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Supporting Information

A Cancer Cell Membrane Vesicle-packaged DNA Nanomachine for Intracellular microRNAs Imaging

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Experimental Section

Materials and regents

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (China). Dimethyl sulphoxide (DMSO) was obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Hoechst 33342, calcein-AM and propidium iodide (PI) were obtained from Yeasen Biotech. Co., Ltd. (Shanghai, China). Phosphate buffer saline (PBS, pH 7.4), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA and penicillin-streptomycin were purchased from Gibco Life Technologies (AG, Switzerland). All other chemicals used in this study were analytical reagent grade and used without further purification. The ultrapure water was obtained from a Millipore water purification system (18 MΩ, Milli-Q, Millipore, USA). All the DNA sequences were purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The RNA sequences purified using high-performance liquid chromatography were obtained from Gene Pharma (Shanghai, China). All the miRNA sequences were diluted in diethyl pyrocarbonate (DEPC)-treated water for experiments, and all the experiments were performed in a laminar flow bench for a clean environment to control the influence of RNase. Before the MB were carried out experiment, they were all annealed (heat at 95 °C for 5 min, gradually cool to 25 °C at 5 °C/min, and stand at 25 °C for 1 h at least) in PBS (10 mM, pH = 7.4, with 137 mM NaCl), ensuring the desirable hairpin structures.

Synthesis of AuNPs

The Au NPs were first synthesized according to previous reports.[1] First, 150 mL of sodium citrate (2.2 mM) was heated to boiling with vigorous stirring, then 1 mL of $HAuCl_4$ solution (25 mM) was added, the color of the solution changed from yellow to soft pink within 10 min, and gold seeds (10 nm) were formed. The solution was then cooled down to 90 °C, 1 mL of $HAuCl_4$ solution (25 mM) was added, and the new solution reacted for another 30 min; this process was repeated once more. Subsequently, 55 mL of the solution was extracted and 53 mL of ultrapure water and 2 mL of sodium citrate (60 mM) were added; after the temperature reached 90 °C, 1 mL of $HAuCl_4$ solution (25 mM) was injected and subsequent solution reacted for 20 min to obtain the Au NPs (25 nm). Then

the obtained AuNPs were used as seed solution and stage two was repeated to obtain the final AuNPs product.

Synthesis AuNP-DNA

300 μL of AuNP solution was mixed with 15 μL of 100 μM poly (A)-tagged DNA probes and then frozen at –20 °C or –80 °C. After thawing, the solution was washed three times with Buffer A (0.1 M NaCl in 0.01 M phosphate buffer, pH 7.4) by centrifugation at 4 °C at 12,000 rpm/min for 30 min. Finally, the pellet was resuspended in Buffer B (0.3 M NaCl in 0.01 M phosphate buffer, pH 7.4) and the resulted poly (A)-DNA-AuNP probes were stored at 4 °C in the dark.

Preparation of AuNP-DNA@CM

MCF-7 cell membranes were collected according to our previously published method with brief modifications.[2] Ordinary Dulbecco's modified Eagle's medium (DMEM) was used to grow MCF-7 cells to 1 × 107 cells/mL, which was used to extract cell membranes. The cell membranes were extracted using the Cell Membrane Extraction Kit and repeated freezing and thawing method. The extracted cell membrane was freeze-dried and stored in – 80℃ for further use. The extracted cell membrane was squeezed nine times using a cell extrusion apparatus with a 400 nm quasi-polycarbonate porous membrane. The amount of cell membrane and material was mixed in a ratio of 1:1, and then the cell membrane extrusion apparatus was used again. Extrusion was performed 11 times with the extruded film size of 200 nm to obtain cell-membrane-modified AuNP-DNA.

Characterization of AuNP-DNA@CM

The morphologies of AuNP-DNA@CM were examined with a FEIF20 TEM (FEI, USA) and a JSM-6700FSEM) (JEOL, Japan. AFM measurement was carried out on NanoscopeIIIa (Digital Instrument, USA) under tapping mode. The UV-vis-NIR absorption was acquired with a UV-1800 spectrophotometer (Shimadzu, Japan) and processed with Origin Lab software. The size distribution and zeta potential analysis were performed using a Zetasizer Nano ZS system (Malvern, UK), and the 633 nm laser was used for the DLS. The confocal laser scanning microscopy (CLSM) images were acquired on a FV1200 microscope (Olympus). The temperature was measured by a digital thermometer

with a thermocouple probe and recorded once every 2 s. An infrared thermal imaging camera (Fluke TiS65, USA) was used to monitor the temperature change.

