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Supporting Information for

Programmable DNA Triple-Helix Molecular Switch in Biosensing: From Homogenous Solution to Living Cells

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EXPERIMENTAL SECTION

Materials and instruments. Oligonucleotides were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China) and the solution was prepared respectively with an 18 MΩ ultrapure water (sterile Millipore water). The concentration of the solution was calculated using UV-Vis absorption with reported sequence-specific absorption coefficients.[1] Unless stated, all chemicals were used as prepared. All cells were grown in DMEM supplemented with 12 % inactivated fetal bovine serum, 100 U/mL 1% penicillin and streptomycin solution and maintained in a humidified CO_2 incubator with 5% CO_2 at 37°C. The concentrations of the solution were estimated by UV-Vis absorption using published sequence-dependent absorption coefficients. Transmission electron microscopy images obtained on JEM-100CXII microscope (JEOL, Ltd., Japan). UV-Vis absorption spectra were recorded on a Hitachi U-4100 UV/Vis spectrophotometer (Kyoto, Japan). Fluorescence images of cells were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan).The pH was measured with a model 868 pH meter (Orion).

Preparation of A/G-Rich DNA Probe-Modified AuNPs. The 13 nm AuNPs were synthesized according to the previous report.[2] Briefly, 100 mL, 0.01 % chloroauric acid ($HAuCl_4$) was promoted to boiling, after that, 4.0 mL sodium citrate solution (1 %) was added quickly while stirring. Next, a color change from pale yellow to wine red appeared in solution. Finally, the solution was kept boiling for extra 10 min and then cooled to room temperature. The prepared AuNPs were stored at 4 °C for further use and the concentration was calculated by its extinction at 519 nm ($\varepsilon = 2.7 \times 10^8$ L mol^{-1} cm^{-1}). The synthesized AuNPs were then modified with A/G-rich DNA probe

on the basis of the reported methods.[3] Briefly, the A/G-rich DNA probe were incubated with the solution containing AuNPs for 12 h. Then, 2 M sodium chloride solution was added to the mixture solution drop by drop at every 6 h period while the final concentration was achieved to 0.3 M. Finally, the solution was centrifuged for 30 min (13000 g) and resuspended in 0.01 M phosphate buffer saline (0.1 M NaCl, pH 7.0) three times. Then the A/G-rich DNA probe-modified AuNPs were resuspended in 0.01 M phosphate buffer saline (PBS) buffer and stored at 4 °C in the dark.

Characterization of DNA Conjugated to the AuNPs. The conjugation of A/G-rich DNA probe on the AuNPs surface was calculated by UV-Vis absorption measurement. The maximal absorption peak (approximately at 260 nm) of the supernatant, with free A/G-rich DNA probe isolated from the AuNPs, was transformed to DNA molar concentrations by UV-Vis absorption. Finally, to acquire the mean number of oligonucleotides on one single particle, we divided the conjugated oligonucleotide concentration by the initial concentration of AuNPs. The concentration of the capture probes on the AuNPs surface was measured by fluorescence experiment with fluorophore-labeled oligonucleotides. The supernatant's maximal fluorescence peak, with free capture probes isolated from the particles, was transformed to molar concentrations of the fluorophore modifying on oligonucleotide by comparison to a standard linear calibration curve. Standard curves were measured with given concentrations of fluorophore-labeled single-stranded DNA using uniform buffer pH and salt concentrations.

Nuclease Assay. Two sets of the nanocomposites (bare AuNPs, AuNPs-integrated THMS, both were 1 nM) were added within replicate 96-well microplate at 37 °C. Then, 2 μL of DNase I (1 U/L) in prepared buffer was dropoed into one set. These samples were monitored for 1 h with its fluorescence intensity. The fluorescence was carried out at appropriate excitation wavelengths, while the solution was cooled to room temperature.

MTT Assay. The human breast cancer cells (MCF-7) cells (1×10^6 cells/well) were placed in replicate 96-well microtiterplates to a final volume of 200 μL. After overnight culture, the original medium was taken out. MCF-7 cells were incubated with AuNPs-integrated THMS or AuNPs for 24 h. Subsequently, 100 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (0.5 mg/mL in PBS) was added to every well with incubation for 4 h at 37 °C. Then, the remaining MTT solution was taken out. To dissolve the formazan crystals, 150 μL of dimethyl

sulphoxide (DMSO) was added to each well, then the assay plate was shaken at room temperature for 10 min. The absorbance was measured at 490 nm with a RT 6000 microplate reader.

