Supporting Information

MicroRNA-induced cascaded and catalytic self-assembly of DNA nanostructures for enzyme-free and sensitive fluorescent detection of microRNA from cancer cells

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Experimental Section

Materials and reagents: The HPLC-purified synthetic miRNAs and other oligonucleotides were all purchased from Invitrogen Biotechnology Co., Ltd (Shanghai, China), and the sequences of the oligonucleotides were listed in Table 1. All reagents were analytical grade and used without further purification.

Table S1 Sequences of the oligonucleotides used in the experiments.

Strand name	Sequence (5'-3')
DNA1	TGCCA GGCAG GAGCA GTCGC A
DNA2	TGCGA CTGCT TGGCA CCTGC C
HP1	AAATG GTCTG TCACA ATACC TTCAA CATTG TGACA GACCA TTTCT ACCAT CCTGC GACTG CTTGG CACCT GCC
HP2	TGCCA GGCAG GAGCA GTCGC AGTTG AAGGT AT(FAM)TGT GACAG ACCAT TTGGA TGGTA GAAAT GGTCT GTCAC

	AAT(DABCYL)
miR-141	UAACA CUGUC UGGUA AAGAU GG
SM*	UAACA CUGUC UGGUA AUGAU GG
miR-200b	UAAUA CUGCC UGGUA AUGAU GA
miR-429	UAAUA CUGUC UGGUA AAACC GU
miR-21	UAGCU UAUCA GACUG AUGUU GA
let-7d	AGAGG UAGUA GGUUG CAUAG UU

* SM indicates the one-base mismatched sequence.

Agarose gel electrophoresis: The 2% agarose gel electrophoresis was used to confirm the formation of the DNA nanowheel nanostructures. Electrophoresis was performed with 1×TBE and run at the same buffer at a constant voltage of 100V for 50 min. The gels were stained with ethidium bromide for 20 min and photographed with a digital camera under a UV illumination.

Native polyacrylamide gel electrophoresis (PAGE): After the sample solutions were prepared, 10 μL of each sample was mixed with 2 μL of 6×loading buffer, and then 10 μL of the mixed solution was loaded into the notches of the freshly prepared 8% native polyacrylamide gel for electrophoresis analysis. Electrophoresis was performed at a constant voltage of 100V for 80 min in 1×TBE buffer (89 mM Tris, 89 mM BoricAcid, 2.0 mM EDTA, pH 8.3). The gels were then stained with ethidium bromide for 20 min, followed by photographing with a digital camera under UV irradiation.

Cell culture and total RNA extraction: Human breast (MCF-7), prostate(22Rv1)and cervical cancer cell lines (HeLa) were all obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). MCF-7, HeLa and 22 Rvl

cells were routinely cultured in RPMI 1640 medium (Thermo Scientific Hyclone), supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 37 °C in a humidified 5% CO_2 incubator, respectively. Total RNA samples were extracted from each cell line by using Trizol Reagent (Invitrogen Biotechnology Co., Ltd) according to the manufacturer's protocol with minor modification. In brief, an appropriate amount of TRizol Reagent was added into cell pellets and centrifugated for 10 min. The upper aqueous phase was carefully transferred to a new centrifuge tube and RNA extraction was precipitated by using isopropyl alcohol. The extracted RNA solution was used immediately.

MicroRNA-triggered assembly of DNA nanowheel nanostructures for sensitive detection of miR-141: The motifs (M1 and M2) used to assemble the DNA nanowheel nanostructures were first prepared by thermal annealing of the mixture of DNA1 (1 μM) and HP1 (1 μM) (or DNA2 and HP2) as follows: 95 ºC (3 min), 65 ºC (30 min), 50 ºC (30 min), 37 ºC (30 min) and 22 ºC (30 min) in TAE- Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, pH 8.0). Then, different concentrations of miR-141 were added to the mixtures of M1 (100 nM) and M2 (100 nM) to initiate the self-assembly process at 20 °C with a total volume of 200 μL.

Fluorescence Measurements: Fluorescence measurements were performed on a FR-5301-PC spectrophotometer (Shimadzu, Tokyo, Japan) at room temperature with a 150 W Xenon lamp (Ushio Inc., Japan) as the excitation source. The emission spectra were acquired by exciting the samples at 490 nm, and fluorescence data were collected from 450 nm to 650 nm. The slit widths of both the excitation and emission were set at 5 nm.

Atomic force microscopy (AFM) imaging: A drop of 5 µL sample solution was spotted onto the freshly cleaved mica that was already treated with 50 µL HEPES buffer (10 mM HEPES, 2 mM Ni^{2+}) to increase the adsorption of the DNA nanostructures, and the sample was allowed to adsorb to the mica surface for 3 min. The mica was then rinsed with ultrapure water and dried with nitrogen. AFM imaging was performed in air with a tapping mode on the JPK NanoWizard 3 Bioscience AFM. Tap300Al-G silicon probes (Budget Sensors, Bulgaria) with resonant frequency of 320 kHz and force constant of 42 N/m were used in the experiments.

Dynamic light scattering (DLS) analysis: The DLS measurement was performed on a Malvern Zetasizer Nano ZS apparatus (Malvern, UK).

