Supporting Information for

Facile Assembly/Disassembly of DNA Nanostructures Anchored on Cell-Mimicking Giant Vesicles

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Methods and Materials

1. General Materials

All HPLC-purified oligonucleotides and various DNA nanostructure samples were assembled in 1×reaction buffer (20 mM Tris, pH=7.4, 2 mM EDTA, 12.5 mM Mg^{2+}) for test tube experiments, but assembled in 10×reaction buffer (pH=8.0) for imaging experiments.

Stains-All was purchased from Sigma-Aldrich Co., Ltd., and 20-bp DNA ladder was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China).

2. DNA Synthesis and Purification

DNA sequences were synthesized on a DNA synthesizer (PolyGen GmbH, Langen, Germany). The synthesis protocol was set up according to the requirements specified by the reagents' manufacturers. After on-machine synthesis, the DNA products were deprotected and cleaved from CPG at 65 °C in a water bath and incubated with 2 mL of AMA (ammonium hydroxide and 40% methylamine, 1:1) for normal deprotection for 30min. However, for Cy3- and Cy5-modified DNA strands, 2 mL of mixed solution (methanol: tert-butylamine: water in 1:1:2 ratio) was used to cleave DNA products from CPG in an incubation lasting 3 to 4 hours. The cleaved DNA product was transferred to a 15 mL centrifuge tube and mixed with 200 μL of 3 M NaCl and 5 mL of ethanol, after which the sample was placed in a freezer at −20 °C for ethanol precipitation. Afterwards, the DNA product was spun at 4000 rpm at 4 °C for 30 min. The supernatant was removed, and the precipitated DNA product was dissolved in 400 μL of 0.1 M triethylamine acetate (TEAA) for HPLC purification.

HPLC purification was performed with a cleaned C18 column (Inertsil ODS-3, 5 μm, 4.6×250 mm, GL Science Inc., Japan) and Agilent 1260 Infinity Quaternary LC (Agilent Technologies, Germany). The collected DNA product was dried and processed for detritylation by dissolving and incubating in 200 μL of 80% acetic acid for 20 min. The detritylated DNA product was mixed with 20 μL of 3 M NaCl and 500 μL of ethanol and placed in a freezer at −20 °C for 30 min. This was followed by spinning the DNA product at 14000 rpm at 4 °C for 5 min. The DNA product was dried by a vacuum dryer and redissolved in ultrapure water, followed by desalting with desalting columns.

The cholesterol-labeled and Alexa Fluor 488-labeled DNA strands were synthesized and

purified by Sangon Biotech (Shanghai) Co., Ltd. All DNA products were quantified and stored in ultrapure water (Milli-Q) for subsequent experiments. The detailed sequences are given in Table S1.

3. Construction of DNA Nanoprisms

All DNA nanoprisms were self-assembled in a test tube by annealing from 95 °C to room temperature over 2 h. Three 96-base DNA sequences containing four 20-base edges (6.8 nm), separated by four thymine vertices, self-assembled into the triangular nanoprism scaffold. Each edge of the equilateral triangle possesses a 20-nt binding region. However, on the top face, we built a 15-nt-long overhanging single strand to hybridize with the added linker strand, while a cholesterol-labeled anchor strand was loaded on the bottom face in order to anchor the DNA nanoprism to the giant vesicle membrane.[1-2]

4. Gel Electrophoresis

For agarose gel electrophoresis, reaction mixtures were eventually quantified in a volume of 8 μL to give a desired concentration, and then each sample was added to 1.6 μL 6× loading buffer and used for electrophoresis experiments directly. The gels of 3% native agarose were stained with ethidium bromide (EB). Electrophoresis was carried out in fresh 1×Tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 110 V at room temperature.

For native polyacrylamide gel electrophoresis (N-PAGE), electrophoresis was carried out in fresh 1×Tris-acetic acid-EDTA/Mg^{2+} buffer (1×TAE/Mg^{2+}, 40 mM Tris-acetic acid, 1 mM EDTA and 12.5 mM magnesium acetate, adjusted to pH 7.4) at 110 V surrounded by an ice/water bath. Finally, the DNA bands were stained by Stains-All for 10 min, and then the gel was washed with water.

