides (Table S1, Supporting Information) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China), the amine-modified DNAs were labeled with Ce6 and purified by HPLC (Agilent Model 1260 HPLC system). A solution of 0.1 M triethylamine acetate (pH 6.5) was used as HPLC buffer A, and HPLC-grade acetonitrile (Sigma Aldrich) was used as HPLC buffer B. The graphene oxide (GO) was bought from XFNANO Materials Tech Co., Ltd. (Nanjing, China), then the GO nanosheets (1 mg/mL) was obtained through ultrasound according to the instruction from the manufacturer. Unless otherwise specified, all other reagents used

in this work were of analytical grade, commercially purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and used without further treatment.

Preparation of HCR/GO mixture

All the hairpin DNAs were annealed to form a stable hairpin structure. Then the HCR/GO mixture was readily prepared by incubating hairpin DNAs with GO nanosheets in 1X PBS buffer (0.75 M NaCl and 50 mM Na₂HPO₄, pH 7.4) at room temperature for 30 min. Then the mixture was centrifuged at 10000 rpm for 10 min to remove the excess DNA. According to the result of Figure S3, 500 nM rH₁ and H₂ were incubated with 500 µg/mL GO to prepare the final mixture, and the concentration of GO was employed to represent the concentration of the mixture.

Hybridization Chain Reaction

The hybridization chain reaction (HCR) was conducted in 100 µL reaction solution containing 25 µg/mL HCR/GO mixture, 1X PBS buffer (0.75 M NaCl and 50 mM Na₂HPO₄, pH 7.4), and target DNA at different concentrations. After incubation for 6 h at 37°C, the solution was used for analysis.

Gel Electrophoresis Analysis.

Reaction mixtures after HCR were finally quantified in a volume of 10 µL for electrophoresis experiments directly. The gels of 4% agarose were stained with ethidium bromide (EB). Electrophoresis was carried out in 1X Tris-borate-EDTA

(TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 110 V.

The gels were imaged and analyzed using a Bio-Rad ChemiDoc XRS System.

Fluorescence Measurements

The fluorescence measurements were conducted on a Fluoromax-4 spectrofluorometer (HORIBA JobinYvon, Edison, NJ) at room temperature. For all fluorescence detection, the excitation wavelength was set at 495 nm (excitation wavelength of FAM) with a 2 nm slit width.

Cell culture

The HeLa cells (human cervical carcinoma cell line) and HEK-293 (human embryonic kidney cell line) were cultured in DMEM medium with the addition of 10% FBS (fetal bovine serum, Invitrogen, Carlsbad, CA, USA) and 0.5 mg/mL penicillin-streptomycin (KeyGEN Biotech, Nanjing, China) in a 5% CO₂ environment at 37 °C.

Confocal laser scanning microscopy (CLSM) analysis

1×10⁵ HeLa or HEK-293 cells were seeded in a 35-mm confocal laser culture dish for 24 h. The cells were washed 3 times with DPBS buffer, then incubated with 1 mL medium contains 25 μg/mL HCR/GO mixture for 2h in the incubator. Then the cells were washed for another 3 times to remove the excess mixture and incubated in the whole medium for 6 hours. Then the cells were stained with 1μM lysotracker green

for 20 min. After changing the medium to DPBS, the samples were analyzed using a Zeiss LSM 880 high-resolution confocal microscope at 63× magnification. Cy3 and lysotracker green were chosen as the excitation channels (laser at 488 nm and 543 nm). The green signal was collected from 493 to 556 nm and the red signal was collected from 550 to 728 nm.

Flow Cytometry

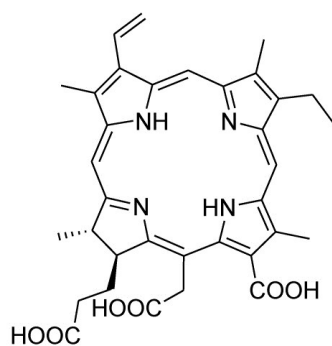
2×10^5 HeLa or HEK-293 cells were seeded in 6 wells plate 24 h prior to the experiment. The cells were washed 3 times with DPBS buffer, then incubated with 1 mL medium contains 25 $\mu\text{g}/\text{mL}$ HCR/GO mixture for 2h in the incubator. Then the cells were washed for another 3 times to remove the excess mixture and incubated in the whole medium for 6 hours. For flow cytometry, the cells were washed with DPBS and detached by 0.05% trypsin. The suspended cells were centrifuged and dispersed in PBS for flow cytometry. Flow cytometry was obtained using a BD FACSVerser system by counting 10,000 cells per sample (FITC channel).

