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Supporting Information

Photocontrolled and self-powered bipedal DNA walking machine for intracellular microRNA imaging

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Reagents and materials

$HAuCl_4 \cdot 4H_2O$, Tween 20, and tris(2-carboxyethyl) phosphine hydrochloride (TCEP·HCl) were obtained from Sigma-Aldrich (St. Louis, MO, USA). $MnCl_2$, $MgCl_2$, NaCl, dithiothreitol (DTT), tris (hydroxymethyl) aminomethane (Tris), and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). MicrONhsa-miR-21-5p mimic and micrOFFhsa-miR-21-5p inhibitors were obtained from RiboBio Company (Guangzhou, China). CCK-8 reagent was supplied by Dojindo Laboratories (Kumamoto, Japan). All oligonucleotides listed in Table S1 were synthesized and HPLC-purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA), with an electric resistance of >18.3 MΩ.

Apparatus.

UV-vis absorption spectra were studied on an UV-2550 spectrometer (Shimadzu, Japan). Transmission electron microscopy (TEM) images were taken on a JEM-2100 transmission electron microscope (JEOL, Japan). Fluorescence spectra and the real-time fluorescence date were recorded with a Hitachi F-7000 fluorescence spectrometer (Hitachi, Japan). Zeta potential and dynamic light scattering (DLS) were measured with the Malvern Zetasizer Nano ZS90 (Malvern, USA). Confocal images of cells were acquired with a Nikon confocal laser scanning fluorescence microscope (Nikon, Japan).

Synthesis of citrate capped AuNPs.

The preparation of the 13 nm AuNPs were reported previously.[1] In brief, 100 mL $HAuCl_4$ aqueous (0.01%) was heated to boiling under reflux. Then, 3.5 mL of the trisodium citrate solution (1%) was quickly added under stirring and kept it boiling for another 10 min. The solution changed from pale yellow to deep red. After the solution reached to room-temperature, the colloid was filtrated through a millipore membrane filter (0.45 μm). The prepared AuNPs were kept at 4 °C for future use. The concentration of these AuNPs was ~10 nM by measuring their UV-vis absorption at 520 nm ($\varepsilon = 2.7 \times 10^8\ M^{-1}\ cm^{-1}$).

Fabrication of bipedal DNA walking machine.

The bipedal DNA walking machine was prepared as described below. Firstly, the thiol-modified DNA strands were reduced by TCEP·HCl at a molar ratio of 1: 100 for 1 h to reduce the disulfide bond before use. Then, the blocker-DNAzyme strands were prepared by incubation of the 12.5 μL of 10 μM DNAzyme 1, 15.0 μL of 10 μM DNAzyme 2, and 55 μL of 10 μM blocker in the 25 mM Tris-HCl (pH 7.4) with 137 mM NaCl at 37°C for 2 h. The excess blocker strands were to confirm the DNAzymes were silenced by the blocker. Subsequently, the above mixture and 250 μL of 10 μM fluorescence substrate were added to the 500 μL AuNPs (10 nM) with the molar ratio of 1:25:500 for the AuNPs, the blocker-DNAzyme strands, and the fluorescence substrate. After shaken at room temperature for 16 h, 31 μL of 1% Tween 20 was added to the above solution to reduce the adsorption and aggregation of AuNPs. Then, the phosphate buffer (0.1 mM PB, pH = 7.4) was added to the above solution to reach 0.01 M, and NaCl solution (2.0 M) was slowly added to the solution to reach 0.3 M NaCl over an 8 h period four times. Finally, the mixture was centrifuged (13000 rpm, 30 min) and washed with Tris-HCl buffer three times. The solution was stored in the 25 mM Tris-HCl (pH 7.4) with 137 mM NaCl.

Measurement of substrates loading on AuNP.

The substrate strands on surface of AuNP were determined by the previous method.[2] 10 μL of 100 mM DTT was added to the 10 μL of 10 nM DNA-functionalized AuNPs solution, and the above solution was diluted to 100 μL by 1× PBS buffer. After the solution was shaken overnight, released FAM-labeled substrate strands were separated via centrifugation (13000 rpm, 10 min). The fluorescence of the solution was measured by F7000. By comparing to the standard curve of FAM-labeled substrate, we speculated ~78 substrate strands loading on AuNPs. On the basis of a molar ratio of 1:20 for the DNAzyme strand and the FAM-labeled substrate strand used together in the conjugation reaction, average ~ 4 DNAzyme strands were modified on each AuNPs.

PAGE electrophoresis.

All samples were prepared in advance for PAGE electrophoresis assay. For example, an aliquot of solution (7 μL) containing DNAzyme 3 (2.86 μM), blocker (3.14 μM) were incubated at 37°C for 2 h. Then, 1 μL substrate (20 μM), 1 μL miRNA-21 target (22 μM), and 1 μL Mn^{2+} (7500 μM) were added to the above solution and incubated at 37°C for 2 h. Finally, the products were analyzed by 20% denaturing polyacrylamide gel. Electrophoresis was carried out at 120 V

for 2.5 h.

Evaluation of miRNA-initiated bipedal DNA walking machine operation *in vitro*.

The miRNA-21 target with the same sequence of miRNA-21 was applied to initiate the operation of the walking machine. The bipedal DNA walking machine (2.5 nM) were incubated with the different concentrations of miRNA-21 in 25 mM Tris-HCl buffer containing 137 mM NaCl at 37°C for 1 h. Then, upon irradiation with 302 nm light for 5 min, 750 μM $MnCl_2$ were added to the above operating solutions. After incubation at 37°C for 2 h, the fluorescence was monitored with excitation at 488 nm and emission at 525 nm.

Cell culture.

HeLa, MCF-7 and L02 cells were grown in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL streptomycin, and 100 U/mL penicillin. All cells were maintained at a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Cell viability assay.