Feasibility of intracellular miRNA-155 accurate detection

According to previous report, we have design 2 types of MB to test the capability to accurate detect intracellular miRNAs.[3] The miRNAs MB (50 nM) incubated with miRNA (50 nM) at 37 °C for 60 min, and detect by fluorescence spectrometer.

Cell culture

Two kind of cancer cells (A549 cells and MCF-7 cells) were used in the whole experiment. All the cells were incubated in DMEM culture containing 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

In vitro cytotoxicity assay

MCF-7 cells (5.0×10^4) were cultured for 12 h in a 96-well plate containing DMEM (100 μL) in each well, and then the medium was replaced with fresh serumfree medium (Opti-MEM) alone or medium containing AuNP-DNA or Au-DNA@CM and incubated for another 4 h. Next, MTT (20 μL, 5 mg/mL) with fresh DMEM (100 μL) was then added to each well. The media was removed 4 h later, and DMSO (100 μL) was added to solubilize the formazan dye. After shocking (37 °C, 120 rpm) for 15 min, the absorbance of each well was measured using a Tecan Sunrise at 488 nm. The cytotoxicity of AuNP-DNA@CM was estimated by the percentage of growth inhibition calculated with the formula.

Growth inhibition % = (1 - Atext/Acontrol) 100%

microRNA Imaging in Living Cells

MCF-7 cells were cultured in a confocal dish for 24 h in the same manner described above. Then the medium was removed and fresh Opti-MEM containing AuNP-DNA and AuNP-DNA@CM (100 μg/mL) was added. After incubation for 4 h, the cells were washed with PBS twice and fresh DMEM was added for further incubation. Twelve hours later, the cells were imaged with a CLSM.

Table S1. DNA sequences used in this work.

Name	Oligonucleotide sequences (5'-3')
miRNA-155	TTA ATG CTA ATC GTG ATA GGG GT
H1	AAA AAA AAA AAC CCC TAT CAC GAT TAG CAA TCG AGG CTA ATC GTG
H2	CGA TTA GCC TCG ATT GCT AAT CGT GTA GCA ATC GAG G
H1-FAM	AAA AAA AAA AAC CCC TAT CAC GAT TAG CAA TCG AGG CTA ATC GTG-FAM
BHQ-H1-FAM	BHQ-AAA AAA AAA AAC CCC TAT CAC GAT TAG CAA TCG AGG CTA ATC GTG-FAM
1 mismatch	TTA ATG CTA ATC GTG ATA GGG GA
2 mismatch	TTA ATG CTA ATC GTG ATA GGG CA
miRNA-21	TAG CTT ATC AGA CTG ATG TTG A
miRNA-1246	AAT GGA TTT TTG GAG CAG G

Figure

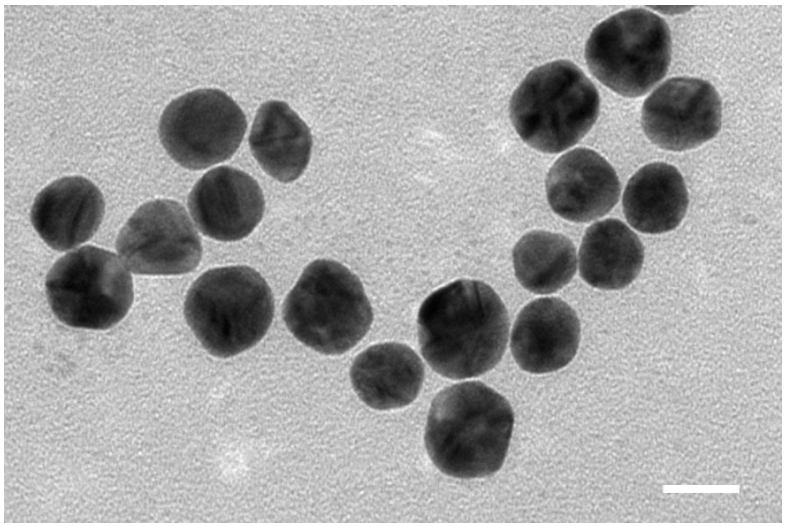

Fig.S1 TEM image of Au-DNA. Scale bar: 25 nm.

