Supporting Information for

Endogenous Glutathione-Activated DNA Nanoprobes for Spatially Controllable Imaging of MicroRNA in Living Cells

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Materials and methods

Materials

All chemicals were acquired without further purification. Zirconium chloride, anhydrous ($ZrCl_4$) was purchased from Innochem. N,N-dimethyformamide (DMF), and Methanol absolute were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. 2-Aminoterephthalic Acid (BDC-NH_2) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Acetic acid and triethylamine were purchased from Kmart (Tianjin) Chemical Technology Co., Ltd. Ethanol absolute was purchased Tianjin Yuanli Chemical Co., Ltd. Dulbecco's modified eagle medium (DMEM), Penicillin-Streptomycin (P/S), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution Gentamycin sulfate (50 mg/mL), 4',6-diamidino-2-phenylindole (DAPI) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Fetal bovine serum (FBS) was purchased from Melone Pharmaceutical Co., Ltd. Glutathione (GSH), Piperazine-1-ethane sulfonic acid (HEPES) and all oligonucleotides (Table S1) were purchased from Sangon Biotech (Shanghai) Co., Ltd.

Instrumentation: Transmission electron microscopy (TEM) measurements were measured on HITACHI H-8100 EM and JEOL JEM-2100 F. Scanning electron microscope (SEM) images were performed on a Hitachi SU8010 scanning electron microscope (JPN). Crystalline patterns of the materials were identified by X-ray diffraction (XRD) using Cu Kα radiation (λ =1.5418 Å) (X'Pert PRO MPD). The Zeta potential of the sample was measured on a nano-particle size and Zeta potential analyzer (Zetasizer Nano ZS90, Malvern Panalytical). Fourier infrared spectra (FTIR) were recorded on Nicolet is5 (Thermo, USA). FS05 steady-state transient fluorescence spectrometer (Thermo Field, USA) was used for fluorescence analysis. The absorbance intensity was acquired by a Biotech SYNERGY H1 microplate reader (Bio Tek, USA). Confocal microscopic images were acquired using a Nikon $A1R^+$ confocal microscope.

Pretreatment of HP-SS-BT.

2 μM of HP-SS-BT in HEPES buffer (10 mM, 20 mM $MgCl_2$, pH = 7.2) was annealed at 95 ℃ for 5 min and then cooled down to 4 ℃, followed by equilibrium at 25 ℃ for 2 hours.

Preparation of UiO66-NH_2.

2-Aminoterephthalic Acid (0.0543 g) was dissolved in 5 mL of DMF. Then 30 μL of triethylamine was added. The mixture was labeled as solution A. $ZrCl_4$ (0.0668 g) was dissolved in 5 mL of DMF as solution B. Acetic acid (1.38 mL) was dissolved in 5 mL of DMF as solution C. Subsequently, A, B, and C were mixed in a reactor and reacted at 85 ℃ for 24 h. Then UiO66-NH_2 was collected by centrifugation and washed with DMF, CH_3OH, and H_2O, respectively. UiO66-NH_2 was dispersed in H_2O for further use.

Synthesis of the HP-SS-BT@UiO66-NH_2.

Briefly, HP-SS-BT (200 nM), 1 ×HEPES buffer (10 mM HEPES, and 150 mM NaCl, pH = 7.2, 1 mL) were mixed with 100 μg/mL of UiO66-NH_2 and reacted at room temperature overnight. This reaction system was centrifuged and washed with 1 ×HEPES buffer three times. The obtained HP-SS-BT@UiO66-NH_2 nanoprobe was stored in 100 μL 1 ×HEPES buffer (10 mM HEPES, and 150 mM NaCl, pH = 7.2) at 4 ℃.

Loading efficiency of HP-SS-TAMRA.

We conducted a quantitative measurement of the load efficiency (LE) of DNA hairpin probes on MOF, using unquenched HP-SS-TAMRA. Specifically, we dispersed 100 μg/mL MOF in HEPES (pH 7.4) and incubated it with 50 nM HP-SS-TAMRA. The resulting HP-SS-TAMRA@UiO66-NH2 suspension was then centrifuged at 15000 rpm for 8 minutes, and the amount of free HP-SS-TAMRA in the supernatant was determined using fluorimetry, based on the standard curve (TAMRA, λex = 555 nm, λem = 580 nm).