Dark-Field Microscopy. Typically, the MCF-7 cells were first incubated on glass coverslips putted at the Petri dish at 37 ℃ for 24 h to ensure sufficient cell attachment and then incubated with 1 mL culture medium containing 2 nM pi-THMS or AuNPs at 37 °C for 1 h. After incubation, the coverslips were taken out and rinsed three times with PBS. The coverslip was shifted to the top of a glass slide and then absorbed in a thin layer of PBS solution for dark-filed imaging. Dark-field microscopy (DFM) images were carried out on an upright optical microscope Nikon 80i (Japan). The sample slantly was focused on by the halogen lamp with white light to through an oil immersion dark-field condenser (NA 1.43-1.20). The nanocomposites with scattered light was gathered using a 60×objective and then acquired using a color CCD camera (DP72, Olympus).

Confocal Fluorescence Imaging. Cancer cells and normal cells were placed on chamber slides over 20 h. The AuNPs-integrated THMS (2 nM) then was respectively delivered into human normal mammary epithelial cells (MCF-10A), MCF-7 and human cervical cancer cells (HeLa) in a DMEM culture medium in 5% CO_2 at 37°C for 3 h. The fluorescence from cells were measured by confocal laser scanning microscopy (CLSM) on specific laser transmitters. As for the measurement with the tumor mRNAs of the levels of expression in MCF-7 cell, treating with β-estradiol (10^{-8} mol/L) for one group while the other with tamoxifen (10^{-6} mol/L) for 48 h.

RT-PCR. Total cellular RNA was extracted from the corresponding cells using Trizol reagent (Sangon Co.Ltd., Shanghai, China) according to the indicated protocol. The cDNA samples were prepared by using the reverse transcription (RT) reaction with AMV First Strand cDNA Synthesis Kit (BBI, Toronto, Canada). RT-PCR analysis of mRNA was performed with SG Fast qPCR Master Mix (2X) (BBI), according to the indicated protocol on a LightCycler480 Software Setup (Roche). The primers (from 5′ to 3′) used in this experiment were:

TK1 forward: CTCCTACCCACTGGTCTGCTTA;

TK1 reverse: CAGGGAGAACAGAAACTCAGCA;

GalNAc-T forward: CCAAGACCTTCCTCCGTTAT

GalNAc-T reverse: AACCGTTGGGTAGAAGC

Table S1. Oligonucleotides Used in This Work*

Entry	Sequence (5′-3′)
P_0	GGGGGA GAA GG AA GAAA AG
P	GGGGGA GAA GG AA GAAA AG-TAMAR
CP1 (75 %)	TAMAR-CTTCTTTT GCGAGTGTCTTTGGCATACTC TTTTCTTC-BHQ1
CP2 (62.5 %)	TAMAR-CCTTCTTT GCGAGTGTCTTTGGCATACTC TTTCTTCC-BHQ1
CP3 (50 %)	TAMAR-CTTCCTTC GCGAGTGTCTTTGGCATACTC CTTCCTTC-BHQ1
CP4 (25 %)	TAMAR-CCCCCTCT GCGAGTGTCTTTGGCATACTC TCTCCCCC-BHQ1
P_1	GGGGGA GAA GG AA GAAA AGTTTTTT-SH
pi-CP	TAMAR-CTTCTTTT AGCGGTGTCTTTGGCATCATC CTTCTTTT
p-CP	TAMAR-CCCCCTCT AGCGGTGTCTTTGGCATCATC TCTCCCCC
P_2	Cy5-CTTCTTTT GCGAGTGTCTTTGGCATACTT TTTTCTTC
P_3	ROX-CTTCTTTT TCTTATGCGGATAGTGAAAGC TTTTCTTC
P_4	Alexa Fluor-CTTCTTTT TAGAGATGCGGTGGTC TTTTCTTC
TK1	AAGTATGCCAAAGACACTCGC
GalNAc-T	GCTTTCACTATCCGCATAAGA
Survivin	GACCACCGCATCTCTA
TK1 (SM)	AAGTATGCCACAGACACTCGC
GalNAc-T (SM)	GCTTTCACTAGCCGCATAAGA
c-myc	CCTCAACGTTAGCTTCACCAA
K-ras	TGGAGCTGGTGGCGTAG
Her-2/neu	CACAGACATGAAGCTGC

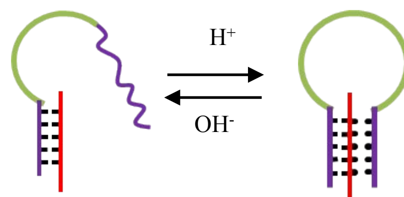

Figure S1. Schematic illustration of the programmable THMS that form an intramolecular triplex structure through the formation of Watson-Crick (dashed) and Hoogsteen (dots) base.

