Supporting Information

DNA Nanotweezers for Stabilizing and Dynamic Lighting up Lipid Raft on Living Cell membrane and Activation of T-Cell

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Materials

Culture medium, fetal bovine serum (FBS), trypsin-EDTA solution, alexa 488 labeled Cholera Toxin Subunit B (C22841) and FITC labeled CD59 Monoclonal Antibody (MHCD5901) were purchased from Thermal Fisher Scientific. APC labeled CD3 and CD28 antibodies were purchased from eBioscience. HRP labeled goat anti-rabbit IgG (D110058) was purchased from Sangon Biotech. IL2 was purchased from BioLegend. CFSE kit was purchased from Invitrogen.α-Tubulin antibody (2144), LAT antibody (9166) and phospho-LAT (Tyr191) antibody (3584) were purchased from Cell Signaling Technology (CST). Chlorpromazine (CPZ), methyl-beta-cyclodextrin (MβCD), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), hoechst 33258 were purchased from Sigma-Aldrich. All DNA strands were synthesized by Takara Bio.

Experimental Section

Cell Culture

Jurkat (E6-1 clone) cell and L1210 cell (Murine lymphocyte leukemia cell line) were cultured in RPMI-1640 medium. HeLa cell was cultured in DMEM medium. 10% v/v FBS (fetal calf serum), 1% penicillin, 1% streptomycin and 2 mM glutamine were supplemented in both mediums. Cells were maintained in tissue culture dish with 10 mL of medium and were incubated at 37°C in an incubator with 5% v/v CO_2 in humidified atmosphere. Every 2 days the cells were subcultured by splitting the culture with fresh medium.

Toxicity measurements

The toxicity of cholesterol-functionalized DNA and CPZ to cells was determined via cellular viability assay based on MTT colorimetry (Figure S1). After certain treatments, the cells were seeded in a 48 well-plate (10^4 cells per well, 5 wells per treatment) and incubated in complete medium for 48 h. Then, 50 μL MTT (5 mg/mL) was added to each well and incubated at 37°C for 4 h. After that, 400 μL 10% SDS in

deionized water was introduced into each well and incubated overnight. The cellular viability was validated by microplate reader (BioTek, Synergy H1).

DNA Nanotweezers induced clustering on live cells

2×10^6 cells (Jurkat cells or L1210 cells) were suspended in 100 μL serum free 1640 medium and mixed with 5 μL of 100 μM 5'chol- and 3'Cy3 anchor ss-DNA. In order to restore more anchor ss-DNA on cell surfaces, CPZ was added into medium with recommended concentration to inhibit endocytic. After incubating with shock on thermostatic oscillator (400 rpm) at 37°C for 15 min, excess anchor ss-DNA was removed by twice centrifugation (1300 rpm, 3 min). The anchor ss-DNA carrying cells were then resuspended in 100 μL serum free medium and incubated with 5 μL 5'chol-cDNA (100 μM) for another 15 min. Then the cells were transferred into a 35 mm dish with glass bottom for confocal microscopy imaging. If MβCD treatment was needed, the cells were preincubated in complete medium containing MβCD with a recommending concentration for 30 min. Then the cells were imaged after removing excess MβCD with centrifugation.

For HeLa cell, 50,000 HeLa cells were added to a small dish with glass bottom and cultured overnight. Then DMEM medium was removed and 5 μL 5'chol- and 3'Cy3 anchor ss-DNA (100 μM) was added. After incubating with shock on thermostatic oscillator (400 rpm) at 37°C for 15 min, excess anchor ss-DNA was washed twice with PBS. The anchor DNA carrying HeLa cells were then incubated with 5 μL of 100 μM 5'chol-cDNA for another 15 min. Then the cells in the small dish were taken for confocal microscopy imaging.

The dynamic process of DNA nanotweezers induced fluorescent aggregates was recoded in Video S1 with a rate of 1 frame per second.

For the strand displacement reaction, the process of cells incubated with 5'chol 3'Cy3 anchor ss-DNA and 5'chol-cDNA was the same with above. After that, Jurkat cells were mixed with 1 mL serum free 1640 medium, excess 5'cholesterol-cDNA was removed by twice centrifugations. Then the cells were suspended in 100 μL serum free

1640 medium and mixed with 5 μL of 100 μM trigger strand DNA and incubated at 25℃ for 20 min. Then the cells were transferred into 35 mm dish with glass bottom for imaging.

The dynamic process of strand displacement reaction induced redistribution of fluorescent aggregates were recorded in Video S2 with a frame rate of 1 frame per min.

Cell imaging was conducted with confocal fluorescence microscopy (Leica, TCS SP8). Leica application suite advanced fluorescence software and ImageJ were used for image analysis.

Recruitment GM1 into DNA nanotweezers seized region

For Jurkat cells, 2×10^6 cells were suspended in 100 μL serum free 1640 medium and incubated with a mixture of 5'chol- and 3'Cy3 anchor ss-DNA (100 μM) and alexa 488 labeled Cholera Toxin Subunit B (2 μL,1mg/mL in PBS). CPZ was added into medium with recommended concentration. After incubating with shock on thermostatic oscillator (400 rpm) at 37°C for 15 min, excess anchor ss-DNA was removed by twice centrifugation (1300 rpm, 3 min). Then the anchor ss-DNA and Cholera Toxin Subunit B modified cells were resuspended in 100 μL serum free medium and incubated with 5 μL 5'chol-cDNA for 20 min. 488 nm and 561 nm laser were used to excite Alexa 488 and Cy3, respectively.

Recruitment CD59 into DNA nanotweezers seized region

For Jurkat cells, 2×10^6 cells were suspended in 100 μL serum free 1640 medium and incubated with a mixture of 5'chol- and 3'Cy3-functionalized anchor ss-DNA (100 μM) and FITC-CD59 antibody (2 μL). CPZ was added into medium with recommending concentration. After incubating with shock at 37°C for 15 min, excess anchor ss-DNA was removed by two times centrifugation (1300 rpm, 3 min). Then the anchor ss-DNA and CD59 antibody modified cells were resuspended in 100 μL serum free medium and incubated with 5 μL 5'chol-cDNA (100 μM) for 20 min. Then the cells were transferred into 35 mm dish with glass bottom for imaging. 488 nm and 561

nm laser were used to excite FITC and Cy3, respectively.

Recruitment TCR-CD3 complexes into DNA nanotweezers seized region

For Jurkat cells, 2×10^6 cells were suspended in 100 μL serum free 1640 medium containing CPZ and mixed with 5 μL of 5'chol and 3'Cy3 anchor ss-DNA (100 μM). After incubating with shock for 15 min at 37 °C, excess anchor ss-DNA was removed by two times centrifugation (1300 rpm, 3 min). Then the anchor ss-DNA modified cells were resuspended in 100 μL serum free medium and incubated with 5 μL 5'chol-cDNA (100 μM) for 20 min.