The loading efficiency was calculated using the formula: LE (%)=(HP-SS-TAMRA$_{initial}$ - HP-SS-TAMRA$_{residual}$)/HP-SS-TAMRA$_{initial}$, where HP-SS-TAMRA$_{initial}$ represents the total content of HP-SS-TAMRA before the addition of MOF, and HP-SS-TAMRA$_{residual}$ represents the amount of HP-SS-TAMRA in the supernatant obtained by centrifugation after incubation with MOF.

Cell culture: MCF-7cells (human breast cancer cells) and HEK293 cells (human embryonic kidney cells) were cultured in Dulbecco's Modified Eagle Medium (H) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 1% (v/v) Penicillin-Streptomycin (P/S) and 0.1% (v/v) gentamycin sulfate at 37 °C in a humidified 5% CO_2 atmosphere, HeLa cells (a human lung cancer cells) were cultured in F12 supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 1% (v/v) Penicillin-Streptomycin (P/S) and 0.1% (v/v) gentamycin sulfate at 37 °C in a humidified 5% CO_2 atmosphere.

Cell cytotoxicity assay: To investigate the effect of the probe concentration on cell viability, different concentrations of probes (0.1, 0.25, 0.5, 0.8 and 1.0 mg/mL) were incubated with the HeLa cells for 24 h. After that, 100 μL of MTT solution (0.5 mg/mL, dissolved by PBS) was added into each well for another 4 h at 37 °C. Then added 100 μL DMSO and shake for 10 min. Finally, the absorbance value at 490 nm was measured.

Intracellular uptake of the probe: HeLa cells were first seeded into 6-well plates and incubated at 37 ℃ for 12 h. Then, 1 mL of 20 μg/mL probe was added to it. After incubation for different times, the cells were washed three times with PBS and fixed with a 4% fixative solution (paraformaldehyde) for 20 min. Following this, the cells were washed three times with PBS and the cell nuclei were stained with DAPI (1 μg/mL). After 15 min, the cells were washed three with PBS and the images were recorded by confocal microscopy.

Supporting tables and figures

Table S1. Sequences of the nucleic acids used in this work.

Name	Sequence (5′-3′)
HP-SS-BT	TAMRA-CGCGTAGCTTATCAGACTGACTCTAT/iHS-SH/TCAACATCAGTCTGATAAGCTACGCG-BHQ2
HP-SS-TAMRA	TAMRA-CGCGTAGCTTATCAGACTGACTCTAT TCAACATCAGTCTGATAAGCTACGCG
DNA-21	TAGCTTATCAGACTGATGTTGA
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA-197	UUC ACC ACC UUC UCC ACC CAG C
let-7a	UGA GGU AGU AGG UUG UAU AGU U

