Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Cell imaging with multi-color DNA framework probes

Mo Xie,†[a] Linjie Guo,†[bc] Shu Xing,[d] Shuting Cao,[c] Ziwei Zhao,[ag] Kaiyi Liang,[e] Jiang Li,[bc] Shihua Luo,*[f] Yu Zhang,*[bc] Lianhui Wang*[a]

[a] *State Key Laboratory of Organic Electronics and Information Displays & Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications, 9 Wenyuan Road, Nanjing 210023, China.*

[b] *Bioimaging Center, Shanghai Synchrotron Radiation Facility, Zhangjiang Laboratory, The Interdisciplinary Research Center, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China.*

[c] *Division of Physical Biology, CAS Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China.*

[d] *Cixi Institute of Biomedical Engineering, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo 315201, P. R. China.*

[e] *Radiology Department of Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Key Laboratory of Shanghai Municipal Health Commission for Smart Image, Shanghai 201800, China.*

[f] *Department of Traumatology, Rui Jin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China.*

[g] *Shanghai Frontier Innovation Research Institute Co., Ltd., Shanghai 201108, China.*

† These authors contributed equally to this work.

*Corresponding authors

E-mail: jqab@163.com; zhangyu@sari.ac.cn; iamlhwang@njupt.edu.cn

Experiment

Preparation and purification of TDN and FDF nanostructures. Equimolar concentration (1 μM) of four component DNA single strains (S1, S2, S3 and S4, synthesized by Invitrogen, USA) were mixed in TM buffer (10 mM Tris, 5 mM $MgCl_2$, pH 8.0). The mixture was annealed from 95℃ to 4℃ to form TDN-I and TDN-IV. HPLC (Agilent 1260 system; Phenomenex BioSec-SEC-4000 column; the mobile phase was 25 mM Tris-HCl, 450 mM NaCl, pH 7.2 with 1 ml/min flow rate) was carried out to purify the TDN structures. To synthesise FDF, the puridied TDN-I and TDN-IV with a molar ratio of 4:1 were mixed and incubated at 37℃ for 2h. Subsequently, the FDF was again purified with HPLC.

Gel electrophoresis Characterization. 1% agarose (BioRad) gel was used for analysis (100 V, 1h). The electrophoresis buffer was 1× TBE (tris-borate-EDTA). DNA was stained with GelRed (Biotium, USA) for further analysis on a chemiluminescence imaging system (G:Box Chemi-XL).

AFM Characterization. Freshly cleaved mica surface was treated with 0.5% APTES (Sigma-Aldrich) for 2 min, washed off by Milli-Q water (18 MΩ·cm) and dried with compressed air. 10 nM of the DNA structures were deposited on mica surface. After absorption for 5 min, 30 μL TM buffer was added. The sample was imaged using an SNL-10 tip (Veeco Inc., USA) in tapping mode in fluid.

Fluorescence spectroscopic measurement. Fluorescence spectrophotometer (Edinburgh FS920) was carried out for measure the fluorescence intensity of Alexa647 labled FDF structure probes (sgc8-FDF(100) and sgc8-FDF(400)). 10 nM of the FDF probe in 100 μL TM buffer was pipetted into a quartz cuvette, then the fluorescence emission spectra were collected from 655 to 750 nm with excitation at 650 nm.

Cell lines. Michigan Cancer Foundation-7 breast cancer cells (MCF-7), cervical carcinoma HeLa cells (HeLa), human leukemic cells (CCRF-CEM) and human embryonic kidney 293 cells (Hek293) were purchased from the Cell Resource Center, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). MCF-7 and CCRF-CEM cells were grown in RPMI1640 cell culture medium supplemented with 10% fetal bovine serum (FBS). HeLa cells were grown in MEM cell culture medium supplemented with 10% FBS. Hek293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. The medium and FBS were purchased from Gibco/Life Technologies (Grand Island, NY, USA). The cell lines were all incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