After excess 5'chol-cDNA was removed by centrifugation (1300 rpm, 3 min), the cells were then resuspended in 100 μL serum free medium containing 5 μL APC labeled CD3 antibodies and incubated at 4°C for 30 min. Then the excess antibodies were removed by centrifugation (1300 rpm, 3 min), and the cells were transferred into 35 mm dish with glass bottom for imaging. 561 nm and 633 nm laser were used to excite Cy3 and APC, respectively.

DNA nanotweezers induced raft components clustering enhance the phosphorylation level of LAT

For assaying the phosphorylation level of LAT, 2×10^6 Jurkat cells were suspended in 100 μL serum free 1640 medium and mixed with 5 μL of 5'chol- and 3'Cy3-functionalized anchor ss-DNA (100 μM). After incubating with shock for 15 min at 37 °C, excess anchor ss-DNA was removed by twice centrifugation (1300 rpm, 3 min). Then the cells were resuspended in 100 μL serum free medium and incubated with 5 μL 5'chol-cDNA (100 μM) for 20 min. After that, the cells were collected by centrifugation (1300 rpm, 3 min) and mixed with 200 μL SDS-PAGE loading buffer. Control experiments with desired treatment were conducted at the same time. Then, western blotting was performed to detected the phosphorylation level of LAT with a sample volume of 15 μL for every treatment. α-Tubulin antibody, LAT antibody and phospho-LAT were used with 1:1000 dilution. HRP labeled goat anti-rabbit IgG was

used with 1:10000 dilution.

Native T cells activation by DNA nanotweezer

Native $CD8^+$ T cells were obtained from spleen cells of Balb/c mouse with flow sorter. 1×10^6 T cells were firstly stained by CFSE with recommended concentration, then cells were suspended in 100 μL serum free 1640 medium and mixed with 5 μL of 5'chol- and 3'Cy3 anchor ss-DNA (100 μM). After incubating with shock for 15 min at 37°C, excess anchor ss-DNA was removed by two times centrifugation (1300 rpm, 3 min). Then the cells were resuspended in 100 μL serum free medium and incubated with 1 μL 5'chol-cDNA (100 μM) for 20 min. After that, the cells were cultured for 72 h with 1640 medium containing 5μg/mL CD28 antibodies, 50ng/ml IL2. Control experiments with desired treatment were conducted at the same time. Then the CFSE dilution was detected by flow cytometry.

Computation Details

The quantum mechanics calculation was carried out in the Gaussian09 software package.[1] The geometrical structures of monomer and dimer of cholesterol were optimized in the gas and solution phase, respectively, using the semiempirical PM6 method[2] in Gaussian09. The solvent effect of environment is described by using the PCM model[3]. The frequency analysis was also performed at the PM6 level to make sure that the stable structures and free energies were obtained. The calculated Gibbs free energy is in kcal/mol at the temperature of 298.15 K.

Supporting table

Table S1. The sequence of DNA nanotweezers.

5’chol- and 3’Cy3 anchor ss-DNA: Chol- EG3-5’-AATACCGGACTACTTCCGTTAACTA TTT-3’-Cy3
5’chol-cDNA: Chol-EG3-5’-AAATAGTTAACGGAA-3’
trigger strand: 5’-AAATAGTTAACGGAAGTAGTCCGGTATT-3’
5’chol-3’chol-anchor-ss-DNA：Chol-EG3-5’-AATACCGGACT(FAM)ACTTCCGTTAACT ATTT-3’-Chol
cDNA of anchor ss-DNA：5’-AAATAGTTAACGGAA-3’
5’chol-Cy5-cDNA: Chol-EG3-5’-AAAT(Cy5)AGTTAACGGAA-3’
3’chol -cDNA of anchor ss-DNA：5’-AAATAGTTAACGGAA-3’ -EG3-Chol

Egn：polyethylene glycol, n=3.

Supporting Figures

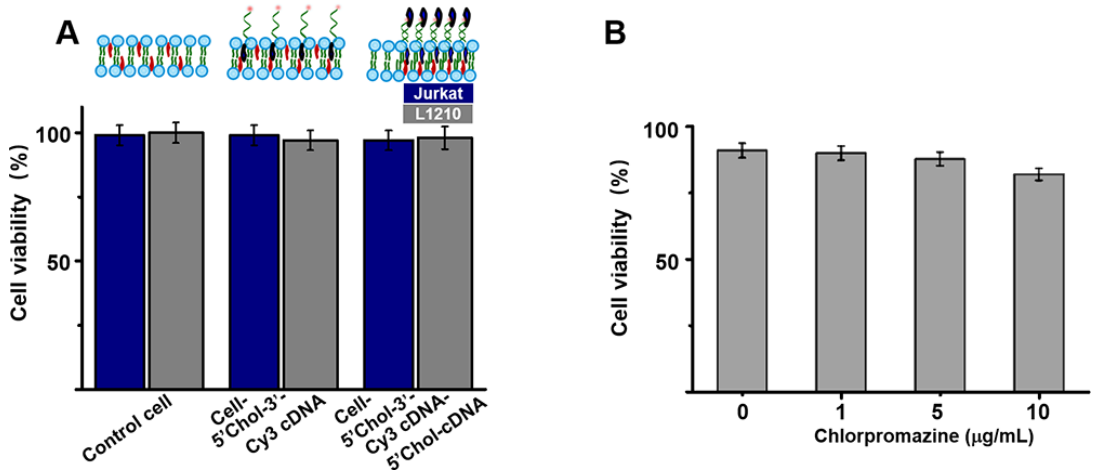

Figure S1. (A) Viability assays of Jurkat and L1210 cells after incubating with 5'chol-3'Cy3 anchor ss-DNA and then with 5'chol-cDNA. (B) Viability assays of Jurkat after incubating with different concentrations of chlorpromazine.

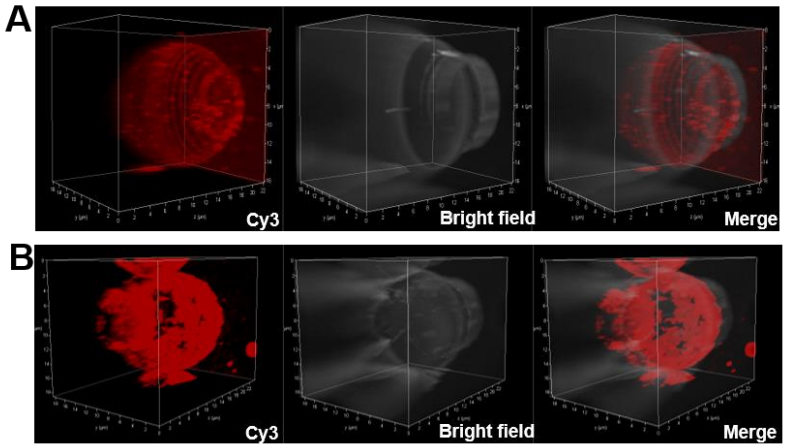

Figure S2. (A) 3D Confocal microscopy images of Jurkat cells upon incubating of 5'chol-3'Cy3 anchor ss-DNA. (B) 3D Confocal microscopy images of Jurkat cells upon incubating of 5'chol-3'Cy3 anchor ss-DNA and 5'chol-cDNA.