All gels were imaged and analyzed using a Bio-Rad ChemiDoc XRS System.

5. Cell Culture and Preparation of Micron-Scale Giant Membrane Vesicles

HeLa cells were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) and cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, heat inactivated) and penicillin (100 U/ml) -streptomycin (100 μg/ml) in a cell culture incubator at 37 °C with 5% CO_2. Cell density was determined using a hemocytometer. For adherent HeLa cells,

short-term (30 s–1 min) trypsin treatment was adopted to dissociate cells from the culture flask or dish. MVs were derived from HeLa cells as previously reported.[3] Briefly, cells were first incubated with carboxylfullerenes for 4 hours and then washed three times with DPBS. Then, culture medium was added, and the cells were irradiated under white light for another 4 hours. After overnight incubation, micron-scale giant membrane vesicles (MVs) were suspended in solution and used as prepared.

6. Preparation of Giant Phospholipid Vesicles

A gentle hydration method was employed to generate giant phospholipid vesicles, which served as a general model of chemical synthetic giant vesicles. A thin dry film of phosphatidylcholines was deposited from 3 mL chloroform solution on the glass surface at the bottom of a flat-bottomed 500 mL flask. Before hydration, the lipid film was carefully dried. Hydration of the film was initiated by 3-4 mL circulating water through the flask overnight. The water was gently poured down the side of the flask.

7. Confocal Laser Scanning Microscopy Imaging

For the colocalization experiment, a 400 μL solution of MVs was plated in a 20 mm confocal dish and incubated with 22.2 μL 9 μM nanoprisms mixture at 37 °C for 30 min. After incubation, MVs were directly subjected to confocal laser scanning microscope imaging using the FV1000 confocal microscope (Olympus). The confocal fluorescence images were collected with an objective lens (60X, UplanApo N.A. 1.35, Olympus) with 65% laser intensity for FRET experiments and 50% laser intensity for the colocalization experiments.

8. Theoretical Estimation of the distance between DNA nanoprisms of Assembly

As shown in Scheme S1. If viewing two neighbor nanoprisms as a particle of "O" and "O'", the average distance (d) between TP-Cy3 and TP-Cy5 is the length of OO'. The distance between two dyes can be ignored, since fluorescent dyes were covalent modified at the end of arm strands that hybridized with a linker and the FRET efficiency is extremely distance dependence with a $1/d^4$.

As top and bottom faces of nanoprism are equilateral triangles, If the edge length of these faces

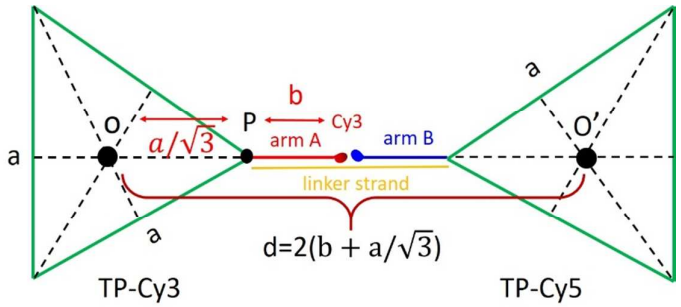
is “a”, the height should be $\sqrt{3}a/2$, thus the distance of their central point (O) to a vertex (P) is $a/\sqrt{3}$.

Both nanoprism and arms are negative charged. Basically, the arm strands are stretched, if the length of elongate section of arm is “b”, therefore, $d = 2\left(a/\sqrt{3} + b\right)$

Here, a=20 nt=6.8 nm, b=15 nt=5.1 nm

So, $d=2\times(6.8/\sqrt{3}+5.1)$=18.1 nm.

If directly estimate the distance between two nanoprisms, the theoretical value could be d=2b=30 nt=10.2 nm



Scheme S1. Theoretical estimation of the distance between TP-Cy3 and TP-Cy5 when FRET. Scheme represent the top view of the assembly of TP-Cy3 (red) and TP-Cy5(blue).

Table S1. Sequences used in the experiment.

