Supporting Information for

Reconstructing Soma-Soma Synapse-like Vesicular Exocytosis with DNA Origami

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1. Experiment section
Reagents and solutions
Oligonucleotides modified with fluorescence were synthesized and purified with HPLC by Invitrogen (Shanghai, China), and cholesterol modified oligonucleotides were synthesized and purified with HPLC by TaKaRa (Dalian, China), and other oligonucleotides were synthesized and purified with HPLC by Sangon (Shanghai, China). M13mp18 was purchased from New England Biolabs (Ipswich, MA, US). HEPES were purchased from Sigma. All other chemicals were reagent grade and were used without further purification. No unexpected or unusually high safety hazards were encountered in this work.

The buffers used were as follows:

Tyrode solution (containing, in mM, 150 NaCl, 4 KCl, 2 CaCl2, 2 MgCl2, 10 glucose, 10 HEPES (310-315 mosm), with pH set at 7.35 with HCl and NaOH).

High K+ stimulation solution (high K+, containing, in mM, 64 NaCl, 90 KCl, 2 CaCl2, 2 MgCl2, 10 glucose, 10 HEPES (310-315 mosm), with pH set at 7.35 with HCl and NaOH) were used.

Preparation of 6-helix bundle (HB) DNA origami nanostructures (DONs)
DONs were assembled with short staple strands and scaffold strands (M13mp18 ssDNA) in 1× TAE-Mg2+ buffer by slowly cooling down from 90 °C to 20 °C. The final concentration of the scaffold strands and staple strands was 5 nM and 50 nM, respectively. To remove the extra short strands, DONs were filtered for three times with 100 kDa (MWCO) centrifuge filters.

Cell culture
PC12 cells were purchased from Shanghai Institute of Biological Sciences, were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% heat inactivated FBS (Gibco), 100 units/mL penicillin (Invitrogen), 100 mg/mL streptomycin (Invitrogen), and 2 mM L-glutamine (Invitrogen) at 37 °C in humidified air containing 5% CO2.

Fluorescence labeling and imaging
PC12 cells were washed three times with 1×PBS. Next, 1 mL trypsin (Invitrogen) was added to each sample and the samples were incubated for 1 min. Then, 1 mL 1640 medium was added, and then the cell suspensions were transferred to tubes washed three times with 1×PBS. Then cells were incubated with chol-DNA for the 30 minutes at room temperature, and washed three times with 1×PBS. Then cells were hybridized with complementary fluorescence sequence or DONs added fluorescence sequence for 30 min at 4 °C, and washed three times with 1×PBS for confocal laser scanning microscopy (CLSM) and flow cytometry experiments.

Real time cellular analysis (RTCA)
PC12 cells were incubated in RTCA system (ACEA, xCELLigence RTCA DP, USA) for 5 days. Chol-DNA and DNA origami were added 45 hours after seeding cells (1.5 × 10^4 cells/well). Cell impedances were measured every 9 min.

Cell conjugation
PC12 cells were labeled with two dyes (CellTracker, Green and Deep Red, Invitrogen) respectively in 1640 medium for 30 min at 37°, and then washed three
times with 1×PBS. Next, 1 mL trypsin (Invitrogen) was added to each sample and the samples were incubated for 1 min. Then, 1 mL 1640 medium was added, and then the cell suspensions were transferred to tubes washed three times with 1×PBS. Then cells were anchored different ssDNA respectively and washed three times with 1×PBS. After cells were mixed, added DONs can conjugate two kinds of cells for 2 hours at 37℃. The conjugation cells were detected with amnis flow cytometer and CLSM.

**Intercellular membrane vesicles transport**

DiI, DiO (both 5 μM) cell labeling solutions (Invitrogen) were used for labeling vesicles in adherent PC12 for 2 h, and then made cell conjugation as above. The connected cells were cultured in RPMI 1640 medium for different time and imaged with CLSM. Transfer was calculated form percentage of DiO fluorescence had been transferred from the donor cell to the recipient cell. Data were analyzed using ImageJ software.

**Confocal laser scanning microscopy**

Images of PC12 cells were obtained at TCS SP8 confocal microscope. Alexa 647-DNAs and CellTracker Deep Red were excited with a 633 nm laser, while DiI were excited with a 561 nm laser, and DiO and CellTracker Green were excited with a 488 nm laser respectively. The imaging channels were set at 650-690, 570-620, and 500-550 nm, respectively. Bright field images were obtained after fluorescence imaging. Data were analyzed using ImageJ software.

**Flow cytometry experiments**

Cells were labeled fluorescence were analyzed in using an amnis flow cytometer (Merck Millipore). The cell conjugation and fluorescence labeling cells suspensions were transferred to tubes for flow cytometry test. At least 5000 cells were analyzed. Consistent gating based on cell size (forward scatter) and granularity (side scatter) were applied to select counted cells. Alexa 647-DNAs and CellTracker Deep Red were excited with a 633 nm laser, and CellTracker Green were excited with a 488 nm laser respectively.

**Carbon fiber nanoelectrode (CFNE) fabrication**

We obtained carbon fiber (7 μm in diameter) from Toray Industries (Japan). Borosilicate glass capillary (1.2 mm o.d., 0.69 mm i.d.) was got from Sutter Instrument (USA). Cooper wire (0.5 mm in diameter) was purchased from Sinopharm Chemical Reagent (Shanghai, China). Graphite powder conductive paint was purchased from Ted Pella (USA). Epoxyresin were purchased from Sigma.

