Supporting information for

Programming Split G-Quadruplex with DNA Nanocage and MicroRNA Imaging in Live Cells

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Experimental Section

Materials and Reagents: Oligonucleotides used here were obtained from Sangon Biotech Co., Ltd (Shanghai, China) and with HPLC purification. MiRNA (mir-122a) were synthesized by TaKaRa (Dalian, China). DNA sequences (Supplementary Table S1) were designed with the help of the Integrated DNA Technologies. Tris (hydroxyl methyl) amino methane (Tris), potassium chloride, and ThT was purchased from Aladdin Reagent Corporation (Shanghai, China). Tris-acetic acid-magnesium (1×TAM) buffer (45 mM Tris-acetic acid and 7.6 mM magnesium acetate, pH 8.0) was used for all reaction systems. All reagents were used as received without further purification. Diethypyrocarbonate (DEPC) treated water and LipoHigh transfection reagent were purchased from Sangon Biotech. Co., Ltd. Stock solutions of ThT (400 μM) were prepared in H_2O, stored in the dark at −20℃, and diluted before use with 1×TAM buffer.

Apparatus: Oligonucleotide quantification was done with a UV1800PC spectrophotometer (Shanghai, China). Fluorescence signals were measured 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 acquired 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.

Preparation of DNA Prism: The strands composing the prism were mixed at a concentration of 0.6 μM in one vessel in a Tris-acetic acid-magnesium (TAM) buffer and annealed from 95 ℃ to 4 ℃ over the course of 3.5 hours. The resulting samples were then analyzed with 6% PAGE. The

electrophoresis was conducted in 1 × TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0). The gels were stained with 4S Red prior to imaging.

Fluorometric assays: The annealed prism device was first incubated with different target sequences for 1 h. Then 10 μL KCl (500 mM) and 1 μL ThT (0.4 mM) were added to the 100 μL reaction system. The resulting samples were incubated for 1 h at room temperature before the ThT fluorescence signals were recorded from 450 to 600 nm at an excitation wavelength of 405 nm in a 100 μL quartz cuvette.

AFM Imaging: After incubation with 40 μL ((3-aminopropyl) triethoxysilane, APTES, 0.5%) for 5 min, the mica was washed by Milli-Q water, and dried by N_2. 10 μL prism dissolved in 1×TAM buffer was incubated for 5 min on treated mica. After washing, 1×TAM buffer was added to a total volume of 100 μL. The DNA prism was then scanned in a Dimension Icon (Bruker) for AFM imaging.

Dnase I assay: To assess the stability of the prism device, various concentrations of Dnase I were incubated with 0.5 μM DNA prism samples for 0, 10, 20, 30, 40, 50, or 60 min, respectively. The samples were then characterized with 6% PAGE.

Cell Culture and Lysis Preparation: Human cervical carcinoma cells (HeLa) and human breast cancer cells (MCF-7) were cultured in DMEM and RPMI 1640 medium, respectively, which was supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 ℃ in a 5% CO_2 incubator. Exosomes were harvested from MCF-7 cells according to the literature[1,2] by differential centrifugation method.

Confocal Laser-Scanning Microscopy Imaging: HeLa cells seeded in confocal dish (15 mm)

were incubated for 24 h. After washing three time, 160 μL DMEM with 75 nM R-prism (1×TAM, 7 mM KCl and 7 μM) was added. Two hours later, the cells were washed three times to remove the probes that were not internalized by the cells. To monitor the miRNA in cells, mir-122a sequences (140 nM) were delivered into cells with LipoHigh transfection reagent according to the operating instruction. After washing, the mir-122a-loaded cells were imaged with confocal laser-scanning microscopy after incubation with the corresponding DNA devices for 2 h.

References

1. Skog, J.; Wuerdinger, T.; van Rijn, S.; Meijer, D. H.; Gainche, L.; Sena-Esteves, M.; Curry, W. T., Jr.; Carter, B. S.; Krichevsky, A. M.; Breakefield, X. O., *Nat. Cell Bio.* **2008,** *10* (12), 1470-U209.