Name	DNA Sequences (5'-3')
Strand 1	TCGCTGAGTAttttGCCTGGCCTTGGTCCATTTGttttGCAAGTGTGGGCAC GCACACttttCGCACCGCGACTGCGAGGACttttCACAAATCTG
Strand 2	CACTGGTCAGttttCCACCAGCTAGATGTTGAAGtttTACTCAGCGACAG ATTTGTGttttCGCTCTTCTATACTGGCGGAttttGGTTTGCTGA
Strand 3	CCACACTTGCttttGTCGACACAGTAGCAGTGTGttttCTGACCAGTGTCA GCAAACCttttCCATGACGATGCACTACATGttttGTGTGCGTGC
Alexa Fluor 488-anchor	Alexa Fluor 488-GTCCTCGCAGTCGCGGTGCGTTTTTTTTTT-Cholesterol
anchor	GTCCTCGCAGTCGCGGTGCGTTTTTTTTTT-Cholesterol
Arm A	CAAATGGACCAAGGCCAGGCCTGATAGCAGCTCGT
Arm B	ACCAGTCGATGTACGCAAATGGACCAAGGCCAGGC
Cy3	CAAATGGACCAAGGCCAGGCCTGATAGCAGCTCGT-Cy3
Cy5	Cy5-ACCAGTCGATGTACGCAAATGGACCAAGGCCAGGC
linker strand	TGACCACGTACATCGACTGGTTTTACGAGCTGCTATCAG
displacement strand	CTGATAGCAGCTCGTAAAACCAGTCGATGTACGTGGTCA

Supporting Figures

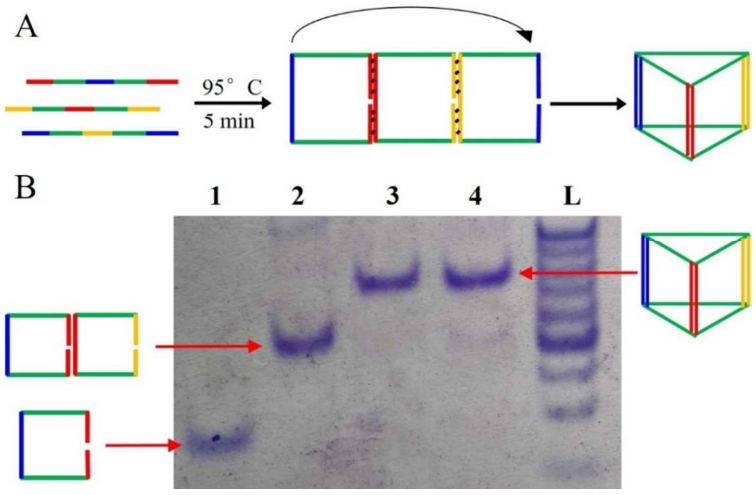


Figure S1. The formation of the nanoprism scaffold in buffer solution. (A) Schematic representation of self-assembly strategy of 3D DNA triangular prism. (B) 5% native polyacrylamide gel electrophoresis (N-PAGE) results of self-assembly of nanoprism, run at 110 V for 1.5 h. Lane 1: Strand 1; Lane 2: Strands of 1+2; Lane 3: Strands of 1+2+3; Lane 4: Strands of 1+2+3 (Thermocycled); L: 20 bp ladder consisting of double strands of DNA with length increase in 20 bp steps. DNA bands were stained by Stains-All.

