Supporting Information:

Autonomous Operation of 3D DNA Walkers in Living Cells for MicroRNA Imaging

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S1. Experimental Section

Chemicals and Materials. Boric acid (≥99.5%), N,N,N,N-tetramethylethylenediamine (TEMED, 99%), Ethylene Diamine Tetraacetic Acid (EDTA, 99.0%), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, 99%), Tris (hydroxymethyl) aminomethane (Tris) and methanol (CH_3OH, 99%) were obtained from Sinopharm Chemical Reagent Company Ltd. (Shanghai, China). All the reagents and solvents were acquired from commercial sources with starting analytical grade and used as received without further purification. Biological sample "MCF-7 cells" were purchased from National Collection of Authenticated Cell Cultures (Shanghai, China). "MDA-MD-231 cells" were purchased from Ruijie Biotech Co. Ltd (Hefei, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), 4S Red Plus, penicillin/streptomycin, glutamine (Gln,100×), 6× Glycerol Gel Loading Buffer (with Xylene Cyanol, BPB), phosphate buffer (PBS, cell culture grade, pH=7.4), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Cell Proliferation, Cytotoxicity Assay Kit, and DNA and RNA (listed below) purified by high performance liquid chromatography were purchased from Sangon Biotech Co. Ltd (Shanghai, China). After dissolved in a Tris-HCl buffer solution, DNA or RNA was stored at 4 °C until use. Lysosome fluorescent trackers (Lyso@tracker) was purchased from Solarbio science□technology Co. Ltd (Beijing, China). MicrONhsa-miR-10b-5p mimics and micrOFFhsa-miR-10b-5p inhibitors were purchased from RiboBio Company (Guangzhou, China). All the experimental solution was prepared with ultrapure water (18.2 MΩ•cm^{-1}) obtained by water purification system of PSDK2-10-C (Beijing, China).

Substrate: 5'-SH-TTT TTT TTT TTT TTG GGC CAC TAT rAGG AAT-carboxyfluorescein (FAM)-3'

Locker: 5'-TTGAAGCACAAATTCGGTTCTACAGGGTA-3'

DNAzyme: 5'-SH-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTA TTC TTC CGA CCG GTC GAA AAT AGT GGC CCG AAT TTG TGC TTC AA

MicroRNA-10b (miRNA-10b): 5'-UACCCUGUAGAACCGAAUUUGUG-3'

Apparatus. Scanning electron microscope (SEM) images analyses were performed on a Hitachi S-8100 (Hitachi, Japan) with an accelerating voltage of 10 kV. Transmission electron microscopy (TEM) images were obtained from a field emission transmission electron microscope (Hitachi HT7700, Japan) under the accelerating voltage of 120 kV. Ultraviolet-visible (UV-vis) absorption spectra were carried out with U-4100 spectrometer (Hitachi, Japan). Fourier Transform Infrared Spectroscopy (FT-IR) was measured using an FT-IR-8400S spectrometer (Shimadzu, Japan) in 400-4000 cm^{-1} region using powdered sample on a KBr plate. The Power X-ray diffraction (XRD, PANalytical X'Pert diffractometer with Cu Kα irradiation) patterns were implemented to receive the samples' crystalline structure and morphology. The surface areas of the products were obtained according to the N_2 adsorption-desorption isotherm using the Quadrasorb SI (Quantachrome Instrument, USA) surface area and porosity analyzer through the Brunauer-Emmett-Teller (BET) method. Fluorescent spectrum analyses were performed on Luminous spectrofluorometer (Thermo Fisher Scientific, China). The quartz cuvette has a thickness of 1 cm and both excitation and emission slits were set at 5.0 nm with a 500 V PMT voltage. The poly-acrylamide gel (PAGE) electrophoresis was run at the DYY-4C electrophoresis system (Liuyi Biological Technology, China) after the gels stained, the images were recorded using a Tanon 2500R gel imaging system (Shanghai, China). Study by inductively coupled plasma mass spectrometry (ICP-MS) were implemented on Agilent 7700x ICP-MS of Agilent Technologies Inc. (California, U.S.A.) Cell imaging were operated by a confocal laser scanning microscope (Olympus FluoView 1000, Japan). Flow cytometry detection was carried out by Flow Cytometer with model of EPICS XL made by Beckman Coulter, Inc. (The U.S.A.)

