Electronic Supplementary Information (ESI) for Chemical Communications

Sensitive detection of intracellular microRNA based on flowerlike vector with catalytic hairpin assembly

Jintong Liu,[a] Ping Du,[a] Jing Zhang,*[b] Hong Shen[a] and Jianping Lei*[a]

[a] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

[b] School of Petrochemical Engineering, School of Food Science and Technology, Changzhou University, Changzhou 213164, China.

Experimental

Materials and reagents. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dopamine and HEPES were purchased from Sigma-Aldrich (USA). LysoTracker Deep Red, Lysosensor yellow/blue DND-160 and serum-free medium (Opti-MEM) were obtained from Life Technologies (USA). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(poly ethylene glycol)-2000] (M.W. 2000, DSPE-PEG-FA) was obtained from Nanocos (USA). All water using in aqueous solutions was ultrapured (≥ 18 MΩ·cm, Milli-Q, Millipore). All RNA and DNA sequences were purchased from Shanghai GenePharma Co., Ltd. and Sangon Biological Engineering Technology Co., Ltd. (Shanghai, China), respectively. The DNA and RNA sequences included:

Hairpin DNA 1 (H1): 5'-FITC-TTTTCAACATCAGTCTGATAAGCTACCATGTGTAGATAG CTTATCAGACTCTACTCA-3';

Hairpin DNA 2 (H2): 5'-TAAGCTATCTACACATGGTAGCTTATCAGACTCCATGTGTAG

A-3’;

Thiolated DNA (tDNA): 5’-HS-AAAAAAAAAATACTCGTCAACATCAGTCTGATAAGCT ACGAGTA-FITC-3’;

AF488-hairpin DNA: 5’-AF488-TTTTCAACATCAGTCTGATAAGCTACCATGTGTAGAT AGCTTATCAGACTCTACTCA-3’;

MiRNA-21: 5’-UAGCUUAUCAGACUGAUGUUGA-3’;

MiRNA-21 inhibitor: 5’-UCAACAUCAGUCUGAUAAGCUA-3’;

Single-base mismatched strand 1 (sm-RNA1): 5’-UAGCUUAUCAGACUGAUGUU<u>U</u>A-3’;

Single-base mismatched strand 2 (sm-RNA2): 5’-UAG<u>G</u>UUAUCAGACUGAUGUUGA-3’;

Single-base mismatched strand 3 (sm-RNA3): 5’-UAG<u>A</u>UUAUCAGACUGAUGUUGA-3’;

Single-base mismatched strand 4 (sm-RNA4): 5’-UAG<u>U</u>UUAUCAGACUGAUGUUGA-3’;

Two-base mismatched strand 1 (tm-RNA1): 5’-UAGCUUAUCAGACUGAU<u>U</u>UUU<u>U</u>A-3’;

The underlined letters represent the mismatched base.

Apparatus. The UV-vis-NIR absorption spectra were recorded with a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu Co., Kyoto, Japan). The transmission electron microscopic (TEM) images were acquired by a Model JEM-2100 high-resolution transmission electron microscope (JEOL Ltd., Japan). Zeta potential analysis was performed on Zetasizer (Nano-Z, Malvern, UK). Dynamic light scattering (DLS) was observed on a 90 Plus/BI-MAS equipment (Brook Haven, USA). Laser scanning confocal microscopic (LSCM) images were gained on a TCS SP5 laser scanning confocal microscope (Leica, Germany). Flow cytometric analysis was performed on a Coulter FC-500 flow cytometer (Beckman-Coulter, USA). MTT assay was performed on a microplate reader (Varioskan Flash, ThermoFisher Scientific, USA). Cell number was determined using a Petroff-Hausser cell counter (USA).