Phototoxicity study

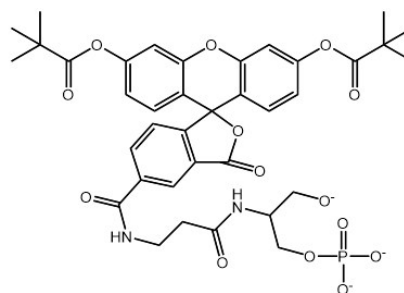
HeLa or HEK-293 cells were seeded in a 96-well plate at a density of 4×10^3 cells per well and incubated for 24 h. After removing the culture medium, cells were incubated with different concentrations of Ce6-HCR/GO or Ce6-rH₁/GO nanosystem, at 37 °C for 4 h. Then the culture medium was replaced with 200 μL of fresh culture medium. Prior to the irradiation, the cells were incubated in dark for another 3h. For PDT, cells were irradiated with white light (36 watts) for different durations. After 24 h

incubation, the cell medium was replaced with 100 μL of fresh culture medium and 20 μL of MTS solution. After a 20-min incubation, cell viability of cells after PDT was assayed by measuring the absorbance at 490 nm, using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT).

Structures of Ce6 and FAM



Chlorin e6 – Ce6



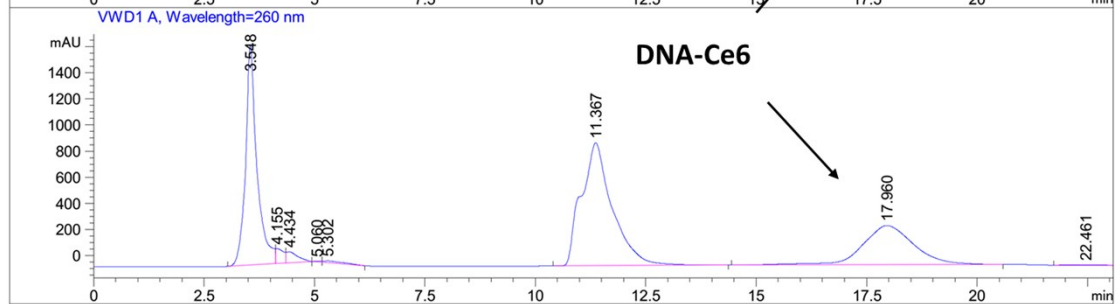
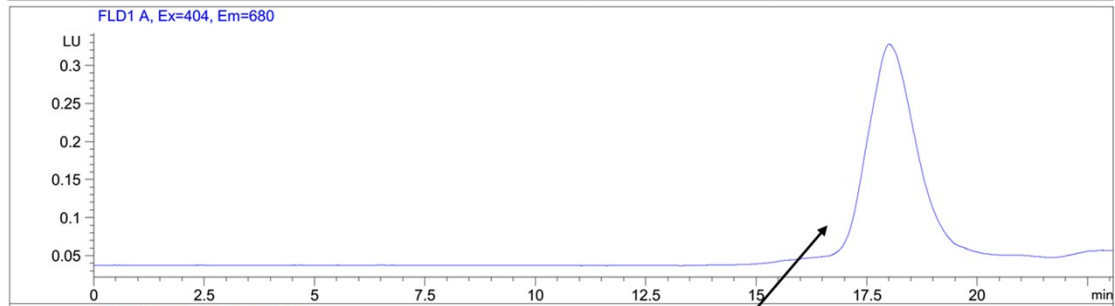
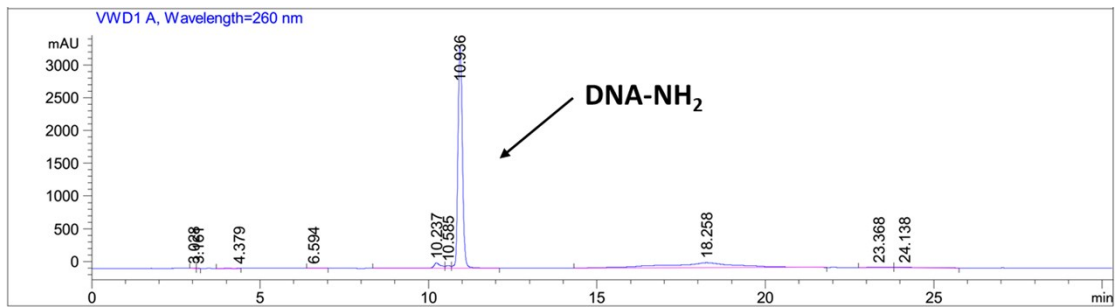
FAM

