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Supporting Information

Fluorescence resonance energy transfer-based DNA framework assembled split G-quadruplex nanodevices for microRNA sensing in living cells

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1. Experimental details

1.1 Materials and Reagents.

Oligonucleotides used here were bought from Sangon Biotech Co., Ltd (Shanghai, China) and was purified by HPLC. MiRNA (mir-122a) was synthesized by TaKaRa (Dalian, China). DNA sequences (Supplementary Table S1) were designed with the help of the Integrated DNA Technologies. All reaction systems used Tris-acetic acid-magnesium (1×TAM) buffer (45 mM Tris -acetic acid, 7.6 mM magnesium acetate and 50 mM KCl, pH 8.0). All reagents were used as received without further purification. Diethypyrocarbonate (DEPC) treated water and LipoHigh transfection reagent were bought from Sangon Biotech. Co., Ltd.

1.2 Apparatus

UV1800PC spectrophotometer (Shanghai, China) was used to quantify Oligonucleotide. Fluorescence signals were got on a RF-5301PC (Shimadzu) spectrofluorophotometer. All DNA samples were annealed on an Applied Biosystems 96-well thermocycler. The confocal microscope images of the cells were obtained with a Leica TCS SP8 confocal laser scanning microscope. Native polyacrylamide gel electrophoresis (PAGE) of DNA were imaged with a Syngene gel imaging system under UV light.

1.3 Preparation of DNA framework

DNA framework (F) was composed of C1, C2, C3, C4, C5 and C6 strands as listed in Table S1. G4-F consisted of C1, C2, C3, C4, C5, C6, C7a and C7b strands. When preparing the DNA framework or G4-F, equal amounts of the constituent

strands were added and mixed in 1 × TAM buffer and the final concentration of each strand was 0.6 μM. The resulting mixed strands (0.6 μM each) were annealed in a PCR tube from 95℃ to 4℃ over the course of 3.5 hours to get the DNA framework or G4-F structure. 6% PAGE was used to characterize the resulting samples. The electrophoresis was proceeded in 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0). The gels were stained with 4S Red before imaging.

1.4 Fluorometric assays

The annealed G4-F device was first incubated with different target sequences for 1 h before the FRET signals were recorded from 530 to 750 nm at an excitation wavelength of 488 nm in a 100 μL quartz cuvette.

1.5 AFM imaging

After incubation with 40 μL ((3-aminopropyl) triethoxysilane, APTES) (0.5%) for 5 min, the mica was flushed by Milli-Q water, and dried by N_2. 10 μL sample dissolved in 1×TAM buffer was incubated for 5 min on treated mica. After flushing, 1×TAM buffer was added to a total volume of 100 μL. The G4-F nanodevices were scanned in a Dimension Icon (Bruker) mode for AFM imaging.

1.6 Dnase I and FBS assay

To evaluate the stability of the G4-F nanodevice, 0.3 U/ml Dnase I and 10% FBS were incubated with 0.5 μM samples for 0, 10, 20, 30, 40, 50, or 60 min, respectively. The samples were characterized with 6 % PAGE.

1.7 Cell Culture and Lysis

Human cervical carcinoma cells (HeLa) were cultured in DMEM medium, which

was supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37°C in a 5% CO_2 incubator. HeLa cells (1×10^6 mL^{-1}) were lysed via low-frequency ultrasonic wave.

1.8 Confocal Laser-Scanning Microscopy Imaging

HeLa cells seeded in confocal dish (15 mm) were incubated for 24 h. After flushing three time, 160 μL DMEM with 75 nM DNA framework (1×TAM, 7 mM KCl and 7 μM) was added. After incubation for 2 h later, the cells were flushed three times to remove the probes that were not internalized by the cells. To monitor the miRNA response in cells, mir-122a sequences (140 nM) were delivered into cells with LipoHigh transfection reagent based on the manufacture's operating instruction. After flushing, the confocal laser-scanning microscopy was used to image mir-122a-loaded cells which was incubated with the corresponding DNA devices for 2 h.

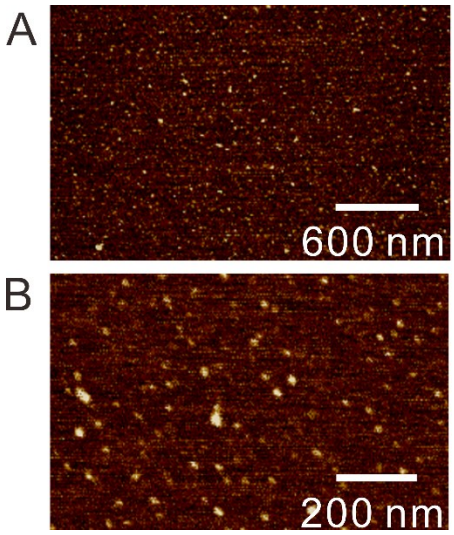

Figure S1 AFM characterization of the DNA nanocube.