Synthesis of AuNF, DA-AuNF and HDA-AuNF. According to a typical method, AuNFs were synthesized by confining the crystal growth in the limited ligand protection region.[S1] The AuNPs of 40 nm diameter were obtained by reducing chloroauric acid with trisodium citrate according to a previous report.[S2] DA-AuNF with a thin PDA shell was obtained via in-situ polymerization of dopamine on the AuNFs. Briefly, 1.0 mL of the AuNF stock solution (~10 nM) was mixed with dopamine solution (0.010 mg/mL, buffered in 10 mM Tris at pH 8.5) to decorate the gold core with a poly-dopamine shell, under continuous sonication for 1 h at room temperature. The as-prepared DA-AuNF was purified by repeated centrifugation at 7000 rpm for 15 min. The purified DA-AuNF was redispersed in the ultrapure water.

The HDA-AuNF vector was prepared via the noncovalent assembly of H1 and H2 on the surface of DA-AuNF. Firstly, DA-AuNF solution (500 μL, 10 nM) was concentrated by centrifugation, and the precipitate was dispersed by H1 (100 μM, 40 μL), H2 (100 μM, 40 μL), DSPE-PEG-FA (1 mg/mL, 20 μL) in 400 μL Tris/HCl (pH =7.4, 10 mM), and incubated at room temperature for 5 h. After centrifugation for two times, the precipitate was redisposed in pH 7.4 phosphate buffer saline (PBS, 10 mM) prepared from ultrapure water for further use. Similar to the above described method, DA-AuNP and HDA-AuNP were synthesized using AuNP instead of AuNF.

For comparison, the thiolated DNA was directly immobilized on the surface of AuNF and AuNP to form tDNA-AuNF and tDNA-AuNP via the Au−S bond, respectively, which were applied as the nanocarriers for the control experiment.

Gel electrophoresis experiments. The 12% native polyacrylamide gel electrophoresis (PAGE) was carried out via using 1×Tris-borate-EDTA buffer (TBE). The loading sample was obtained from DNA sample (7 μL), 6 × loading buffer (1.5 μL) and UltraPowerTM dye (1.5 μL), placed

for 3 min for the complete integration with DNA, and applied onto the lane. The gel was scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA).

Cell culture. A549 and HeLa cell lines were cultured in a flask in Dulbecco's modified Eagle's medium (DMEM), and MCF-7 cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 containing 10% fetal calf serum (FCS, Gibco), 1% penicillin, and 1% streptomycin at 37 °C in the humidified atmosphere containing 5% CO_2.

Cell lysis. 5×10^8 cells were pretreated with miRNA-21 inhibitor for 6 h, dispensed in a 15-mL EP tube, washed twice with ice-cold pH 7.4 PBS, and resuspended in 2 mL of ice-cold CHAPS lysis buffer containing 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EDTA, 0.1 mM PMSF, 0.5% CHAPS and 10% glycerol. The mixture was incubated for 30 min on ice and centrifuged at 16000 rpm at 4 °C for 20 min. The supernatant was collected as cell lysis for experiments or frozen at -80 °C.

Cytotoxicity evaluation. The cytotoxicity of the HDA-AuNF or HDA-AuNP to cells was evaluated by MTT assays. In brief, HeLa cells (1.0×10^5) were seeded in 96-well plate for 24 h, rinsed with PBS and incubated with fresh DMEM containing HDA-AuNF or HDA-AuNP (20 μL) for 5 h. Then the medium was removed and 20 μL of MTT stock solution (12 mM) was added to wells along with 200 μL of phenol free DMEM. The cells were incubated at 37 °C for 4 h, and the MTT containing medium was replaced with 150 μL DMSO. The absorbance at 560 nm of the solution was recorded by a microplate reader after incubated 37 °C for 15 min. The cell viability was then determined by $(A_{test}/A_{control}) \times 100\%$.

Colocalization assay. HeLa cells were seeded into 35-mm confocal dishes (5×10^4), and incubated for 12 h at 37 °C. For colocalization assay of the nanovector-transfected HeLa cells,

the LysoTracker Deep Red solution was diluted from stock solutions to 75 nM with complete growth medium, and then freshly placed over the cells for 15 min after 5 h incubation of HDA-AuNF. After the cells were washed twice with PBS, fluorescence images were obtained from 660 to 700 nm under the excitation of 633 nm for LysoTracker Deep Red and 500 to 550 nm under the excitation of 488 nm for HDA-AuNF.

