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Supporting Information

Three-dimensional bipedal DNA walker enabled logic gates responding to telomerase and miRNA

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Experimental

Materials and chemicals

Trisodium citrate, manganese (II) chloride ($MnCl_2$), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid (CHAPS), tetramethylammonium hydroxide, phenylmethylsulfonyl fluoride (PMSF), diethypyrocarbonate (DEPC), and ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic Acid (EGTA) were ordered from Sigma (USA). 3'-Azido-3'-deoxythymidine (AZT) was obtained from Aladdin Biological Technology Co., Ltd. (Shanghai, China). Deoxynucleotide triphosphates (dNTPs) were purchased from New England Biolabs Ltd. (Beijing, China). HeLa cells were from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). All oligonucleotides and were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences were listed in Table S1.

Preparation of AuNPs

Bare AuNPs were prepared by sodium citrate reduction of $HAuCl_4$.[1] Briefly, 100 mL of $HAuCl_4$ solution was firstly prepared with the concentration of 0.01% (w/v). Then, it was boiled. 10 mL of sodium citrate with the concentration of 38.8 mM was spiked rapidly. The resulted solution was stirred with the reflux condenser. After reacting for 0.5 h, the heat was removed and the solution was slowly cooled to room

temperature. The AuNPs were then centrifuged at 12000 rpm for 30 min and resuspended in pure water.

All DNA probes are firstly prepared in the 10 mM phosphate buffered saline (pH 7.4) containing 10 mM TCEP. For AND gate, 100 μL of Probe S1 (30 μM) was mixed with 900 μL of AuNPs. After 16 h incubation, the nanoconjugates were "aged" in 10 mM phosphate (pH 7.0) containing 0.1 M NaCl for 24 h. Probe L1 and L2 were added in the S1 modified AuNPs with the final concentration of 3 μM for 2 h. Afterward, Excess reagents were removed by centrifuging at 12000 rpm for 0.5 h. For OR gate, Probe S2 modified AuNPs were achieved with the same manner and Probe L3 (3 μM) was added in the nanoconjugates for the hybridization.

Preparation of MnO_2 nanosheets

MnO_2 nanosheets were prepared according to a previous report.[2] Briefly, the solution of tetramethylammonium hydroxide and H_2O_2 was prepared with the concentrations of 0.6 M and 3%, respectively. 20 mL of the above solution was mixed with 10 mL of $MnCl_2$ with the concentration of 0.3 M. The solution then turned to dark brown, which was further stirred overnight. To purify the obtained nanosheets, the bulk MnO_2 was centrifuged at 2000 rpm for 10 min and further washed by pure water and methanol, respectively. Next, 5 mg of dried MnO_2 nanosheets was dispersed in 20 mL of pure water, followed by ultrasonicating for 10 h. Further, 20 μL of MnO_2 nanosheets was mixed with 5 μL of TP (50 μM), 2 μL of W1 (50 μM) and 2 μL of W2 (50 μM) to form the DNA-MnO_2 nanocomposites.

Telomerase extraction

HeLa cells were cultured in DMEM containing 10% FBS at 37 °C in an atmosphere of 5% CO_2. 10^6 cells were firstly collected and resuspended in 200 μL of ice cold 0.5% (w/v) CHAPS lysis buffer (pH 7.5) containing 10 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF and 10% (v/v) glycerol. After incubating on ice for 30 min, the solution was pipetted for several times to thoroughly lyse the cells. The extract was then separated by centrifuging at 4 °C (12000 rpm for 30 min). The supernatant was collected and transferred into a fresh RNase-free tube. Next, the extracted telomerase was ready for assay or stored frozen at −80°C.

Bipedal DNA walking for logic gate construction

The samples were mixed with Probe TP (2 μM), W1 (2 μM) and W2 (2 μM). The solution was incubated at 37°C for 2 h, which was used as logic inputs. 10 μL of the above solution was blended with 100 μL of DNA-AuNPs nanocomposites (AND and OR gates) containing Mn^{2+} (2 μM) for 40 min. Afterward, fluorescence emission spectra were recorded (excited at 494 nm). To check the selectivity of the DNAzyme, a series of other metal ions (Mg^{2+}, Zn^{2+}, Ca^{2+}, Ba^{2+}, Cu^{2+}, Pb^{2+}) with the concentrations of 2 μM were used to replace Mn^{2+}. Then, the obtained fluorescece spectra were compared.