HeLa cells (2×10^3) were dispersed into 96-well plates in 200 μL culture medium. After incubation at 37°C for 24 h, the cells were treated with different concentrations of AuNPs (2.5 nM, 5.0 nM and 7.5 nM) in 100 μL RPMI-1640 medium at 37°C for different times (6 h, 12 h, 18 h and 24 h). Then, the cells were washed three times with PBS, and incubated with 10 μL of CCK-8 reagent in 100 μL culture medium for 2 h. Following that, the cell viability was evaluated by measuring the absorbance at 450 nm by using an ELx800™ microplate reader.

Evaluation of intracellular operation of the bipedal walking machine.

The cells were seeded on a 35 mm Petri dish with a 10 mm well in 2 mL of culture medium at 37°C for 24 h. Then, the cells were washed with PBS and incubated with 1 mL of culture medium containing 2.5 nM DNA-functionalized AuNPs at 37°C for 6 h. Then, the cells were treated with light irradiation for 5 min, washed with PBS, and incubated with 1 mL of culture medium containing 250 μM Mn^{2+} at 37°C for 2 h. The cells were washed three times with cold PBS before imaging.

To investigate the miRNA-21 expression fluctuations in living cells, the HeLa cells were firstly treated with 0.2 mM MicrONhsa-miR-21-5p mimic or 0.5 mM MicrOFFhsa-miR-21-5p inhibitor and 6 μL of transfection reagent in 1 mL culture medium at 37°C for 24 h. Then the cells

were washed three times and incubated with 1 mL of culture medium containing 2.5 nM DNA-functionalized AuNPs at 37°C. After incubation for 6 h, the cells were treated with light irradiation for 5 min, washed with PBS, and incubated with 1 mL of culture medium containing 250 μM Mn^{2+} at 37°C for 2 h. The cells were washed three times with cold PBS before imaging. All fluorescence images were acquired using an oil dipping objective (100×, 1.25 NA) on a Nikon confocal laser scanning fluorescence microscope.

Flow cytometric assay.

Cells (10^5 cells) were incubated with 1 mL of culture medium containing 2.5 nM DNA-functionalized AuNPs at 37°C for 6 h. Then the cells were treated with light irradiation for 5 min, washed with PBS, and incubated with 1 mL of culture medium containing 250 μM Mn^{2+} at 37°C for 2 h. Subsequently, the cells were washed with PBS, and detached from culture dishes using 0.25% trypsin for 2 min. Cells were centrifuged (3000 rpm, 4 min) and washed with Tris-HCl buffer three times. The cells were then resuspended in 1 mL PBS followed by flow cytometry assay in FITC channel on a CytoFLEX flow cytometry system (Beckman Coulter, USA).

Table S1. Sequence information for oligonucleotides and miRNAs used in this work

Name	Sequence (5′-3′)
blocker	AAATGTCACTCATGTTCAACATCAGTCTGATAAGCTA
nonspecific blocker	AAATGTCACTCATGTTCAACATCAGTCTGAT*GCTATC*
DNAzyme 1	ATCAGACTGATGTTGAACAGGCTAGCTACAACGAGAGTGACA TTTTTTTTTGGCTTGGCTTGCTTTTTTT(PC linker)TTTTTTT-SH
DNAzyme 2	ATCAGACTGATGTTGAACAGGCTAGCTACAACGAGAGTGACA TTTTTTTTTGCAAGCCAAGCC
DNAzyme 3 for PAGE analysis	ATCAGACTGATGTTGAACAGGCTAGCTACAACGAGAGTGACA TTTTTTTTT
fluorescence substrate	FAM-TGTCACTCrAUGTTCAACATCAGTTTTTTTTTTTTTTT-SH
fluorescence substrate 1	FAM-GTCACTCrAUGTTCAACATCAGTTTTTTTTTTTTTTT-SH
substrate for PAGE analysis	TTTTTGTCACTCrAUGTTCAACATCAGTTTTT
miRNA-21 target	TAGCTTATCAGACTGATGTTGA
miRNA-222	AGCUACAUCUGGCUACUGGGUCUC
miRNA-141	UAACACUGUCUGGUAAAGAUGG
single-base mismatch target	TAG*T*TTATCAGACTGATGTTGA
two-base mismatch target	TAG*T*TTATCAGAC*A*GATGTTGA
three-base mismatch target	TAG*T*TTATCAGAC*A*GAT*C*TTGA

