

Supporting Information

A cubic DNA nanocage probe for in situ analysis of miRNA-10b in tumor-derived extracellular vesicles

Xiaoyan Sun¹, Yafei Chen¹, Haiyan Li¹, Xing Wei¹, Mingli Chen^{1,2*},
Jianhua Wang¹, Lei Ye^{2*}

1. Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, BOX 332, Shenyang, Liaoning, 110819, P.R.China.
2. Division of Pure and Applied Biochemistry, Department of Chemistry, Lund University, Box124, 22100 Lund, Sweden.

E-mail: chenml@mail.neu.edu.cn (M.-L. Chen); lei.ye@tbiokem.lth.se (L. Ye)

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Experimental Details

Materials.

All DNA sequences, low MW DNA Marker (20-500 bp) 1× PBS, and 4% paraformaldehyde solution (PFA) solution, were bought from Sangon Biotech Co., Ltd. (Shanghai, China). Magnesium chloride (MgCl₂) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), phosphotungstic acid, and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Solarbio Life Sciences Co., Ltd. (Beijing, China). The BCA protein concentration determination kit was from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8) was from ApexBio (USA). 4S red plus nucleic acid gel stain was purchased from BBI Life Sciences (Markham, Ontario, Canada). The MCF-10A cells and MDA-MB-231 cells, and their corresponding special culture medium were purchased from Procell Life Science & Technology Co., Ltd. (Procell, Wuhan, China).

Instrumentations.

The morphology of the cubic DNA nanocage was characterized with atomic force microscopy (Bruker Nano, USA). The fluorescence spectra were measured with an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The morphology of extracellular vesicles was characterized with a Tecnai G20 transmission electron microscope (FEI, USA). The number of extracellular vesicles was measured with nanoparticle tracking analysis (NanoSight 300, UK). The size of extracellular vesicles was measured with ZSE Nano Zetasizer (Malvern, UK). Electrophoretograms were captured by an ultraviolet illumination gel imaging analysis system (Tanon-4600SF, China). A multimodal microplate reader (BIO-TEK, USA) was used to measure the cell viability and the protein content of extracellular vesicles. A confocal laser scanning microscope (Olympus-FV 1200, Japan) was used for the *in situ* analysis of extracellular vesicles. Total internal reflection fluorescence microscopy (Olympus, Japan) was used for the visualization of probe labeled EVs.

Cell culture

MDA-MB-231 cells were cultured in Leibovitz's L-15 supplemented with 10% fetal bovine serum and 1% P/S. MCF-10A cells were cultured in DMEM/F12 supplemented with 5% horse serum, 20 ng mL⁻¹ of growth factor, 0.5 µg mL⁻¹ of hydrocortisone, 10 µg mL⁻¹ of insulin, 1% NEAA and 1% P/S. All cells were in a humidified atmosphere containing 5% CO₂ at 37 °C.

Extracellular vesicles isolation

Typically, After the MDA-MB-231 cells and MCF-10A cells were cultured in a serum-free medium for 24 h, the cell starvation medium was collected for extracellular vesicles isolation. The obtained cell starvation medium was centrifuged at 2,000 g for 20 min and then at 10,000 g for 30 min. The obtained supernatant was filtered using a 0.22 µm filter membrane and then was ultracentrifuged at 100,000 g for 120 min twice. It was noted that all centrifugation operations were performed at 4 °C. Finally, the sediment was resuspended in PBS solution and stored at -80 °C for further use.

Extracellular vesicles characterization

TEM. The morphology of EVs was characterized by a transmission electron microscope. 5 µL of extracellular vesicles were dripped on copper mesh for 15 min and then washed with PBS solution three times. Then, fix with 4% paraformaldehyde for 10 min and wash with water three times. Finally, it was stained with 2% phosphotungstic acid for 5 min, washed with water three times, and dried for measurement.

NTA. The concentration of EVs was characterized by a nanoparticle tracking analysis. The EVs were diluted one hundred times before measurement.

Total protein concentration. The concentration of total proteins in the EVs was quantified with a BCA protein concentration determination kit.

Assembly of cubic DNA nanocage

All sequences of DNA used are listed in Table S1. The DNA strands (T1-4, S1-4) were dissolved in buffer (1× PBS, 5 mM MgCl₂) with the final concentrations of 10 µM. All DNA strands were mixed at equimolar concentrations. The mixture was

heated to 95 °C for 10 min, and then gradually cooled to 4 °C at a rate of 1 °C/min.

Atomic force microscopy (AFM).