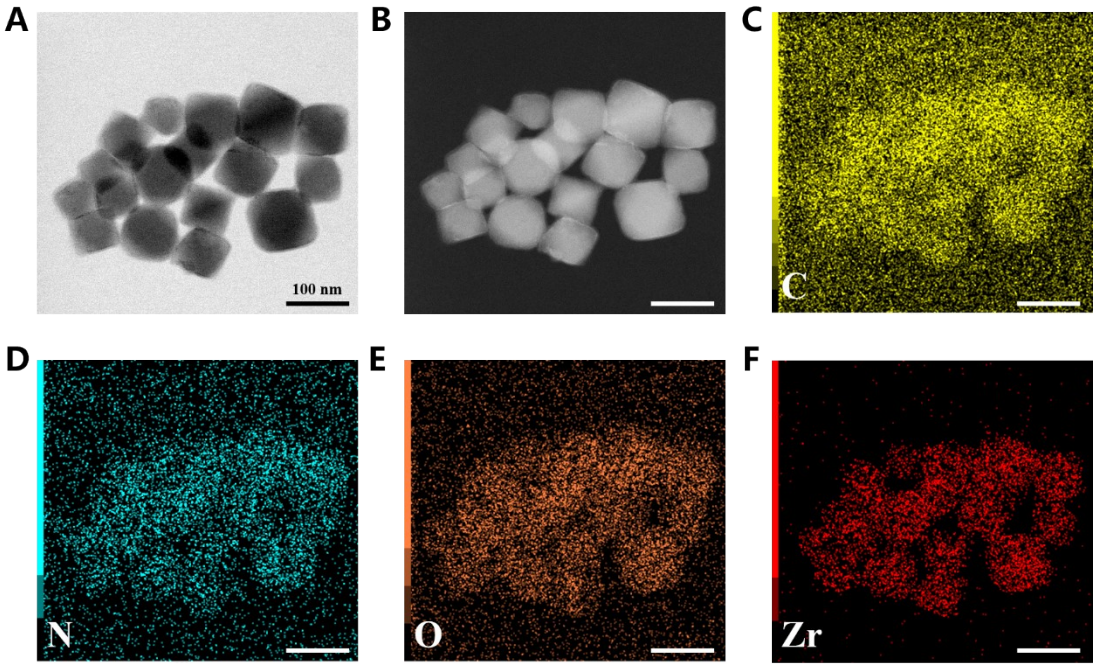

Figure S1.TEM images of (A) UiO66-NH2 and (B-F) Energy-dispersive spectroscopy mappings images of C, N, O, and Zr in UiO66-NH_2. Scale bar is 100 nm.

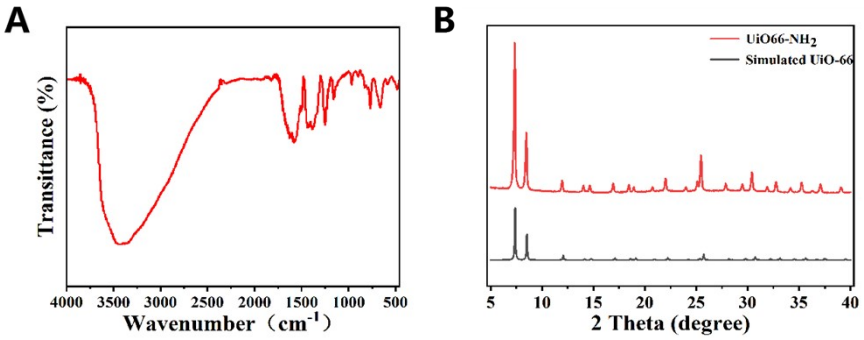

Figure S2. (A) XRD spectra of UiO66-NH_2. (B) FTIR spectra of UiO66-NH_2.

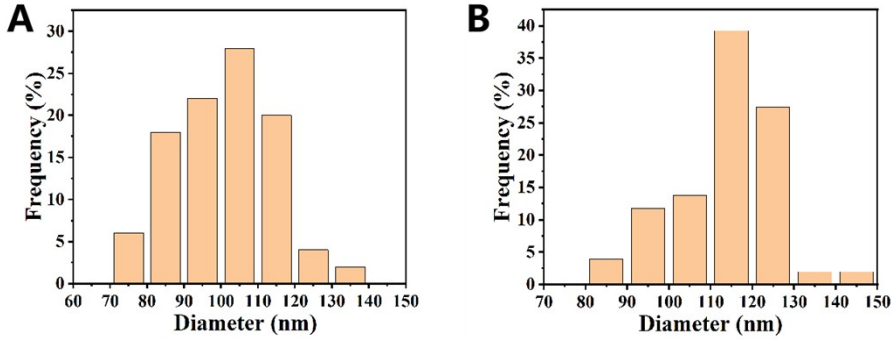

Figure S3. Size distribution of UiO66-NH_2 (A) and HP-SS-BT@UiO66-NH_2 (B).