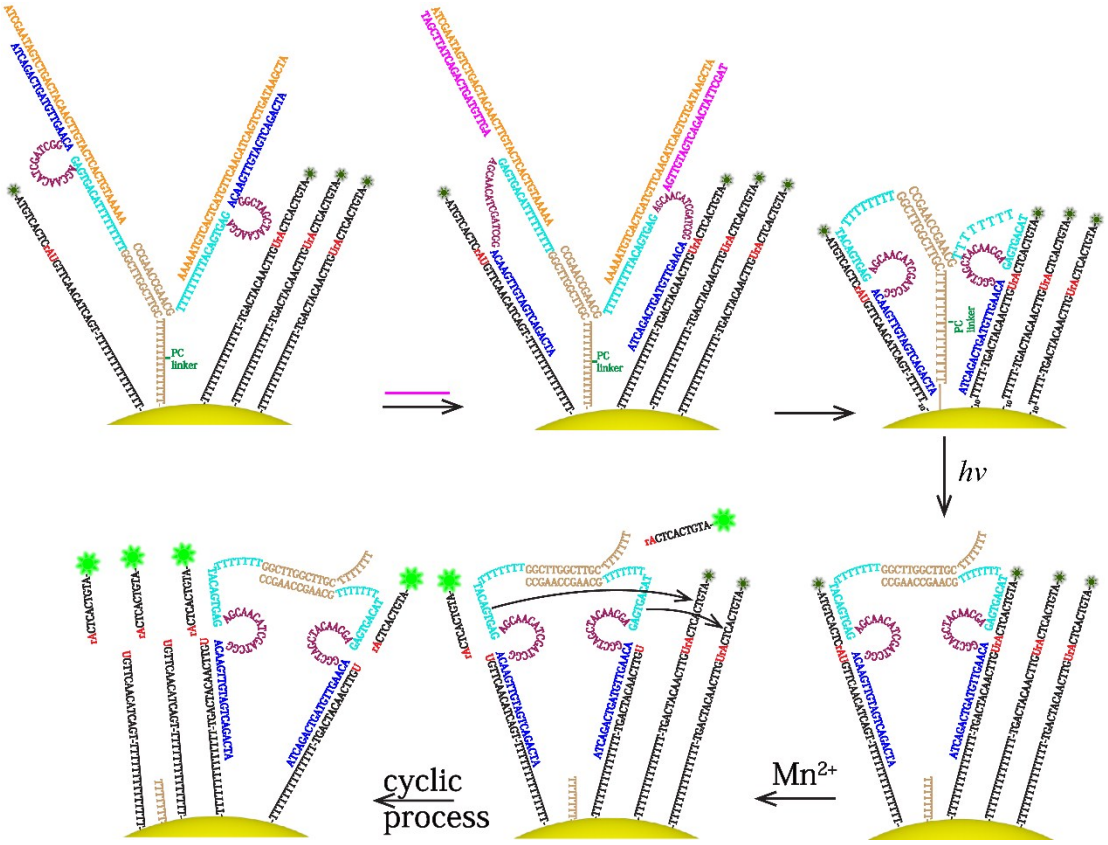

Fig. S1 Schematic illustration of the operation of the miRNA-initiated self-powered bipedal DNA walking machine.

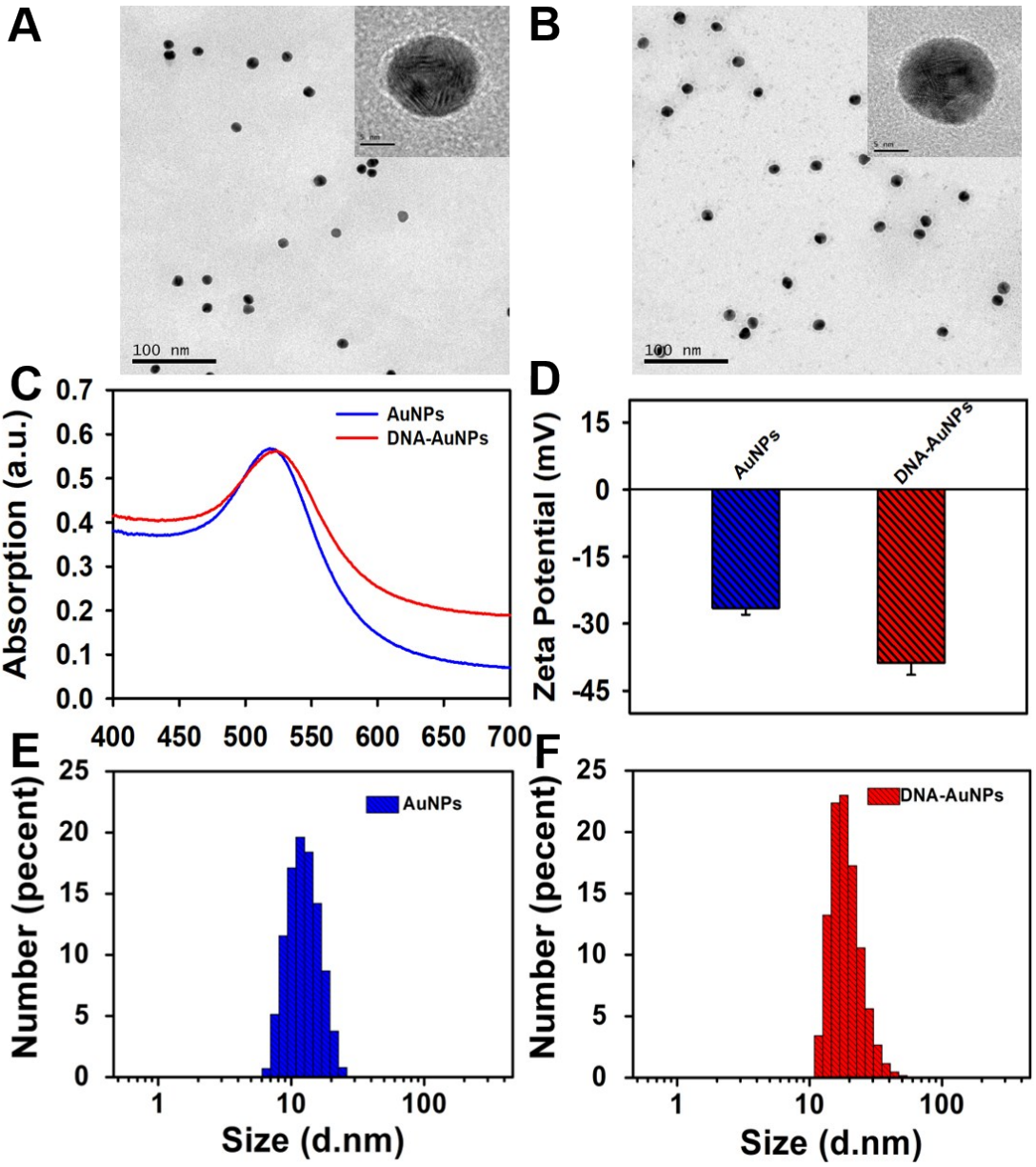

Fig. S2 Characterization of the bare AuNPs and the DNA-modified with AuNPs. TEM images of (A) bare AuNPs and (B) the DNA-modified with AuNPs. (C) UV-vis absorption spectra of bare AuNPs and (blue line) the DNA-modified with AuNPs (red line). (D) Zeta-potential of bare AuNPs and the DNA-modified with AuNPs. The DLS of (E) bare AuNPs and (F) the DNA-modified with AuNPs.