The cubic DNA nanocage (2 μL, 10 nM) was dropped on a freshly uncovered mica sheet for 10 min, followed by washing with H₂O three times.

Agarose gel electrophoresis

1% agarose gel was used to verify the successful assembly of cubic DNA nanocage. The DNA samples were mixed with 6× DNA loading buffers and loaded into 1% agarose gel. The electrophoresis was performed at 120 V for 70 min. The obtained agarose gel was stained with 4S red plus nucleic acid gel stain.

TIRF imaging

EVs from MDA-MB-231 cells and MCF-10A cells were incubated with the CDN probe at 37 °C for 2h, and stained with Dil for 15 min respectively. The unconjugated probe and dye were removed by ultrafiltration. TIRF was used to visualize the binding of miRNA-10b with probes in EVs.

Detection of miRNA-10b from extracellular vesicles

5 μL cubic DNA nanocage was mixed with 5 μL EVs and incubated at 37 °C for 2 h. The fluorescence emission wavelength from 510 nm to 650 nm was recorded under an excitation wavelength of 494 nm.

Detection of other miRNAs with the probe

Some coexisting miRNAs were chosen as representative interferents to investigate the selectivity of probes. 5 μL cubic DNA nanocage was mixed with 5 μL miRNAs and incubated at 37 °C for 2 h. The fluorescence emission wavelength from 510 nm to 650 nm was recorded under an excitation wavelength of 494 nm.

Tracing miRNA-10b intracellular communication

For the tracing of extracellular vesicles, cubic DNA nanocage was incubated with EVs for 2 h at 37 °C and then free cubic DNA nanocage was removed by ultrafiltration using an ultrafiltration tube (100 kDa molecular weight cutoff). The labeled EVs were incubated with MCF-10A cells from 2-10 h. Then, cells were stained with DAPI and were imaged using confocal microscopy to evaluate the cellular uptake behavior of EVs.

CCK-8 assay

To investigate the effect of EVs from MDA-MB-231 cells on the proliferative capacity of MCF-10A cells, a CCK-8 assay was performed. 100 μL of 1×10^5 cells MCF-10A cells were grown in 96-well plates for 24 h. Then, the cells were treated with EVs with a final concentration of $10 \text{ mg } \mu\text{L}^{-1}$ for 0, 24, 48, and 72 h. Add 10 μL CCK-8 solution to each well and absorbance at 450 nm was measured after incubation for 1.5 h to evaluate cell activity.

Cell migration

The logarithmic growth phase MCF-10A cells were cultured in six-well plates for 24 h and delineated using 200 μL pipet tips. EVs with a final concentration of $10 \text{ mg } \mu\text{L}^{-1}$ were added to cells and 0, 12, 24, and 48 h were selected for images. The blank group was cultured with a serum-free medium.

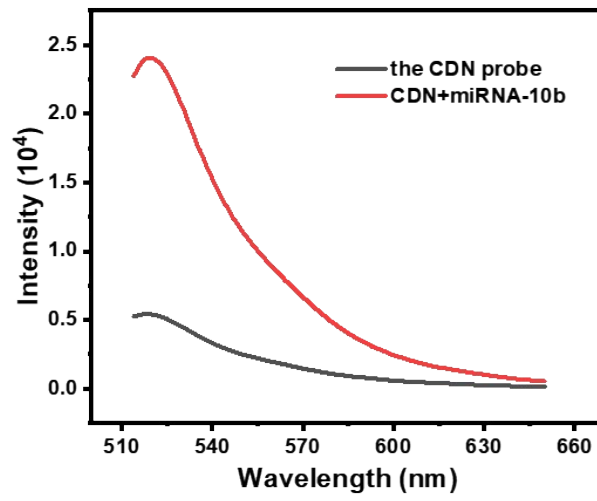


Fig. S1. Fluorescence spectra of the CDN probe incubated with miRNA-10b. CDN alone was used as the negative control.