Preparation of AuNPs@ZIF-8. ZIF-8 solid power was prepared according to the literature[1] simply by stirring the mixture of $Zn(NO_3)_2 \cdot 6H_2O$ and 2-methylimidazole in methanol at room temperature. In order to further load the AuNPs, 20 mg ZIF-8 solid was dispersed in 10 mL methanol by ultrasonic treatment for 10 min. 300 μL $HAuCl_4$ aqueous solution (50 mM) was added into the ZIF-8 solution drop by drop under stirring for 6 h at room temperature. After that, 1 mL methanol containing 7.2 mg $NaBH_4$ was added into the system quickly to reduce the precursors for 1 h. Subsequently, the obtained AuNPs@ZIF-8 samples were separated from the turbid liquid by centrifugation at 8000 rpm, followed by washed with fresh methanol three times. At last, the products were dried in vacuum at 80 °C for 12 h.

Construction of the 3D DNA walker on AuNPs@ZIF-8. Before conjugation with the AuNPs@ZIF-8, thiolated DNA was incubated with TCEP·HCl for one hour to break the disulfide bond. Subsequently, 30 μL of 10 μM locker DNA was hybridized with 10 μL of 10 μM DNAzyme in 25 mM Tris-HCl (pH 7.4) with 137 mM NaCl at 37 °C for 2 h to form the locker-DNAzyme. After that, 50 μL of the fluorescence substrate DNA (20 μM) and 20 μL of the prepared locker-DNAzyme (2.5 μM) were added to the AuNPs@ZIF-8 solution (430 μL, 30 μg/mL) and vibrated for 24 h. During the process, 25 μL 1% Tween 20 was introduced into the solution after vibrating for 16 h and then, the

NaCl solution (2 M) was added into the solution for four times in the following 8 h to increase the salt concentration to 0.3 M. Furthermore, the superfluous DNA was separated by centrifuging the resulting solution (13000 rpm, 30 min) and then rinsed 3 times with 10 mM Tris-HCl (pH 7.4) containing 0.05% Tween 20. At last, AuNPs@ZIF-8 co-loaded with the substrate DNA and locker-DNAzyme was obtained, shortly named as DNA/AuNPs@ZIF-8.

Fluorescence Detection of miRNA-10b in Vitro. Briefly, the prepared 3D DNA walker was dispersed in 25 mM Tris-HCl buffer containing 137 mM NaCl (pH=5.5) as a concentration of 30 μg/mL. 100 μL of the AuNPs@ZIF-8 solution were incubated with different concentrations of miRNA-10b (5 μL) at 37 °C for 2 h. The fluorescence spectra were scanned with excitation wavelengths of 490 nm. To investigate the specificity, other miRNAs (100 nM) were used to replace miRNA-10b target for the same experimental process.

PAGE electrophoresis. First, 10 mL of 30% acrylamide/bis-acrylamide gel solution at the molar ratio of 29 : 1, 5 mL of 5×Tris–borate–EDTA (TBE) buffer (450 mM Tris, 450 mM boric acid, and 10 mM EDTA, pH 8.0), 0.18 mL of 10% APS, 0.016 mL of TEMED and 9.8 mL ultrapure water were mixed for the preparation of the hydrogel at room temperature. After 30 min, the gel was soaked in 1×TBE buffer (pH 8.0). Furthermore, 12 μL different samples were mixed with 2 μL loading buffer and transferred to the prepared 12% native poly-acrylamide gel for electrophoresis. The PAGE electrophoresis was operated in 1×TBE buffer at the voltage of 170 V for 5 min and 110 V for about 45 min. Finally, the gels were stained with diluted 4S Red Plus for 10 min and further investigated by gel imaging system.

