Supporting Information

Dual Types of Biomarkers Activated DNA-Au Nanomachine for Multi-Site Imaging and Gene Silencing

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EXPERIMENTAL SECTION

Chemicals and Materials. Gold (III) chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$, 99.9%) was purchased from Shanghai Bojing Chemical Co., Ltd. (Shanghai, China). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP·HCl), and mercaptoethanol were bought from Shanghai Shaen Chemical Co., Ltd. (Shanghai, China). (2-(4-(Azidomethyl)phenyl)ethene-1,1,2-triyl)tribenzene (TPE-N_3) was purchased from Shenzhen Aijin Technology Co., Ltd. (Shenzhen, China). Tris-HCl solution and all the oligonucleotides used in this work were supplied by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were shown in Table S1. All the above reagents were of analytical grade and were used directly without further purification. The water used in the whole experiment was distilled water.

Table S1. The oligonucleotide sequences used in this work.

Name	Sequences*(5′→3′)
aptamer	GCAGTTGATCCTTTGGATACCCTGG-$(CH_2)_6$-DBCO
Cy5-reporter	Cy5-TAGCTTATCAGAC
anti-DNA	CCAGGGTATCCATTTT-$(CH_2)_{12}$TCAACATCAGTCTGATAAGCTA-$(CH_2)_{12}$-dithiol
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A

Apparatus and Measurements. The UV-Vis absorption spectrum of the chimeric DNA-functionalized Au nanoparticles was recorded with a U-4100 spectrophotometer (Hitachi Co., Ltd., Japan). The fluorescence spectrum was carried out with Edinburgh FS (Edinburgh Instruments Ltd., Britain). The Transmission electron microscopy (TEM) images of nanoparticles and chimeric DNA-functionalized Au nanoparticles were measured using a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The potential of nanoparticles and chimeric DNA-functionalized Au nanoparticles were performed on a Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom). Fluorescence images of breast cancer cells with both the blue channel and red channel were recorded on an inverted fluorescence microscope DS-Ri2 (Nikon Instruments Ltd. Japan), while the fluorescence images with only red channel images were imaged in a FV3000 confocal microscope (Olympus Co., Ltd., Japan). The toxicity of breast cancer cells by a multi-plate reader Varioskan Flash (Thermo Fisher Scientific Co., Ltd.). The apoptosis status of breast cancer cells was recorded with a CytoFLEX Flow cytometry (Beckman Coulter, Inc., USA).

Cell Culture. The cancer cell lines used in this work **(**MCF-7 human breast cancer cells, HL-7702 human normal liver cells, MCF-10A human normal epithelial breast cells,

Hela cells human cervical cancer cells) were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Science (Shanghai, China). MCF-7, HL-7702 cells, and Hela cells were cultured in DMEM medium containing 1% non-essential amino acids, 100 U/mL penicillin and 10% fetal bovine serum (FBS) at 37 °C with a humidified atmosphere (95% air and 5% CO_2). MCF-10A were cultured in specific medium purchased from Procell Life Science&Technology Co., Ltd.

Synthesis of TPE-Aptamer with a Click Chemistry Reaction. As shown in Figure S1A, the ligation of alkynyl-TPE (TPE-N_3) with dibenzocyclooctyne (DBCO) modified aptamer (DBCO-aptamer) was carried out with a click chemistry reaction by mixing 200 μL TPE-N_3 (2000 μM) and 400 μL DBCO-aptamer (50 μM), followed by incubating at room temperature 24 h under a gentle stirring. Afterward, the mixture was purified by high performance liquid chromatography (HPLC), and the mixture solutions exhibited two chromatographic peaks at 18.8 min and 29.3 min (Figure S1B). The mass spectrum (MS) analysis of the chromatographic peak at 29.3 min showcased a peak at *m/z* 8576.6, which was corresponding to the molecular weight of TPE-aptamer with the yield of 79.5%, suggesting the successful synthesis of TPE-aptamer. Moreover, the UV-vis spectrum of the synthetic TPE-aptamer exhibited the characteristic absorptions of both the DBCO-aptamer (at 260 nm) and TPE-N_3 (at 334 nm) with a tiny blue-shifted (Figure S1C), further indicating the TPE-aptamer has been successfully prepared.