We developed method of fabricating CFNE according to the protocol of Huang et al. Carbon fiber was cleaned in acetone, alcohol and ultrapure water firstly. Then, 37℃ thermotank was prepared for drying the carbon fiber for 3 days. The borosilicate glass capillary was pulled into two separated electrodes with a model P-2000 micropipette puller (Sutter, USA) firstly. A selected single carbon fiber was attached on the end of a copper wire with graphite powder conductive paint and then inserted into aborosilicate glass capillary carefully. To etch the CFNE, the Carbon fiber in electrode was placed on the flame of the alcohol lamp, and then the other end of the glass capillary was sealed by epoxyresin with copper wire exposed outside. As general result, our CFNE size was about 80 nm nanotip in diameter and 30 μm in
Electrochemical characterization of CFNEs

The CFNEs were tested by cyclic voltammetry (CV) in solution of 0.01 M \( K_3[Fe(CN)]_6 \) containing 0.5 M KCl. The CV was on the electrochemical workstation (CHI660A, CH Instruments, Shanghai, China), and the voltage ranged from -0.2 V to 0.5 V, and the scanning rate was 50 mV/s. The concentration of the KCl solution for the reference electrode of Ag/AgCl was 3 M. We found steady state volt-ampere characteristic curves, indicating that CFNEs were successfully constructed with diffusion-limited mass transport exhibited.

Atomic force microscope (AFM)

DONs were imaged in AFM (Vecco/Digital Instruments). For AFM imaging, 2 \( \mu L \) sample solution was deposited onto mica to adsorption for 2 min. The mica was then washed three times with Milli-Q water. Then the sample was scanned under tapping mode. Artificial soma-soma synapse of cell conjugation was imaged in AFM (Resolve-AFM, Bruker). After cultured for 8 hours, cell conjugations were scanned in medium. The applied force was precisely tuned to acquire high resolution cell images.

Scanning Electron Microscope (SEM) image

CFNEs were sputter-coated with gold. Immediately. The cultured connected cells were washed with 1×PBS three times before fixed in 2.5% of paraformaldehyde solution overnight at 4℃. Then cells were dehydrated with a series of graded ethanol solution. Then the cells were dried in hexamethyldisilazane (HMDS) and sputter-coated with gold. CFNEs and cells were imaged in SEM (JEOL, Japan).

Imaging of slipping CFNE inside soma-soma synapse-like junction

When an artificial soma-soma synapse-like junction is successfully identified under the TCS SP8 confocal microscope with 40 × oil immersion objective, the nanotip of the CFNE was firstly placed nearby the of the single junction, and then was slowly inserted into the synapse and withdrawal from synapse with a micromanipulator (Eppendorf, Germany). The micromanipulator has a two-speed joystick, moving step by step and continuously, and has a programmed Z-axis limit (about 100 nm in fine adjustment), which enables CFNE nanotip can target the small synapse-like junction. All the micrographs were acquired with high resolution (1024 × 1024 pixels).

Electrochemical characterization of CFNE inside junction

We added 0.01 M \( K_3[Fe(CN)]_6 \) containing 0.5 M KCl in the Tyrode solution to check the seal of short (2-3 \( \mu m \)) active electrode by soma membrane. We detected currents in different states with patch clamp (DL Naturegene Life Sciences, China) in voltage of -200mV vs. Ag/AgCl reference electrode.

Amperometric measurements, data acquisition and analysis

All amperometric monitoring experiments were taken artificial soma-soma synapse-like junction on an inverted microscope (Nikon) coupled with patch clamp at room temperature in Tyrode solution. Detection system placed in a Faraday cage, and all apparatuses were grounded. After a CFNE was successfully inside a synapse-like junction by a micromanipulator, the high K+ stimulation solution delivered through a manual piston pump (Cell Tram Oil, Eppendorf, Germany) at about 50 \( \mu m \) away from
the synapse by another micromanipulator. All the electrical measurements were carried out versus an Ag/AgCl reference electrode submerged in Tyrode solution in voltage-clamp mode in patch clamp. The amperometric spikes were recorded at a constant potential of 700 mV vs. Ag/AgCl reference electrode. Signals were sampled at 20 kHz, and bessel filtered was at 2.0 kHz. Amperometric data were collected during 2 min and then analyzed according to previous methods. [1] Firstly, the root-mean-square (r.m.s.) noise of the dI/dt was measured in a trace segment that did not contain peaks. Then, dI/dt was to detect simple event that were one peak with 5 time larger than the r.m.s. noise. If there was more than one peak within an event and the dI/dt of the peaks was 3 time larger than the r.m.s. noise, then the event was identified as complex event. The duration of simple event was calculated as full width at half maximum of peak. the duration of complex event was calculated as:

\[
   t_{1/2}^{\text{complex}} = [t(f_n) - t(f_1) + t_{1/2}(f_1) + t_{1/2}(f_n)]/2
\]

Where \( t(f_1) \) and \( t(f_n) \) are the times at peaks, and \( t_{1/2}(f_1) \) and \( t_{1/2}(f_n) \) are the duration of the first and the last peak. The relationship between the current peak and absolute quantity of catecholamine was calculated from Faraday law. Moreover, the spikes measured is a diffusion-controlled process and sites of exocytosis on the cell surface could lead to the measured would be expected to be spatially localized with electrode, [2] so catecholamine measurement is surmised only from the CFNEs nanotip in our research.

References:
(1). Li, Y. T.; Zhang, S. H.; Wang, L.; Xiao, R. R.; Liu, W.; Zhang, X. W.; Zhou, Z.; Amatore, C.; Huang, W. H. Nanoelectrode for amperometric monitoring of individual vesicular exocytosis inside single synapses. Angew. Chem., Int. Ed. 2014, 53, 12456-12460.

