Cascaded catalytic hairpin 3D DNA walker-assisted dual-targeting biomimetic sensor for intracellular microRNA imaging

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Experimental section:

1. *Materials*

Gold(III) chloride hydrate ($HAuCl_4 \cdot 4H_2O$) and sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8) was offered by Yeasen Biotechnology Co., Ltd. (Shanghai, China). The 1 × TE buffer [10 mmol L^{-1} Tris-HCl, 1.0 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), pH 7.8-8.2] and DEPC water were purchased from Sangon Biotechnology Inc. (Shanghai, China). All DNA and RNA sequences (Table S2) were synthesized and purified by Sangon Biotechnology Inc. (Shanghai, China).

2. *Apparatus and measurements*

After ambient air-drying, the morphology of prepared materials was characterized using transmission electron microscope (TEM; JEOL JEM-2100Plus; Tokyo, Japan; 200 kV) and scanning electron microscope (SEM; JEOL JCM-5700; Welwyn Garden City, UK). The hydrodynamic size and zeta potential of prepared materials were measured in PBS (PH 7.4) at 25 ℃ using dynamic light scattering (DLS; Malvern Zetasizer Nano ZSE; Malvern, UK). The UV-vis absorption spectra were recorded on a UV-3200 spectrophotometer (Mapada, Shanghai, China). Fluorescence spectra were obtained on a RF-6000 fluorophotometer (Shimadzu, Kyoto, Japan). Confocal laser scanning microscopy (CLSM; TCS SP8 STED 3X, Leica; Solms, Germany) was used to obtain cell images.

3. *Cell culture*

MCF-7 and MCF-10A cells were obtained from Wuhan Pricella Biotechnology Co., Ltd. (Wuhan, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin−streptomycin and 10% fetal bovine serum (FBS) at 37 °C in a humidified 5% CO_2 incubator.

Supporting figures

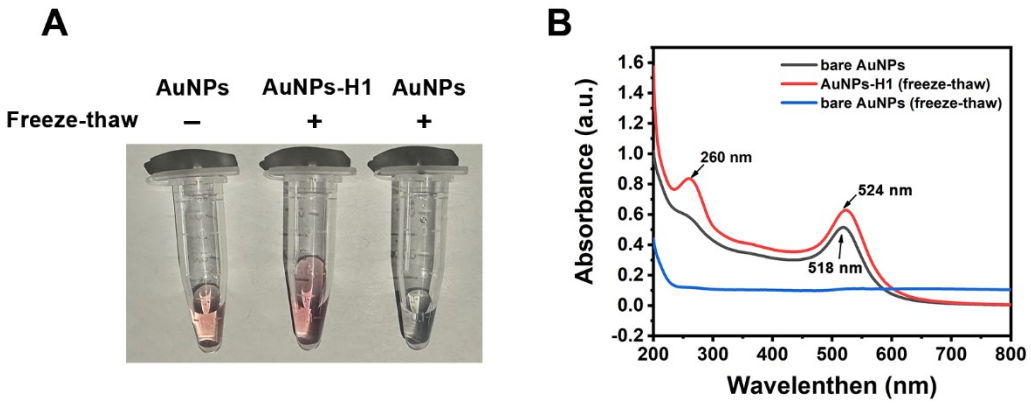

Figure S1. Characterization of AuNPs-H1. (A) Photographs showing the color of AuNPs before and after a freeze−thaw cycle in the presence or absence of H1. (B) UV–vis absorption spectra of bare AuNPs, bare AuNPs after freeze-thaw, and AuNPs-H1 after freeze-thaw.