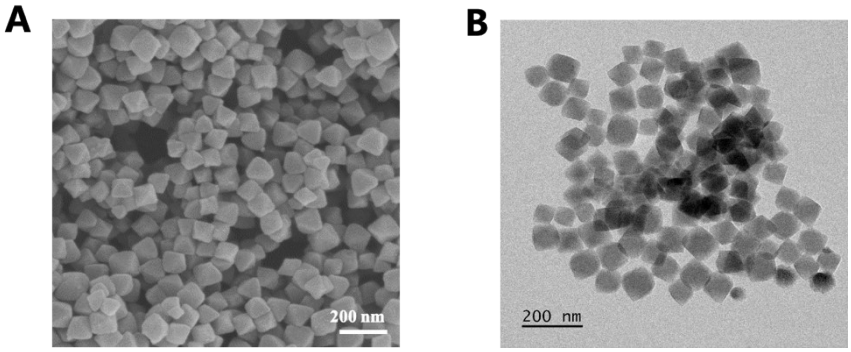

Figure S4. (A) SEM image of HP-SS-BT@UiO66-NH_2; (B) TEM image of HP-SS-BT@UiO66-NH_2.

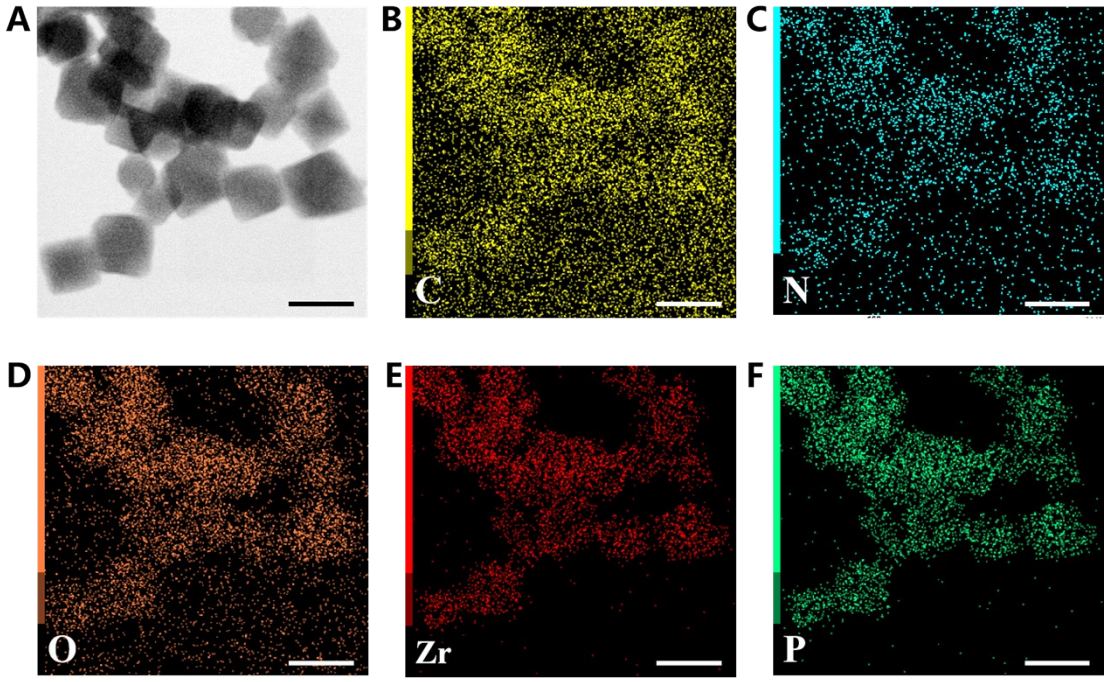

Figure S5.TEM image of (A) HP-SS-BT@UiO66-NH_2 and (B-F) Energy-dispersive spectroscopy mapping images of C, N, O, Zr, and P in HP-SS-BT@UiO66-NH_2. Scale bar is 100 nm.

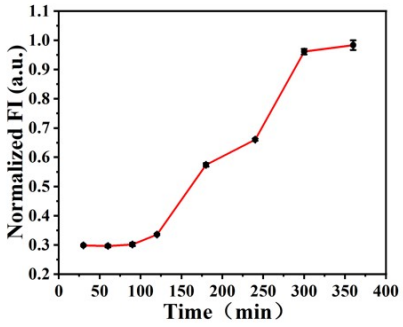

Figure S6. Time-dependent fluorescence spectral changes of HP-SS-BT with 5nM miRNA-21 and 5mM GSH.