Cell imaging and discrimination. For cell imaging and discrimination, MCF-7, HeLa, CCRF-CEM and Hek293 cells were plated in glass-bottom dishes (Cellvis) at a density of 50,000 per dish respectively. After culturing for 24 h, cells were incubated with the mixture of AS1411-F4,1(410), SYL3C-F4,1(041) and sgc8-F4,1(104) (final

concentration, 1 nM each) at 37 °C for 30 min. Before imaging, cells were washed with PBS for 3 times. Total internal reflection fluorescence (TIRF) imaging was performed on a commercial super-resolution microscope (N-STORM, Nikon) with a 100x objective lens and an electron multiplying charge-coupled device (EMCCD) camera. Images were recorded in three fluorescence channels (excitation: 488, 561, and 647 nm, respectively). Fluorescence image analysis and statistics were carried out using the software on the microscopic system. The regions of individual cells (ROIs) were outlined manually according to the brightfield images. Then the mean fluorescence intensity of a cell in each channel was obtained by dividing the cumulative fluorescence intensity of the cell by the area of the cell.

Supporting Tables

Table S1. DNA Sequences

Sequences for building TDN. Underlined sequences are the linker sequences between TDN.	
Name	Sequence (5’ to 3’)
L1-S1	GTGCTTGGTAACATAGGTGCACAGCCAGTTGAGACGAACATTCCTAAGTCTG AAATTTATCACCCGCCATAGTAGACGTATCACCAGG
L1-S2	GTGCTTGGTAACATAGGTGCACAGCGCTACACGATTCAGACTTAGGAATGTT CGACATGCGAGGGTCCAATACCGACGATTACAGCTT
L1-S3	GTGCTTGGTAACATAGGTGCACAGCGTGATAAAACGTGTAGCAAGCTGTAA TCGACGGGAAGAGCATGCCCATCCACTACTATGGCGG
L1-S4	GTGCTTGGTAACATAGGTGCACAGCCTCGCATGACTCAACTGCCTGGTGATA CGAGGATGGGCATGCTCTTCCCGACGGTATTGGACC
L1’-S1	GCTGTGCACCTATGTTACCAAGCACCAGTTGAGACGAACATTCCTAAGTCTG AAATTTATCACCCGCCATAGTAGACGTATCACCAGG
S2	GCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGA CGATTACAGCTT
S3	GTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCA CTACTATGGCGG
S4	CTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGAC GGTATTGGACC
Sequences for cell discrimination. Aptamer sequences sgc8, AS1411, and SYL3C are appended on the 5’ end of S2.	
sgc8-S2	*ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA*TTTTT GCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGA CGATTACAGCTT
AS1411-S2	*TTGGTGGTGGTGGTTGTGGTGGTGGTGG*AAAAA GCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGA CGATTACAGCTT
SYL3C-S2	*CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG*TTTT T GCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGA CGATTACAGCTT

Supporting Figures

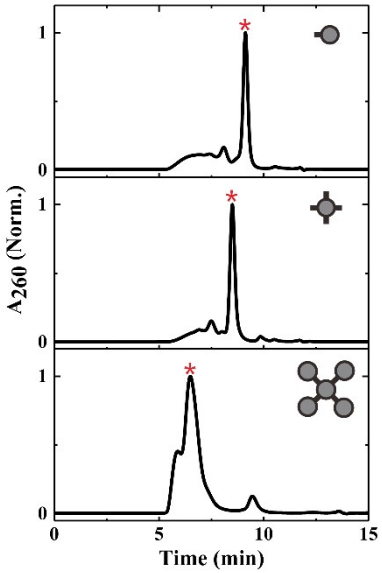

Figure S1. Chromatograms of TDN-I, TDN-IV, and FDF structures.