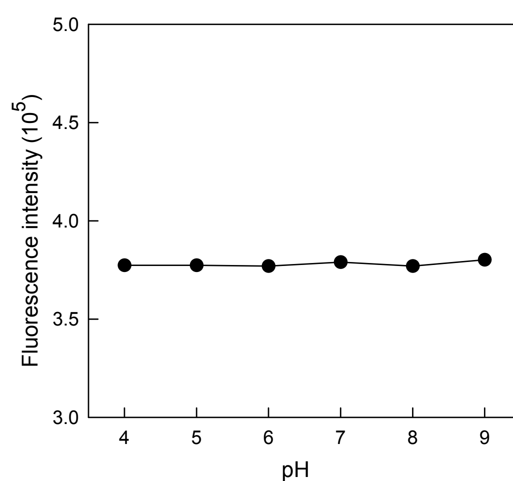

Figure S2. The fluorescence intensity of TAMAR-labeled P (100 nM) as function of different pH (The excitation wavelength was 560 nm and the emission wavelength was recorded at 580 nm).

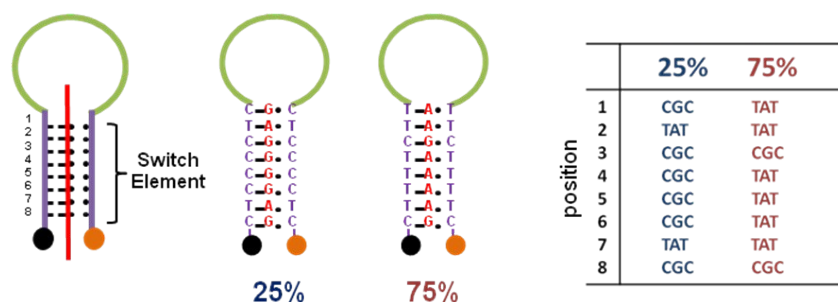

Figure S3. The distribution of different TAT/CGC content in the programmable THMS.

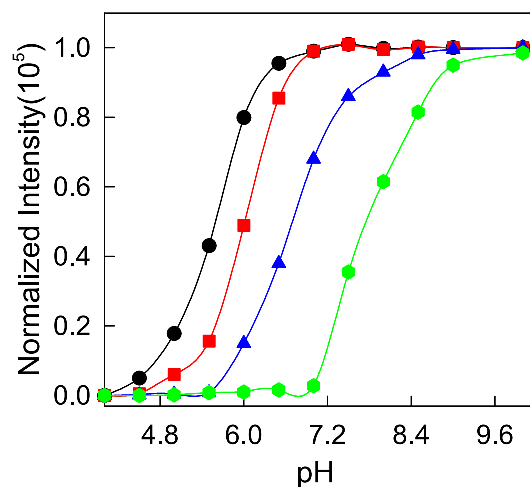

Figure S4. Triplex-to-duplex transition of 25 % TAT (black curve), 50 % TAT (red curve), 62.5 % TAT (blue curve) and 75 % TAT (green curve)-contained THMS are monitored through a TAMAR inserted at the 5′-end and a BHQ-1 labeled at the 3′-end. The maximum fluorescence intensity was normalized to 1.0.