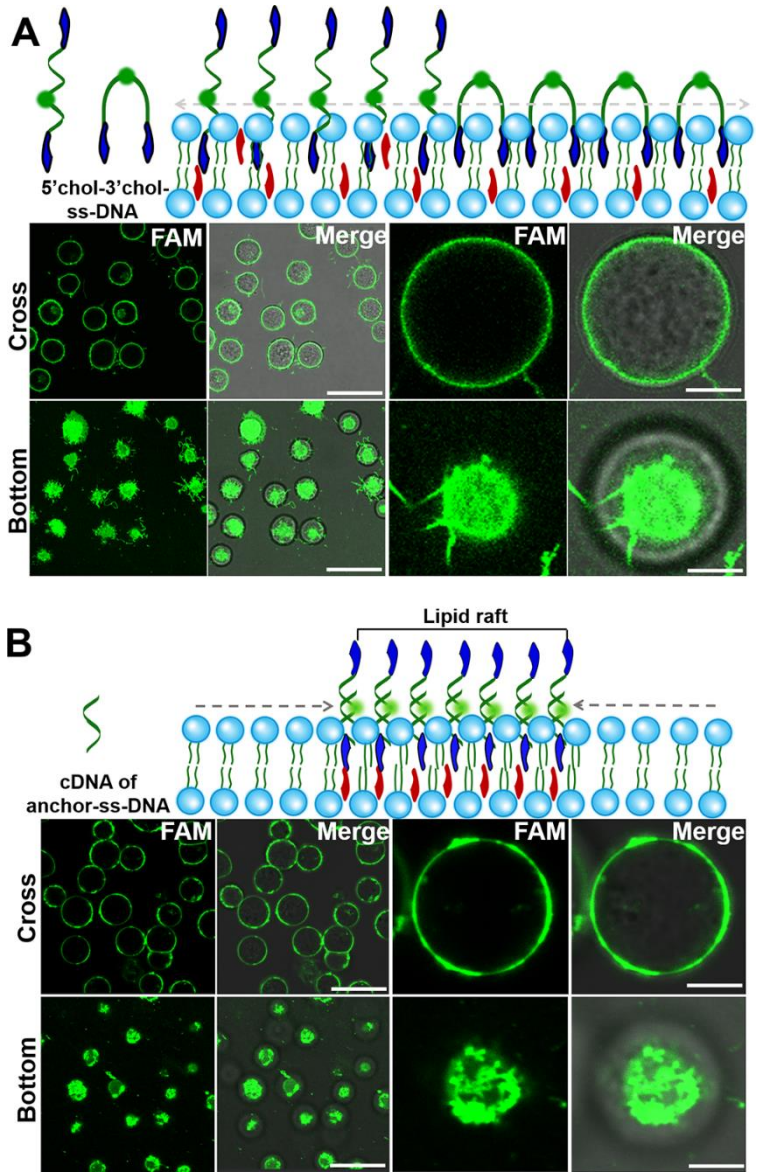

Figure S3. Control experiment suggests the hybridization with cDNA is a prerequisite for the formation of fluorescent aggregates. (A) Upper: schematic illustrating of treating Jurkat cells with 5'chol-3'chol-ss-DNA. FAM was modified in the middle. Lower: Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above-mentioned treatment. (B) Upper: schematic illustrating of treating Jurkat cells with 5'chol-3'chol-ss-DNA and cDNA. Lower: Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above-mentioned treatment. Scale bar: 15 μm in left line, 5 μm in right line.

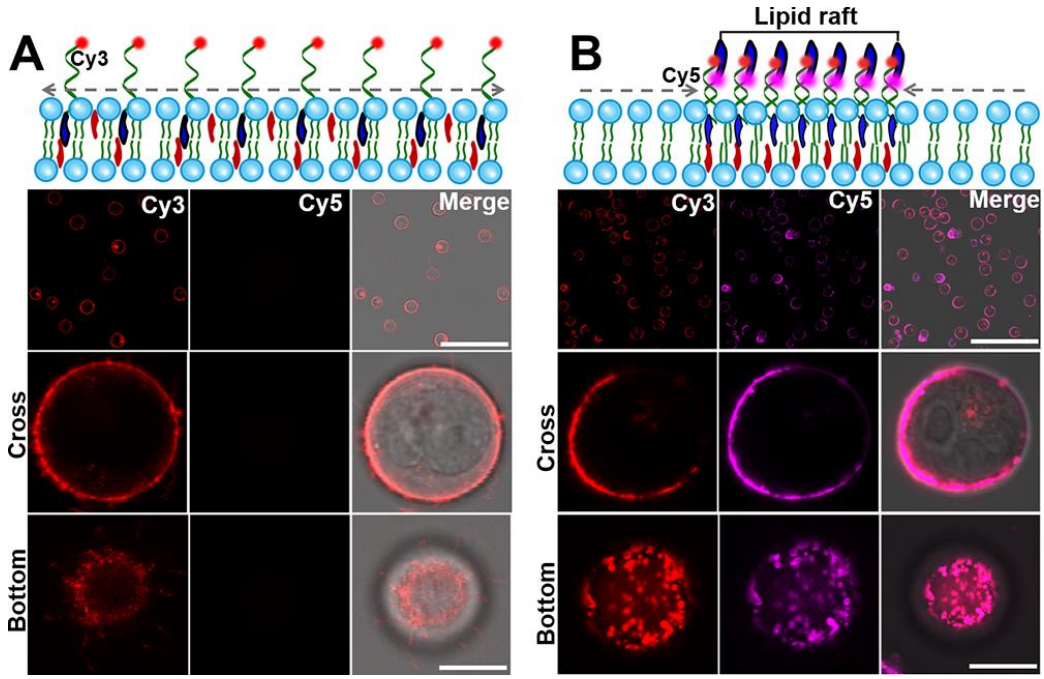

Figure S4. Control experiment suggests the hybridization is indeed happened between 5'chol-3'chol-ss-DNA and cDNA. (A) Upper: Schematic illustrating of treating Jurkat cells with 5'chol-3'Cy3-ss-DNA. Lower: Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above treatment. (B) Upper: Schematic illustrating of treating Jurkat cells with both 5'chol-3'Cy3-ss-DNA and then with 5'chol-cDNA with Cy5 modification near 5' cholesterol. Lower: Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above treatment. Scale bar: 30 μm in the upper images; 5 μm in the lower images.