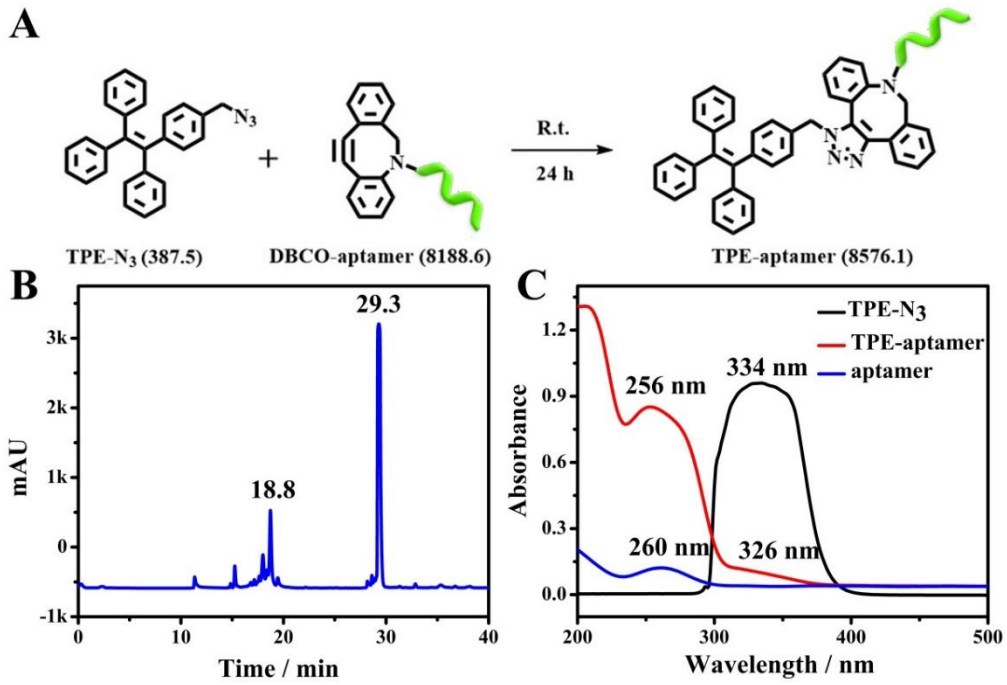

Fig. S1 (A) Synthesis equation of TPE-aptamer with a classical click chemistry reaction. (B) HPLC spectrum of the reaction products. (C) UV-vis absorption spectrum of TPE-N_3, aptamer, and TPE-aptamer.

Preparation of Triblock DNA. The anti-DNA was first incubated with TCEP for 1 h at room temperature reducing the disulfide bond to thiol to prepare reduced anti-DNA. Equimolar reduced anti-DNA, Cy5-reporter, and TPE-aptamer were heated to 95 °C for 5 min, and then progressively cooled down to 37 °C, and then further incubate at 37 °C for 1 h to prepare triblock DNA. Afterward, the mixture DNA solution was treated with a NAP-5 desalting column to remove excess salt, then freeze-dried to remove the solvent, and added sterilized water to make a triblock DNA solution.

Preparation of DNA Nanomachine. Au NPs with a diameter of 13 nm were prepared with the classical sodium citrate reduction method according to the previous work, and the concentration of Au NPs was measured by the maximum absorptions at 519 nm ($\varepsilon=2.7*10^8\ L^{-1}\ mol^{-1}\ cm$), which was estimated to 4 nM. 100 μL of concentrated Au NPs was mixed with 100 μL of triblock DNA solution and put it on a shaker (25°C, 140 rpm) to assemble DNA nanomachine. Then, PBS solution (0.01 M), SDS (0.1%), and NaCl (0.1 M) were incubated with above-mentioned mixture overnight. Afterward, the prepared DNA nanomachine was centrifuged, washed, and stored in a TE buffer.