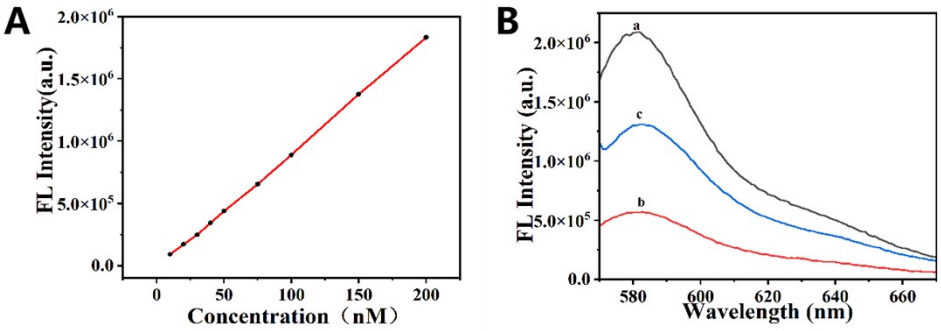

Figure S7. (A) Standard linear calibration curves of HP-SS-TAMRA; (B) Fluorescence spectra of HP-SS-TAMRA (a), HP-SS-TAMRA@UiO66-NH_2 (b) and HP-SS-TAMRA@UiO66-NH_2 in PBS (c).

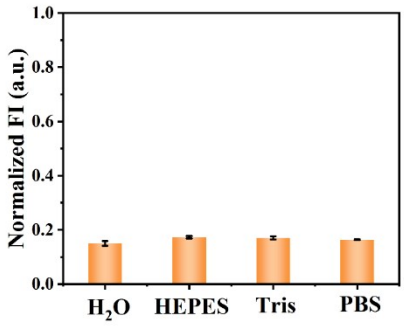

Figure S8. The relative fluorescence intensity changes of HP-SS-BT nanoprobe in different buffers.

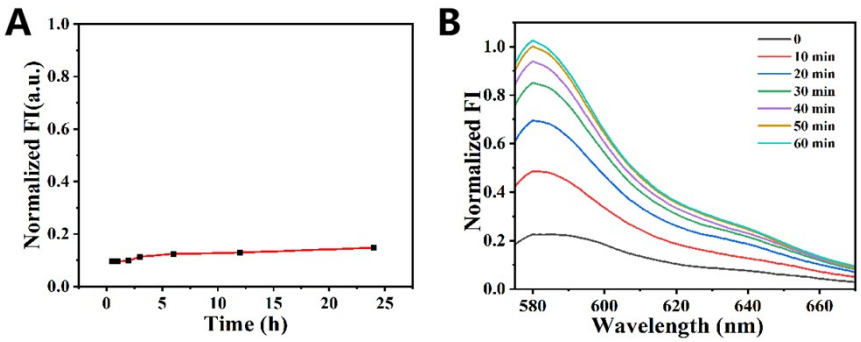

Figure S9. (A) The relative fluorescence intensity changes of the supernatant of HP-SS- TAMRA @UiO66-NH_2 in H_2O for different times; (B) Fluorescence spectra of HP-SS- TAMRA @UiO66-NH_2 in PBS for different times.

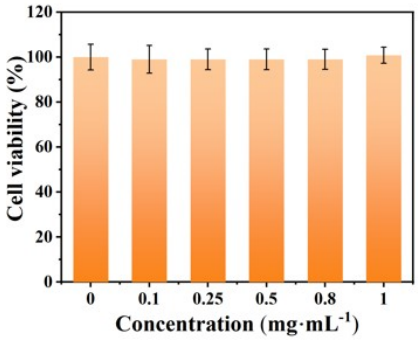

Figure S10. MTT assay of HeLa cells treated with the different concentrations of HP-SS-BT@UiO66-NH_2 for 24 h.