ICP-MS. 1 mg AuNPs@ZIF-8 were dispersed with 1 mL of 100 mM HEPES buffer (pH 7.4 or 5.5) for different period of time. Afterwards, the mixture was first centrifuged at 10000 rpm for 10 min. and then diluted to 1:2500 with ultrapure water for further investigation. Besides, HeLa cells (~5.62×10^4 cells) were incubated with 1 mL of RPMI-1640 containing 10 μg mL^{-1} AuNPs@ZIF-8 for 3 h at 37 ℃. The cells were further collected and lysed by 200 μL of cell lysate, and were 1:50 diluted using ultrapure water. Finally, the ICP-MS was performed to determine the content of Zn^{2+} in one cell. The Zn^{2+} concentration in HeLa cells with or without treatment of AuNPs@ZIF-8 was calculated to be about 18.25 mM and 0.08 mM.

Cell Culture. MDA-MB-231 (Human metastatic breast cancer cells) and MCF-7 cells (Human non-metastatic breast cancer cells) were expanded in the growth media (DMEM containing 1% Gln, 10% FBS and 1%) in a 5% CO_2 humidified incubator at 37 °C. The culture solution was replaced every 48 h. The cells were cultured until 80–90% confluence for further use.

Cell Viability Assay. The cell viability assays were implemented in the light of MTT assay as standard protocol. MDA-MB-231 and MCF-7 cells (5×10^5 cells/well) were seeded in a 96 well microplate and cultivated in DMEM overnight under 5% CO_2 at 37 °C. Furthermore, different concentrations of 3D DNA walker were introduced and co-incubated with cells for 24 h under the same conditions. After that, 10 μL MTT solution (5 mg/mL) in PBS was incubated for 2 h to form formazan. The medium was aspirated and replaced with 100 μL DMSO in each well to dissolve the formazan salt. The whole experiments were repreated three times. The percentage of cell viability of the control was suggested as 100%.

Cell Staining and Imaging. Two types of cells (MCF-7 and MDA-MD-231) were seeded into Laser confocal special dishes (5×10^5 cells/well) under 5% CO_2 at 37 °C and then cultured overnight to get adhesion. Furthermore, the cells were co-incubated with 3D DNA walker (30 μg/mL) for 0.5 to 8 h. After that, the cells were rinsed with PBS for 3 times and fixed by 4% paraformaldehyde at room temperature for ten minutes. Finally, the cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (1 μg/mL) in PBS for ten min and further rinsed with PBS for 3 times. Similarly, MDA-MB-231 cells were incubated with 3D DNA walker under the same conditions and then co-stained with lyso@tracker (75 nM) for 2 h.

To regulate the miRNA-10b expression level in the living cells, MDA-MB-231 cells were first transfected with 10 μL of MicrONhsa-miR-10b-5p mimic or MicrOFFhsa-miR-10b-5p inhibitor or (0.2 mM) in 1 mL culture medium at 37 °C for 24 h. Then the cells were incubated with 1 mL culture solution containing 30 μg/mL 3D DNA walker at 37 °C for 6 h. All fluorescence images were obtained by a confocal laser scanning microscope and flow cytometry was used to detect the fluorescence intensity versus counts of cells.

S2. Figures and tables

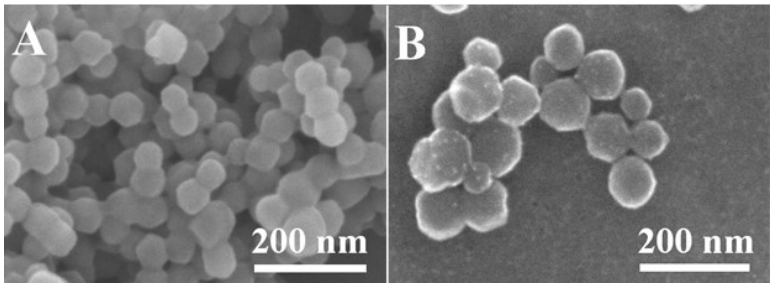

Figure S1. SEM images of ZIF-8 (A) and AuNPs@ZIF-8 (B).