2. Pablo Tosar, J.; Gambaro, F.; Sanguinetti, J.; Bonilla, B.; Witwer, K. W.; Cayota, A., *Nucleic Acids Res.* **2015,** *43* (11), 5601-5616.

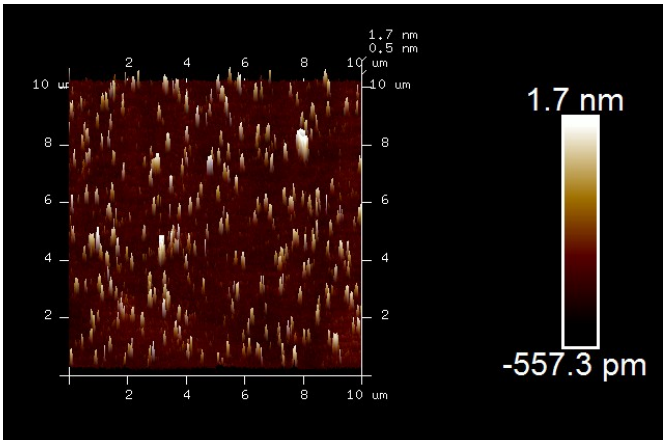

Fig. S1. Height of the assembled empty prism was estimated to be 1.7 nm by the height sensor from the AFM.

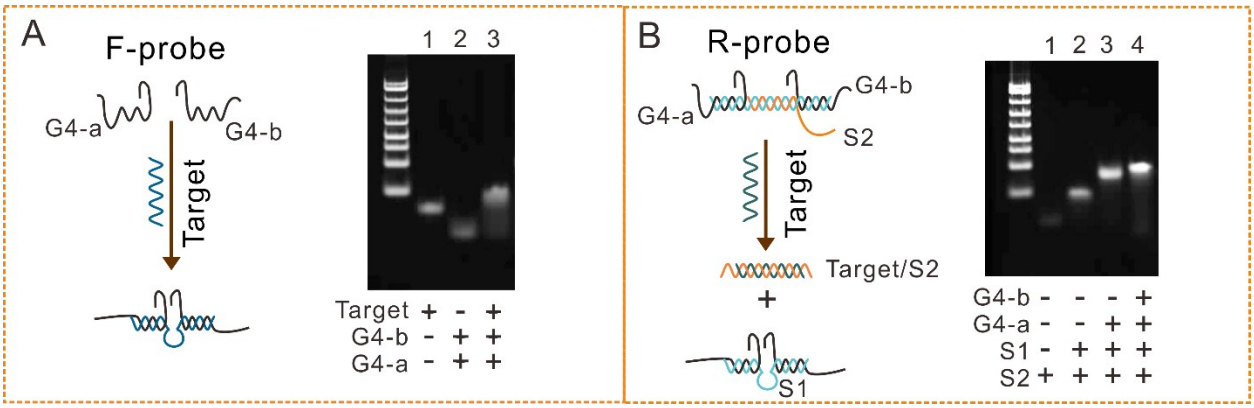

Fig. S2. 8 % PAGE analysis of the assembly of F-probe (A) and R-probe (B).