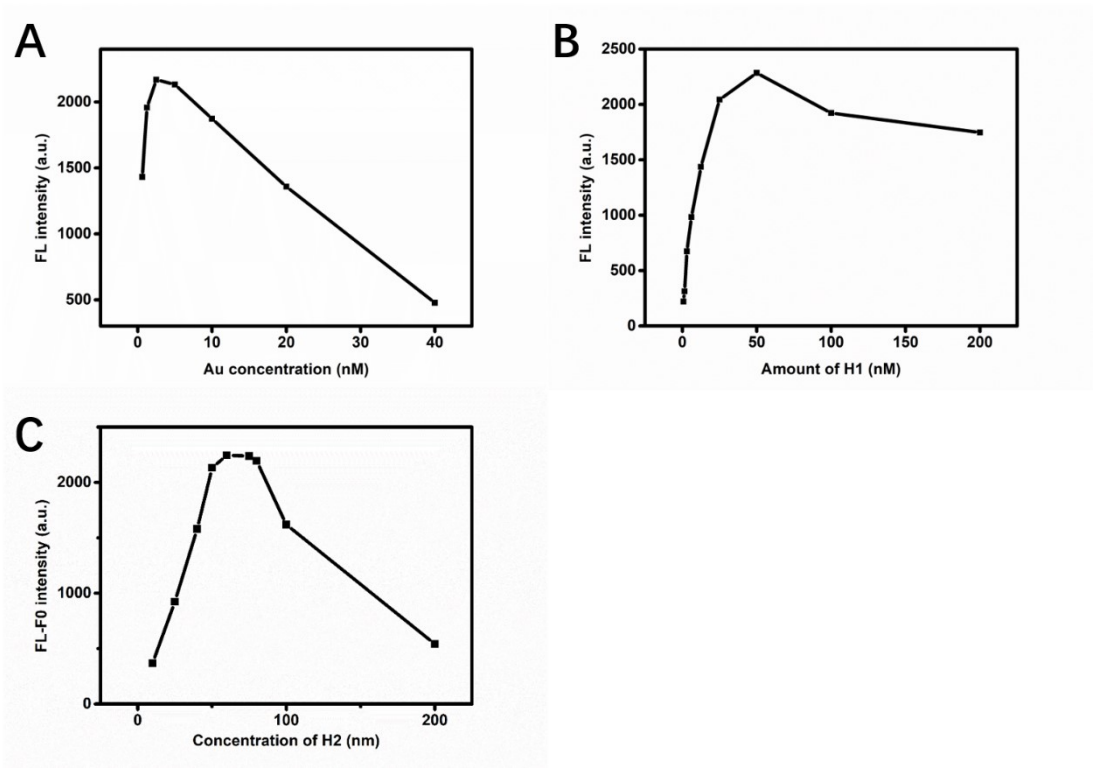

Fig.S2 (A) optimization of system concentration, the fluorescence intensity of AuNP-H1-FAM concentration with excess H2 and miRNA. (B) the fluorescence intensity of H1-FAM concentration with AuNP (2.5 nM) and excess H2 and miRNA. (C) the fluorescence intensity of H2 concentration with AuNP-H1-FAM (2.5 nM) and excess miRNA-155. the fluorescence intensity of H2 concentration with AuNP-H1-FAM (2.5 nM) and excess miRNA-155.