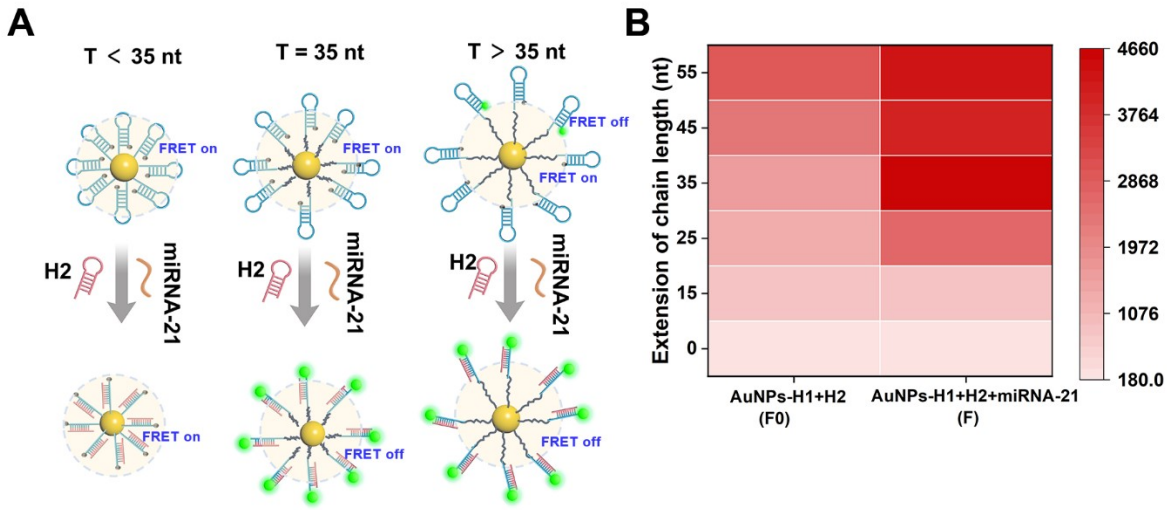

Figure S2. The effect of different numbers of bases of H1 5' end extension on the detection efficiency of AuNPs-H1. (A) Schematic diagram. (B) Fluorescence intensity of the DNA walker with different length of H1 strand.

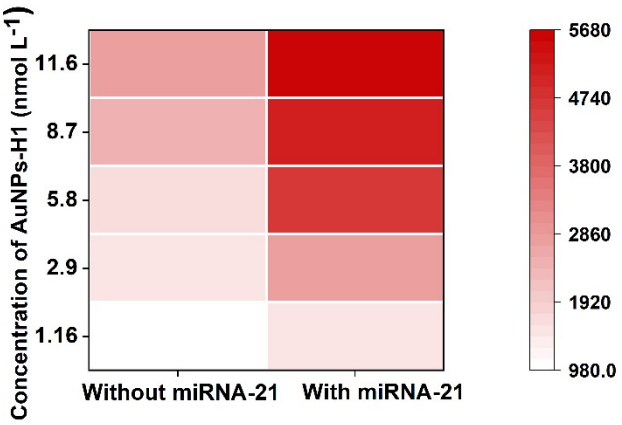

Figure. S3. The Fluorescence intensity of the DNA walker in the presence or absence of miRNA-21.

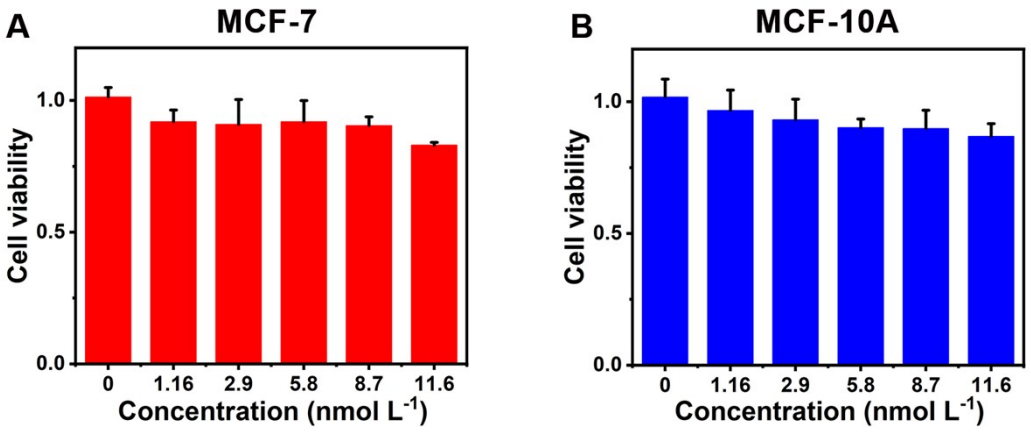

Figure S4. Cell viability of (A) MCF-7 and (B) MCF-10A cells treated with different concentrations of AuNPs-H1/H2@TCM-apt. All bars represent means ± SD (n = 3).