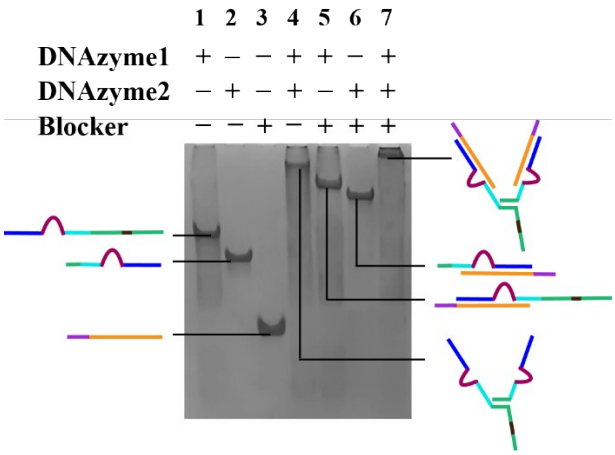

Fig. S3 PAGE gel electrophoresis image.

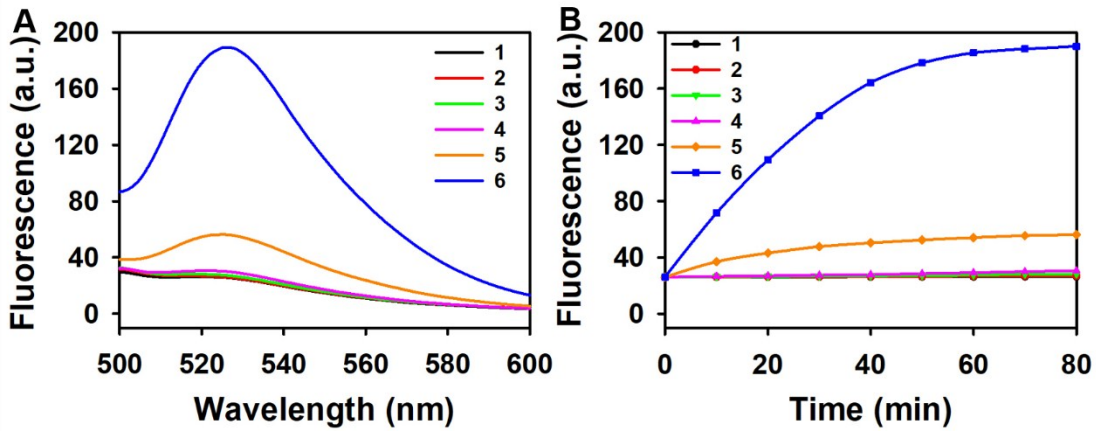

Fig. S4 (A) Fluorescence spectra and (B) real-time fluorescence of (1) the bipedal DNA walking machine, (2) the walking machine upon the light irradiation, (3) the walking machine incubated with miRNA-21 upon the light irradiation, (4) the walking machine incubated with Mn^{2+} upon the light irradiation, (5) the walking machine incubated with miRNA-21 and Mn^{2+}, (6) the walking machine incubated with miRNA-21 and Mn^{2+} upon the light irradiation.

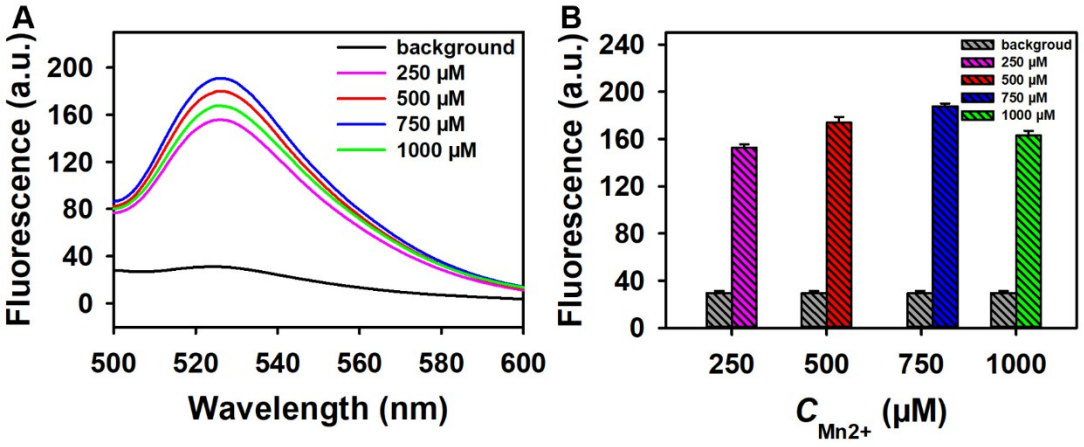

Fig. S5 Effect of the cofactor (Mn^{2+}) on the operation of the bipedal DNA walker. (A) Fluorescence spectral responses of 2.5 nM DNA-functionalized AuNPs to the different concentrations of the cofactor Mn^{2+} (250, 500, 750, and 1000 μM) upon the light irradiation. (B) Fluorescence intensities at 525 nm versus different concentrations of the cofactor Mn^{2+} (the excitation at 488 nm) upon the light irradiation.