(2) Jankoski, J.A.; Kennedy, R.T.; Kawagoe, K.T.; Schroeder, T.J.; Leszczyszyn, D.J.; Near, J.A.; Diliberto, JR, E.J.; Viveros, O.H. Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. Proc. Natl. Acad. Sci. U. S. A. 1991, 88, 10754-10758.
2. Supporting Results

Figure S1. The models of linear 6 HB DONs monomer nanostructure reconstructed by Cando software.
Figure S2. AFM images of 6HB DONs monomers.
Figure S3. Length of 6HB DONs oligomers.
Figure S4. Flow cytometry of different concentration of chol-DNA anchoring on PC12 cell membrane.
Figure S5. Curvatures $K$ of DONs (left, Data was analyzed by using ImageJ software) and great circle of spherical cells (right).
**Figure S6.** DONs anchoring on small circles (green) rather than great circles (blue) of spherical cells. Scale bar, 10 μm.
Figure S7. CLSM images of PC12 cell after anchoring DONs and chol-DNA at 37°C for 2 hours. Scale bars, 10 μm.
**Figure S8.** The effect of chol-DNA and DONs on PC12 cell proliferation was monitored by RTCA Instrument.
Figure S9. Flow cytometry of cell adhesion without DONs.
Figure S10. (a) CLSM images of cells without DONs. Scale bar, 50 μm. (b) CLSM image of cultured cells after cells mixing without DONs. Scale bar, 50 μm.
Figure S11. Flow cytometry of cell adhesion by using cholesterol anchored strands adhesion system.
Figure S12. CLSM images of cellular uptake of DiO and Dil by membrane vesicles. Scale bars, 40 μm.
Figure S13. CLSM images of membrane vesicles transport between soma-soma synapse-like junction. Scale bars, 20 μm.
Figure S14. Characterization of CFNE. (a) Scheme for the fabrication of CFNEs. (b) CV of four different CFNEs fabricated at different time in K₃[Fe(CN)₆] solution.
**Figure S15.** SEM image of carbon fiber, Scale bar, 10 μm.
Figure S16. The process of a CFNE sliding inside soma-soma synapse-like junction (1→2→3) and withdrawal from synapse (4→5→6). Scale bar, 20 μm.
Figure S17. CV of short CFNE in K$_3$[Fe(CN)$_6$] solution.
Figure S18. High K⁺-induced amperometric spike.
Figure S19. Example of a simple amperometric current trace and a complex amperometric current trace.
Figure S20. First derivative (dI/dt) of the current trace. (a) First derivative (dI/dt) of the amperometric spike of simple event. (b) First derivative (dI/dt) of the amperometric spike of complex event. The dotted and solid plane lines are the threshold of 3×rms noise and 5×rms noise respectively.
Table S1. Basic DNA sequences used to form 6-HB DON.