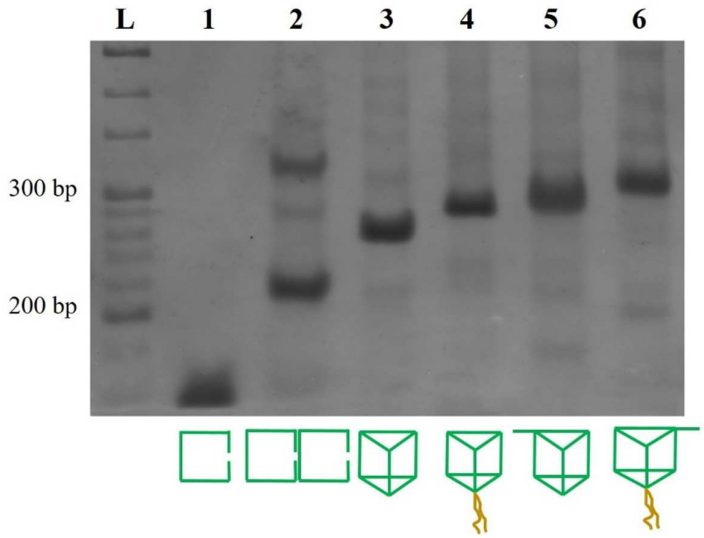


Figure S2. N-PAGE (5%) results of self-assembly of DNA nanoprisms, run at 20 V for 4 h. Lanes 1-3 show the stepwise assembly of DNA nanoprism scaffold, demonstrating that the 3D scaffold was the major product finally shown with a single band in lane 3. Lanes 4-6 represent this scaffold loading a cholesterol-labeled DNA anchor on the bottom face, an overhang DNA arm on the top face, and both sequences on both faces, respectively. The gels were imaged using a Bio-Rad ChemiDoc XRS System.

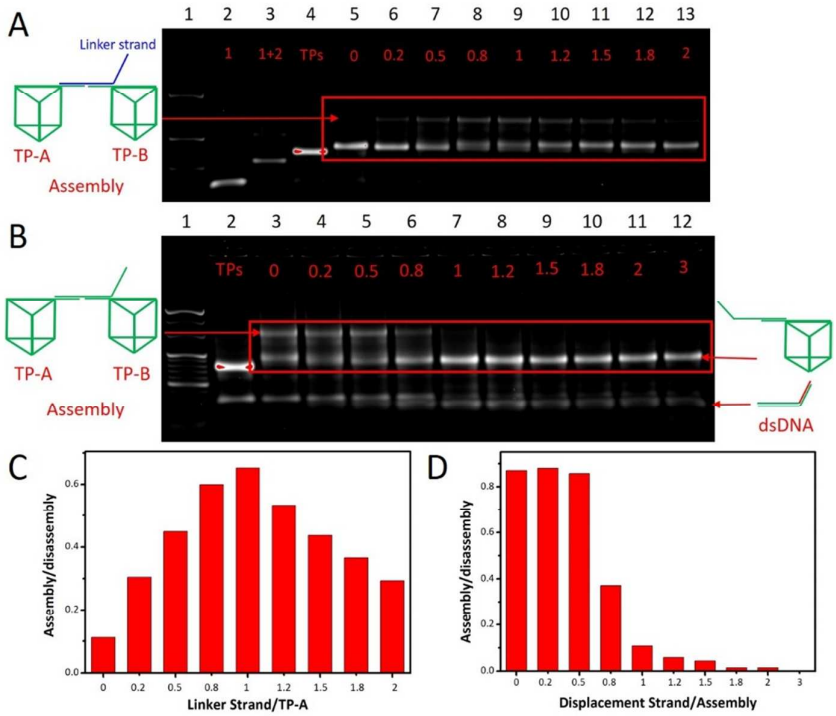


Figure S3. Agarose gel electrophoresis (3%) to optimize the conditions of assembly/disassembly of DNA nanoprisms in the test tube, running at 110 V for 1 h. (A) The efficiency of different concentrations of linker strand to assemble two TPs (TP-A, TP-B); Lane 1: 20-bp DNA ladder. Lane 2: strand 1. Lane 3: strands of 2+3. Lane 4: self-assembled TP scaffolds with strands of 1+2+3 after annealing. Lane 5: the equivalent TP-A and TP-B. Lane 6~13: different ratios (0.2, 0.5, 0.8, 1, 1.2, 1.5, 1.8, 2) of linker strand/TP-A (or TP-B) for assembly. (B) The displacement efficiency of displacement strand to disassemble the dimeric nanoprism (the assembly of TP-A and TP-B). Lane 1: DNA ladder. Lane 2: DNA nanoprism scaffold. Lane 3~12: different ratios (0, 0.3, 0.5, 0.8, 1, 1.2, 1.5, 1.8, 2, 3) of displacement strand/assembly of TP-A and TP-B. (C) (D) show quantitation of the results shown in A and B, respectively.