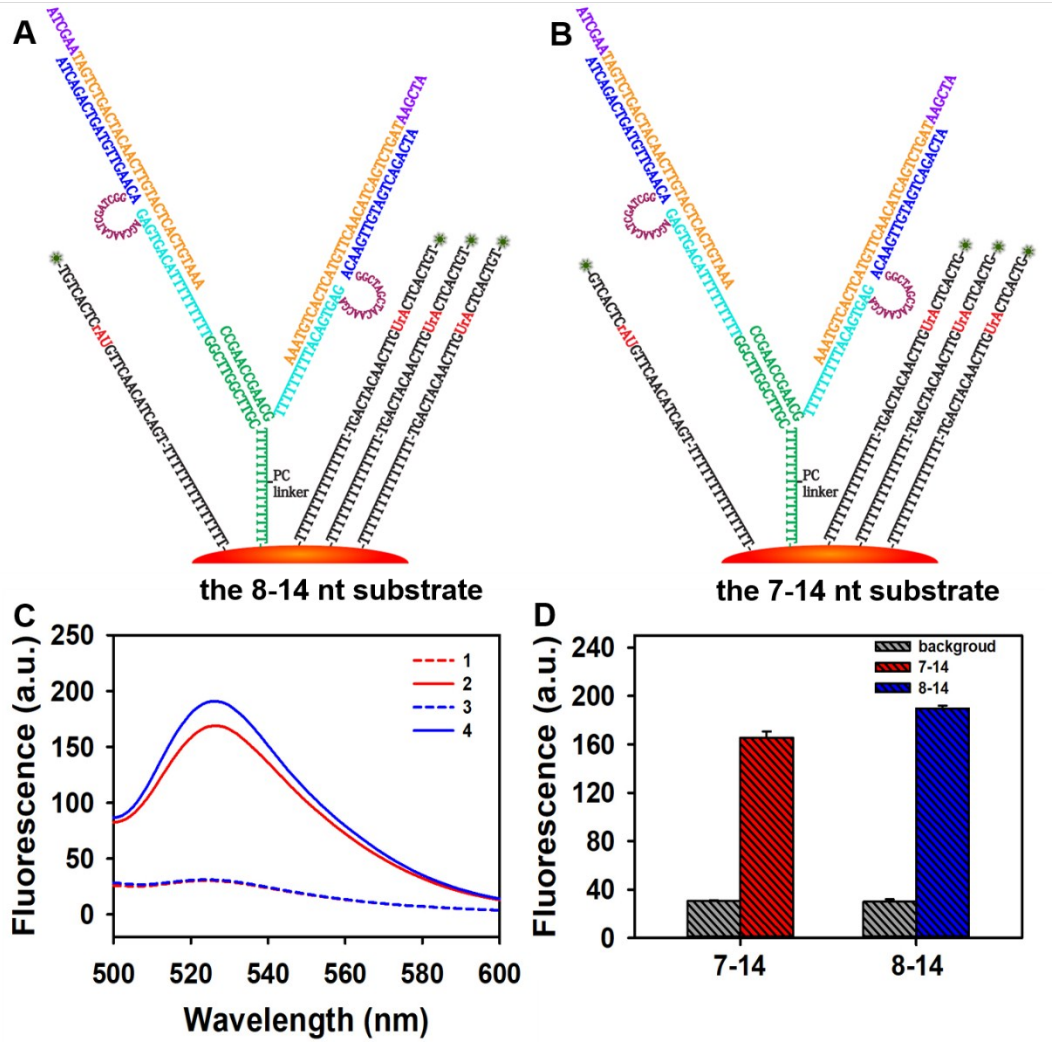

Fig. S6 Construction of (A) the 8-14 nt substrate strands and (B) the 7-14 nt substrate strands-modified on the AuNPs. (C) Fluorescence spectra of the 7-14 fluorescently labelled substrate strands-modified AuNPs before (1) and after (2) addition of miRNA-21 target, and 8-14 fluorescently labelled substrate strands-modified AuNPs before (3) and after (4) addition of miRNA-21 target in the presence of Mn^{2+}, upon the light irradiation. (D) Fluorescence intensities versus varying length of the substrate.

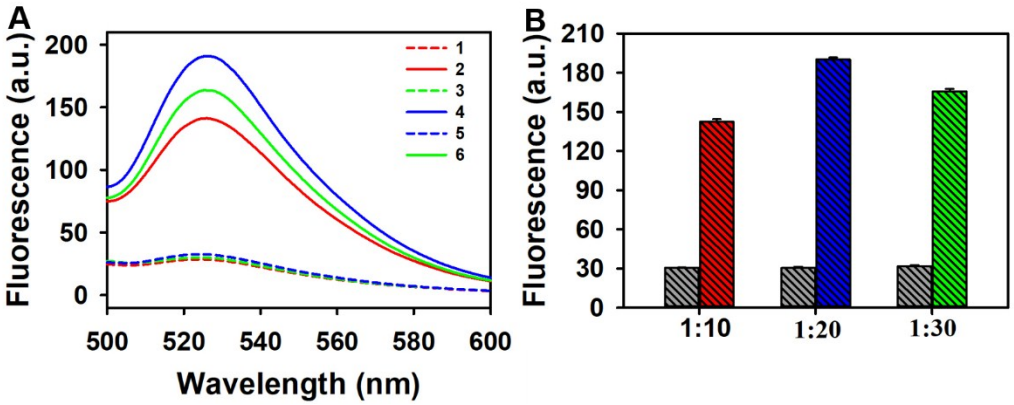

Fig. S7 The bipedal DNA walking machine constructed with different ratio of the DNAzyme walking strands to substrate strands in response to the miRNA-21 in the presence of Mn^{2+} upon the light irradiation.

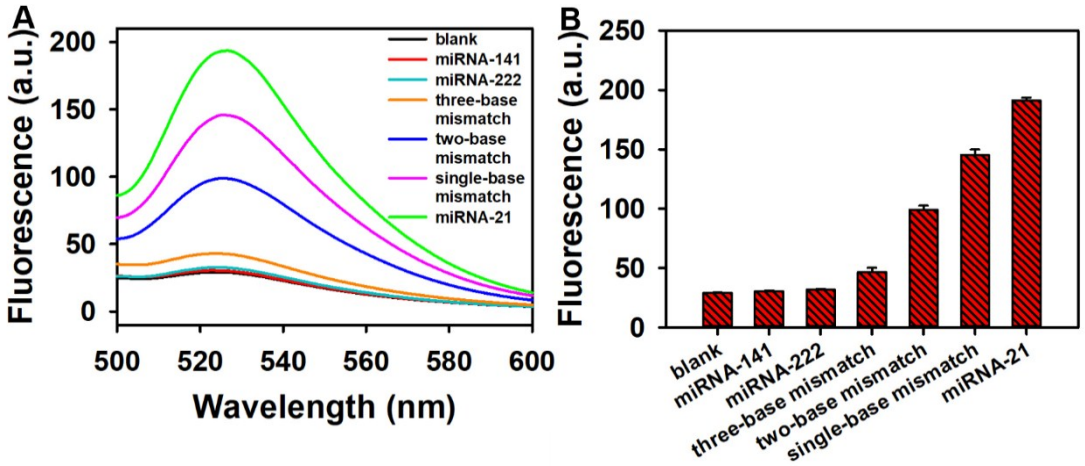

Fig. S8 (A) Fluorescence spectra and (B) intensities of the bipedal DNA walking machine in response to the different targets in the presence of Mn^{2+} (750 μM) upon the light irradiation.