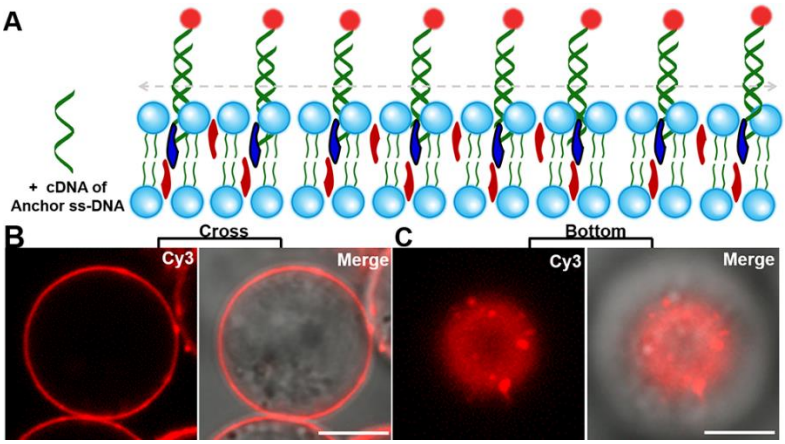

Figure S5. Control experiment suggests a 5' chol functionalization of cDNA is another prerequisite for the formation of fluorescent aggregates. (A) Schematic illustrating of incubating Jurkat cells with 5'chol-3'Cy3 anchor ss-DNA (A) and then incubated with cDNA. (B): Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above-mentioned treatment. Scale bar: 5 μm.

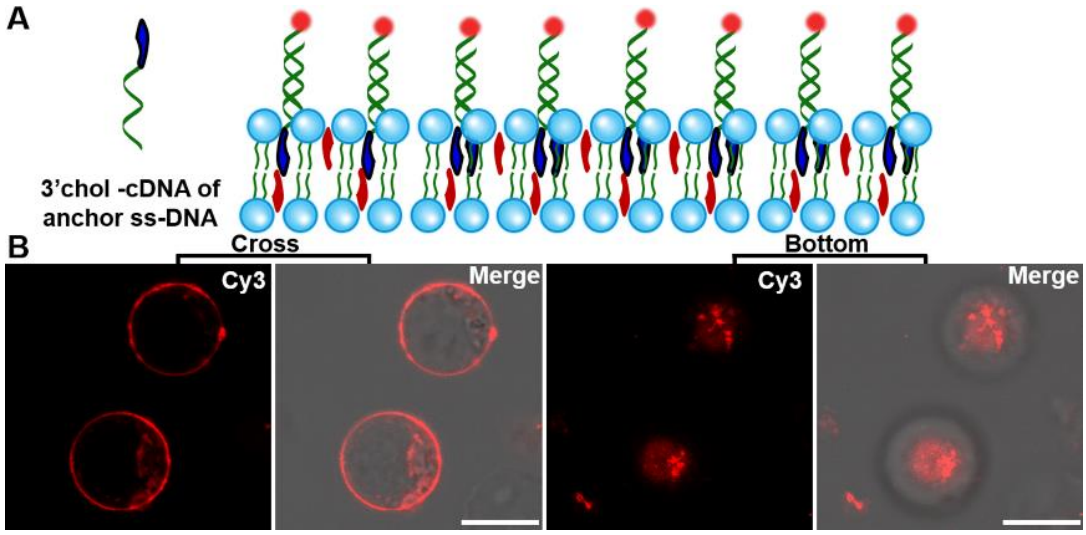

Figure S6. The aggregation of DNA duplex on cell membrane is dependent on the 5'chol-functionalization of cDNA instead of 3'chol-functionlization. (A) Schematic illustrating of incubating Jurkat cells with 5'chol-3'Cy3 anchor ss-DNA and then with 3' chol-cDNA. (B) Confocal microscopy images (cross and bottom view) of Jurkat cells upon the above-mentioned treatment Scale bar: 10 μm.

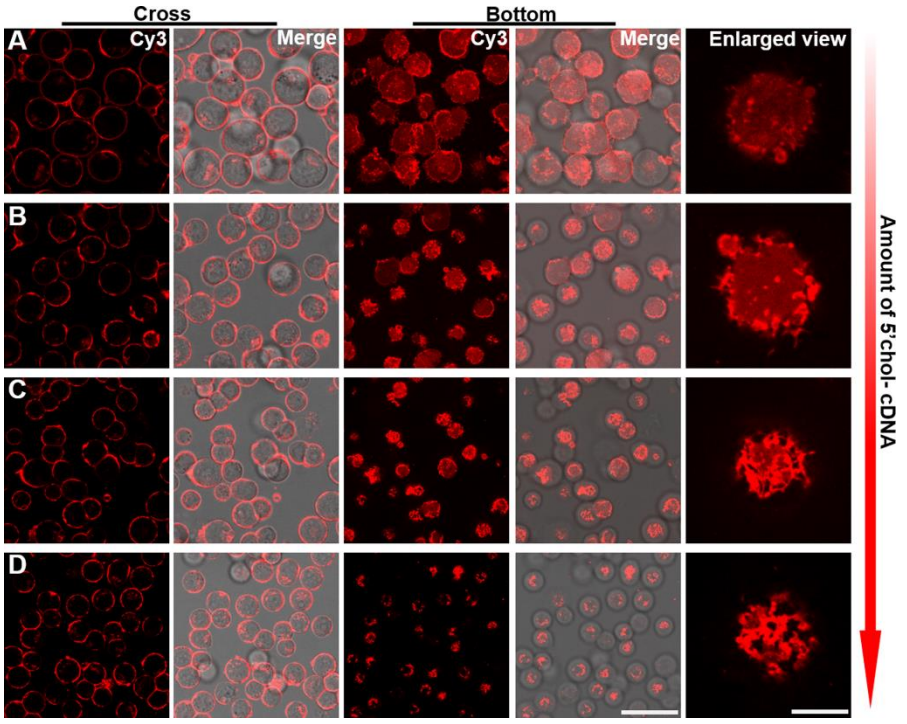

Figure S7. Control experiment suggests the aggregation of DNA duplex on cell membrane is dependent on the amounts of 5'chol-cDNA. Confocal microscopy images of Jurkat cells upon treatment with 5μM anchor ss-DNA, then incubating with 0.75 μM (A), 1.5 μM (B), 2.5 μM (C) and 5 μM (D) of 5'chol-cDNA. Scale bar: 20 μm in the left four columns; 5 μm in the rightmost column