Table S1. The oligonucleotides sequences.c

Name	Sequence
mMTH1 Target	5' - GAA GTA CCC GTG GAA TTT C - 3'
rH₁	5' - FAM-AGA AGA AAT TCC ACG GGT ACT TCA ACG GCC TGA AGT ACC CGT GGA ATT -3'
H₂	5' - FAM-TGA AGT ACC CGT GGA ATT TCT TCT AAT TCC ACG GGT ACT TCA GGC CGT- 3'
rH₁-Cy3	5' - AGA AGA AAT TCC ACG GGT ACT TCA ACG GCC TGA AGT ACC CGT GGA ATT-Cy3 -3'
H₂-Cy3	5' - TGA AGT ACC CGT GGA ATT TCT TCT AAT TCC ACG GGT ACT TCA GGC CGT-Cy3- 3'
rH₁-NH₂	5' - H₂N-AGA AGA AAT TCC ACG GGT ACT TCA ACG GCC TGA AGT ACC CGT GGA ATT -3'
H₂-NH₂	5' - H₂N-TGA AGT ACC CGT GGA ATT TCT TCT AAT TCC ACG GGT ACT TCA GGC CGT- 3'
rH₁-Ce6	5' - Ce6-AGA AGA AAT TCC ACG GGT ACT TCA ACG GCC TGA AGT ACC CGT GGA ATT- 3'
H₂-Ce6	5' - Ce6-TGA AGT ACC CGT GGA ATT TCT TCT AAT TCC ACG GGT ACT TCA GGC CGT- 3'

Synthesis of Photosensitizer labeled DNA (Ce6-DNA)

The Ce6 labeled DNA was synthesized according to our previous method with slightly modification.¹ To a 2ml PE tube, 5.97mg Ce6 (10 μ mole), 2.06mg Dicyclohexylcarbodiimide (DCC) and 1.16mg N-Hydroxysuccinimide (NHS) was dissolved in 250 μ L DMF for activation reaction with 1-h stirring at 37 $^{\circ}$ C. Then, 10 OD DNA was dissolved in 250 μ L NaHCO₃ solution (0.1 M) and mixed with the activated Ce6 for coupling with a strong stirring overnight at 37 $^{\circ}$ C. After the reaction, 50 μ L NaCl (3M) and 1.25 mL cold ethanol were added for precipitation at -20 $^{\circ}$ C for 2h. Then the precipitated oligonucleotides were collected by centrifugation, dissolved in 0.1 M triethylammonium acetate (TEAA), and then purified by HPLC (Agilent Model 1260 HPLC system, Alltech C18 column) using acetonitrile and 0.1 M TEAA buffer and acetonitrile (Sigma Aldrich) as the eluents. After drying, the collected Ce6-labeled DNA product was dissolved in sterile water, quantified by determining the UV absorption at 260 nm, and stored in the freezer at -20 $^{\circ}$ C for future experiments. The result of purification from HPLC was shown as follows. The UV-vis absorption of oligonucleotides (at 260 nm) and fluorescence signal of Ce6 (Em = 680 nm) were used to verify the structure of all sequences. Before the reaction, DNA-NH₂ showed a UV-vis absorption (\sim 260 nm, absorption of DNA oligonucleotides) at \sim 12 min only. However, a new DNA absorption peak appeared at \sim 17.5 min and simultaneously a fluorescence emission of Ce6 was observed, which demonstrated the successfully synthesized of Ce6-DNA.²