| Staple strand | Sequences (5'-3') |
|---------------|-------------------|
| 6HB-1         | GCGCGTAGTCACCAGCATAACCATTAACCCGGAACCCAGACGGG |
| 6HB-2         | GCGTAACGCTGTTACATTACAGTTGTCAGAGCCTTTT |
| 6HB-3         | GACCCGGTAGTCACCAGTGGTTACATTACAGTTACAGAGCCTTTT |
| 6HB-4         | AGCCGGCATCACCAGTGGTTACATTACAGTTACAGAGCCTTTT |
| 6HB-5         | GAAACCCAAAACCCGTGAGATGGTTAAAAACACCACCTTTAC |
| 6HB-6         | ACATTATAGCAAGCCTGAGTAGTTAGTGAGAGCAGAGCAGAG |
| 6HB-7         | GAAGGGTGATCAGGTTTTGAGA |
| 6HB-8         | AAATCGTTTACCCAGCATACGCAAGGATAAAACGTACTGAGATGAG |
| 6HB-9         | TTAGAAGTAGCTTATACAGCTTACCTGTAACCTCAGAGAGGCC |
| 6HB-10        | ACTAATAGCCAGCTAAAGCCTCAGAGCAGCAGATAGCTCAGAG |
| 6HB-11        | AATTTGAGTCGGAGTTAATCATACAGGCTTCTCAGTAAGAGC |
| 6HB-12        | TCAATCAGATAAGGTTGTCAGCATAATTCTGACAGCCATCTTTT |
| 6HB-13        | AAATCTATCTGCAACCTGTTAGCATTACCTCTCTACCTAGAG |
| 6HB-14        | CGCGCTTGTAGCAGAGTGTAGCTGACAGCAGGCTTCTT |
| 6HB-15        | CCAGCAGAAGACCATCACCATTACATAACGAAAACACTCACCA |
| 6HB-16        | GATAGCCGTGGGAAACATGTTTTAAATAAACATCTCAAATCAC |
| 6HB-17        | GACAAATAGCGTTGTAGCTGAGCTCAGTTTACCTT |
| 6HB-18        | TCAATATGGAACCGTCTTCTAGCTGATGAAAGTTAGTTT |
| 6HB-19        | ATAGAAACTAAGCCAGTACGTCCTTTAATAATGATGAGGTA |
| 6HB-20        | GTCACACCCAGTGCCAGAACCAGACCGACACCGCATGCGCA |
| 6HB-21        | TCAATCGCCCGCCGCGCCGAAAGCTTCAAATTAAAGGTTTTATTT |
| 6HB-22        | GCAACAGTTTCTGTCAGAAGCAGAAGCGACAGCAGTGGCA |
| 6HB-23        | GCCTTGCATACGGAGACCATAAAATCAAAGGAGACATTACCTAGC |
| 6HB-24        | GTAATAATGAGTGAGAATCCCATCCTCAATAATAATAC |
| 6HB-25        | CCATCAGCTTTCCTGGATAGCCTTCAAAGAGGCCGCGCCA |
| 6HB-26        | GTGTTTTTATCGCCGCAAGAAAGTTTGGCCAGCGATTAGCTATC |
| 6HB-27        | AAAGGGACAGGTTGACAGACGGAGTATATCATCAGTTAAGC |
| 6HB-28        | TCCTCTGATTCCTTACAGAGGCATAGTCATTATAATTGA |
| 6HB-29        | GAATTATCCGGTTGAAAAGGCGCCAGACGATTAGCCGCTCAT |
| 6HB-30        | ATTTTCGAAATAACCGAGAGATGAAAGGGAGAAAGAAAATCC |
| 6HB-31        | ATCAGAATTTGGTTTACCTTCATGAAGAGGTTAATAATAC |
| 6HB-32        | CGTTTTAAACAGCCACCAAAATCATACGAGTACTGAGCTTGGG |
| 6HB-33        | ATAACGGGAGTCTCCGATAAGTGCGTGATGATGTTAGAAGAGA |
| 6HB-34        | TAAATCAAACCGAGCTTGGCCCTGACAGAGTTCACACGCTCA |
|   |   |
|---|---|
| **A** | **TCGCGCATTCACAAAAATAGGGTGTATCACAATTTTTAATATTTT** |
| 6HB-35 | **TCGCGCATTCACAAAAATAGGGTGTATCACAATTTTTAATATTTT** |
| 6HB-36 | **GAAGATGATTGACACTCAGAACCAGCCACTACCTTTTTCATTTT** |
| 6HB-37 | **TTTAACACTAGAGACCCCTCAATTTCTGTAAGCTCCTGGCCT** |
| 6HB-38 | **ACATAAAAAGGCGACCAGTACACTGAGAGGCCAAGACGAGTA** |
| 6HB-39 | **ACAGAAGTCACTAGTATTATTTCTGAAAAATAAAATTTAAACAG** |