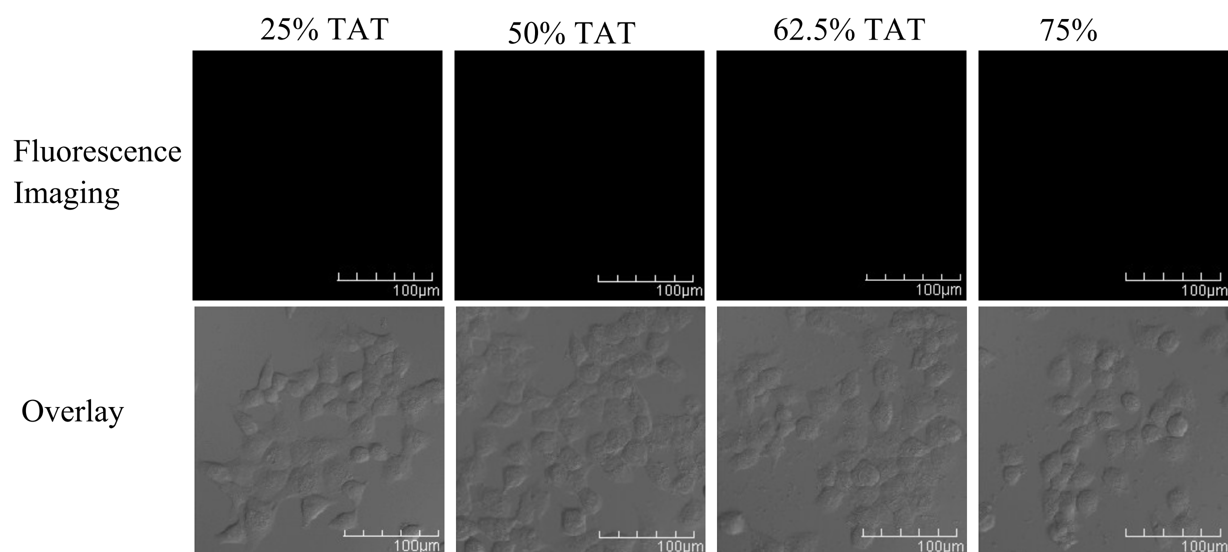

Figure S5. Confocal microscopy images of MCF-7 cells (TAMAR channel) which were incubated with 25%, 50%, 62.5% and 75% TAT-contained THMS (300 nM) for 3 h and then washed with PBS buffer for three times, respectively.

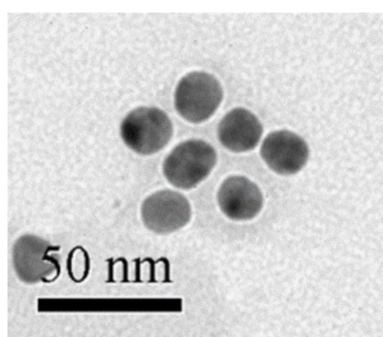

Figure S6. TEM images of 13 nm AuNP.

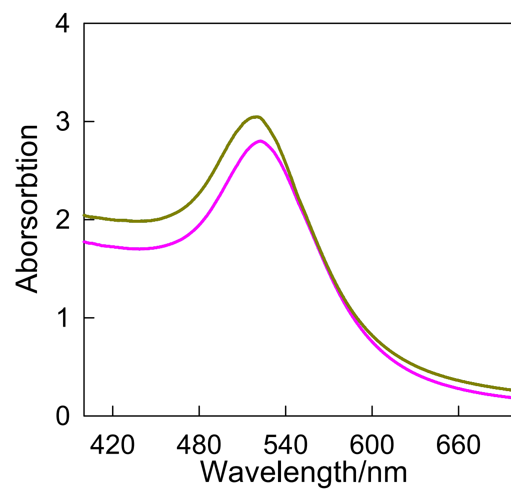

Figure S7. Absorption spectra of AuNPs (grass green curve) and AuNP-P_1 (pink curve).

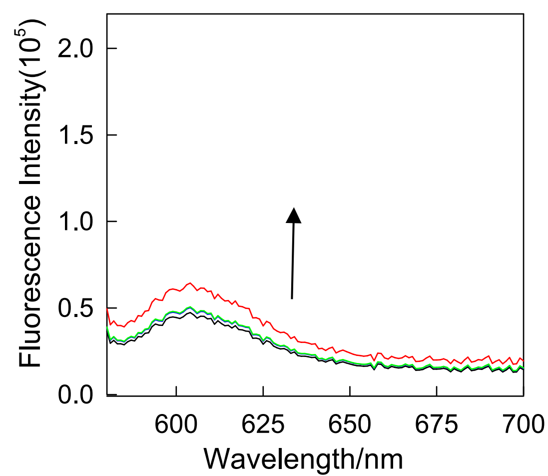

Figure S8. Representative fluorescence spectrum of p-THMS (2 nM) as functions of different pH. The arrows indicate the pH (4.5, 5.5, 6.5, 7.5).

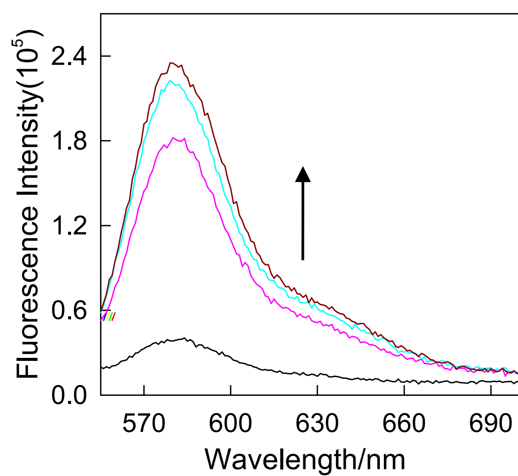

Figure S9. Representative fluorescence spectrum of pi-THMS (2 nM) as functions of different pH. The arrows indicate the pH from 4.5 to 7.5 (4.5, 5.5, 6.5, 7.5).