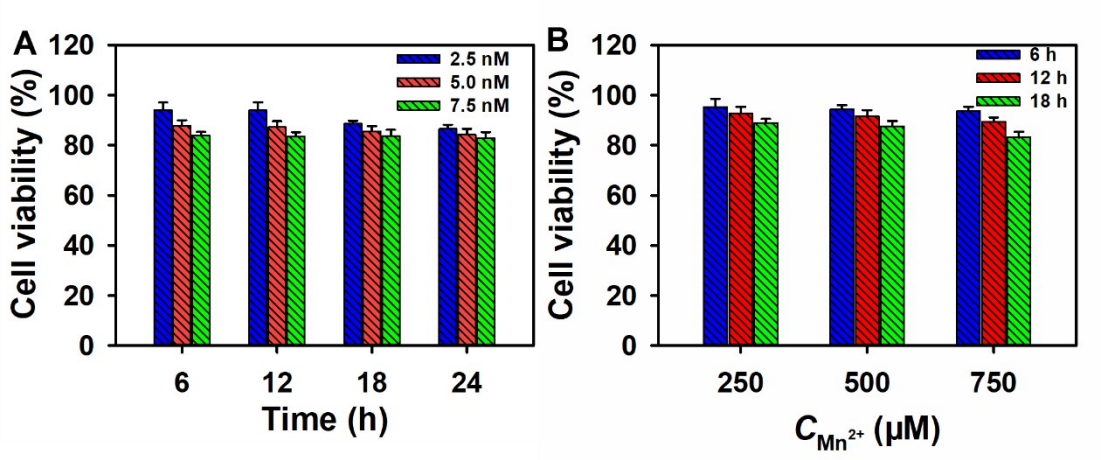

Fig. S9 Cell viability determined by CCK-8 assay in HeLa cells. HeLa cells treated with (A) different concentrations of DNA-functionalized AuNPs (2.5, 5.0, and 7.5 nM) and 250 μM Mn^{2+} for different times (6, 12, 18, and 24 h), (B) different concentrations of Mn^{2+} for different times (6, 12 and 18 h).

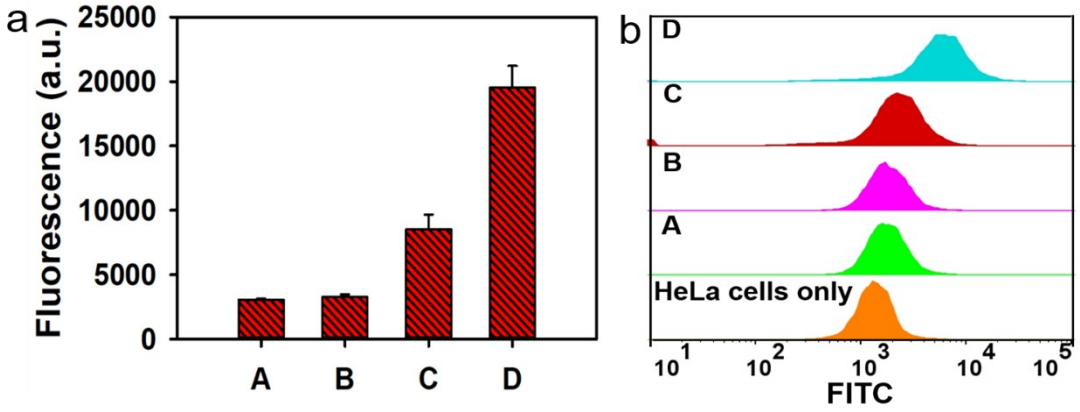

Fig. S10 (a) Mean fluorescence values in HeLa imaging after incubation with (A) bipedal DNA walking machine only, (B) nonspecific walking machine and 250 μM Mn^{2+}, upon light irradiation, (C) bipedal walking machine and 250 μM Mn^{2+}, (D) bipedal walking machine and 250 μM Mn^{2+}, upon light irradiation. (b) Flow cytometry analysis of HeLa cells and HeLa cells treated with above different treatment.

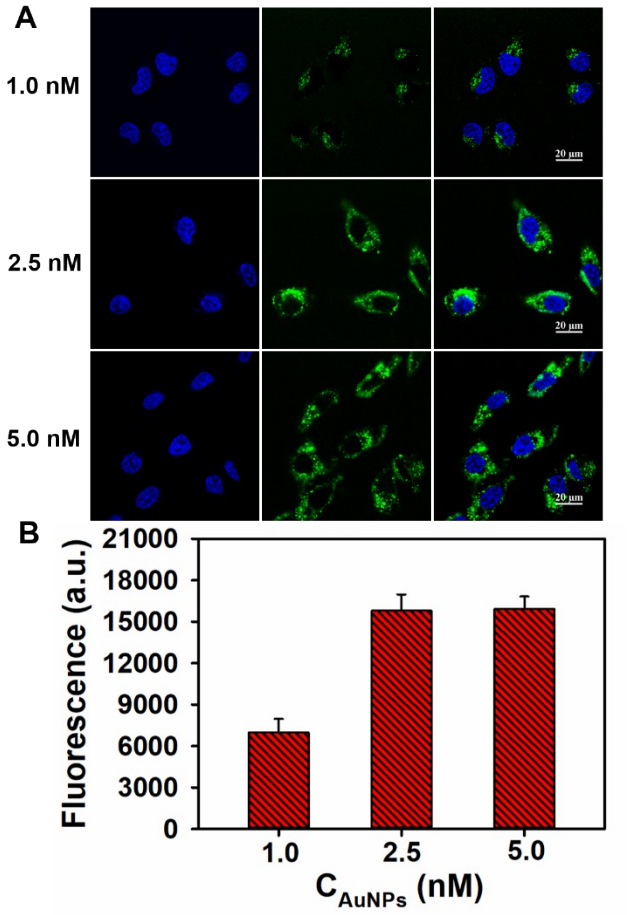

Fig. S11 (A) The Fluorescence images and (B) mean fluorescence values of HeLa cells incubated with different concentrations of the bipedal DNA walking machine in the presence of Mn^{2+} (250 μM) upon the light irradiation.