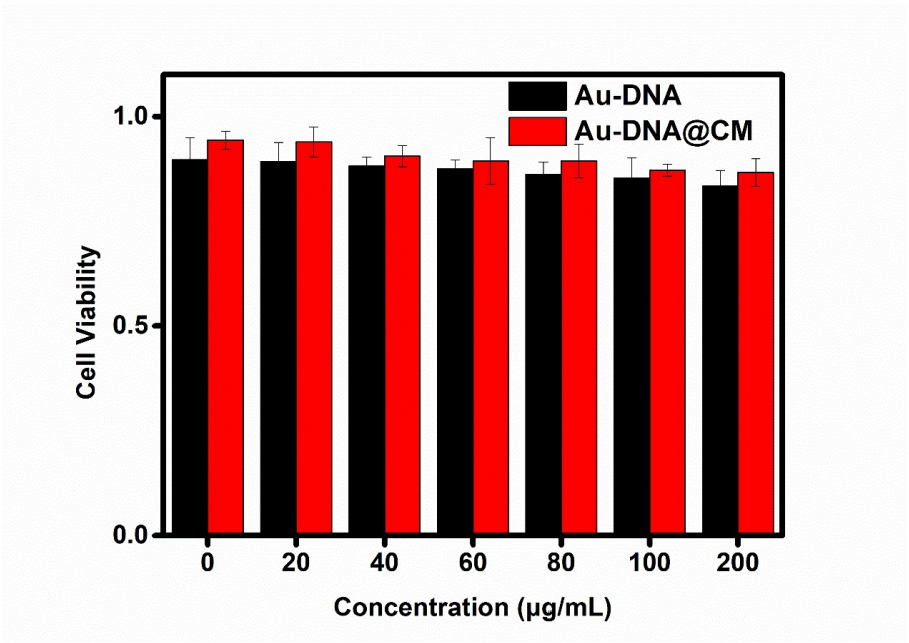

Fig.S3 MTT cytotoxicity test. 4 h of Au-DNA and Au-DNA@CM treated MCF-7 with different concentration from 0-200 μg/mL. The data error bars indicate means ± SD (n=3).

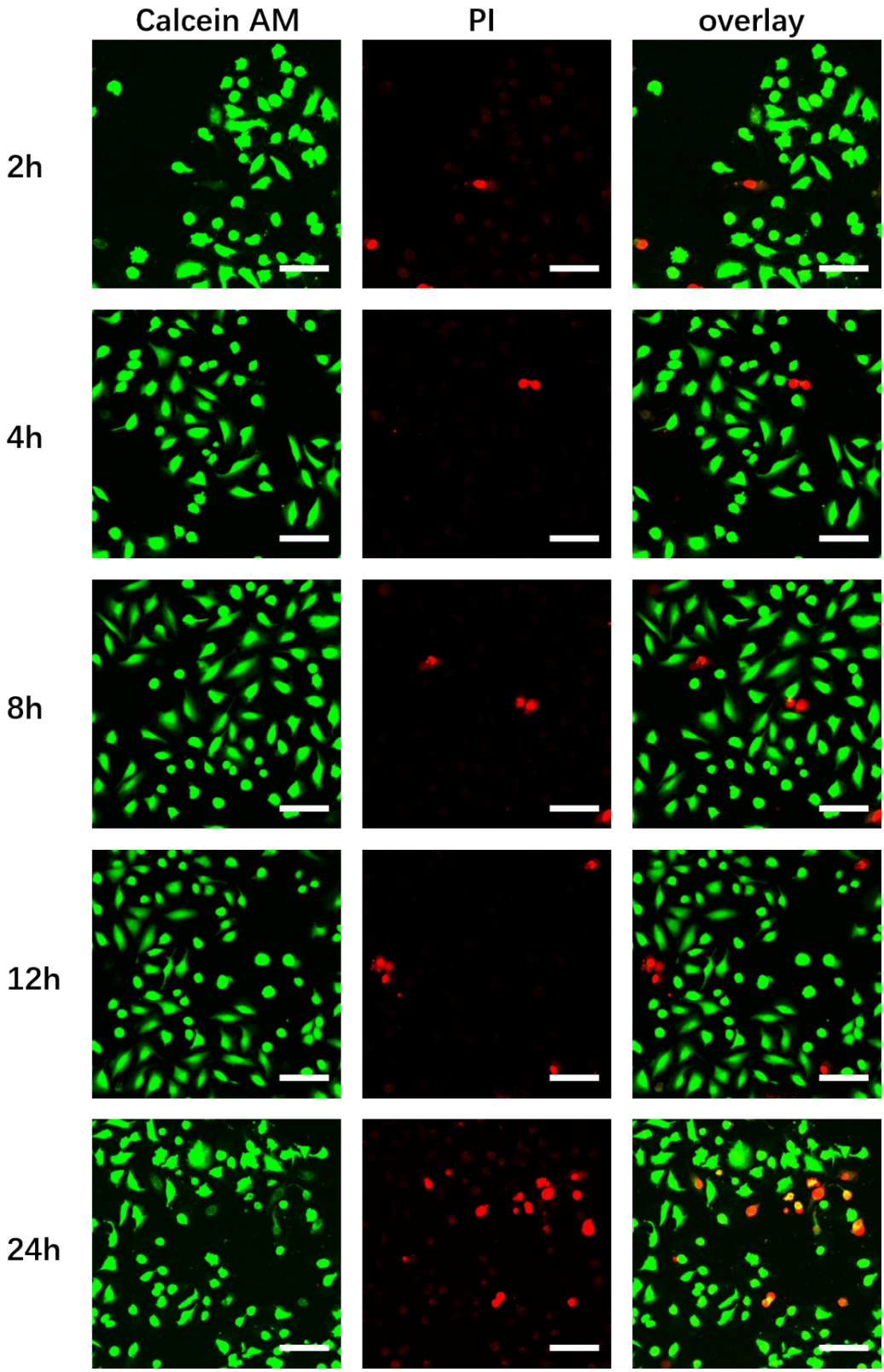

Fig.S4 Fluorescence images of calcein AM/PI-costained MCF-7 cells incubated with the Au-DNA@ CM at different time points. Scale bar: 100 μm.