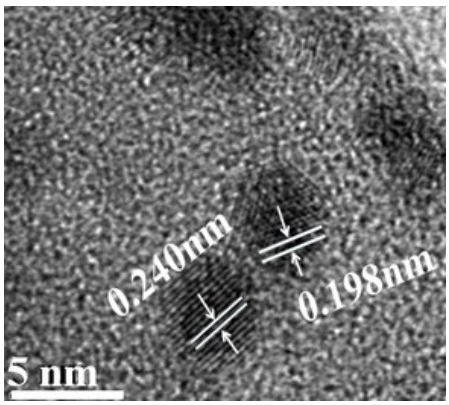

Figure S2. HRTEM of AuNPs@ZIF-8.

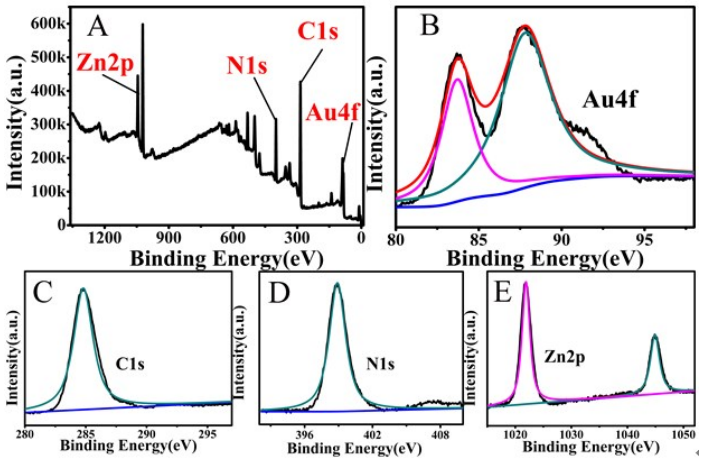

Figure S3. XPS spectra of AuNPs@ ZIF-8: survey spectrum (A); Au 4f (B); C1s (C); N1s (D); Zn2p (E).

The surface elemental compositions and valence states of the prepared samples were determined by X-ray photoelectron spectroscopy (XPS) technology. The XPS survey spectrum in **Figure S3** exhibits evidence that element Au, C, N and Zn are indeed in the hybrid AuNPs@ZIF-8 which coincides with the observation from TEM-mapping, further verifying the successful preparation of AuNPs@ZIF-8.

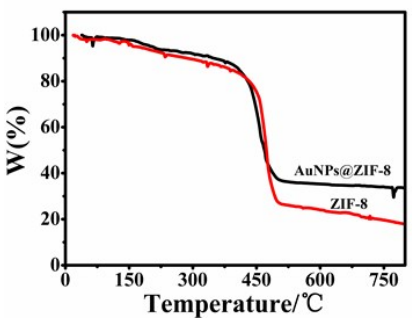

Figure S4. Thermogravimetric analysis (TGA) analysis of AuNPs@ZIF-8 and ZIF-8.

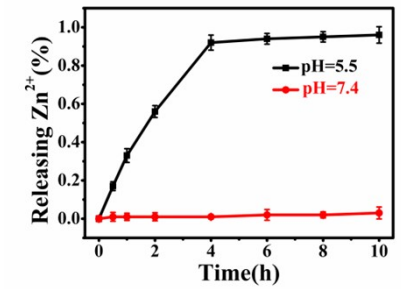

Figure S5. The proportion of released Zn^{2+} with time at pH 5.5 and pH 7.4.