| 6HB-40 | **ATCGTCGAAAAATCATGTAGCATCACAACATATAATTTGACC** |
| 6HB-41 | **ATAGCGGGTCAATAAAAGGTTTGCTACTTCTGATTTTTCAATCGTAA** |
| 6HB-42 | **GAATTAGCCTTTTATGATGGGATTTTGATTAGATATCGGCC** |
| 6HB-43 | **CTCCGGCATCGATAGTGGAGATAGAAGGCTGAGATCTCGGCCT** |
| 6HB-44 | **TGGTATGACGAGAAATTCTTTTAAACGCGACCCTCTTGAGTA** |
| 6HB-45 | **CAATAATTTGAGCCAGCCTTTTAATTGTAATTTGCTCGCTA** |
| 6HB-46 | **AGAAATAAGGCGACAAACAAACCCTCAGGCCAGCACCAGTTGAA** |
| 6HB-47 | **AGTATCAATCAATAAGGCTTTGCAAGGAGATACGCGACTCT** |
| 6HB-48 | **ACGCTCAGAAAAACCCCTCACTCAGCGCAAGATAGCGCCTAG** |
| 6HB-49 | **GATGAATTTTTGATAAGAGAAGGATTAGGTCAAACAATCAT** |
| 6HB-50 | **AACGCCAAGCAAACCTACAGAGGCTTTGAACGCGGAAATCC** |
| 6HB-51 | **CAAATATTTGACGACCCTTTAATGTAATTGCTTCGCTA** |
| 6HB-52 | **AGAAATAAGGCGACAAACAAACCCTCAGGCCAGCACCAGTTGAA** |
| 6HB-53 | **TCAAATATGCCCCGTACCCTGC** |
| 6HB-54 | **GTCCAGACCAGAAGCCAAACCCTAAACGATACTCGTGCCGCT** |
| 6HB-55 | **ACGCGCCAGCCCTCTCATCTTTGACCCCCAGAGGGAGGCTGC** |
| 6HB-56 | **ACAGGAGATCGTAGGGCCGCGCGCTTTAACAGGGGCTTCCAT** |
| 6HB-57 | **ACGATTTATAAGGCGCGCTGCAGTGTAATCTTCCCTTAAAC** |
| 6HB-58 | **AAAACAAACTGGAACAGGCTATTACATAATATGCAGATGGCG** |
| 6HB-59 | **CGCCAGCATGAAACCTTTACAAACAATTATCAGCTGGAG** |
| 6HB-60 | **CTCCCTCTCAATATATCTTAAATATCTTATGTGAGGaATTAG** |
| 6HB-61 | **CTTGATAGAGGCGAAACGTTATTAATTTACGTTAGAACCCCT** |
| 6HB-62 | **CAAAATAGCGAACCAGCAGAAGGAGGAGGTTGTTCTCATT** |
| 6HB-63 | **AAACCCAAACCTCAACAGGTAACTCATAATATGCGGATTGC** |
| 6HB-64 | **AGAATTAGTACGCGTCGCTTTGACGAGCAGCTGAGAGGATAC** |
| 6HB-65 | **CAGAGATCGTAGGGCCGCGCGCTTTAACAGGGGCTTCCAT** |
| 6HB-66 | **ACGATTTATAAGGCGCGCTGCAGTGTAATCTTCCCTTAAAC** |
| 6HB-67 | **CACCCAAATCATCAGCGCAGTATATTATCGCGTAATACCG** |
| 6HB-68 | **CTCCCTCTCAATATATCTTAAATATCTTATGTGAGGaATTAG** |
| 6HB-69 | **CTTGATAGAGGCGAAACGTTATTAATTTACGTTAGAACCCCT** |
| 6HB-70 | CATTTTCTAGCTTAACCTTGCTGAACCTTGGGCGCTTTGGGG |
| 6HB-71 | CAGTTTTTCAAATGCCCAGCTGAGAGCCGACAGTACATTTC |
| 6HB-72 | TAACCGGTAAGAAATTGTTTTGATTATCTGGAGCATGAGCGG |
| 6HB-73 | TGAACCTTTAGTTAACAGAGGTGAGGCCCGGACTCCCAA |
| 6HB-74 | CAGTAGCCAATCGATCGCCATTTAAAAACGCCATTAAGTAC |
| 6HB-75 | CACCGACATTGTAAGGGCTATATTGCTTGCTGGCTTTAAAT |
| 6HB-76 | AATATTGAAATACCATCCTGAAAGCGTAATGCAAGTGAAG |
| 6HB-77 | AGACAAAAACCCGGAAATAAAAAGGGACATACCGACGTCAAC |
| 6HB-78 | TGTCACTATATGCCTGGATTATTTACATTTGCAGGTTTTAA |
| 6HB-79 | CATATAAACACAGTGGCTCTACATGGAATTATCAAAAAA |
| 6HB-80 | GTATGTATCATGTAATCCAGAAATAATATCCGCTATCTTAC |
| 6HB-81 | ATACCCAATATATGCGTGAAGTGTTAAAGAACAGTGT |
| 6HB-82 | AAAGTTACGACGCACAAACCTGTGGAAATTGCGTGTAACGT |
| 6HB-83 | ACATGGCATACAGGTCTCTGTATTACAGAGCATGTTCAACC |
| 6HB-84 | TTACGCATGTATTTATGAGGCGACACATCGCTGGCTAATAG |