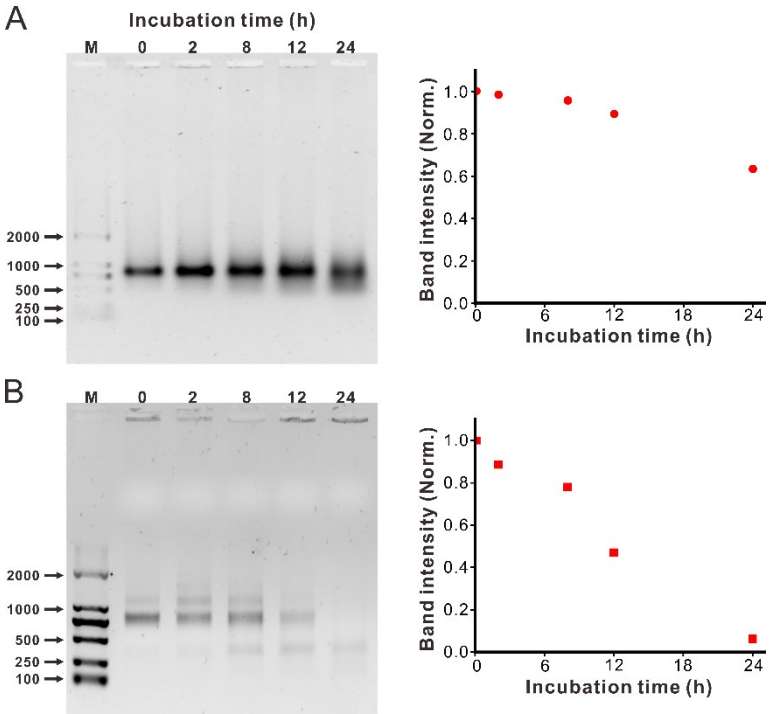

Figure S2. Stability analysis of FDF. Agarose gel images and band intensity quantification (normalized to the intensity profile integral area of the lane, analyzed with ImageJ) of FDF separately incubated in (A) 1640 medium (10% FBS) and (B) cell lysis solution for 0 (control), 2, 8, 12 and 24 h.