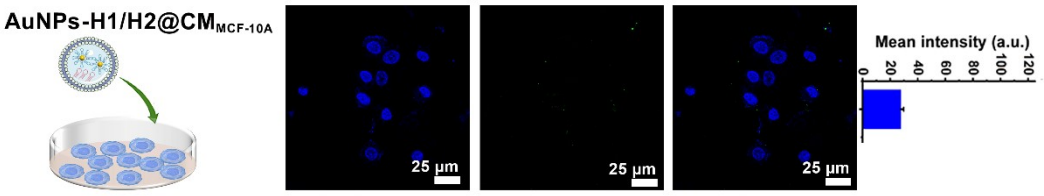

Figure S5. CLSM images and fluorescence intensity of MCF-7 cells treated with AuNPs-H1/H2@$CM_{MCF-10A}$. Scale bar = 25 μm.

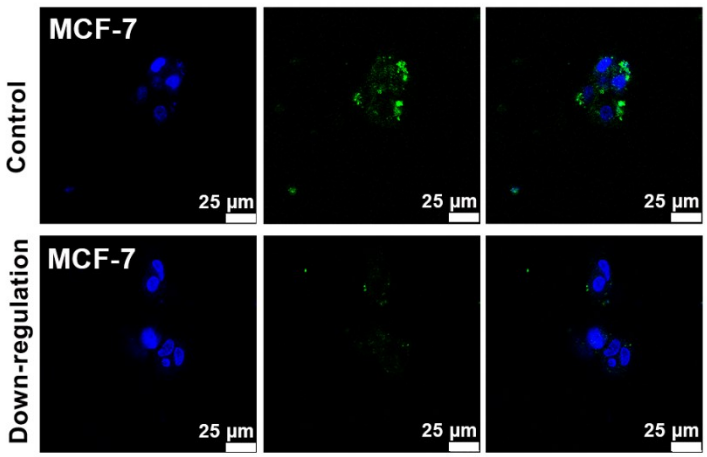

Figure S6. CLSM images of miRNA-21 in MCF-7 cells treated with or without miR-21 inhibitor. Scale bar = 25 μm.

Supporting tables

Table S1 Comparison of different methods for miRNA detection

Technology	Target	Linear range	LOD	Living cells imaging	Ref.
Fluorescence	miRNA-141	5-100 nmol L^{-1}	2.5 nmol L^{-1}	No	[1]
Fluorescence	miRNA-21	------	1.27 nmol L^{-1}	Yes	[2]
Colorimetric	miRNA-155	1-100 nmol L^{-1}	0.7 nmol L^{-1}	No	[3]
Fluorescence	miRNA-21, let7a	0.05-10 nmol L^{-1}, 0.05-4 nmol L^{-1}	16.67 nmol L^{-1}, 7.14 nmol L^{-1}	Yes	[4]
Fluorescence	miRNA-155	0-30 nmol L^{-1}	0.35 nmol L^{-1}	Yes	[5]
Fluorescence	miRNA-21	1-60 nmol L^{-1}	0.69 nmol L^{-1}	Yes	[6]
Fluorescence	miRNA-21	0-10 nmol L^{-1}	0.46 nmol L^{-1}	Yes	This work

Table S2. The details of sequences used in the research

Name	Sequences (5'-3')
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
H1	TCAACATCAGTCTGATAAGCGGTAGGGTAGGGCGCTTATCAGACTGA
T_{35}-H1	SH-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAACATCAGTCTGATAAGCGGTAGGGTAGGGCGCTTATCAGACTGA-FAM
H2	TGATAAGCGCCCTACCCTACCGCTTATCAGACTGAGGTAGGGTAGGGCGGGTTGGG
AS1411 aptamer	GGTGGTGGTGGTTGTGGTGGTGGTGG
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA141	UAACACUGUCUGGUAAAGAUGG
miRNA-122	UGG AGU GUG ACA AUG GUG UUU G
miRNA-210	CUG UGC GUG UGA CAG CGG CUG A

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