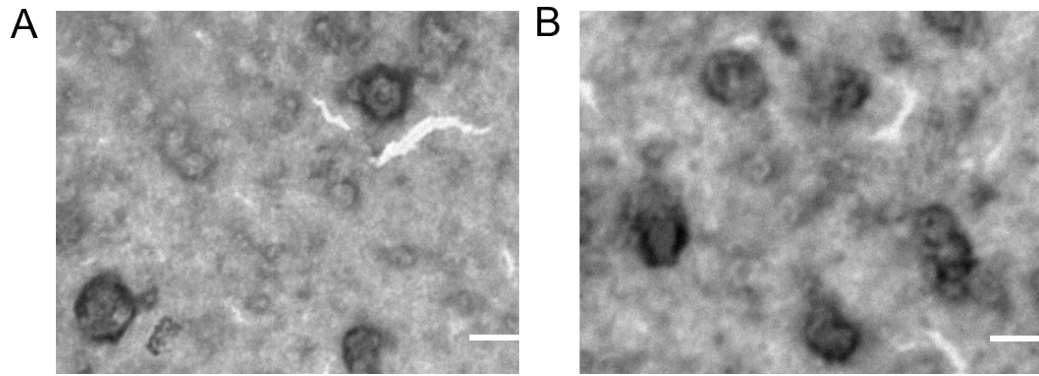


Fig. S2. The TEM image of EVs from MDA-MB-231 cells (A) and EVs from MCF-10A cells (B). Scale bar = 100 nm.

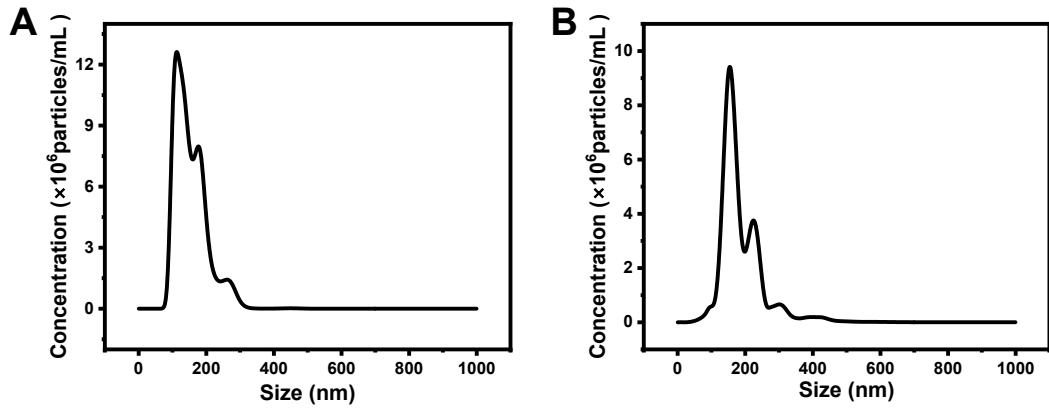


Fig. S3. The NTA analysis of EVs from MDA-MB-231 cells (A) and EVs from MCF-10A cells (B).

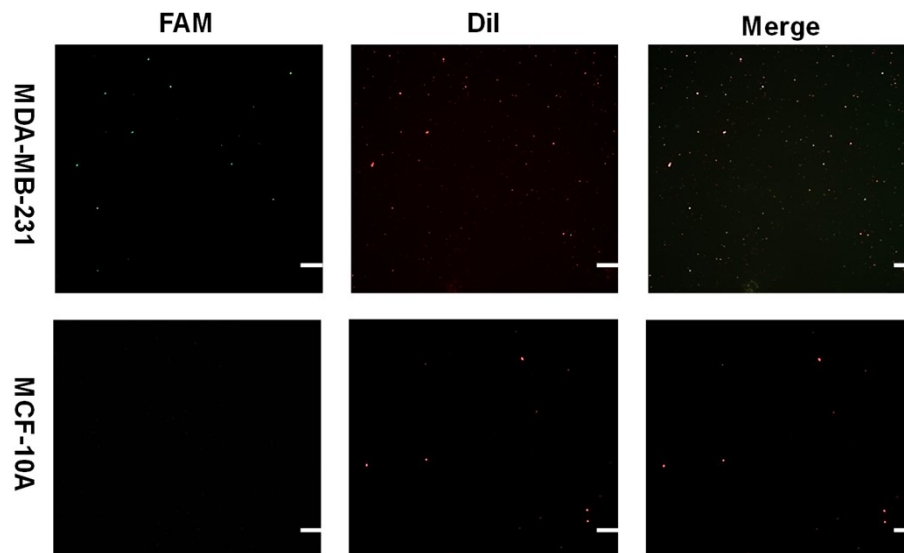


Fig. S4. TIRF images of EVs from MDA-MB-231 cells and MCF-10A cells incubated with the CDN probe. Scale bar = 10 μ m.