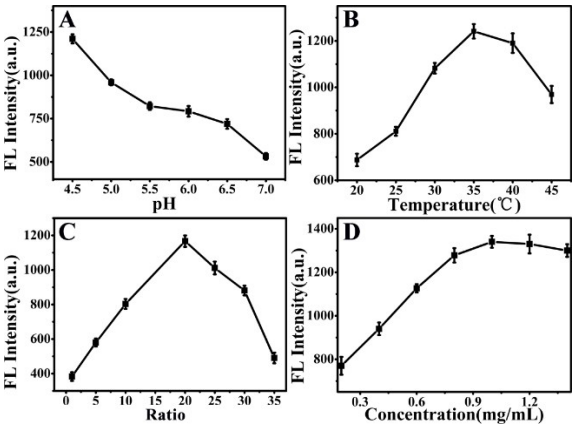

Figure S6. Effects of pH (A); incubation temperature (B); ratio of substrate stand to DNAzyme walking stand (C) and the concentration of the AuNPs@ZIF-8 (D) on the response of miRNA initiated 3D DNA walker.

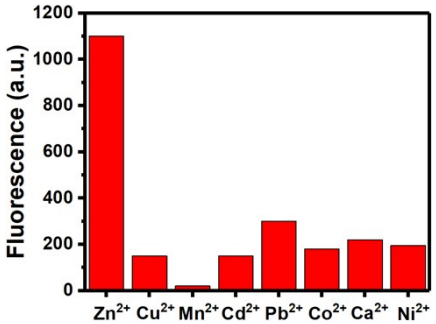

Figure S7. The selectivity of the system response to different divalent metal ions.
Under the same experiment in the presence of 10 nM miR-10b with other divalent metal ions, the fluorescence of the DNA/Au NPs has no obvious change towards other different divalent metal ions except Zn^{2+}, which demonstrated the high specificity of the system towards Zn^{2+}.[2,3,4]

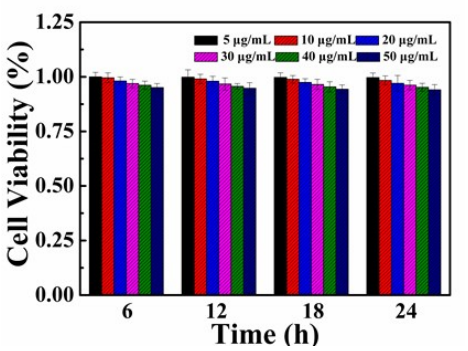

Figure S8. MTT assay of cytotoxicity for the 3D DNA walker in MDA-MB-231 cells. MDA-MB-231 cells were treated with different concentrations (5, 10, 20, 30, 40 and 50 μg/mL) of 3D DNA walker for 6, 12, 18 and 24 h, respectively. It showed the 3D DNA walker had slight toxicity to the cells even at 50 μg/mL after 24 h incubation with the cell viability decreased by ~9%.

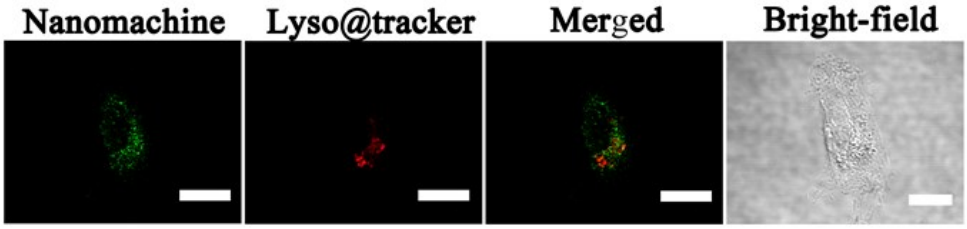

Figure S9. Fluorescence images for co-localization analysis with lysosome in MDA-MB-231 cells. MDA-MB-231 cells were incubated with 30 μg/mL DNA walker, and then treated with 75 nM lyso@tracker.