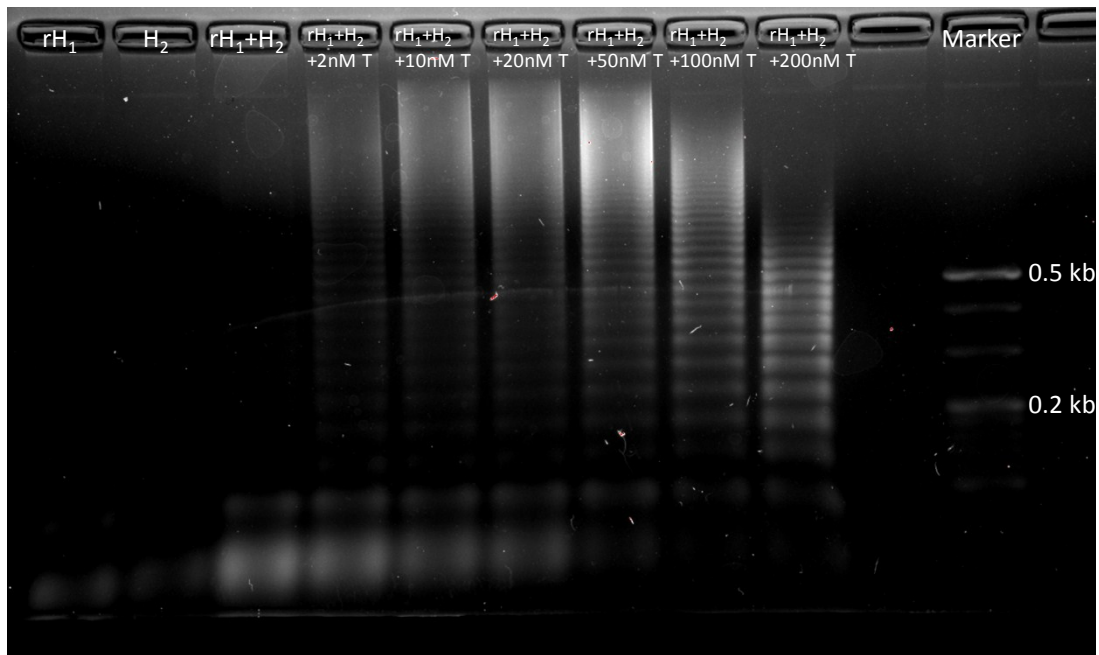


Figure S1. The gel electrophoresis analysis of the HCR when the concentration of mMTH1 target (T) ranged from 0 nM to 200 nM. 1 μ M rH₁ and 1 μ M H₂ were incubated with the mMTH1 target in 1X PBS buffer for 6 hours at 37°C, then 4% agarose gel contained ethidium bromide was used to run the samples.

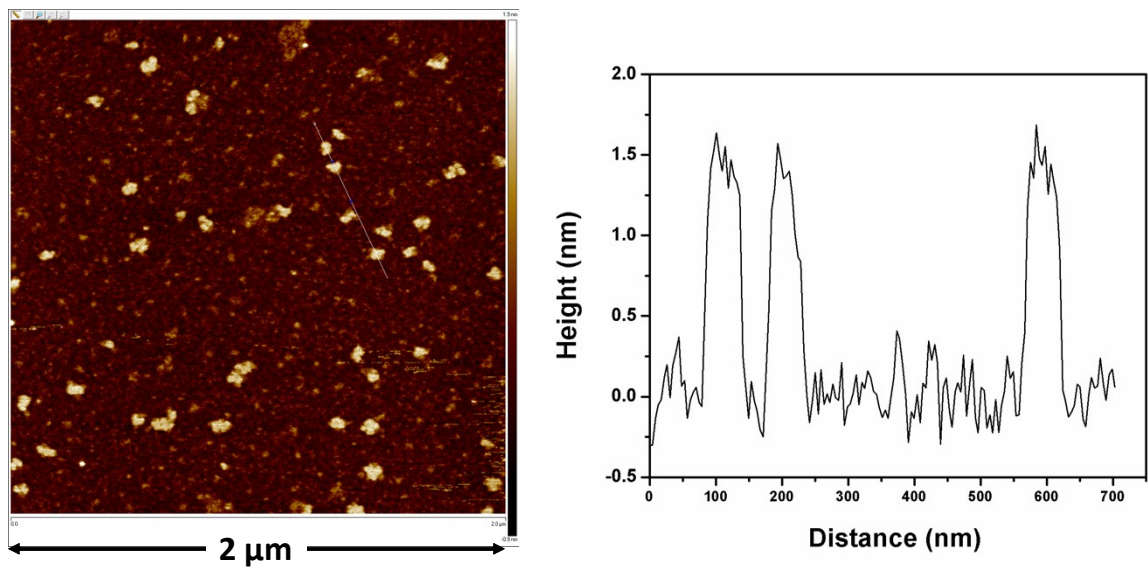


Figure S2. AFM image of graphene oxide nanosheets.