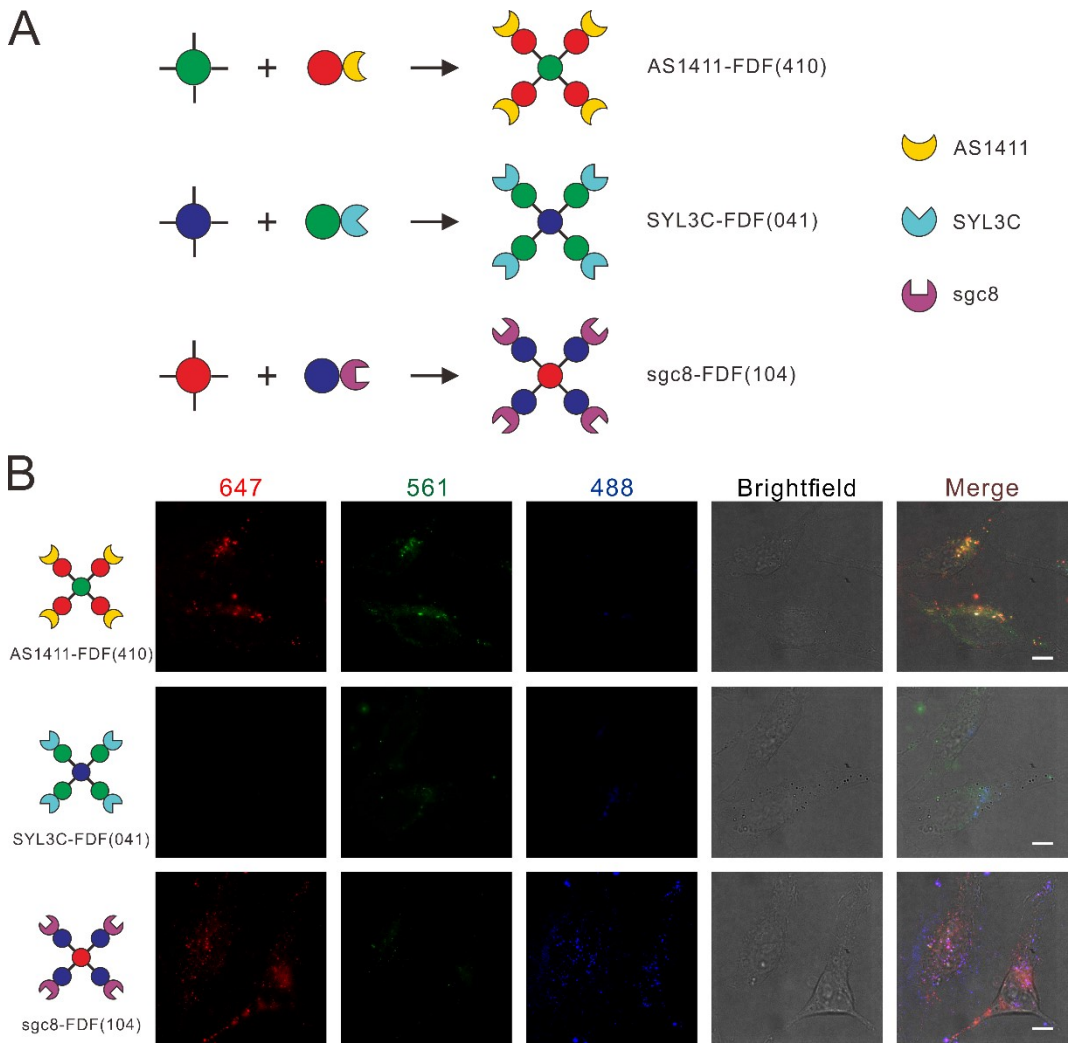

Figure S3. (A) Schematic of AS1411-FDF(401), SYL3C-FDF(041), and sgc8-FDF(104) structures. (B) TIRF images of cells separately incubated FDF-encoded aptamers. From top to bottom, AS1411-FDF(401), SYL3C-FDF(041), and sgc8-FDF(104). From left to right, fluorescence channels of Ex 647, 561 and 488, in addition with brightfield and merged images. Scale bar, 10 μm.

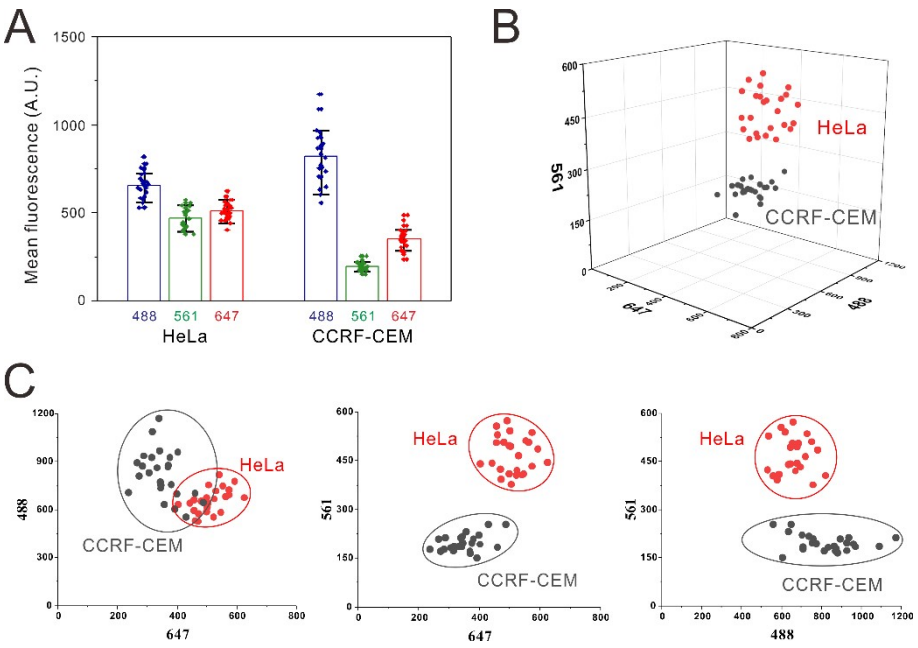

Figure S4. FDF-encoded aptamer fluorescent probes for discriminate two types of cells (HeLa and CCRF-CEM) with high expression of PTK-7. (A) Fluorescence intensity of each color in each type of the FDF-aptamer probe labeled cells. (B) Scatter plot of the cells in three dimensions representing the three fluorescence channels. (C) Two-dimensional projection of (B).