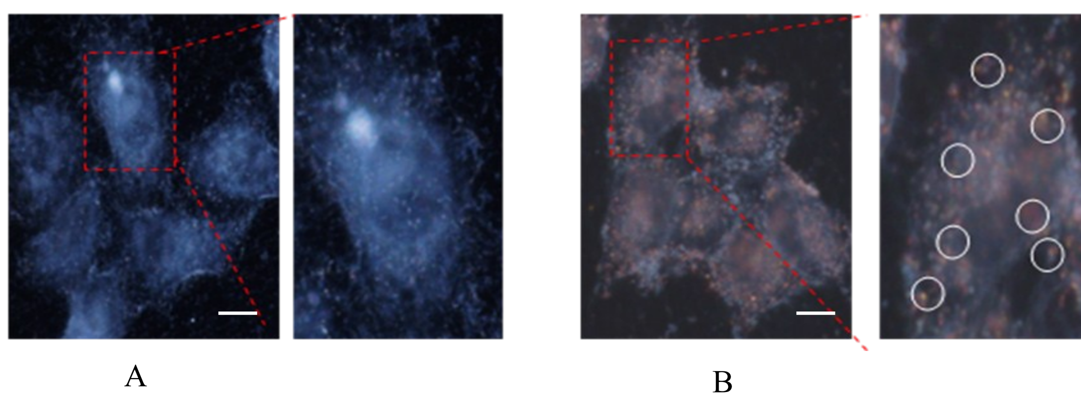

Figure S10. Representative DFM images of MCF-7 cells incubated without (A) and with (B) pi-THMS (2 nM) for 3 h after washing with PBS for three times, respectively. Scale bar: 10 μm.

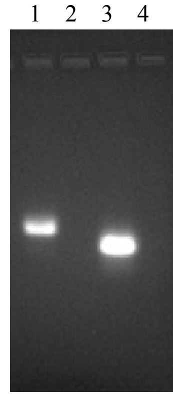

Figure S11. 4% agarose gel electrophoresis image of TK1 mRNA-targeted pi-THMS before and after incubated with TK1 mRNA. Lane 1: Cy5-labeled P_2; pi-THMS without TK1 mRNA incubation;Lane 3: pi-THMS with TK1 mRNA incubation. Lane 4: TK1 mRNA. The excitation wavelength was matched with Cy5 which labeled on the P_2 DNA sequence.

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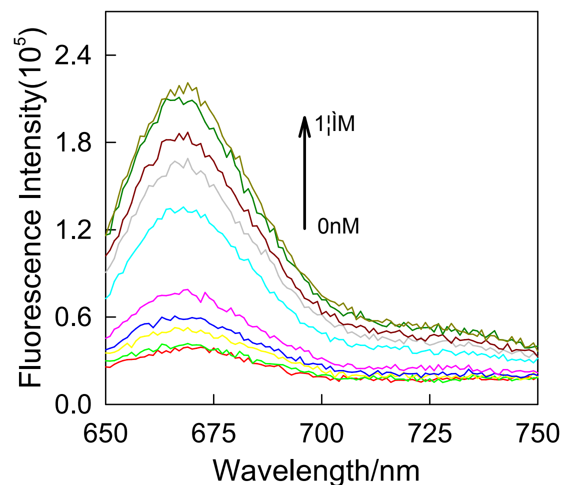

Figure S12. Representative fluorescence spectrum of TK1-targeted pi-THMS in the sodium phosphate buffer by increasing concentrations of TK1 mRNA. The arrows indicate the concentration from 0 to 1 μM (0, 2, 5,10, 25, 50, 100, 200, 500, 1000 nM).