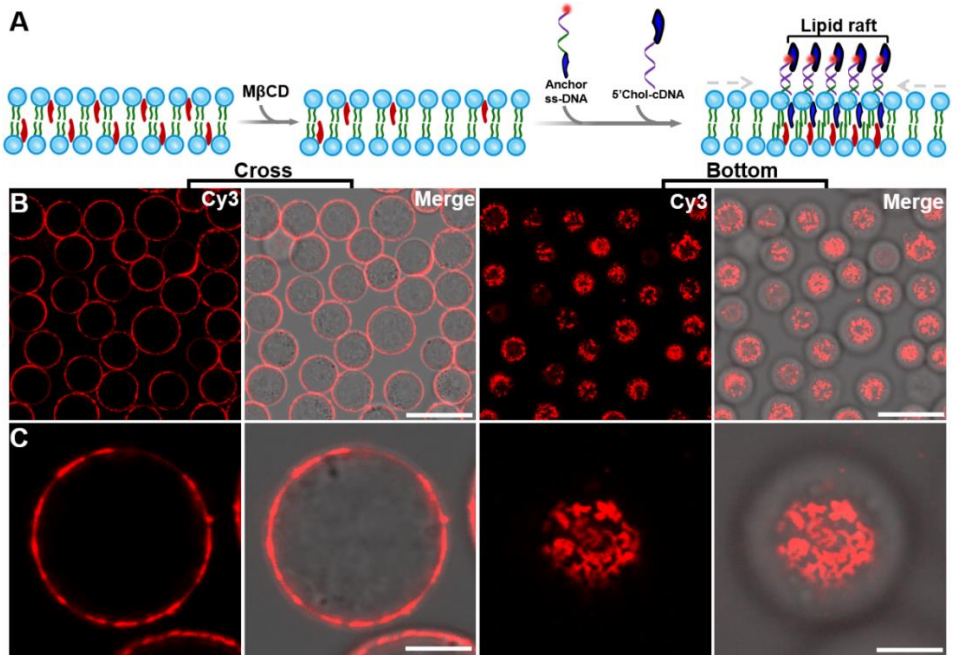

Figure S8. Control experiment with MβCD extraction suggests the aggregating of DNA duplex on cell membrane was largely independent of membrane endogenous cholesterol. (A) Schematic illustrating of incubating Jurkat cells with MβCD to remove some endogenous cholesterol, then treating with 5'chol-3'Cy3 anchor ss- DNA and finally with 5'chol cDNA. (B) Confocal microscopy images (cross and bottom sections of Jurkat cells upon the above-mentioned treatments. Scale bar: 20 μm. (C) Enlarged Confocal microscopy image of a single cell upon the above-mentioned treatments. Scale bar: 5 μm.

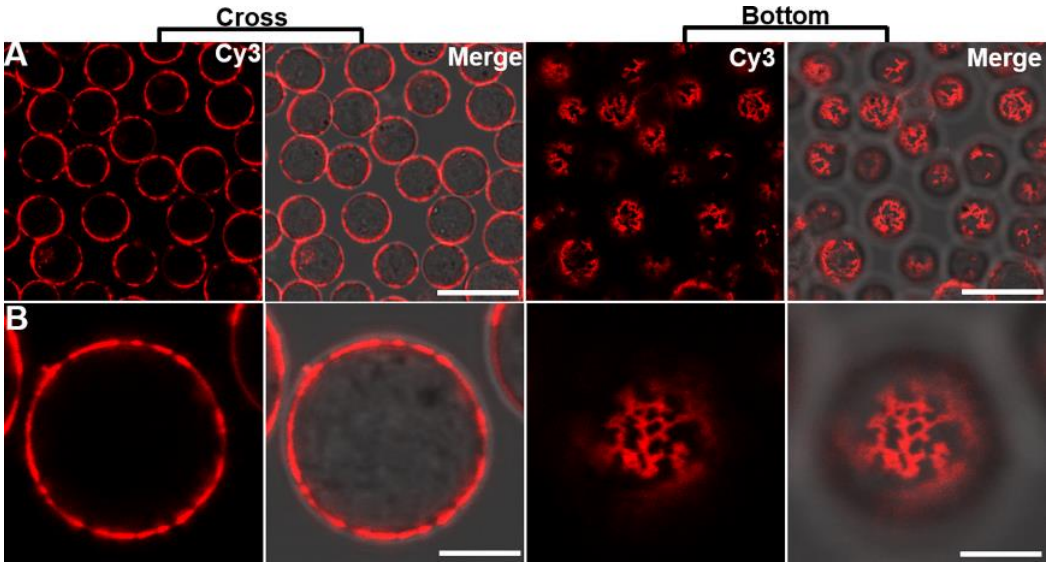

Figure S9. The strategy of using DNA nanotweezers to light up lipid raft could be also applied to other cell line. Confocal microscopy images (cross and bottom sections) of L1210 cells upon incubating with 5'chol-3'Cy3 anchor ss-DNA and subsequently with 5'chol-cDNA. Scale bar: 20 μm in A; 5 μm in B.

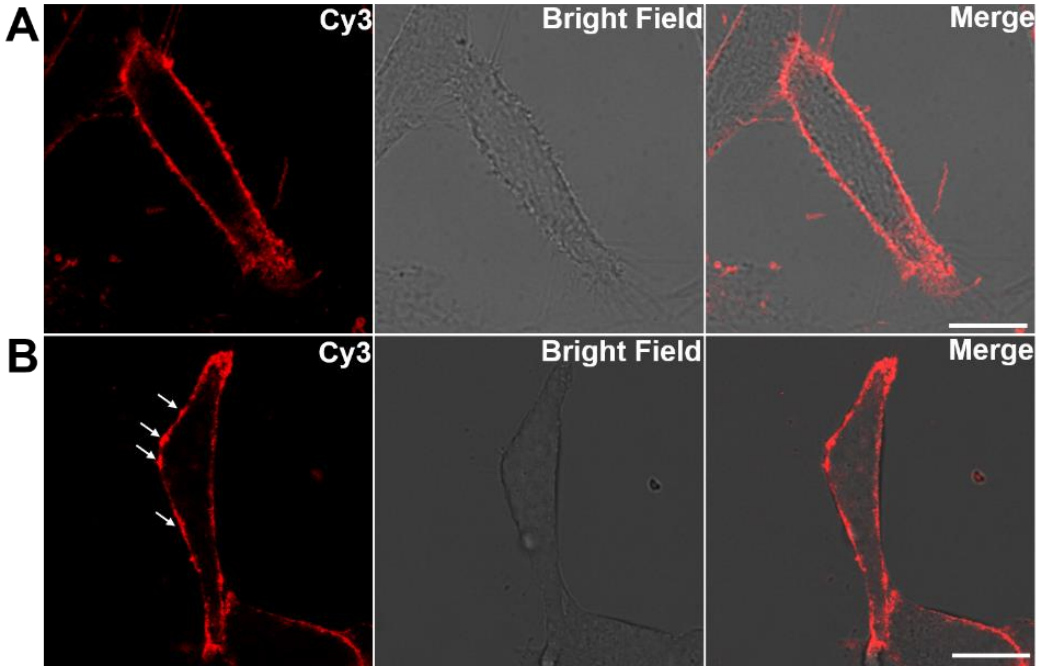

Figure S10. The strategy of using DNA nanotweezers to light up lipid raft could be also applied to HeLa cell. Confocal microscopy images of Hela cells upon incubating with 5'chol-3'Cy3 anchor ss-DNA (A) and then subsequently with 5'chol-cDNA (B). Scale bar: 5 μm.