Quantification of Triblock DNA Loaded on Au NPs. The standard curve method was applied to calculate the concentration of triblock DNA loaded on the DNA nanomachine. Taking the fluorescence signal of Cy5-reporter as standard, the standard curve of Cy5-reporter was first acquired by measuring a series of concentrations of Cy5-reporter. Then, to recover the fluorescence signal of Cy5-reporter in the DNA nanomachine, 20 mM mercaptoethanol was incubated with the DNA nanomachine overnight to make the triblock DNA completely releases from Au NPs. Completely separated these by centrifugation, and then detected the fluorescence of Cy5-reporter of the supernatant under the same conditions. According to the calibration curve, each Au NPs loaded on 55 numbers of Triblock DNA, indicating the Au NPs with the advantage of high loading capacity.

Fluorescence Imaging of Different Types of Breast Cancer Biomarkers with the DNA Nanomachine. MCF-7 cells were introduced into a Petri dish, and immediately cultured in DMEM medium with a humidified atmosphere (5% CO_2) at 37°C for 24 h to reach 80% cell anchorage-dependent rate, and then washed with sterile PBS. Then, the acquired MCF-7 cells were divided into three groups: the blank group, the control group (incubation of TPE-Aptamer), and the sample group (incubation of various concentrations of DNA nanomachine). After incubation for 24 h, MCF-7 cells were washed 3 times with PBS solution to reduce background signals. After washing, the cells were imaged with the inverted microscope at the blue channel, and the red channel, respectively.

The Cytotoxicity Test of the DNA Nanomachine. The cytotoxicity of the DNA nanomachine was investigated using a standard MTT assay with MCF-7 cells and MCF-10A cells. Firstly, the MCF-7 cells or MCF-10A cells were seeded in 96-well microtiter plates and cultured in a humid atmosphere with 5% CO_2 at 37°C for 24 h. 10 μL of different concentrations (0 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM) of DNA nanomachine were incubated with cells. After rinsed by PBS, 10 μL of MTT solution and 90 μL DMEM medium were added, and further incubated at 37 °C for 4 h. Finally, after slightly taking out the solution, 110 μL Formazan solvent was added to each well to dissolve the precipitates, followed by swayed with at 37°C for 10 min and then measured the optical density (OD) of the solutions in each well at 490 nm with a microplate reader. Cell viability was calculated as described by the manufacturer. Then, the viability of MCF-7 cells under the same concentration of the DNA nanomachine at different incubation times (0 h, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h), was investigated.

Apoptosis Assay. The apoptosis induced by the DNA nanomachine was detected by flow cytometry with Annexin V-FITC apoptosis detection kit. After incubating with DNA nanomachine for 24 h, cells were then digested with trypsin and collected by centrifugation. After re-dispersed the cells in 100 μL binding buffer, 5 μL of Annexin V-FITC (single staining), 10 μL of PI (single staining), and both 5 μL Annexin V-FITC and 10 μL PI (double staining), were added respectively. Finally, the cells were incubated at room temperature for 15 minutes, and the apoptosis of different cell samples were analyzed by flow cytometry.

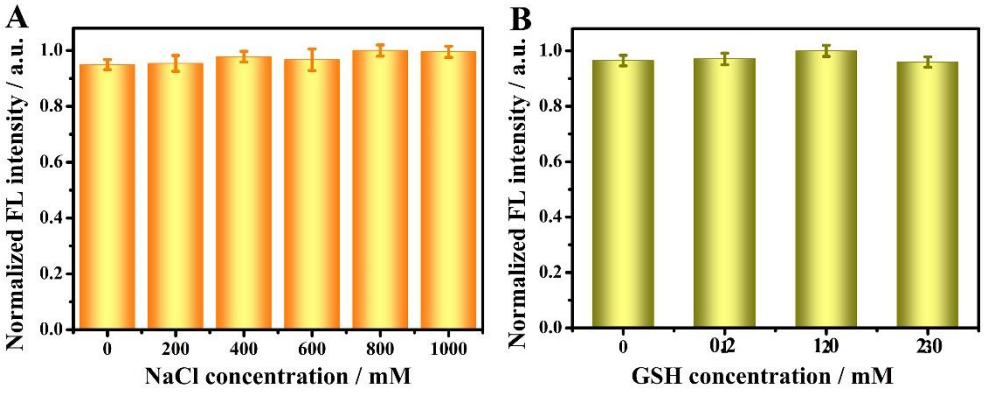

Fig. S2 (A) The stability of DNA-Au nanomachine in 10 mM PBS containing NaCl with various concentrations (0 mM, 200 mM, 400 mM, 600 mM, 800 mM, 1000 mM). (B) The stability of DNA-Au nanomachine in 10 mM PBS containing GSH with different concentrations (0 mM, 0.2 mM, 1.0 mM, 2.0 mM).