The HDA-AuNF subcellular distribution was further evaluated by the local pH pixel analysis.[S3] First, AF488-HDA-AuNF was prepared using AF488-hairpin DNA instead of H1 on the HDA-AuNF. Then, the cells were incubated with the AF488-HDA-AuNF for certain time to conduct the pixel analysis. The local pH pixel was determined by Lysosensor's I_{Blue}/I_{Green} intensity ratio.

In situ quantitative detection of intracellular miRNA-21. Standard curve extrapolation was performed by using miRNA-21 mimic to realize the quantitative detection of intracellular miRNA-21.[S4] The cells were incubated with increasing amounts of miRNA-21 mimic for 6 h, and the quantity of miRNA-21 mimic internalized by the cells was calculated by the absorbance change of the media at 260 nm. Then the cells were further treated by Opti-MEM supplemented with HDA-AuNF for 5 h at 37 ˚C and washed twice with PBS. The confocal fluorescence images were recorded and analyzed at the emission wavelength of 500-550 nm at room temperature by exciting at 488 nm. Flow cytometric analysis was performed using the same procedures and the cells were collected and resuspended in binding buffer for flow assays over FL1 (FITC) channel.

Supporting figures

DLS assays

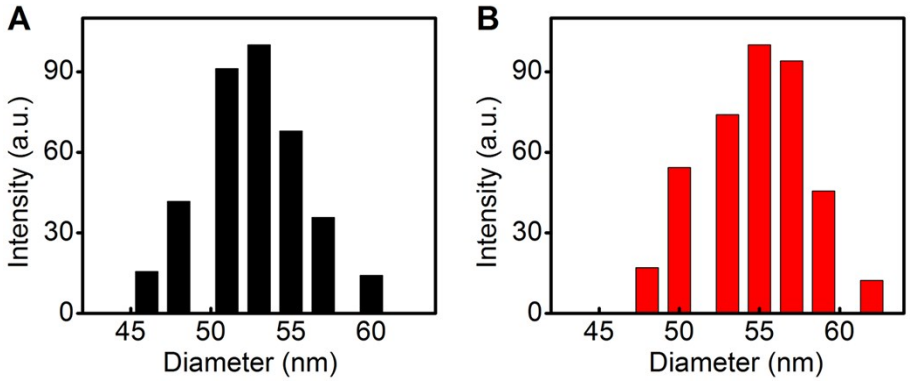

Fig. S1. DLS assays of A) AuNF and B) DA-AuNF.

Comparison of loading efficiency

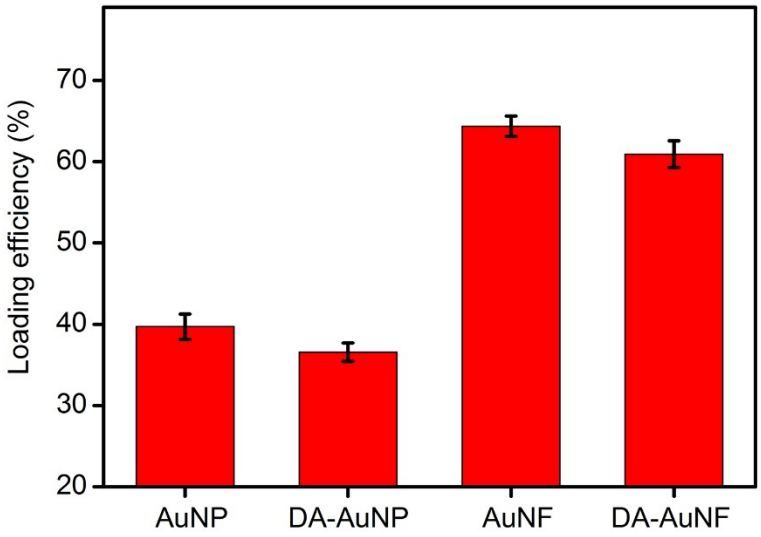

Fig. S2. Dependence of DNA loading efficiency on AuNP, DA-AuNP, AuNF and DA-AuNF in pH 7.4, 10 mM Tris-HCl.