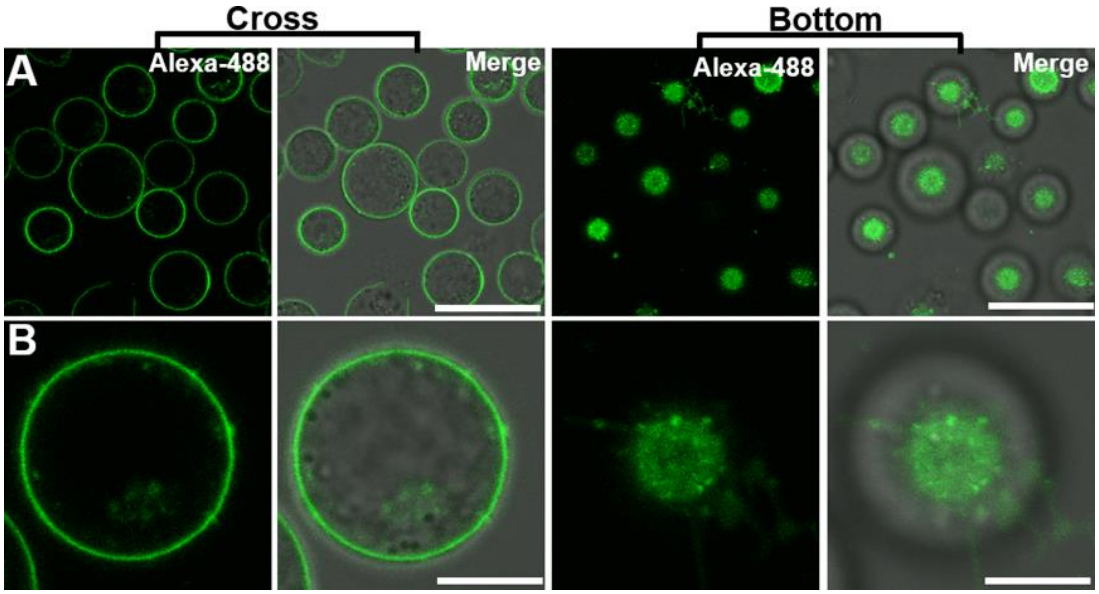

Figure S11. Confocal microscopy images (cross and bottom sections) of Jurkat cells after incubating with alexa488 labeled cholera toxin B. The bottom picture is an enlarged view of the top picture. Scale bar: 20 μm in the top line and 5 μm in the bottom line.

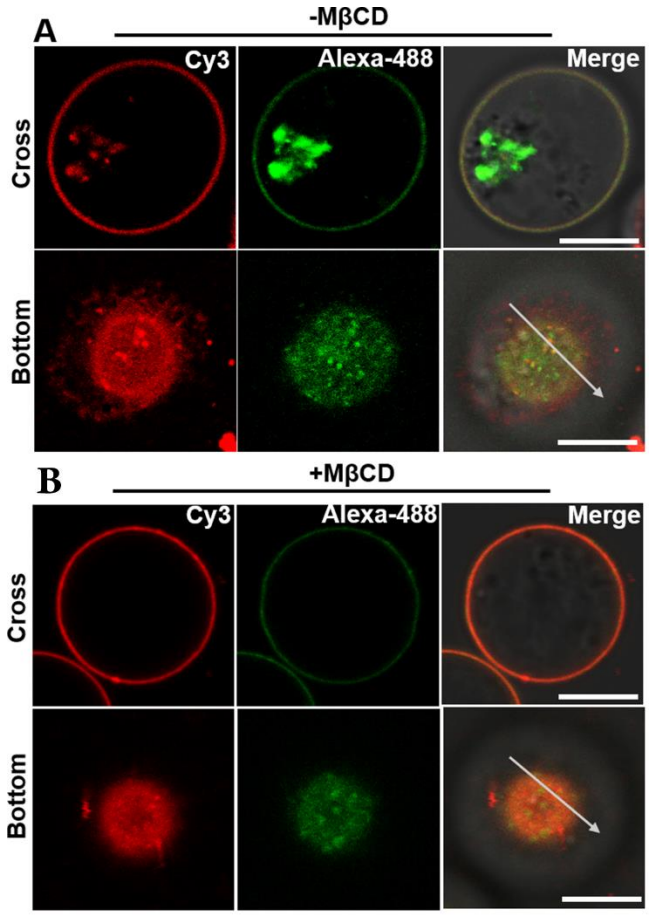

Figure S12. (A) Confocal microscopy images of Jurkat cells upon incubating with 5'chol-3'Cy3 anchor ss-DNA and alexa488 labeled cholera toxin B. (B) Confocal microscopy images of Jurkat cells upon first treatment with MβCD and then incubating with 5'chol-3'Cy3 anchor ss-DNA and alexa488 labeled cholera toxin B. Scale bar: 5 μm.

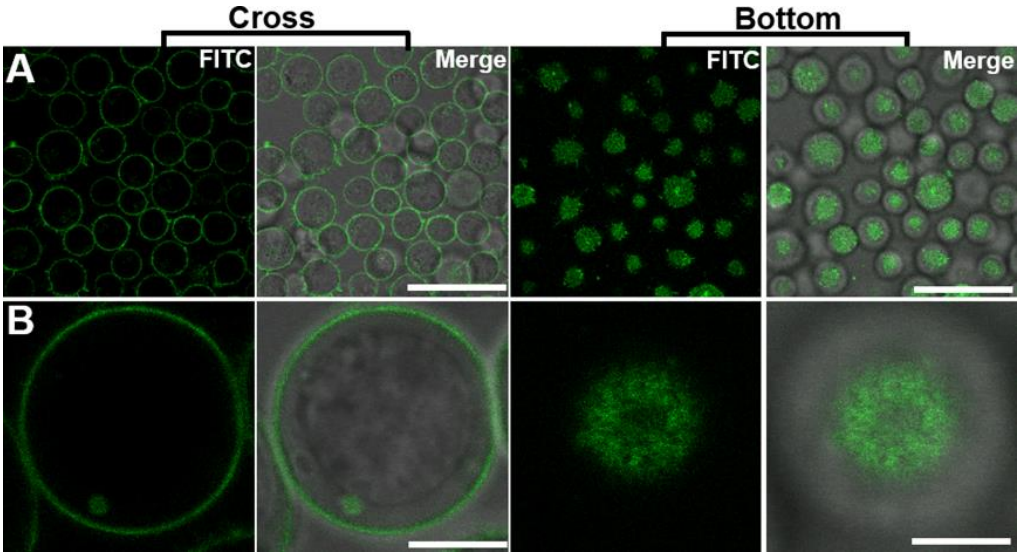

Figure S13. Confocal microscopy images (cross and bottom section) of Jurkat cells upon treatment with FITC-labeled CD59 monoclonal antibodies. The bottom picture is an enlarged view of the top picture. Scale bar: 20 μm in the top line and 5 μm in the bottom line.