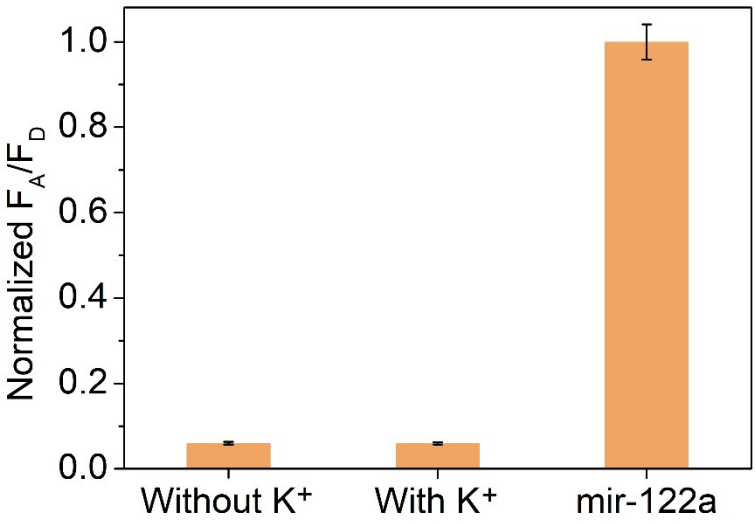

Figure S2 Variations of F_A/F_D in different conditions. The concentration of G4-F used here was 500 nM. Without K^+: 1×TAM buffer; With K^+: 1×TAM buffer and 50 mM KCl; mir-122a: 1×TAM buffer and 50 mM KCl with 250 nM mir-122a.

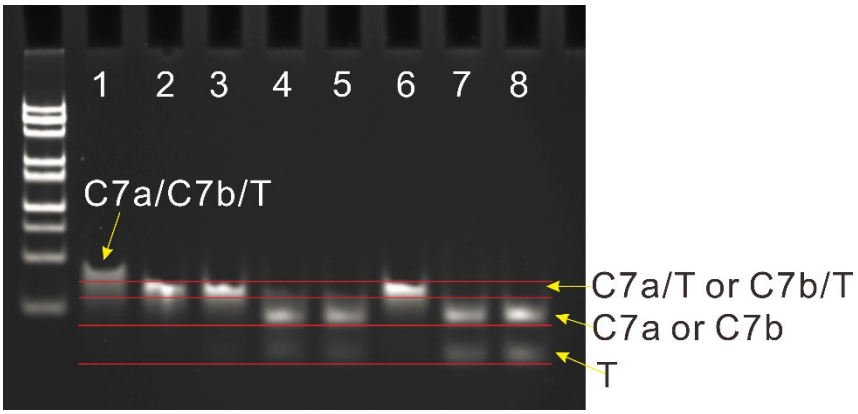

Figure S3 The PAGE characterization for hybridization results of target and spurious strands (T) with G4 segments (C7a and C7b). From lane 1 to lane 8： mir-122a, M4, M8, M15, M19, M4-8, M4-8-15, Random.

Table S1. All the oligonucleotides used in this work.

Name	**5'-3'**
C1	CCAGCCGCCGTTCTCCTGGATCCAAGGCTCTAGGTGGTCATT CAGGTAAGTGGCCATCCAAGCTGCGA
C2	CCACTCCCGTTTCTGGGATGCCATACTCTAACTCAGATTCGCT GATTCTGAATGACCTTTAGCGTTGGCT
C3	GATAAGGATTTAGGTCTGCCCTTTCGCAGCTTGGATGGCCAC TTTTTCAGCGAATCTGAGTTAGAGT
C4	GAGACAGCCAGGAGAAATCAAACAGAGGCCGCATGCTGGGG CCGTACAGTTCCACAAAGGCATCCCAG
C5	AATCCTTATCTTTGCGGCCTCTTTCCGTATATTCACGAAAAGG AGTTCGGCGGCTGGTTGGGCAGACCTA
C6	CTCCTTTTCGTGAATATACGGTATTGATTTCTCCTGGCTGTCT CTTACGGGAGTGGAGCCAACGCTA
C7a	TGGAACTGTACA TGAAA CAAACACCATT TTTGGGTAGGG-Cy3
C7b	Cy5-GGGTTGGGTTT TCACACTCCA TGAAAATTGGATCCAGG
mir-122	UGGAGUGUGACAAUGGUGUUUG
M4	UGGUGUGUGACAAUGGUGUUUG
M8	UGGAGUGAGACAAUGGUGUUUG
M15	UGGAGUGUGACAAUCGUGUUUG
M19	UGGAGUGUGACAAUGGUGAUUG

M15	UGGAGUGUGACAAUCGUGUUUG
M4-8	UGGUGUGAGACAAUGGUGUUUG
M4-8-15	UGGUGUGAGACAAUCGUGUUUG
Random	AACTATACAACCTACTACCTCA