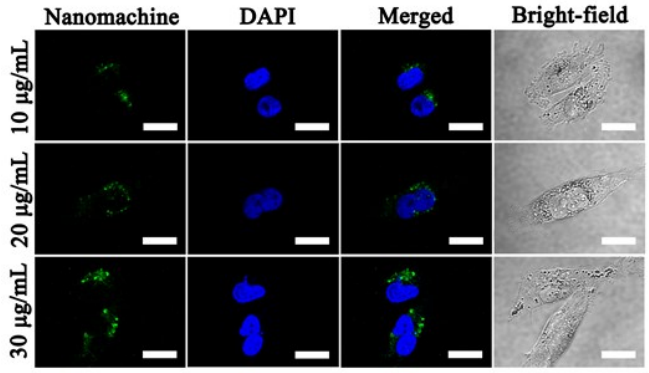

Figure S10. Fluorescence images of the MDA-MB-231 cells treated with different concentrations of the 3D DNA walker (10, 20, and 30 μg/mL) for 2 h.

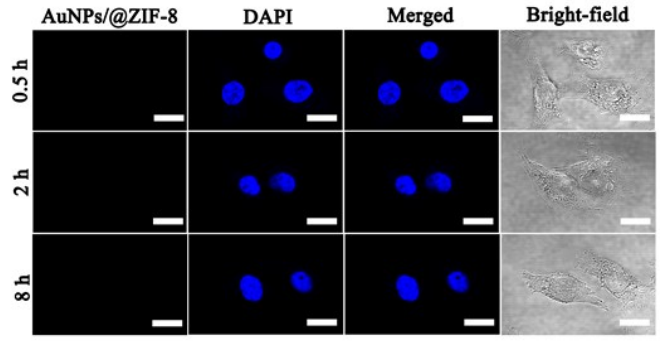

Figure S11. Fluorescence images of the MDA-MB-231 cells treated with 30 μg/mL AuNPs@ZIF-8 for different time.

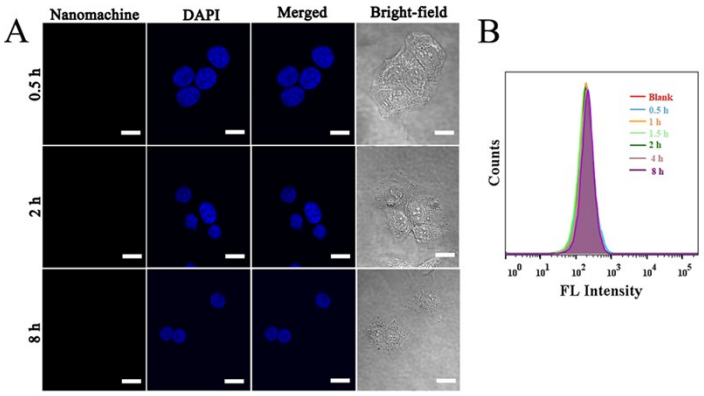

Figure S12. Fluorescence images of the MCF-7 cells treated with 30 μg/mL 3D DNA walker for different time (A) and the corresponding flow cytometry analysis (B).

Method	Driving Force	Linear Range	LOD	Ref.
DNAzyme-based ratiometric fluorescence	Zn^{2+}-DNAzyme	2 to 60 nM	0.68 nM	[5]
Highly Integrated DNA Motor	Mn^{2+}-DNAzyme	2.5 to 20 nM	1.2 nM	[6]
Entropy-driven DNA nanomachine	Toehold-assisted branch migration	20pM to 10 nM	8 pM	[7]
gold nanoparticle loaded split-DNAzyme probe	Mg^{2+}-DNAzyme	0 to 1nM	10 pM	[8]
3D DNA Walker	Zn^{2+}-DNAzyme	0.1 to 100 nM	34pM	This work

Table S1. Comparison of the present 3D DNA walker for miRNA detection with other DNA walkers.

S3. References

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