Intracellular imaging

10^6 cells was firstly seeded in a confocal dish for 24 h. Then, 10 μL of DNA-AuNPs nanocomposites and 20 μL of DNA-MnO_2 nanocomposites were added. The cells were

incubated for another 6 h. Subsequently, the cells were washed with PBS for three times and observed under confocal microscope. For telomerase inhibition investigation, AZT was previously interacted with the cells for 24 h before further logic operations.

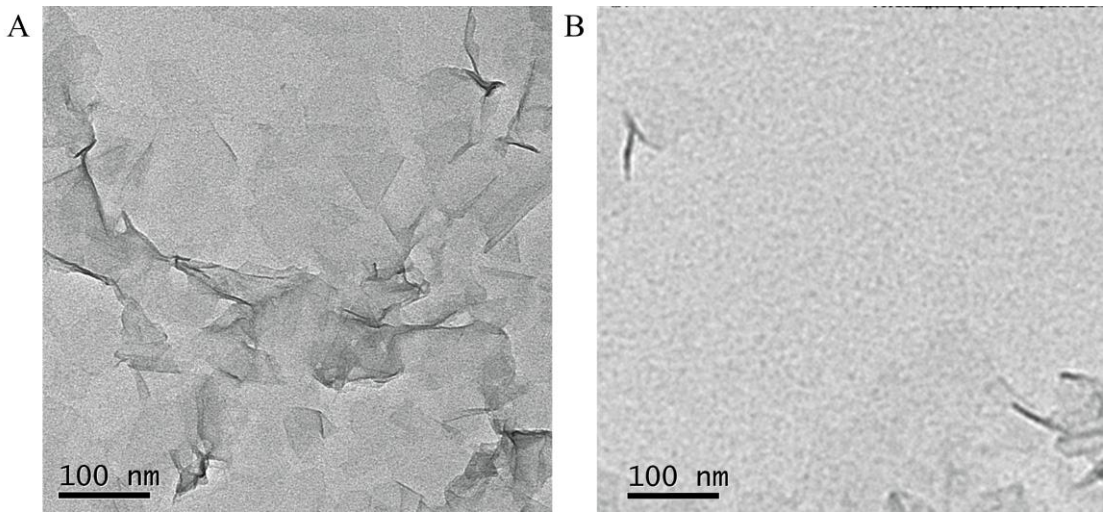

Figure S1. TEM images of MnO_2 nanosheets in the (A) absence and (B) presence of GSH.

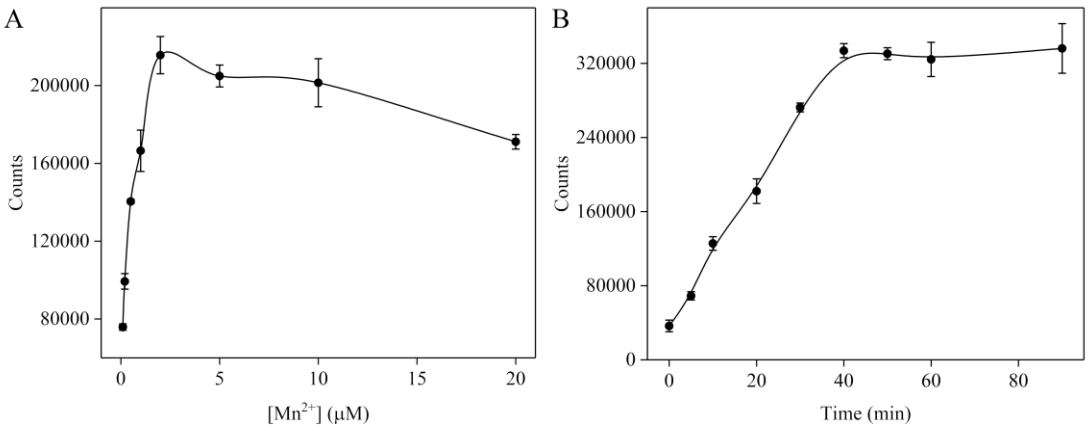

Figure S2. Optimizations of (A) Mn^{2+} concentration and (B) reaction time based on AND gate.