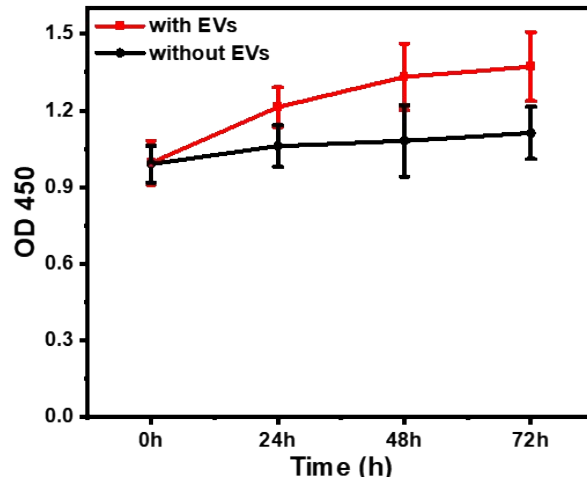


Fig. S5. The effect of EVs on the proliferation of MCF-10A cells.

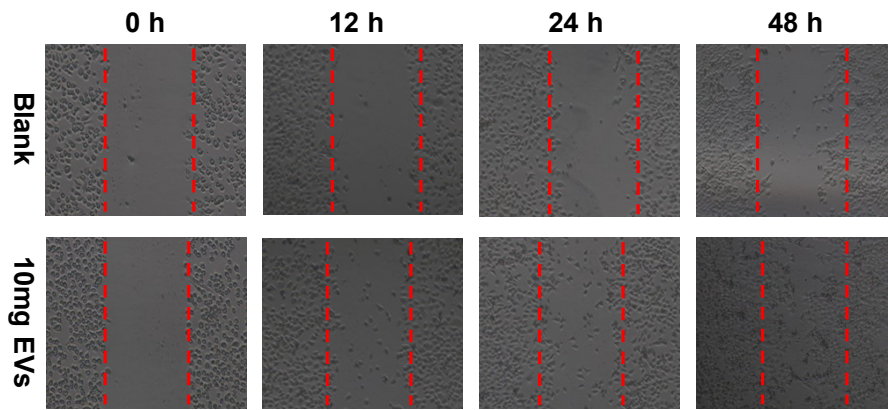


Fig. S6. Cell scratching assay images of MCF-10A cells incubated with serum-free medium and 10 mg EVs.

Table S1. All sequences of DNA used.

Name	Sequence (5' to 3')
T1	CACTGATCTAATGATCAACAGCCTATATCGTTACCAGATACTATGG CGGCTCTTC
T2	GTATTGGACCCTCGCATCAATAGCGCATCGTTACGAAGTGGTAACG ATATAGGCT
T3	TCGTAACGATGCGCTATAACGACACCTAGATGGGAATCTACTCATC TGCTGAACT
T4	GAATTGACAGACGTCGTCATGATCATTAGATCAGTGAGTTCCCATC TAGGTGTCG
S1	FAM-ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAG AGCCGCCATAGTATTTTTACAAATTTCGGTTCTACAGGGTA
S2	ATGCGAGGGTCCAATACCATATCACCAGGCAGTTGAAGGTGTAGC AAGCTGTAAT
S3	FAM-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCAGTT CAGCAGATGAGTATTTTTACAAATTTCGGTTCTACAGGGTA
S4	ACGACGTCTGTCAATTCCATTCAGACTTAGGAATGTAGTTCCACG TAGTGTCGT
cDNA	BHQ-1-GTAGAACCGAAT
miRNA-10b	UACCCUGUAGAACCGAAUUUGUG
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-151	CUAGACUGAAGCUCCUUGAGG
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
miRNA-214	ACAGCAGGCACAGACAGGCAGU

Table S2. Comparison of analytical performance of miRNAs with other probes.

Probes	Method	Linear range (particles mL ⁻¹)	LOD (particles mL ⁻¹)	Ref.
DNA cube	FRET	2.50×10^8 - 1.50×10^{10}	9.80×10^7	[1]
DNA logical device	fluorescence	2.40×10^8 - 1.70×10^9	1.20×10^8	[2]
molecular beacon	fluorescence	5.00×10^8 - 5.00×10^{10}	3.80×10^8	[3]
Au@Pd nanopopcorn	lateral flow strip	10^8 - 10^{10}	1.40×10^7	[4]
CHA probe	FRET	-	10^9	[5]
molecular beacon	simultaneous multiplexed	10^{10} - 10^{12}	10^{10}	[6]
molecular beacon	TIRF	3.00×10^7 - 10^{10}	-	[7]
DNA nanocage	FRET	1.11×10^8 - 5.70×10^{10}	1.77×10^7	This work

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