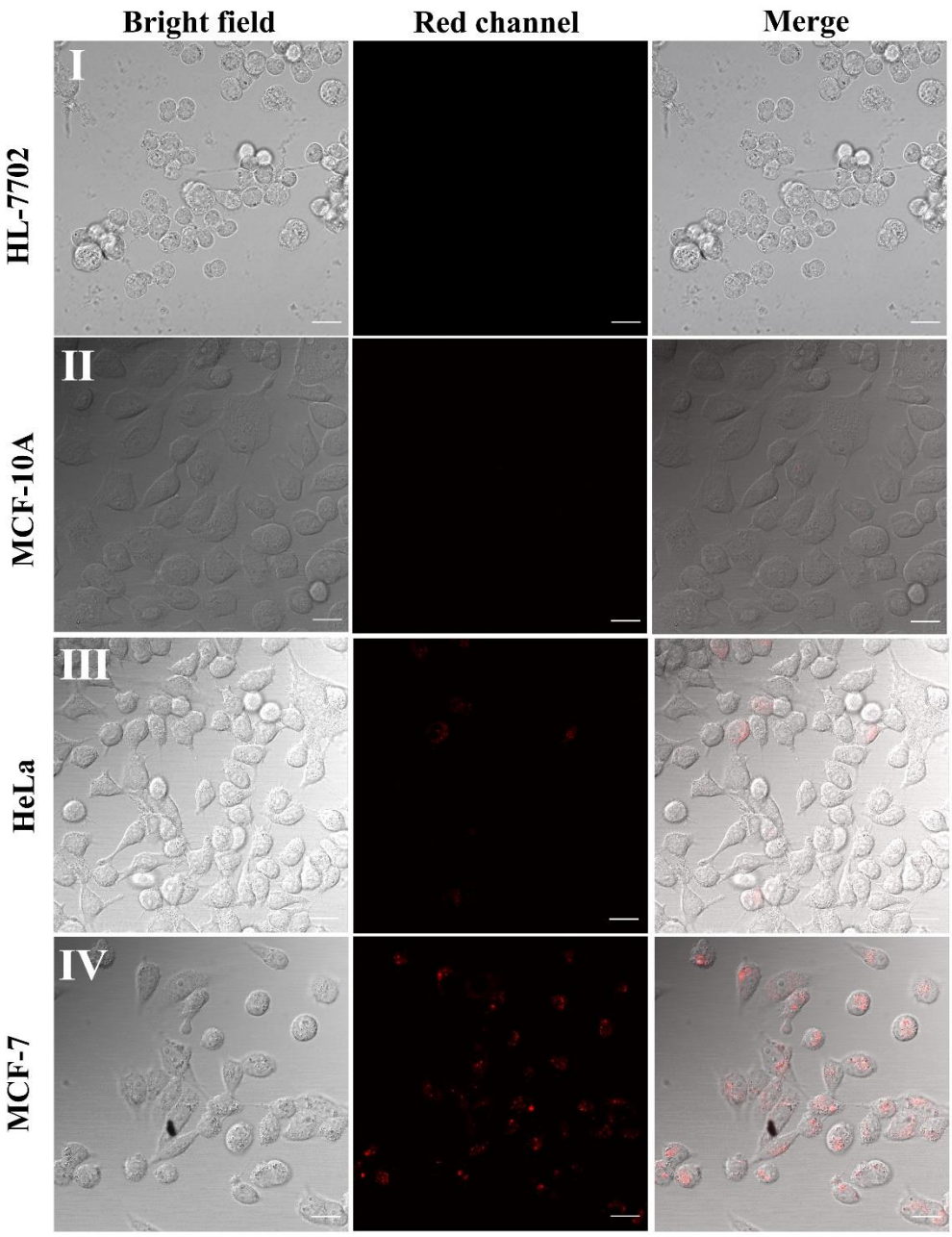

Fig. S3 Bright-field microscopy images, confocal fluorescence microscopy images with red channel, and merge images corresponding to the analysis of HL-7702 cells (entry I), MCF-10A cells (entry II), HeLa cells (entry III), and MCF-7 cells (entry IV) incubated with the DNA-Au nanomachine. Scale bar: 20 μm.