Supplementary Figure:

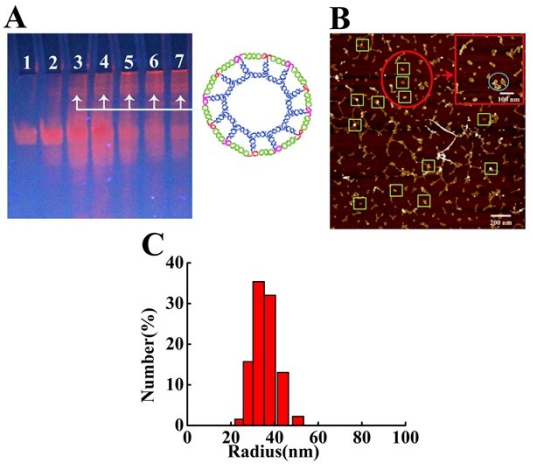

Fig. S1 (A) Native PAGE analysis of different reaction mixtures: Lane 1: M1 motif (1 µM); Lane 2: mixture of M1(1 µM) and M2 (1 µM) motifs; Lane 3: miR-141 (500 nM), M1 motif (1 µM), M2 motif (1 µM); Lane 4: miR-141 (100 nM), M1 motif (1 µM), M2 motif (1 µM); Lane 5: miR-141 (10 nM), M1 motif (1 µM), M2 motif (1 µM); Lane 5: miR-141 (1 nM), M1 motif (1 µM), M2 motif (1 µM); Lane 6: miR-141 (0.5 nM), M1 motif (1 µM), M2 motif (1 µM) and Lane 7: miR-141(0.1 nM), M1 motif (1 µM), M2 motif (1 µM). (B) AFM image showing the distribution of the self-assembled DNA

nanostructures. The independent desired DNA nanostructures were labeled with green squares, and the blue circle indicated the aggregation of DNA nanostructures. (C) Size distribution of the self-assembled DNA nanostructures obtained by DLS measurement.

The miR-141 triggered cascaded and catalytic self-assembly of DNA nanowheel nanostructures was further characterized by 8% polyacrylamide gel electrophoresis (PAGE) and atomic force microscope (AFM). As shown in Fig. S1A, the mixture of M1 and M2 displays one merged band (Lane 2) with similar electrophoretic mobility to that of M1 (Lane 1), indicating the coexistence of M1 and M2. Subsequent incubation of miR-141 with the mixture of M1 and M2 leads to the appearance of a new band with lower electrophoretic mobility (Lane 3), indicating the successfully assembled nanostructures. The assembly of the DNA nanostructures is inversely related to the initiator concentration, which can be observed from the notches (Lane 3 to Lane 7) with gradual decrease in the concentration of miR-141 from 500 nM to 0.1 nM corresponding to gradual increase in the band intensity with the formation of more nanowheel nanostructures. The formation of the DNA nanowheels was also confirmed by AFM. As shown in Fig. S1B, we can clearly see many DNA nanowheels with diameter about 36±8 nm randomly distributed on the mica surface. It should be noted that DNA nanowheels with larger size may be attributed to the adsorption process of the DNA samples to the mice surface. Meanwhile, the formation of long nanowires indicates incomplete assembly during the synchronization of HCR and T-junction cohesion assembly process. The size distribution of the self-assembled nanostructures was also determined by using the dynamic light scattering (DLS) technique. According to Fig. S1C, the size of the DNA nanostructures varies from 24 to 50 nm, and 95% of them falls in the range between 28 to 43 nm, which is consistent with the AFM data.

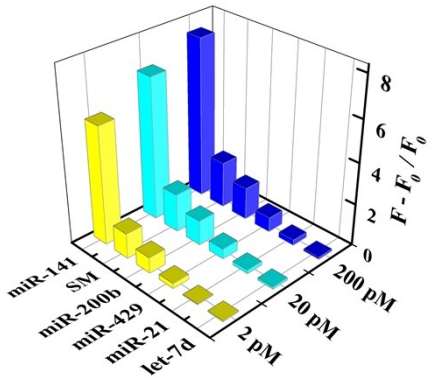

Fig. S2 Specificity evaluation of the proposed method using different miRNAs. Bars represented the fluorescence increase ratio ($F-F_0/F_0$) for different targets of let-7d, miR-21, miR-429, miR-200b, one-base mismatched sequence (SM), and miR-141 at three concentrations of 2 pM, 20 pM, 200 pM, where F and F_0, respectively, were the fluorescent signals in the presence and absence of the miRNA.

The capability of discriminating the target miRNA among the family members is particularly important for understanding their biological functions of individual miRNAs and the relationship with corresponding human diseases. However, it is always a great challenge to achieve this goal owing to their similar sequences with high homology. To examine the specificity of the proposed method, we challenged the sensing system with other control miRNAs, including let-7d, miR-21, miR-429, miR-200b and one-base mismatched sequence (SM) under three concentrations of 200 fM, 2 pM, 20 pM, respectively. The results were shown in Fig. S2, from which we can clearly see that the presence of the control miRNAs causes negligible changes in fluorescence increase ratio ($F- F_0/ F_0$, F and F_0, respectively, are the fluorescent signals in the presence and absence of the miRNA), whereas the addition of the target miR-141 generates significantly enhanced fluorescence increase ratio. The results here indicate the high selectivity of this strategy, in which only the perfectly complementary miRNA can trigger the self-

assembly formation the DNA nanostructures with significantly enhanced fluorescence intensity.