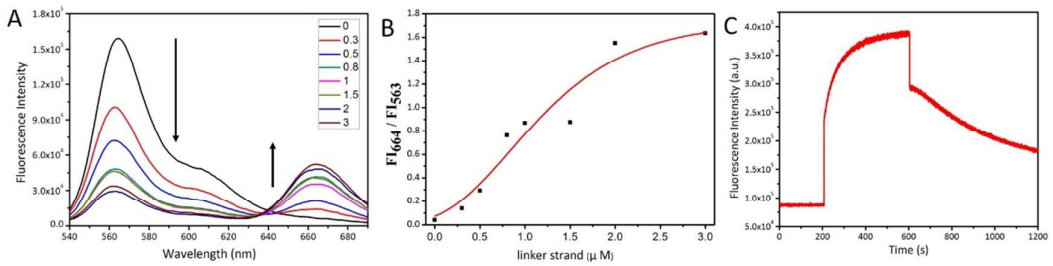


Figure S4. The assembly/disassembly process of DNA nanoprisms in buffer solution. (A) Fluorescence change of 1 μM mixture of TP-A and TP-B labeled with Cy3 and Cy5, respectively, loaded upon addition of a series of concentrations of linker strand. λex=530 nm. Bandwidth=3 nm (B) Fluorescence intensity ratio (FI_{664}/FI_{563}) shows the effect of adding various linker strands. (C) Kinetics analysis of DNA-mediated assembly and disassembly of DNA TPs in the test tube. λex=530 nm, λem=660 nm, Bandwidth=5 nm.

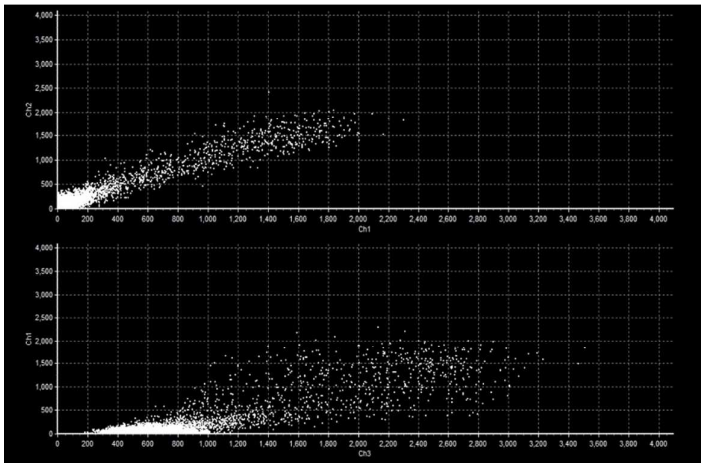


Figure S5. Colocalization coefficients (Pearson's coefficients) of Alexa Fluor 488 and Cy3 are 0.94752 and 0.83745, respectively. Alexa Fluor 488 was labeled on anchor strand; Cy3 and Cy5 were labeled on arm strands of respective nanoprisms. The anchor strand and arm strands were loaded on the DNA nanoprism scaffold. (CH1 is Alexa Fluor 488 channel, CH2 is Cy3 channel, and CH3 is Cy5 channel in graph).