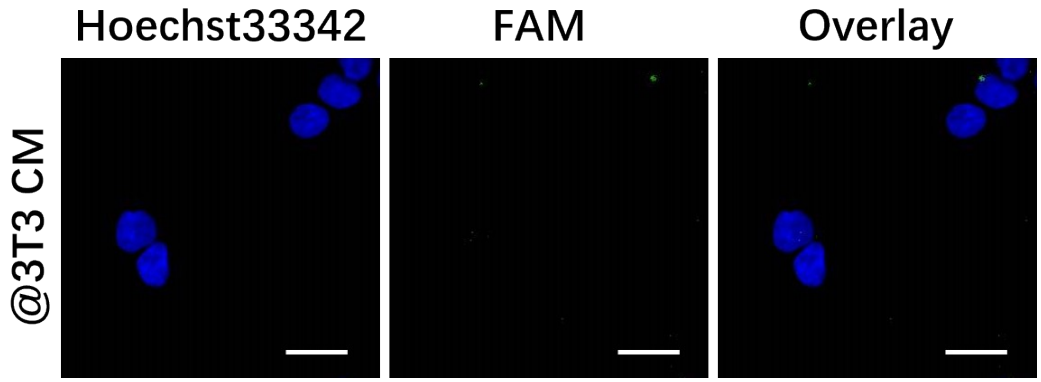

Fig.S5 Confocal laser scanning microscopy images of MCF-7 cells treated with Au-DNA@3T3 CM. scar bar: 50 μm.

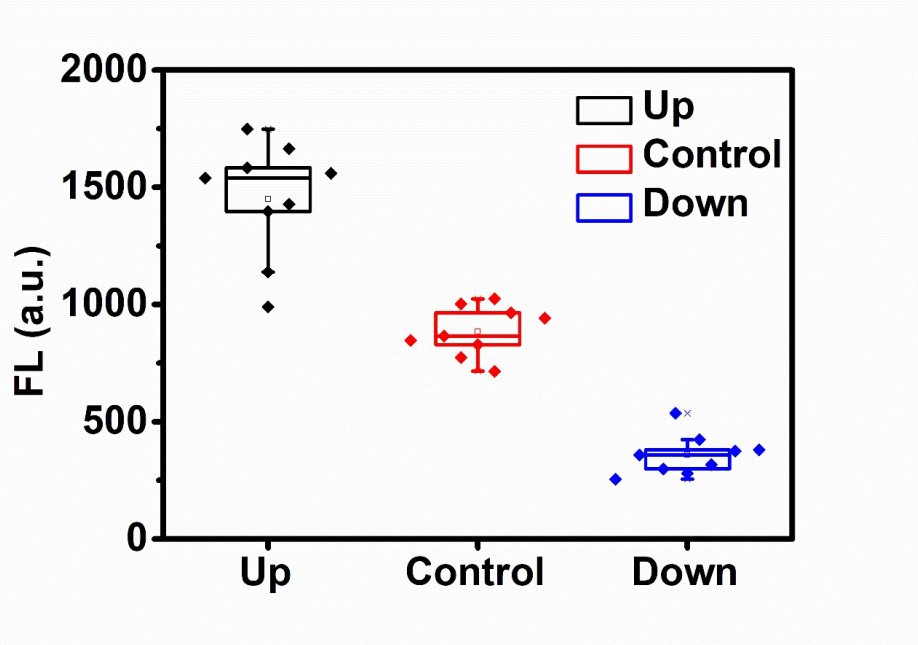

Fig.S6 MCF-7 cells treated with PBS, miRNA-155 siRNA, and miRNA-21 mimics and incubated with Au-DNA@ CM. The data error bars indicate means ± SD (n=3).

Reference

1. N. G. Bastús, J. Comenge and V. Puntes, *Langmuir*, 2011, **27**, 11098-11105.
2. K. Zhang, X. Meng, Z. Yang, Y. Cao, Y. Cheng, D. Wang, H. Lu, Z. Shi, H. Dong and X. Zhang, *Adv. Mate.*, 2019, **31**, 1807888.
3. C. Jung, P. B. Allen and A. D. Ellington, *Nat. Nanotechnol.*, 2016, **11**, 157-163.