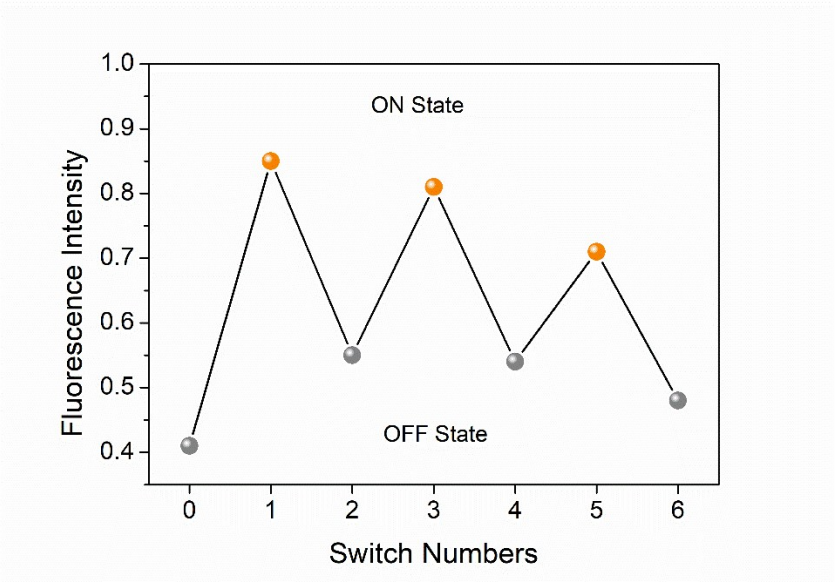

Fig. S3. Fluoresecent analysis of the switching cycles reversible performance of the designed nanodevice between 'OFF' and 'ON' state by alternately adding the S2 and target (mir-122a) into the rigid prism switching system. The concentration of nanodevice was 0.5 μM. 0.5 μL of S2 (100 μM) or mir-122a (100 μM) was added to the reaction systems each time.

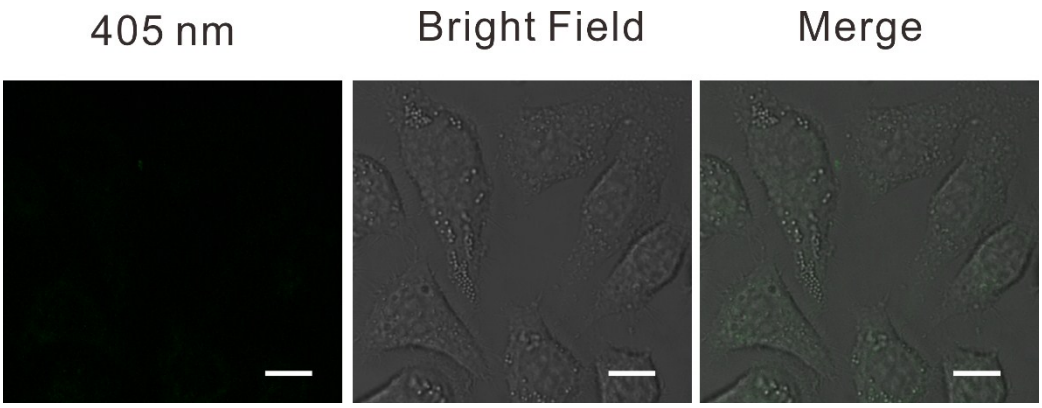

Fig. S4. Confocal microscopy images of HeLa cells treated with ThT alone. Normal HeLa cells were utilized. 500 nM ThT was incubated with cells for 2 h before imaging with confocal laser-scanning microscopy with 405 nm for the excitation wavelength. Scale bar: 10 μm.