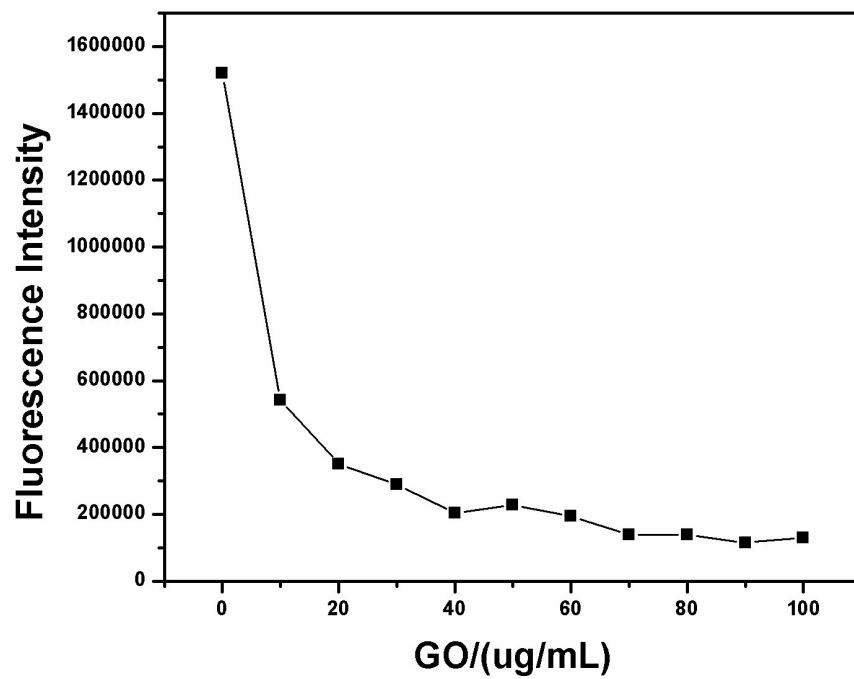


Figure S3. Quenching effect of GO at various concentrations on the fluorescence of rH1/H2. The concentration of rH1/H2 is 10 nM. GO concentrations ranged from 0 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$.

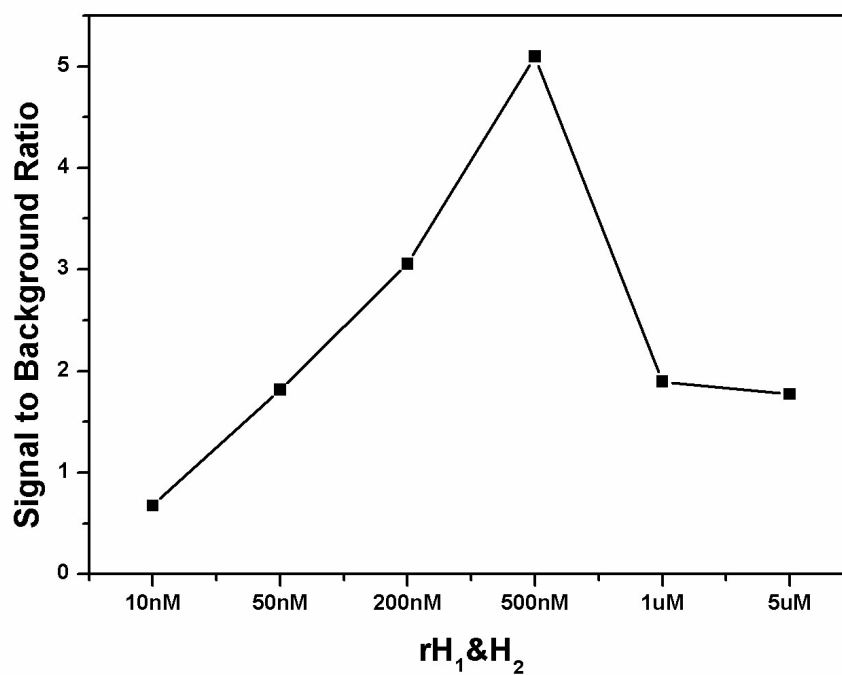


Figure S3. Signal to background ratio of the HCR product after incubation with 50nM or 0nM mMTH1 under different concentration of rH₁/H₂ probes. The concentrations of rH₁ and H₂ are each 10, 50, 200, 500, 1000, 5000 nM. The concentration of GO is 500 μ g/mL.