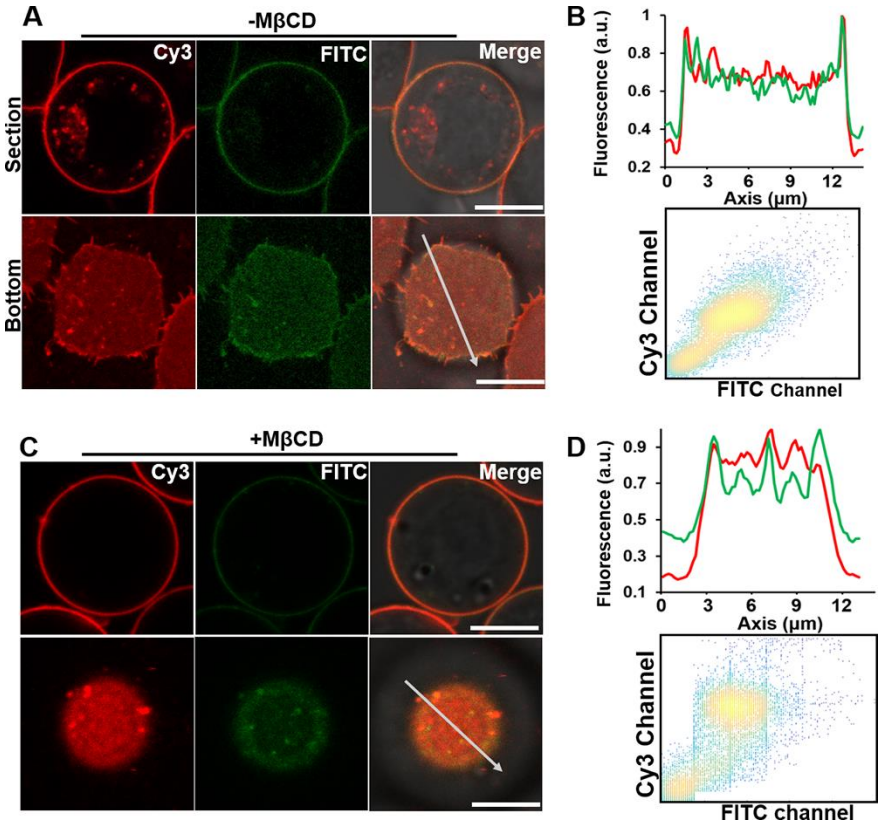

Figure S14. (A) Confocal microscopy images of Jurkat cells upon simultaneous incubating of 5'chol-3'Cy3 anchor ss-DNA and FITC-labeled CD59 monoclonal antibody. (B) Colocalization between anchor ss-DNA and CD59 antibody analyzed by line profiling the fluorescence intensity of Cy3 (red) and Alexa 488 (green) along the line selected in the bottom fluorescence profile. (C) Confocal microscopy images of Jurkat cells upon first treatment with MβCD, and then incubating with 5'chol-3'Cy3 anchor ss-DNA and FITC-labeled CD59 monoclonal antibody simultaneously. (D) Colocalization between anchor ss-DNA and CD59 antibody analyzed by line profiling the fluorescence intensity of Cy3 (red) and Alexa 488 (green) along the line selected in the bottom fluorescence profile. Red channel: the fluorescence of Cy3, green channel: the fluorescence of FITC, merge is red and green channel plus bright field image. Scale bar: 5 μm.

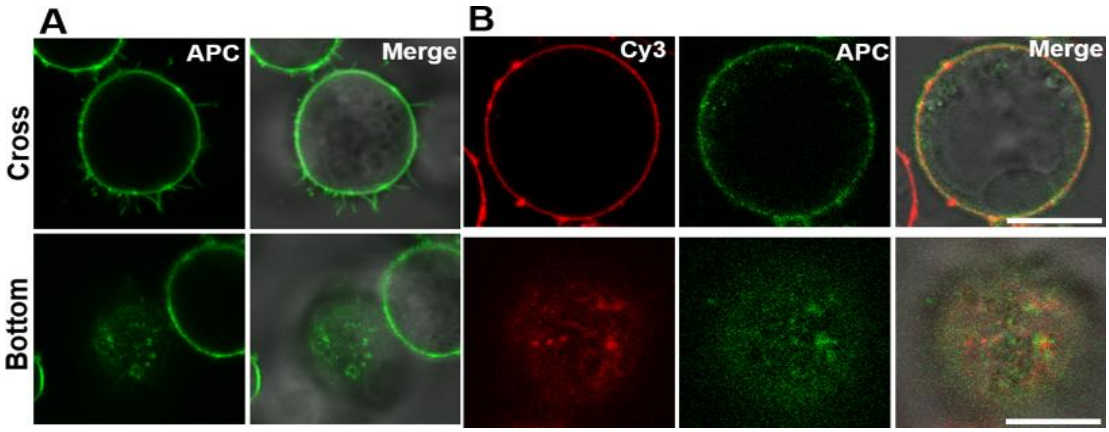

Figure S15. Confocal microscopy images (cross and bottom section) of native CD8+T cells upon treatment with APC-labeled CD3 monoclonal antibody (A) or treatment with both 5'chol-3'Cy3-anchor ss-DNA and APC-labeled CD3 monoclonal antibody (B). The even distribution of APC indicates CD 3 is evenly distribute on the membrane of native CD8+T cells. Scale bar: 5 μm.