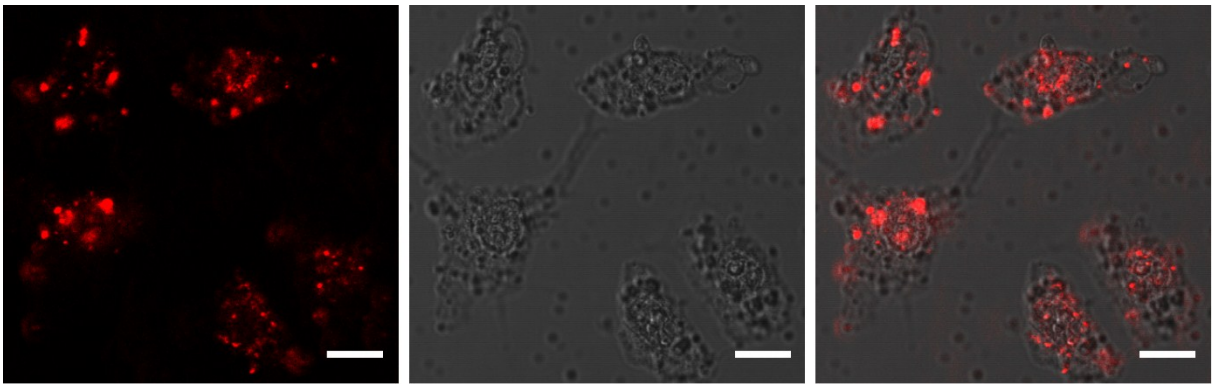

Fig. S5. Fluorescence images of mir-122 loaded HeLa cells prepared with LipoHigh transfection reagent. Cy5 modified mir-122 was used for the purpose of fluorescence imaging. Scale bar: 10 μm. HeLa cells were cultured overnight in 15 mm culture dish at a density of 5×10^4 per well in order to achieve 60%-80% confluence. Prior to the miRNA transfection, cultured cells were washed with PBS and preincubated for 2 h with DMEM without serum. Then we added the mir-122 loaded lipofectamine. The treated cells were incubated in RPMI 1640 medium without serum at 37 °C for 4 h. Then, the serum-free medium was replaced with DMEM containing 10 % FBS and the cells were further incubated with R-prism for another 2 h. Finally, the confocal microscopy images were taken. Scale bar: 10 μm.

Table S1. All the oligonucleotides used in this work.

Name	**5'-3'**
C1	CCAGCCGCCGTTCTCCTGGATCCAAGGCTCTAGGTGGTCAT TCAGGTAAGTGGCCATCCAAGCTGCGA
C2	CCACTCCCGTTTCTGGGATGCCATACTCTAACTCAGATTCG CTGATTCTGAATGACCTTTAGCGTTGGCT
C3	GATAAGGATTTAGGTCTGCCCTTTCGCAGCTTGGATGGCCA CTTTTTCAGCGAATCTGAGTTAGAGT
C4	GAGACAGCCAGGAGAAATCAAACAGAGGCCGCATGCTGG GGCCGTACAGTTCCACAAAGGCATCCCAG
C5	AATCCTTATCTTTGCGGCCTCTTTCCGTATATTCACGAAAA GGAGTTCGGCGGCTGGTTGGGCAGACCTA
C6	CTCCTTTTCGTGAATATACGGTATTGATTTCTCCTGGCTGTC TCTTACGGGAGTGGAGCCAACGCTA
C7a	TGGAACTGTACATGAAAAACGAGACTGTGGGTAGGG
C7b	GGGTTGGGTCGTCACTCCATGAAAATTGGATCCAGG
S1-11 /F-Target	TGGAGTGACGTGGAGTGTGACCAGTCTCGTT
S2	CAAACACCATTGTCACACTCCA
mir-122a	UGGAGUGUGACAAUGGUGUUUG

/R-Target	
mir-122a-Cy5	UGGAGUGUGACAAUGGUGUUUG-Cy5
S1-7	TGGAGTGACGTGGAGTGCAGTCTCGTT
S1-8	TGGAGTGACGTGGAGTGTCAGTCTCGTT
S1-9	TGGAGTGACGTGGAGTGTGCAGTCTCGTT
S1-10	TGGAGTGACGTGGAGTGTGACAGTCTCGTT
S1-11	TGGAGTGACGTGGAGTGTGACCAGTCTCGTT
S1-12	TGGAGTGACGTGGAGTGTGACACAGTCTCGTT
S1-13	TGGAGTGACGTGGAGTGTGACAACAGTCTCGTT
S1-14	TGGAGTGACGTGGAGTGTGACAATCAGTCTCGTT
S1-15	TGGAGTGACGTGGAGTGTGACAATGCAGTCTCGTT