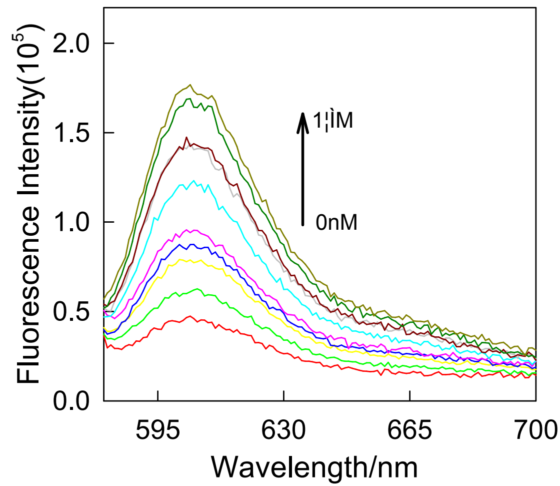

Figure S13. Representative fluorescence spectrum of GlaNAc-T mRNA-targeted pi-THMS in the sodium phosphate buffer by increasing concentrations of GlaNAc-T mRNA. The arrows indicate the concentration from 0 to 1000 nM (0, 2, 5, 10, 25, 50, 100, 200, 500, 1000 nM).

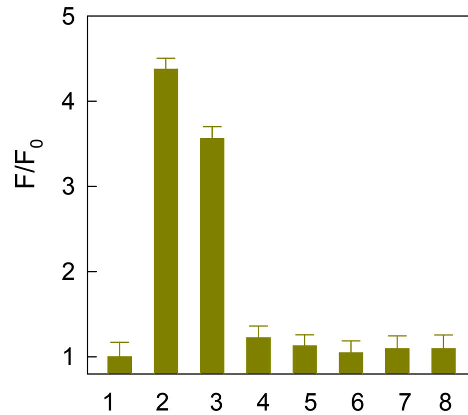

Figure S14. Fluorescence enhancements (F/F_0) of pi-THMS in the sodium phosphate buffer upon addition of TK-1, GalNAc-T and other interfere mRNA. 1-8 represent blank, TK1, GalNAc-T, TK1 single-base mismatched target, GalNAc-T single-base mismatched target, Her-2/neu, K-ras, c-myc (The concentrations of the mRNA were 200 nM).

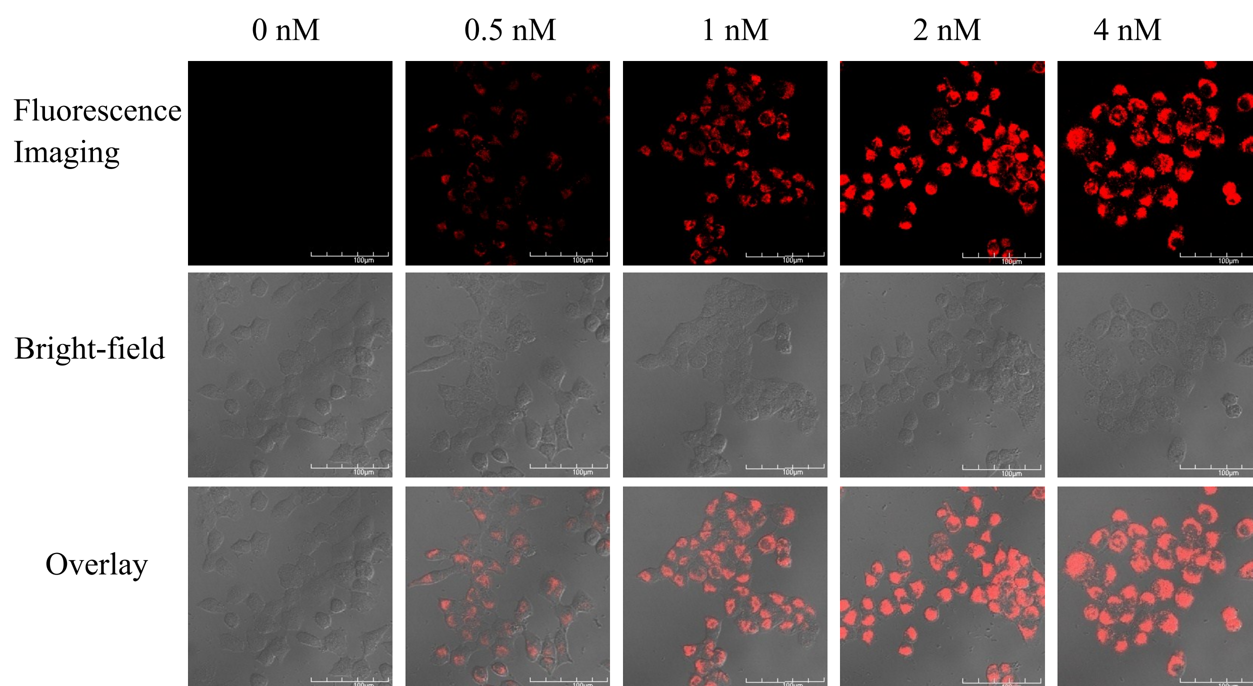

Figure S15. Confocal microscopy images recorded at Cy5 channel of TK1 mRNA-targeted-pi-THMS-treated MCF-7 cells as a function of different concentrations of pi-THMS.