Fluorescence dynamical response

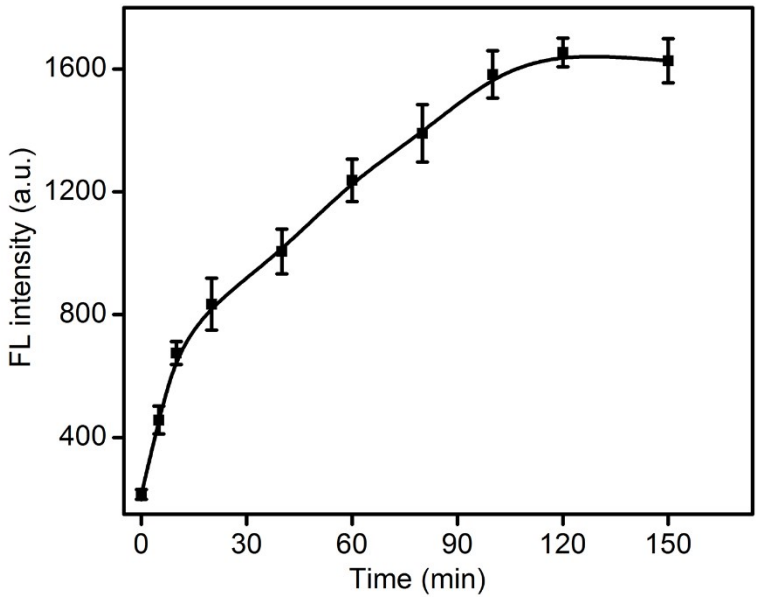

Fig. S3. Time-dependent fluorescence response of HDA-AuNF to miRNA-21 at the concentration of 1.0 nM.

Quantitative analysis of miRNA-21 by tDNA-AuNF

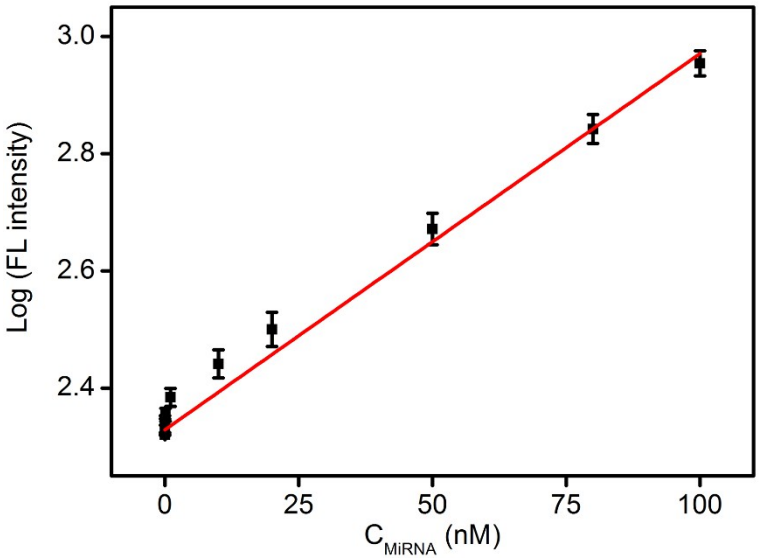

Fig. S4. Logarithm of fluorescence intensities of tDNA-AuNF vs. miRNA-21 concentration.

Specific response of HDA-AuNF to miRNA-21 in cell mimic.