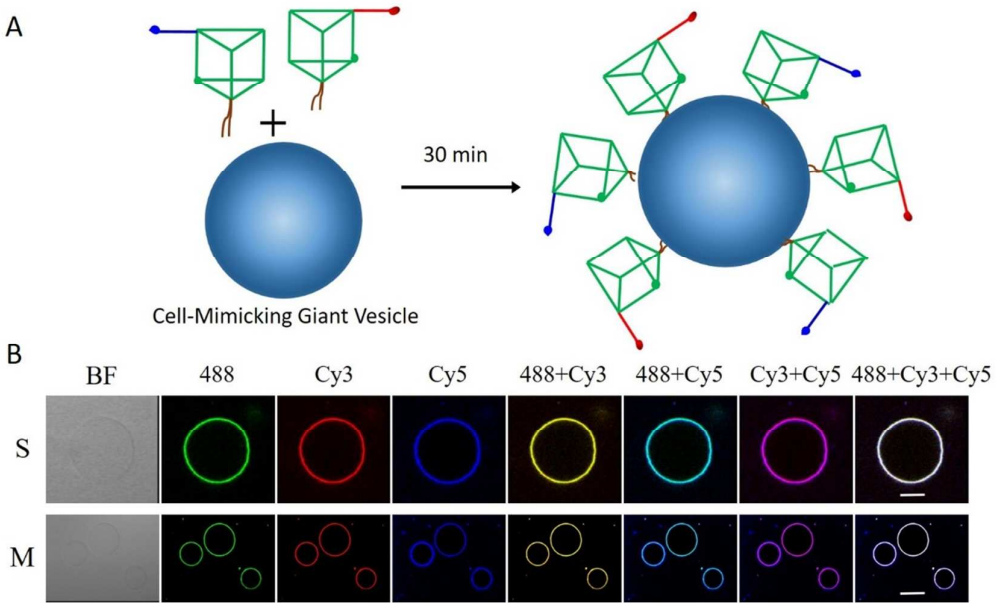


Figure S6. (A) Schematic representation of 3D-DNA nanoprisms anchored on the membranes of cell-mimicking MVs. (B) Confocal imaging of colocalization of TP-Cy3 and TP-Cy5 on the surfaces of MVs. 9 μM 22.2 μL DNA TP mixture was added to 400 μL solution of MVs and incubated at 37 °C for 30 min. Row S shows the single giant vesicle (Scale bar: 5 μm), while row M shows multiple giant vesicles in the observed field (Scale bar: 10 μm).

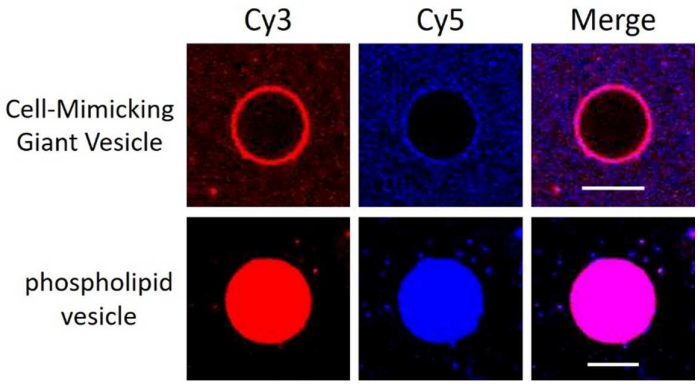


Figure S7. Confocal imaging of colocalization of nanoprisms on cell-mimicking MVs (above) and giant phospholipid vesicles (below), incubated at 37 °C for 30 min. Scale bar: 10 μm.

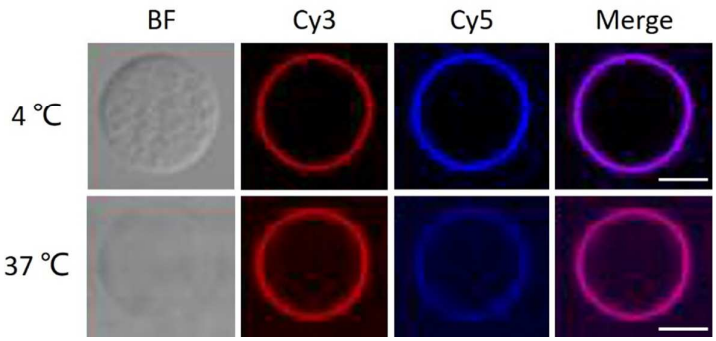


Figure S8. Confocal imaging of different FRET efficiencies on the membranes of cell-mimcking MVs at 4 °C and 37 °C, respectively, indicating that low temperature favors the assembly of TP-Cy3 and TP-Cy5. Scale bar: 5 μm.

Supplementary References

(1) Conway, J. W.; McLaughlin, C. K.; Castor, K. J.; and Sleiman, H. *Chem. Commun.*, **2013**, *49*, 1172-1174.

(2) Conway, J. W.; Madwar, C.; Edwardson, T. G.; McLaughlin, C. K.; Fahkoury, J.; Lennox, R. B.; and Sleiman, H. F. *J. Am. Chem. Soc.* **2014**, *136*, 12987-12997.

(3) Liu, Q. L.; Guan, M. R.; Xu, L.; Shu, C. Y.; Jin, C.; Zheng, J. P.; Fang, X. H.; Yang, Y. J.; Wang, C. R. *Small* **2012**, 8, 2070-2077.