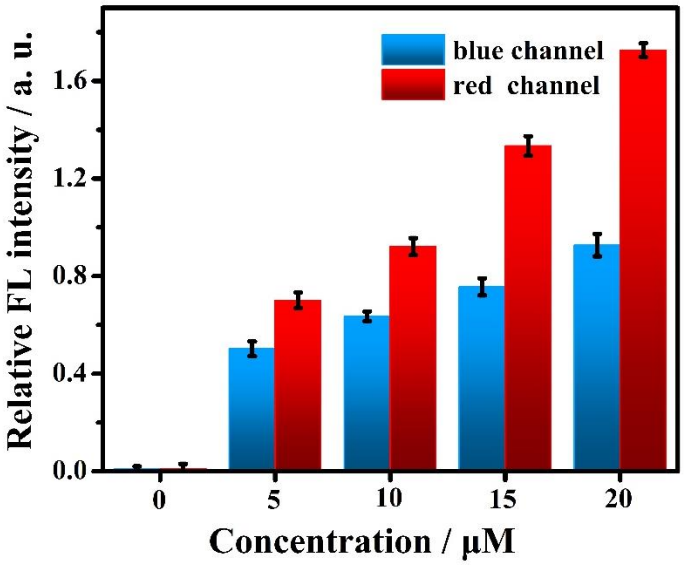

Fig. S4 Using Image J measured the relative mean fluorescence intensities of the blue channel and red channel (a, and c-f), and the data expressed as mean ± standard deviation (n=5).

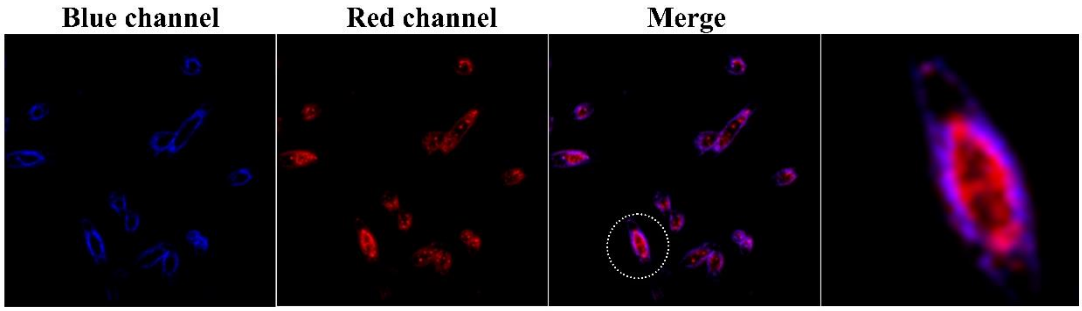

Fig. S5 The inverted fluorescence imaging images for MCF-7 cells incubated with 20 μM DNA-Au nanomachine with blue channel, red channel, merge images and larger images of representative cells of merge images in the circle.

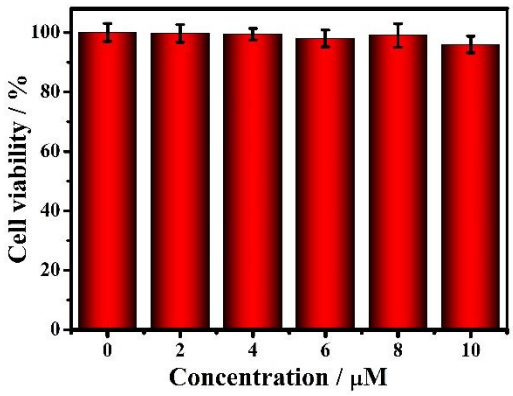

Fig. S6 MTT assay of MCF-10A human normal epithelial breast cells with different concentrations of DNA nanomachine.