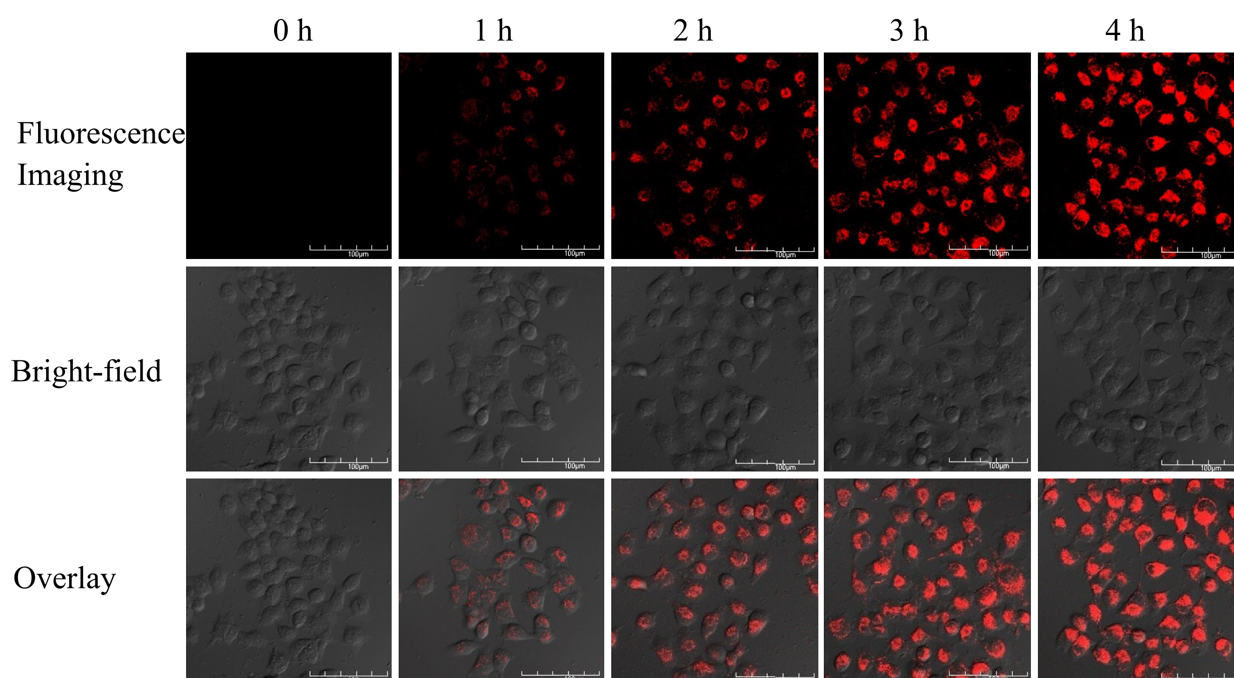

Figure S16. Confocal microscopy images recorded at Cy5 channel of TK1 mRNA-targeted-pi-THMS-treated (2 nM) MCF-7 cells as a function of different incubation time.

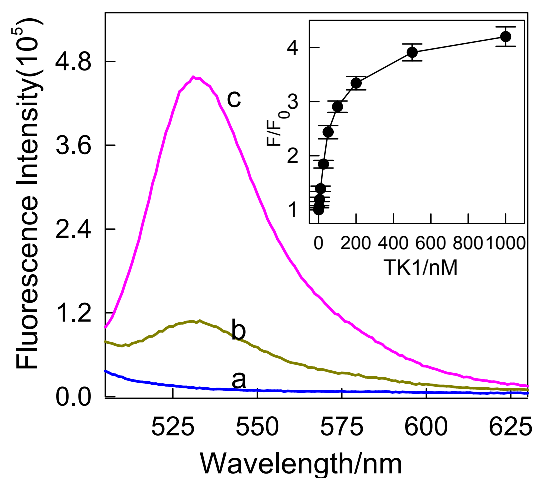

Figure S17. Fluorescence emission spectra of AuNPs-P_1 (a), (a)+P_4 (b), and (b)+200 nM survivin mRNA (c) in the sodium phosphate buffer. Inset: Fluorescence enhancements (F/F_0) of pi-THMS in the sodium phosphate buffer by increasing concentrations of survivin mRNA. P_4: The survivin mRNA corresponding capture sequences were modified with Alexa Fluor 488 dyes.