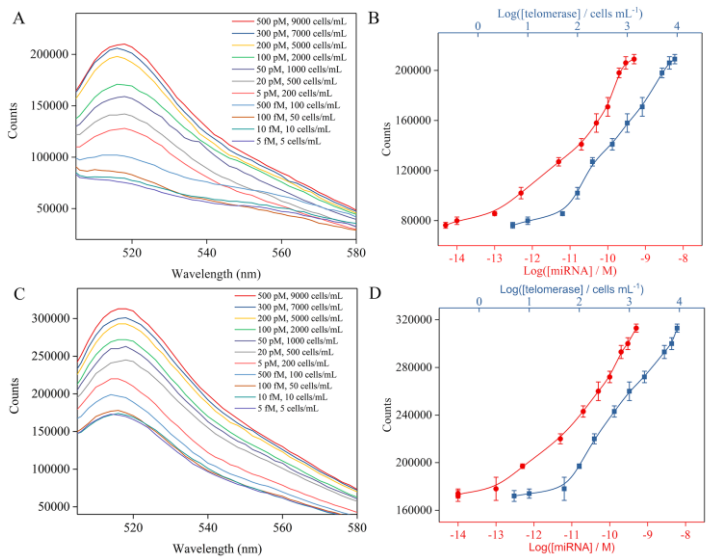

Figure S3. Fluorescence emission spectra of (A) AND gate and (C) OR gate for the measurements of different concentrations of target miRNA and telomerase. (B) and (D) are corresponding calibration curves. The concentration of DNAzyme is 2 μM. The concentrations of S1 and S2 are 3 μM.

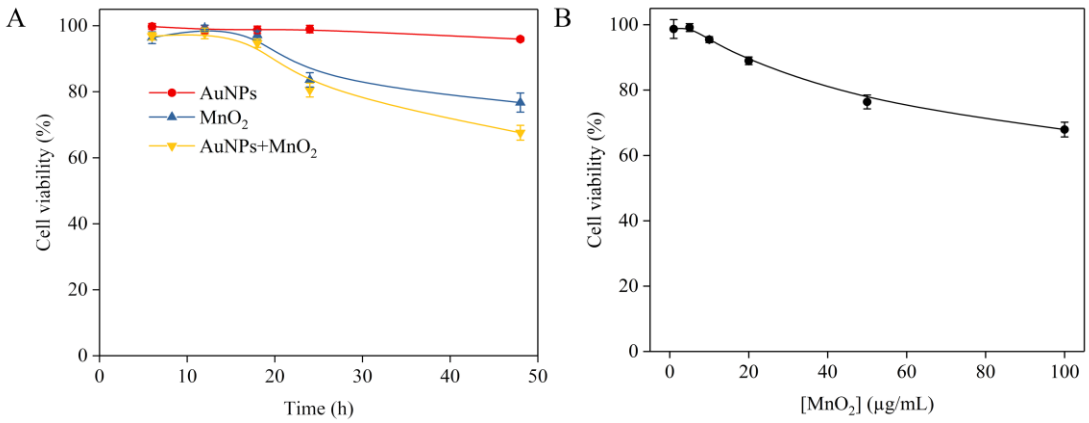

Figure S4. (A) Cell viability after treated with AuNPs, MnO_2 nanosheets and the mixture with different time durations. (B) Cell viability after treated with MnO_2 nanosheets with different concentrations.

Table S1. DNA and RNA sequences used in this work.

Name	Sequence (from 5’ to 3’)
W1	AACCCCTATCACGATTAGCATTAACTAATCGTCTTAATGCTA ATCGTGCTCCGAGCCGGTCGAAACCCTAACGGG
W2	CATTAAGACGATTAGTTAATGCTAATCGTGATAGGGGTATTA ACTAATCGTGCTCCGAGCCGGTCGAAACCCTAACGGG
miR-155	UUAAUGCUAAUCGUGAUAGGGGUU
miR-21	UAGCUUAUCAGACUGAUGUUGA
TP	AATCCGTCGAGCAGAGTT
L1	AACTAATCGTGTTT
L2	CCCTAACCCTAACCCTAAAACTCT
L3	FAM-CCCTAACCCTAAAACTCT
S1	SH-$(CH_2)_6$-CCCGTTTTACCCGTTAGGGT/rA/GGCACGATTAGTT-FAM
S2	SH-$(CH_2)_6$-CCCGTTTTAGGGCCCCGTGCCCGTTAGGGT/rA/GGCACGATTAG-FAM

References

1 P. Miao, Y. Tang, B. Wang, C. Jiang, L. Gao, B. Bo, J. Wang, *Electrochim. Acta.*, 2016, **190**, 396-401.

2 P. Miao, Y. Tang, *Anal. Chem.*, 2020, **92**, 12700-12709.