| 6HB-85 | CCAATAATCCCATCCAGGGAAACGTACGGCGCGGTTAAATAG |
| 6HB-86 | GCCTAAAGGGCTGTCAGAGCGGAGACTACAGTGAACAC |
| 6HB-87 | CCAAACCTTTAGGACTGTTTGGGCCAGTGCGGCGCGTTTTT |
| 6HB-88 | GCATAGGCCGAAACACCCGAAATCGGCAAAAGCGGTCCAAGCA |
| 6HB-89 | ACCGGAATAGGACGTAGATAGGGTTGAGTGAAGAAACCGAG |
| 6HB-90 | TTCAGTGATTTACCTTTATTAAGAAGCAGTGGCTTGAGAAGCCT |
| 6HB-91 | GTAGTAAACATTTTCCAGCTACTAAAC |
| 6HB-92 | TAAGTAATTTAAATATATTTAAATGTATAAAAAGTTACAAAA |
| 6HB-93 | CTAATTTCTACATTTGCCTGAGAAGACTTCTGCAGTAAA |
| 6HB-94 | TTAATGTACCTATTTGCAAATTTTATGTAACCAATGAA |
| 6HB-95 | CCCCTAGAAAACAGCCAATCAAAAATATAATACATTATAA |
| 6HB-96 | ATAGGAAAAAGCAATTTTCTACATGAAATAGGAACACAGT |
| 6HB-97 | CAAACTAATCCATCTCCTGGAAACACACACAGTCTGTAA |
| 6HB-98 | TTACGCTTAAAGACGAGTTGTTGGAATGAAATGAAAC |
| 6HB-99 | GTAATGTGTCATAGTTGAGGAGCGACAGCAGCCTAATAGT |
| 6HB-100 | ACAGTTTACGAGTACCCAGCACGCTTTTGCTGCTTTTTAA |
| 6HB-101 | ATTCGAGGAAAGTTGGCAAAAGCGCCATTACCGAAATGCTG |
| 6HB-102 | AAGGGTCTAGCTCAAGGGGAGCAGGGGCTGCTTTAATGCACCTTTT |
| 6HB-103 | TGCTTTTCTTTTGCGGAAAGGGGGATGTTGAATACGGAAATTTA |
| 6HB-104 | GGCTGAGATTTCTACCTGAAATACCATTGAGCAAGCAGTCA |
| 6HB-105 | TGCGCTGAGGTGAGTTTTTCCAGTTCTGGGCTTTAAATA |
| 6HB-106 | ATATTCTGTCAAAGCAAGCTTTACCTGCCTGGGACACTGTTT |
| 6HB-107 | TGCGGGAAGGAAGCTACCGAGCTCGAATCTACATTAAGAA | A |
| 6HB-108 | GAGGGTAATTATAGTGTGAATTTGTATTACCAGCCATTTAAC | T |
| 6HB-109 | TTTCATGCAGAATCCCGAGGCTGAAACTCAGAAGCCAGGACAG | A |
| 6HB-110 | CCACTACATTCTTAAACTACTATTACTTCTTTTAAATTC | |
| 6HB-111 | TACACTATTAAGCTCGGGAACCTGGTAAAAGATGCAGAAGGGGACG | |
| 6HB-112 | GCGCGAATTGGCAACGCAGGGAGACAGAATCCAAGAA | A |
| 6HB-113 | AAATTTGTCTTCTGTTTCTTTTTCTTTTTACACAGGAGACCAATC | |
| 6HB-114 | GGAACGAAAAAGGAATTCACCGGGCTGGGGCTGTAAGGGGTATT | |
| 6HB-115 | TGCTCAGAGGTTGAAATAATCGAGAAAAGCCACCAGAAGAAC | C |
| 6HB-116 | GTTGAGATGAAATAATGGTATTCCAGAGCAGGTTTTCGGGGAA | |
| 6HB-117 | GAATAATCTGCTCGAGTCACAGTAAAATGACCAATAGACGCTGAC | |
| 6HB-118 | ACCAGTCATTCCATTATATTATTTATCCCTAAGAAGCGAAAGGAG | |
| 6HB-119 | ATTCGAAATACAGGCTTGATTTTACCGTTCATCGGGAGGACCG | |
| 6HB-120 | ATTGTGAATAAAGGGGCTTTCAGAGGACAGGTTTTTCGCGGAAGA | |
| 6HB-121 | ATGGGCTCTCATACA | |
| 6HB-122 | CATATATAGCCCGGACAAATAAATCTCTCAGAAGTTTGAGTA | |
| 6HB-123 | AAGCCTTCGCCACCGAGGTGGAGGAGTTACTCTGTGGATT | |
| 6HB-124 | TTGTACCAGCACCACCCCGACCAGAAGCCAAAATTTTAGGGAAT | |
| 6HB-125 | CAAAAATCCATGTCACCTCAAGACCTTAAATGAGACTAACA | |
| 6HB-126 | AGAGGGTGGAACCTTCCTTGGTAACAGATTATTGTTGCAATAATG | |
| 6HB-127 | CATTAACAGCCGCCGGAACCAAGACCGCCCTTTGGTTGAAAG | |
| 6HB-128 | CGCGAGCAACGATCAGCCCCCTTTATTAGCGAATCTCTCACAACC | |
| 6HB-129 | GCAATGAGATTTCTCGTGCTGAACTCTGGAAGATGTTAAATGAA | |