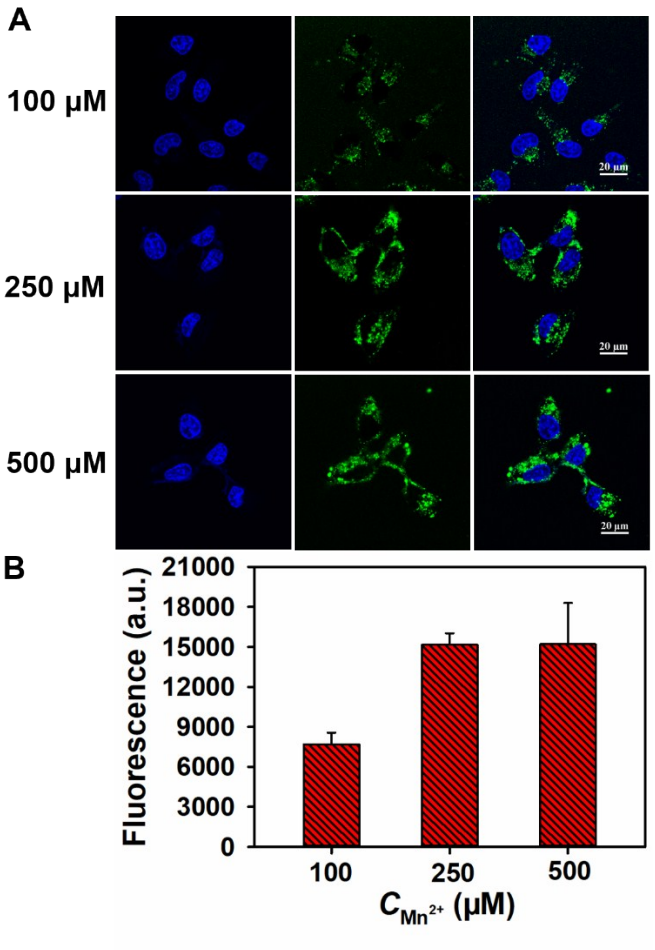

Fig. S12 (A) Fluorescence images and (B) average fluorescence intensity of HeLa cells incubated with the bipedal DNA walking machine and different concentrations of Mn^{2+} upon the light irradiation.