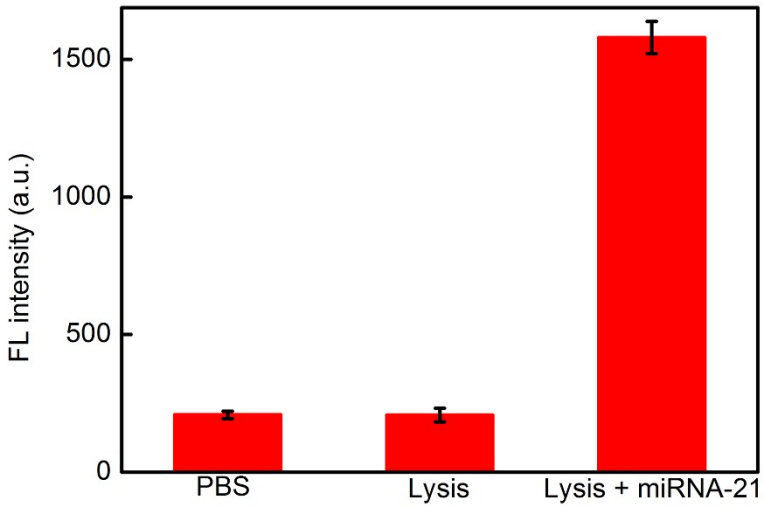

Fig. S5. Fluorescence response of HDA-AuNF in PBS and lysis of inhibitor-pretreated cells in the absence or presence of 1.0 nM miRNA-21.

MTT assays

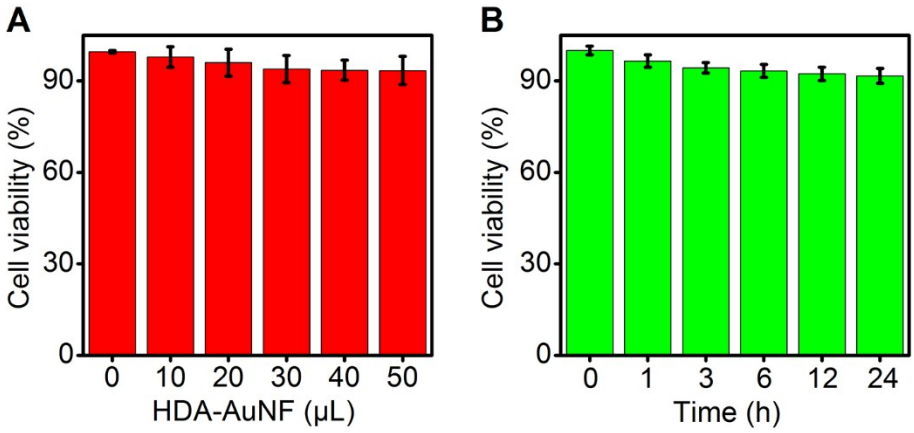

Fig. S6. MTT assays for HeLa cells after incubated with A) increasing amounts of HDA-AuNF for 5 h and B) HDA-AuNF (20 μL) for different times.