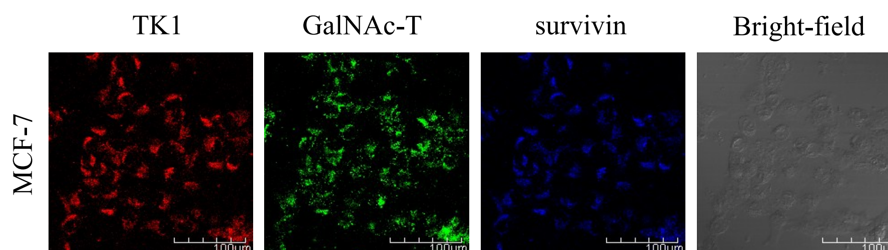

Figure S18. (A) Intracellular imaging of TK1 mRNA, GalNAc-T mRNA and survivin mRNA under CLSM using multiplex pi-THMS. MCF-7 cells were incubated with the multiplex pi-THMS (2 nM) for 3 h at 37 °C. The three mRNAs were recorded by Cy5 with 648 nm excitation, ROX with 568 nm excitation and Alexa Fluor 488 with 488 nm excitation. Scale bars: 100 μm.

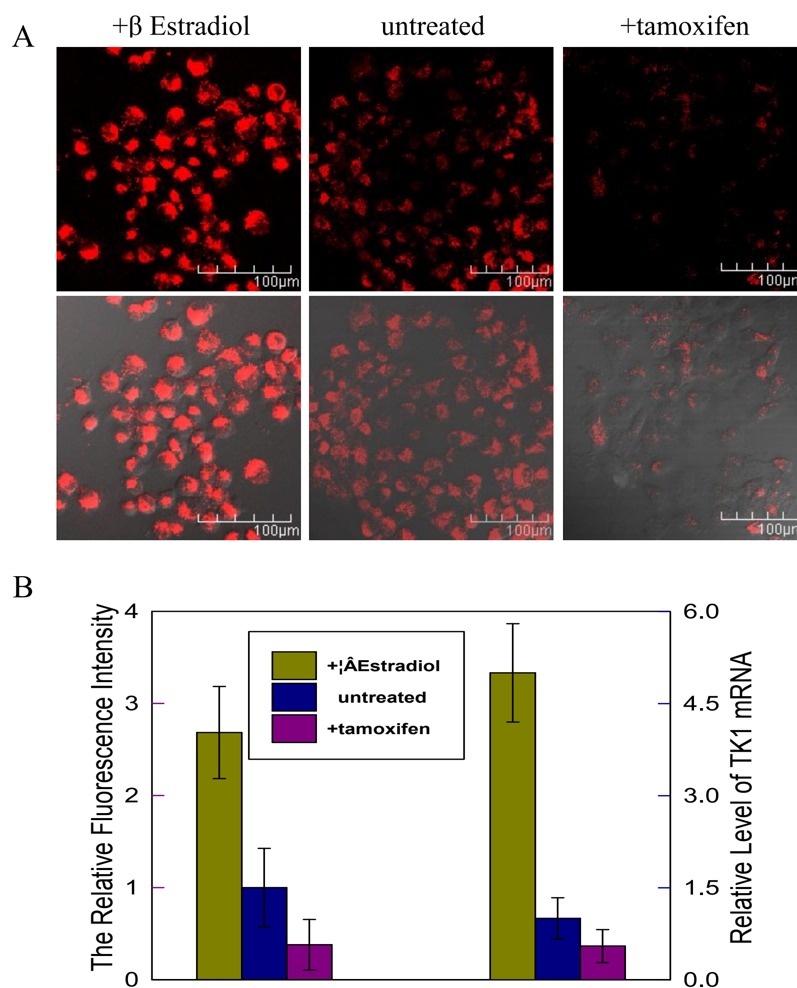

Figure S19. (A) Fluorescence images of TK1 mRNA in MCF-7 cells treated with β-Estradiol or tamoxifen by TK1 mRNA-targeted pi-THMS. Scale bars: 100 μm. (B) Histogram of the relative fluorescence intensity and RT-PCR results of the above three groups.

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[3] J. W. Liu and Y. Lu, *Nat. Protoc.,* 2006, **1**, 246.