| 6HB-130 | TCTGCGACAGGCGGACGACCGTAAATCTACACTTATTTACA | |
| 6HB-131 | GTGTCTATAATACACTTACAGCAGATACTGTCTAGTGAAACCA | |
| 6HB-132 | AATGCTGCAAAAGGATTEGGGGAATAGACAGGAAGAGCGAACAAG | |
| 6HB-133 | AGGTCATGAGGTGTATTATTCATTAAAGGCTTGCACTATGGAACA | |
| 6HB-134 | GGTACGGAATGACATTCAGGGAGATTGTGTAAGGGCGAAGACAAGA | |
| 6HB-135 | TTTAGGGCGTCGCGAGGAAATCATATGGGAAGAGTTGCAACA | |
| 6HB-136 | GATTAAGTCTGTCAGCAGAAAGACACCAGCCAGATTTGTCGCGC | |
| 6HB-137 | CAATATGACTCCTCGATACAGGAGGATGTAACGGTTTCAATTTCAAA | |
| 6HB-138 | GATCTACTATAATGCTCAAACAGTACACCATA | |
| 6HB-139 | CCTGACTGCAACCGGATAGAAATACATATCGCCATAGCCATT | |
| 6HB-140 | CAGAAAAAGGAAGTTGGCAGTATTAAGAATTTTGCGACTAC | |
| 6HB-141 | CATAAATGAAAGGCAGAAACCGAGGAAACGGTAAAGTTGATT |
| 6HB-142 | TAAAATGAAACACTTTTTAAGAAAAAGTAAATCGCTAAGTCTGT |
| 6HB-143 | GAGAGGCACAAAGAAGAAGAATGAAAATCTCTGAACTGAGA |
| 6HB-144 | CATAACCGTGAAGAGATAACCCACAAATGTGAAAGCCGATT |
| 6HB-145 | AACGCCAGGCCGACACTCTGAAACATTGAAGAAGCCTGCT |
| 6HB-146 | AAAAGCCTTGAACCC |
| 6HB-147 | GCAAGCGCCTATGTTACCTATCGAGAACAGGCGCATGAAACTG |
| 6HB-148 | GTTTGATCACAACAAATCTATTACCAGCGAAAAATCGACCAGGC |
| 6HB-149 | AAAAGAAGCGTATGGGCTTTATCCGGTAATTAATCAAGACAGAAG |
| 6HB-150 | GAAACAGAAACAGTGTCCCGACTTGGGTAATTTTGCTGTC |
| 6HB-151 | AGGGCCGATAAAGGGCTTATTTGCACATCCTGAAAGACCA |
| 6HB-152 | GATTGTACATTGTTATGTTGCTTTGAAATATTAAAGGTTGATA |
| 6HB-153 | GTAAAATGCGCCGATATTCTCATTTCAAGTCAGACAGGAGGAT |
| 6HB-154 | TAAACAAATTAGAAACATCAAAGAAAAACCAACACCGGCC |
| 6HB-155 | TCCCTGTAGATATTGAGATTACCTCTTTGACCCACCAAGCCCA |
| 6HB-156 | CAACCCCGGAAGTTATGTGAGTGTAATAAACCCAGCAGCAT |
| 6HB-157 | GTAATTTGATATCGTATGTTATTTCCCTTAGTTTGCCCTCATAG |
| 6HB-158 | CCCTGCAAAAGCATCGATTAAGACGCGCTCAGCCTGAGGTT |
| 6HB-159 | TCAGGAAACAACAGTCATAGGTCTGAGGAGTAGCCGACTTTC |
| 6HB-160 | AGGATCGAATCTCCTGATTGCGTAGATTTTTCTGGATATAAGA |
| 6HB-161 | GGCTGCCAGAGATAAGGGTTATATATAACTAGGAAAACGTAAGG |
| 6HB-162 | CGCAACTCTAAACAAACGAAAAAGGACCCAGCAGCAAAAAA |
| 6HB-163 | TTACGCCCTTTTTGATTAATTTCATCTCTTCTGAATTTAATCACGCT |
| 6HB-164 | AGTTTGGCCCTTCTGAGCCTGTGATAAAAGGGAGGCCGATAG |
| 6HB-165 | ACGACCGGACCAGTAAATCATATAATTACTATTACCAAAACCAGAT |
| 6HB-166 | AGAGGATTCTGAAATAATACAAATTCTTTGAGAATAAGCGGCTTT |
| 6HB-167 | ATAGCTGGAAAAACGGCCTTAAATTGGAACACAATAAGTCGGAA |
| 6HB-168 | ACACAACTGGTAATATTTGAGCCGAGGCGCTCTTAAAGACTT |
| 6HB-169 | TGCTAATACATCAATAGTGACTCCGCAAAAGCAATAACGTAATG |
| 6HB-170 | ACTGCCCGCAAATATATAAAGGAAAGCATGTGAGAATAAAGGAA |
| 6HB-171 | ATGTACCATTCAAACAGTACCTTTTACCGATGAAAGGGGTTT |
| 6HB-172 | TTATGGTTATAATACCAATAGATAATAGCAGATATAACCAA |
| 6HB-173 | TGGGCCCTTTTAGACTAATTTACGGAGCAGAAATTGAGCCTGAT |
### Table S2. Sequences for conjugating DNA-functionalized cells.