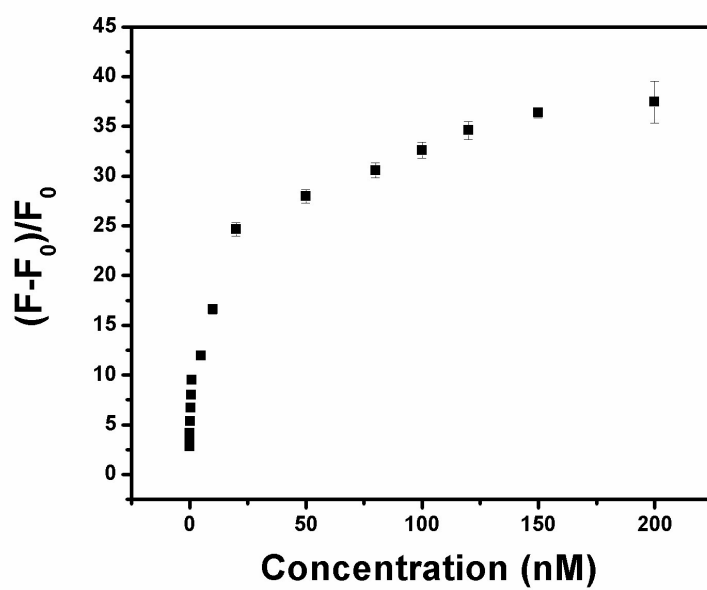


Figure S4. The plot of the ratio for fluorescence enhancement at 518 nm after addition of target at different concentration.

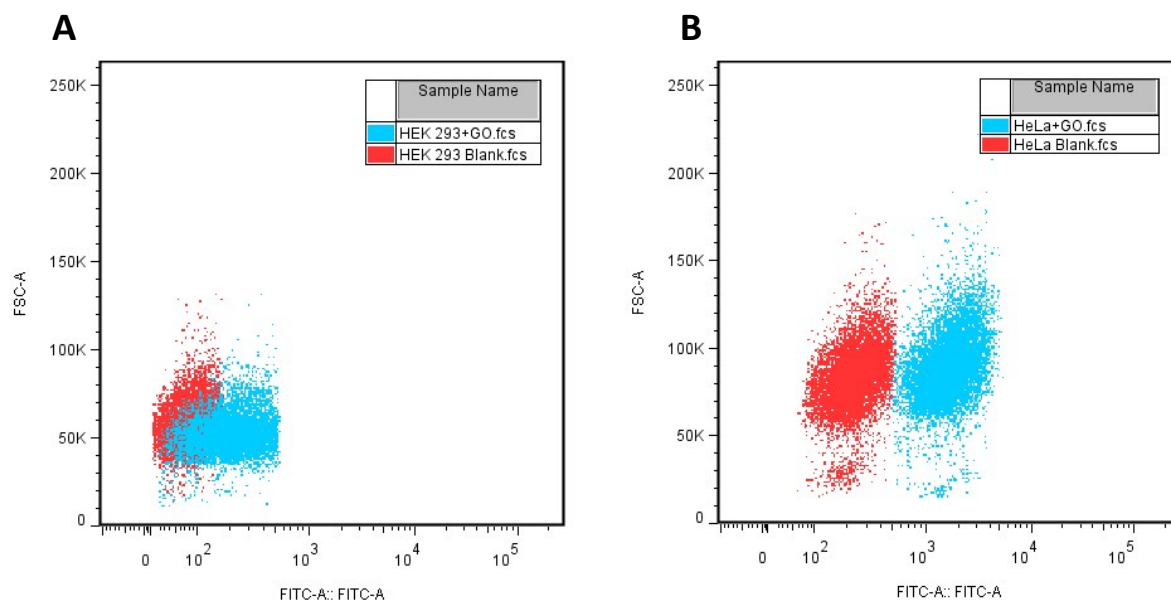


Figure S5. The fluorescent intensity of (A) HEK-293 and (B) HeLa cells when the GO/HCR nanosystem was applied.

References

1. H. Fan, G. Yan, Z. Zhao, X. Hu, W. Zhang, H. Liu, X. Fu, T. Fu, X. B. Zhang and W. Tan, *Angew. Chem. Int. Ed.*, 2016, **55**, 5477.
2. M. Xiong, H. Zhu, Q. Rong, C. Yang, L. Qiu, X. B. Zhang and W. Tan, *Chem Commun*, 2016, **52**, 4679.