HDA-AuNF-treated different cell lines

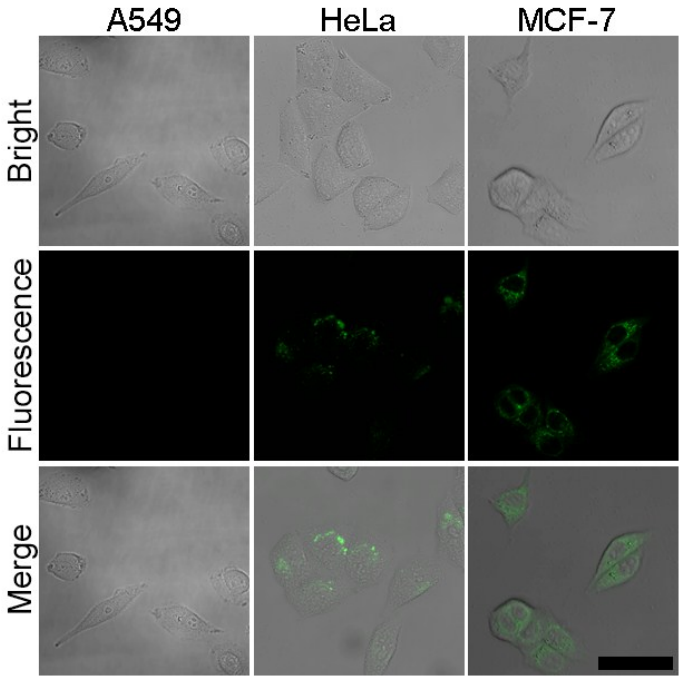

Fig. S7. Confocal fluorescence images of MCF-7, HeLa and A549 cells after transfected with HDA-AuNF at 37 °C for 5 h with $\lambda_{ex/em}$ of 488/500–550 nm. Scale bar: 50 μm.

Colocalization assay

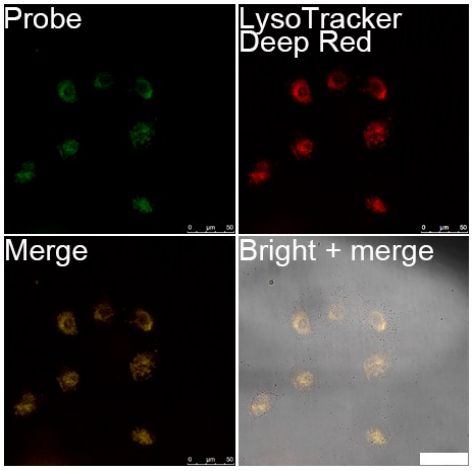

Fig. S8. Colocalization images of HDA-AuNF-incubated HeLa cells with LysoTracker Deep Red. Scale bar: 50 μm.

Distribution of AF488-HDA-AuNF in cell

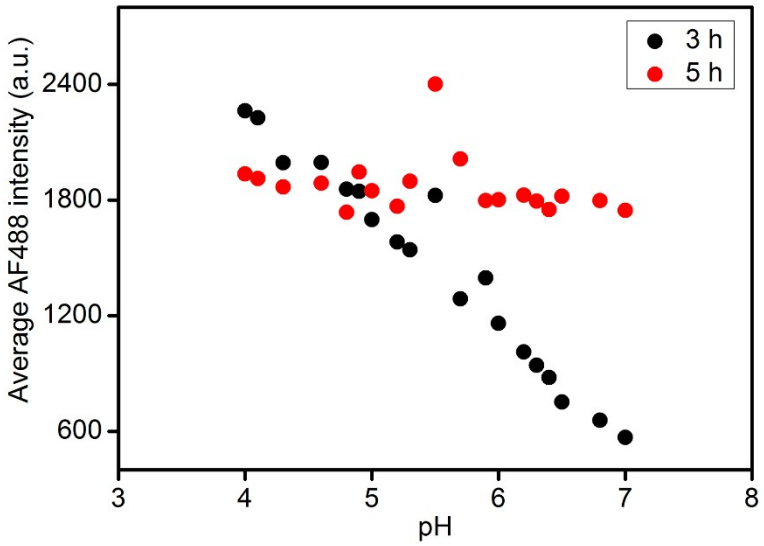

Fig. S9. Distribution of AF488-HDA-AuNF in cells at 3 h- or 5 h- incubation as a function of pH, determined by ratiometric fluorescence imaging of Lysosensor dye.

Supporting references

S1 J. Xie, Q. Zhang, J. Y. Lee and D. I. C. Wang, *ACS Nano*, 2008, **2**, 2473-2480.

S2 C. K. K. Choi, J. M. Li, K. C. Wei, Y. J. Xu, L. W. C. Ho, M. L. Zhu, C. H. Choi and L. M. Bian, *J. Am. Chem. Soc.*, 2015, **137**, 7337-7346.

S3 J. Wang, S. R. MacEwan and A. Chilkoti, *Nano Lett.*, 2017, **17**, 1226-1232.

S4 H. F. Dong, J. P. Lei, H. X. Ju, F. Zhi, H. Wang, W. Guo, Z. Zhu and F. Yan, *Angew. Chem. Int. Ed.*, 2012, **51**, 4607-4612.