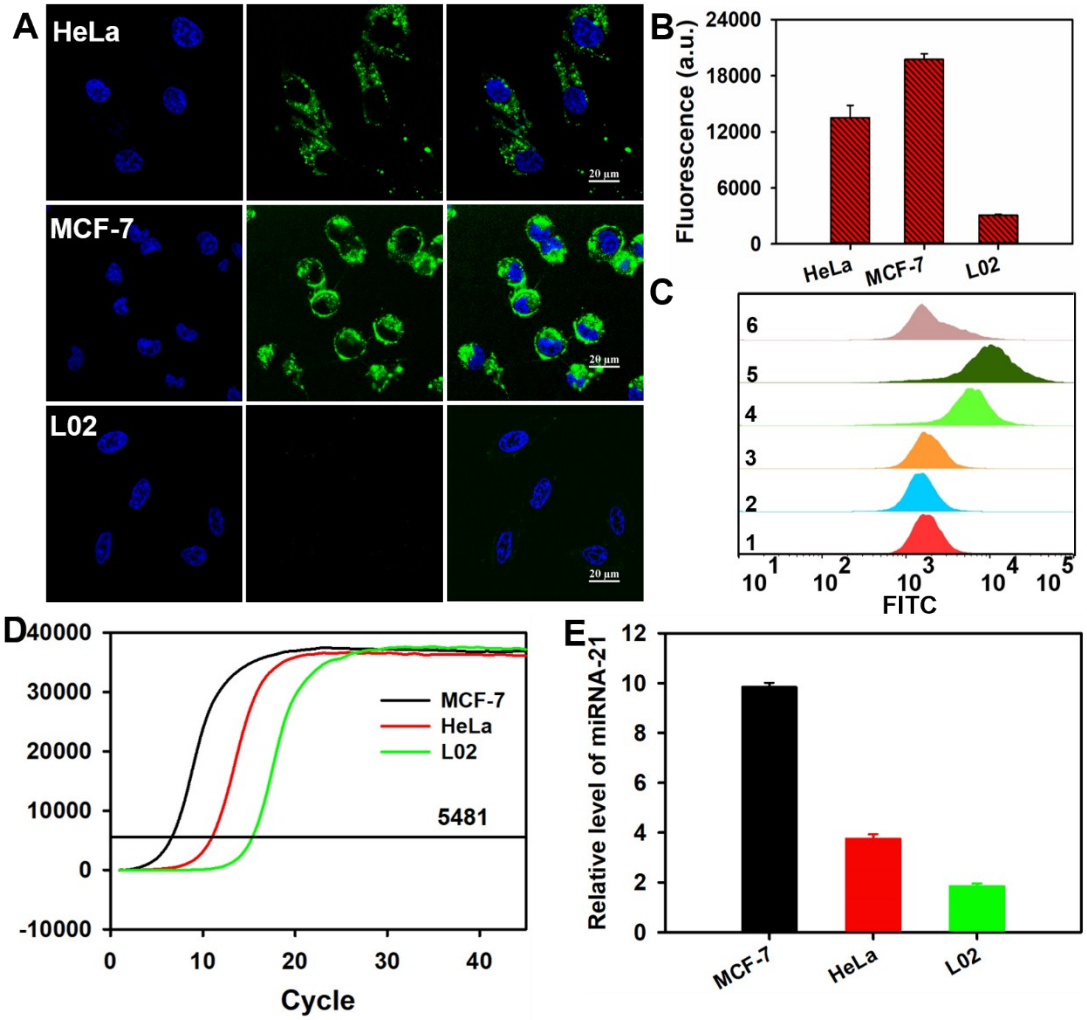

Fig. S13 Operation of the bipedal DNA walking machine in different types of cell lines including HeLa, MCF-7 and L02 cells. (A) Fluorescence images of HeLa, MCF-7 and L02 cells after incubated with the 2.5 nM DNA-functionalized AuNPs and 250 μM Mn^{2+} upon the light irradiation. (B) Average fluorescence intensity of the three different cell lines imaging. (C) Flow cytometry analysis of (1) HeLa, (2) MCF-7, and (3) L02 cells incubated with the bipedal DNA walking machine; and the (4) HeLa, (5) MCF-7, and (6) L02 cells incubated with the bipedal DNA walking machine and Mn^{2+} upon the light irradiation. (D) Real-time fluorescence curves in qRT-PCR analysis, and (E) relative expression levels for miRNA-21 in MCF-7, HeLa and L02 cells.

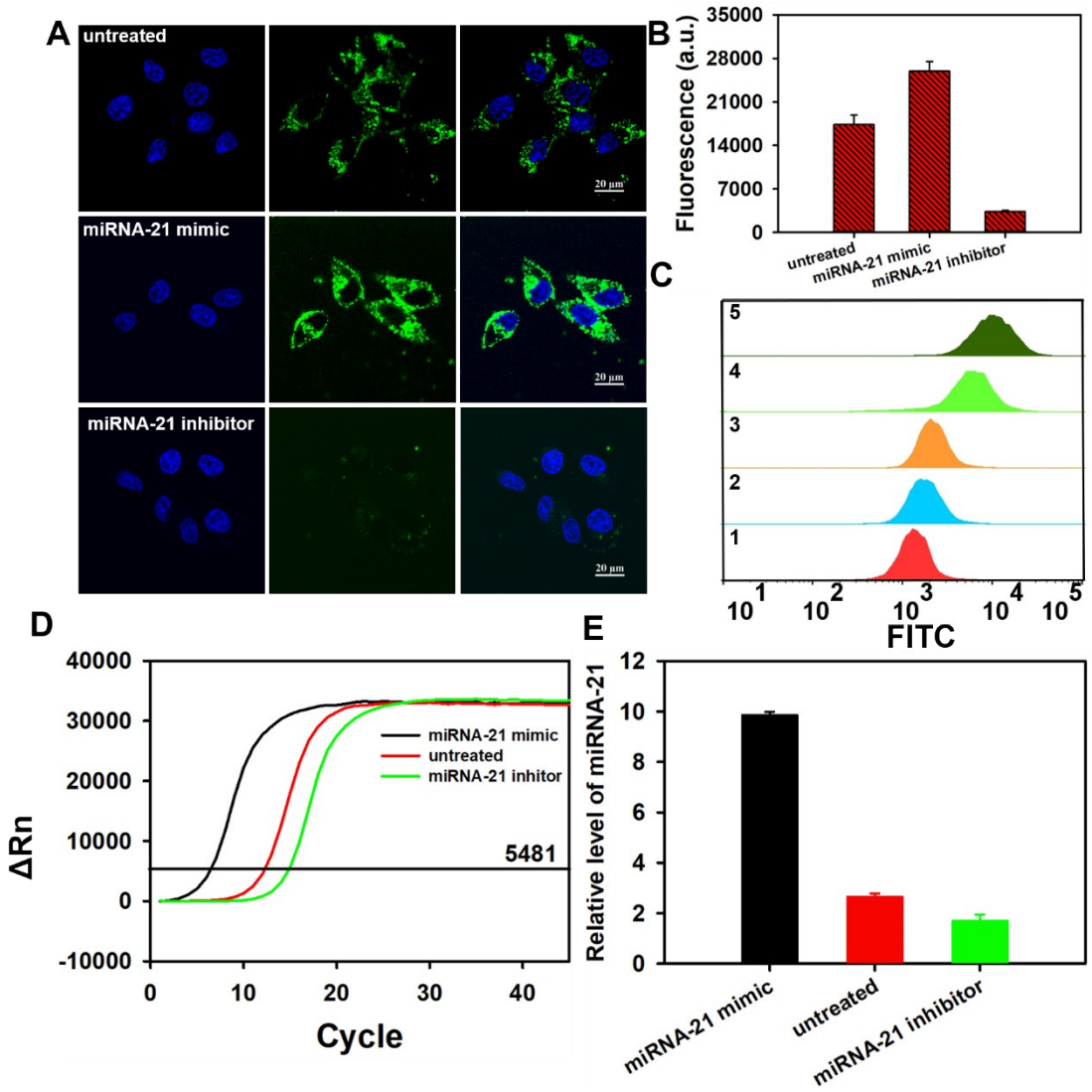

Fig. S14 Operation of the bipedal DNA walking machine to investigate the fluctuation of miRNA-21 expression levels in HeLa cells. (A) Fluorescence images and (B) Mean fluorescence values of HeLa cells incubated with 0.2 mM MicrONTMhsa-miR-21-5p mimic or 0.5 mM MicrOFFTMhsa-miR-21-5p inhibitor for 24 h followed by DNA-functionalized with AuNPs (2.5 nM) and Mn^{2+} (250 μM) upon the light irradiation. (C) Flow cytometry analysis of HeLa cells (1), HeLa cells treated with 2.5 nM DNA-functionalized AuNPs (2), HeLa cells treated with 2.5 nM DNA-functionalized AuNPs and 250 μM Mn^{2+} upon the light irradiation (4), HeLa cells pretreated beforehand with miRNA inhibitor (3) or miRNA mimic (5) and then incubated with bipedal DNA walking machine and Mn^{2+} upon the light irradiation. (D) Real-time fluorescence curves in qRT-PCR analysis, and (E) relative expression levels for miRNA-21 in HeLa cells with different treatment.

References.

(1) K. C. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.*, 1995, **67,** 735-743.

(2) H. Y. Peng, X. F. Li, H. Q. Zhang, X. C. Le, *Nat. Commun.* 2017, **8,** 14378-14390.