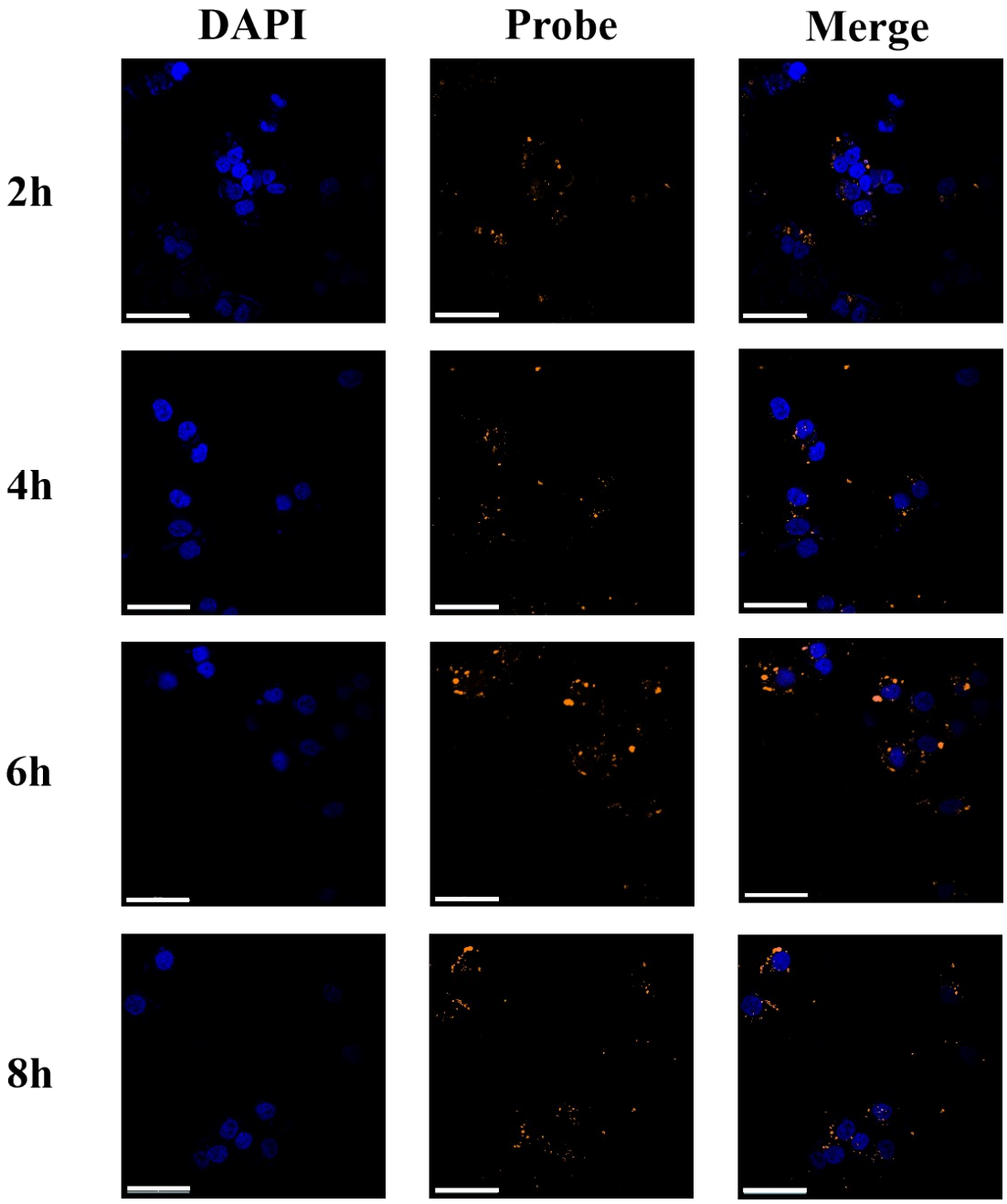

Figure S11. Confocal laser scanning microscopy (CLSM) images of HeLa cells after incubation with HP-SS-TAMRA@UiO66-NH_2 at different times at 37 °C. Scale bar: 50 μm.