| Staple strand | Sequences (5’-3’) |
|---------------|-------------------|
| Linker-6HB -30-A | ATTTTCAGAATAACGGACAGATGAACGGAGAAAGAAAATCCT TTTGCAGTGATGTCATAGAGCGCC |
| Linker-6HB -31-A | ATCAGATTTTTGTTTACTTCTCATAGAGTGCTAGTTAATAAATCTTT TTTGCAGTGATGTCATAGAGCGCC |
| Linker-6HB -32-A | CTTTTTAACAACCACAACCACATAACGTAACTCGGCTATTAGGAATT GTTGAGACCTTACATAGAGCGCC |
| Linker-6HB -33-A | ATAACGGGCAGTCTCGGATAAGTGCCGTATGTGTAACAGGAA TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -34-A | TAAATCAAAACGGCGCTTGCCCTGACAGTCTCAACACGTCAA TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -35-A | TCTGGCATTCACAAATAGGTGTATCACATTCTTTATTATTTTT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -36-A | GAAGATGATTGACACTCAAGAGACCCACGCACTCAGTTTTCTTTT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -37-A | TTTAACACTCGAGACAGCCCTCTTCTTCTGTAAGCTGCTGCTTT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -38-A | ACATAAAAGAGCCGCGACCTGACCTGACTGACGGCAACGAGAAT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -39-A | ACAGAAAGTCAGTGATTATTTCTGAAATACATATATAAAAATAGGTGTATCACATTCTTTATTATTTTT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -40-A | ATCGTCGAAATATGCATATGACATTCACACATATAATGACCT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -41-A | ATAGCGAGGTCATATAAGTTTTGTCGTATTTCTAATCAGTTAATT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB -42-A | GAATTTAGGCTTTTTATGATTGGAATTGTTTGATTGACATATCGGCAATT TTTTGACAGTCTCGGATGTCATAGAGCGCC |
| Linker-6HB | Linker Sequence |
|-----------|----------------|
| -43-A     | CTCCGGCATCGATAGTGAGAATAGAAAGAGTTGATCGCTTCTTT |
| -44-A     | TGCAAAATACATATTTTTTCACGTGAGCAGTACAGGTGCTGTT |
| -45-A     | CAAATATTGGACGCCACCTTTTTAAATGTATATTTGCTGCTATT |
| -46-A     | TGGTGGCAGGCGAATAATTCCCTCCTTTTGGGATTAT |
| -47-A     | AGAATAAAGGCGAGAAGAAGATTTGACATTGT |
| -48-A     | AGTATCAATCAATAGGCTGAGGATATAGCAGTACGCT |
| -49-A     | ACGCTCAAAGAAAACCTCAGCAGCGAAGGATTGACATG |
| -50-A     | GATGAATTGGGTAAGAAGGATTAGAGTCAAAACACATTT |
| -51-A     | AACGCGAAACGAAAACCTCAGCAGCGAAGGATTGACATG |
| -52-A     | AATAAGAAAAAGAACCTTCATATTACAGGATTGAAACCATGCTTTT |
| -53-A     | GTCCAGCTACTGGCTGCAAGGTGCTGCTGCT |
| -54-A     | ACGCCAGCCCTCATTTTGAACCCCCAGAGGGAGCTGCA |
| -55-A     | ACGCCAGCCCTCATTTTGAACCCCCAGAGGGAGCTGCA |
| -56-A     | AATAATATAAGGACAAACGGGATTGAAACCATGCTTTT |
| -57-A     | AATAATCTACTGACCGTGAGCTCGTCAAGAGCAACCGGCA |
| -58-A     | AAACCAAACGTGAC ACATCATGATAGGAGAAGCTGCTGCTG |
| -59-A     | CCAACTTTTAAGGACTGTTTGGCCGAGTGCAGCGCGGCTTTT |
| -60-A     | GACATAGGCCAACACCCGAAATCGGCAAAAGCGGTCCAAGCA |
| -61-A     | ACCGGATAGGACGTAGATGGGTGAGTGAGAAACCGGAG |
| -62-A     | TTCAGTGTCTTTTATGATAGGAGTGGGACTGAGGGCTTG |
| -63-A     | TAAATTTTTTATATATTTTATGTATGATAGGAGTGGGACTGAGGG |
| -64-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -65-A     | TTCAGTGTCTTTTATGATAGGAGTGGGACTGAGGGCTGC |
| -66-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -67-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -68-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -69-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -70-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -71-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -72-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -73-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -74-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -75-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -76-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -77-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -78-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -79-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -80-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -81-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -82-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -83-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -84-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -85-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -86-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -87-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -88-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -89-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -90-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -91-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -92-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -93-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| -94-A     | TTTGCTGTTTTCAGTACTAAGGGCTGC |
| Linker-6HB -95-B | CCCTCAGAAAAAACAGGCATCAAAAAATAAATACTAATTACATTTGCTGTTGTTGACTAAGCGTCG |
|------------------|----------------------------------------------------------------------|
| Linker-6HB -96-B | ATAGGAAAAACATTTTTGTTATCAACATTAATAGGAGCAAACAGTTTCTAGTTTGCTGGTTGACTAAGCGTCG |
| Linker-6HB -97-B | CAAAATATTCATCTTCCGTGGGAACAAAACAGCTTCTGATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -98-B | TTAGCGTTGAAAAAGCACTTCTTCTGTTGAGACAAATATTTGAAAAACAGTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -99-B | GTAATGGTCAATAAGTTTCTGAGGGGAGCAAGGAGCAAGGAGCTTCTGATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -100-B | ACGTTTTACGAGTACTCAGCCAGCTGTGCTAATGACTTTTACTTTCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -101-B | ATTAGGAAAAGCAATTTCATCAACATTAATAGGAGCAAACAGTTTCTGATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -102-B | AAGGCTCTAGCTCAGGCGGTACCTGCTTAACTGACTTTTTACTTTCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -103-B | TGCTTCTTTTGGGAAAGGGGATGTTGGAATACGAAATTTAATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -104-B | GGGCTGAGATATCCGATATCAGCTCAGTTAAATACTATGAGGAGCGCAGTTTCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -105-B | TGCGCCGATTAGAGGAAGGCGCCATTTACCCAAATGCTTCTGCTTAAATATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -106-B | ATATCGTTTCAAGCAAGCCTTTTGCACTGCTGGCAGGACTGGTTTACTTTCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -107-B | TGGCGGAAAGGAAGCTACGACGCTCAGCACTCTACATTAAAGCCAATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -108-B | GAGGGTAAATTAGTGTAATGTTGAAATGTTAATTACGCGCATTTAACCTTTTCTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -109-B | TTTTCTACGGAATCCTCGCAAGCAATTACCTGACTACACCTTTTCTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -110-B | CCACTACATTTCATTGCTAACTACATTTATTACTTTCTTTTTTTTCTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -111-B | TACACTATTTTAGACAGTGCTGGGAACACTTGTTAAAGATGCAAGTTTGGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -112-B | GCGCGAATTTTGCAAACGCGCGGAAAGACAGAAATCCAAGAAATTTGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -113-B | AAATTGTCTCGTTTTTCTTTTTTCGGACAGACCAATCTTCTGGCTGTTGTTGACTAAGCGTCG |
| Linker-6HB -114-B | GGAACGGAAAGGAATTTCACCAGGCTGGCGCCGCTATAAGGGTGATTTTTGGTGGTTGACTAAGCGTCG |
| Linker-6HB -115-B | TGCTCAGAGGGTGTAATAATCAAGAAAGCCACCAGAAAGAACATTTGCTGTTGTTGACTAAGCGTCG |
For construction of DONs monomer, staple strands 6HB-5, 6HB-7, 6HB-53, 6HB-138, 6HB-146, and 6HB-151 were used, and DONs oligomer was assembled with replacing 6HB-5-poly, 6HB-7-poly, 6HB-53- poly, 6HB-138- poly, 6HB-146-poly, and 6HB-151- poly, respectively.

For cell conjugation, additional "TTTTGCAGTGAAGTACAGCGC"(5'-3') sequence was in "Linker-6HB-30-A" to "Linker-6HB-58-A" and additional "TTTTGCTGTTGTACTAACGTCG"(5'-3') sequence was in "Linker-6HB-87-B" to "Linker-6HB-115-B".

Table S3. DNA sequences for cell surface modification.

|          |                  |
|----------|------------------|
| ssDNA-A  | Chol-TTTTATGACTCACTGC |
| ssDNA-B  | Chol-TTTTAGTCAACACAGC |
| ssDNA-M  | Chol-TTTTTTTTTTTTTTTTTTTTTT |
| M-Alexa 647 | Alexa 647-AAAAAAAAAAAAAAAAAAAAA |
| DONs-Alexa 647 | Alexa 647-TTTTAGTCAACACAGC |

The ssDNA-A and ssDNA-B were hybridized with Linker-6HB-XXX- A and B (shown in Table S2) for cell conjugation. ssDNA-M and M-Alexa 647 was used to verify chol-DNA anchoring on cell membranes. DONs-Alexa 647 was used to verify DONs anchoring on cell membranes.