Coordinates of optimized cholesterol dimer in solution phase:

C	4.03662400	4.00353700	-0.72585300
C	5.54773900	3.82801700	-0.88690900
C	6.17188900	3.43881300	0.45528200
C	5.53669900	2.16986800	1.03715200
C	4.03788900	2.28852700	1.12012600
C	3.39896500	1.93627800	2.24440400
C	1.91323100	1.96029100	2.41032500
C	1.17192800	2.06737100	1.06352600
C	1.84613400	3.16159000	0.19095300
C	3.32089400	2.75039500	-0.14729300
C	1.03963300	3.50458000	-1.08156900
C	-0.46465800	3.72236200	-0.84686900
C	-1.07190700	2.53129500	-0.07976000
C	-0.29759500	2.44954600	1.27773300
C	-1.16780400	1.54242900	2.15942200
C	-2.61309500	1.80501500	1.68167500
C	-2.54128500	2.72492200	0.42746200
C	-0.94840500	1.25444700	-0.92233500
C	3.36810900	1.59242100	-1.16041600
C	-3.66110800	2.42381900	-0.59592900
C	-3.58985100	3.39157700	-1.78503200
C	-5.05727700	2.50940300	0.06486600
C	-6.13658800	1.82091900	-0.78271800
C	-7.49210200	1.81385900	-0.06443800
C	-8.59353600	1.11584400	-0.88958700
C	-9.00174400	1.95402900	-2.10593400
C	-9.80997500	0.82187000	-0.00176000
O	7.57031900	3.19696700	0.15351900
H	3.59313500	4.25940200	-1.70782100

H	3.83569100	4.87430300	-0.07081800
H	6.00704300	4.76070900	-1.26614300
H	5.77408900	3.05510600	-1.64775500
H	6.14136200	4.27664900	1.18627000
H	5.97930000	1.94485100	2.02844600
H	5.79350200	1.29462000	0.40106400
H	3.94455600	1.60724000	3.12944700
H	1.63694400	2.80829200	3.07253900
H	1.57520100	1.05006000	2.94710400
H	1.23555800	1.08011500	0.54350800
H	1.89506500	4.09092700	0.81243200
H	1.17462900	2.69658200	-1.83239700
H	1.46473000	4.41556300	-1.54560400
H	-0.98080600	3.85330500	-1.81991900
H	-0.63286100	4.66293300	-0.29186900
H	-0.31239500	3.47059500	1.73985000
H	-1.04282500	1.77118700	3.22872100
H	-0.89833300	0.47724400	2.04112200
H	-3.21149500	2.28019900	2.47469200
H	-3.11310700	0.85057400	1.44333300
H	-2.65721800	3.78327900	0.75672200
H	0.09678500	1.03414600	-1.17240300
H	-1.34347300	0.37564900	-0.38187300
H	-1.49733900	1.34005000	-1.86469100
H	4.38153100	1.44178200	-1.54829500
H	3.04376400	0.63895900	-0.70670200
H	2.71113900	1.78226400	-2.01892500
H	-3.52295600	1.38195300	-0.97700900
H	-2.60122700	3.36949500	-2.26556000

H	-4.33363800	3.13985600	-2.54818900
H	-3.77436100	4.42659200	-1.47806500
H	-5.32736800	3.56725700	0.23982700
H	-5.03781400	2.04487000	1.06890100
H	-5.82901400	0.78238300	-1.01390000
H	-6.22932700	2.32912700	-1.76241800
H	-7.80042600	2.84927100	0.17312100
H	-7.38871100	1.30581900	0.91440400
H	-8.19181100	0.12719900	-1.25711100
H	-8.14308200	2.16508900	-2.75324100
H	-9.75242000	1.43833900	-2.71411800
H	-9.42938800	2.91781400	-1.80950900
H	-10.17258800	1.71908900	0.50938300
H	-10.64513500	0.42008100	-0.58517100
H	-9.56413800	0.07322900	0.77080100
H	8.01514800	2.75066300	0.90163100
C	5.04273700	-1.90664600	-2.05783400
C	6.54320700	-2.10547600	-2.27758100
C	7.31051300	-1.55717700	-1.07282600
C	6.88115300	-2.24323100	0.23022500
C	5.39097500	-2.16771900	0.43364700
C	4.89875300	-1.83672000	1.63541000
C	3.44274500	-1.76912400	1.96401600
C	2.55022100	-2.43052400	0.89672300
C	3.04926700	-2.04379000	-0.52203400
C	4.51070600	-2.55970500	-0.75163600
C	2.08966000	-2.52652500	-1.63035900
C	0.61763700	-2.15530200	-1.38900300
C	0.15165200	-2.63736700	-0.00144300

C	1.09787800	-1.96042600	1.04587500
C	0.38427100	-2.19154800	2.38718500
C	-1.12021300	-2.14640000	2.03070000
C	-1.23305900	-2.09004400	0.47905400
C	0.18263200	-4.17192300	0.06211900
C	4.55048100	-4.09476500	-0.86946300
C	-2.50053200	-2.79136800	-0.05713600
C	-2.55466500	-2.73905900	-1.58980200
C	-3.76069100	-2.11731900	0.53833400
C	-5.05478100	-2.86331900	0.18887100
C	-6.27810900	-2.18434000	0.81945400
C	-7.60324600	-2.84160600	0.37675800
C	-7.93005200	-2.50285400	-1.08297700
C	-8.74916100	-2.40088800	1.29736500
O	8.70040900	-1.86624000	-1.34797500
H	4.48978300	-2.31771900	-2.92509900
H	4.81528300	-0.82205300	-2.04551500
H	6.87030700	-1.59888300	-3.20505700
H	6.78255500	-3.17711900	-2.42408800
H	7.22723700	-0.45107400	-0.99399300
H	7.42915400	-1.80341900	1.08733300
H	7.18870100	-3.31147400	0.20830800
H	5.55615300	-1.57679400	2.46590400
H	3.15694700	-0.70157600	2.09578200
H	3.25287800	-2.24265200	2.94850400
H	2.60635400	-3.53798900	1.01414800
H	3.07942800	-0.91770700	-0.56673500
H	2.17149200	-3.62777200	-1.73900000
H	2.41093000	-2.10651500	-2.60298400

H	-0.01512300	-2.60464000	-2.18132000
H	0.48164800	-1.06187000	-1.48189000
H	1.08677600	-0.85244600	0.85159100
H	0.65163700	-1.42577100	3.13126200
H	0.65927200	-3.16295100	2.82924500
H	-1.60278700	-1.26179500	2.48148600
H	-1.63870300	-3.03023200	2.43559100
H	-1.29753800	-1.00921300	0.17059500
H	1.19785400	-4.55957200	-0.08249300
H	-0.17073300	-4.54149600	1.03013600
H	-0.45108400	-4.61483400	-0.71248300
H	5.56718600	-4.45736600	-1.05430900
H	4.18963900	-4.57240800	0.04849700
H	3.92036600	-4.45190000	-1.69247200
H	-2.48635800	-3.86107700	0.26204200
H	-1.65495700	-3.18054100	-2.04145400
H	-3.41900000	-3.28995300	-1.97653100
H	-2.63034500	-1.71027800	-1.95767300
H	-3.82373600	-1.07043400	0.18370700
H	-3.66750400	-2.05015000	1.63909100
H	-4.98965600	-3.91504200	0.52677600
H	-5.17673800	-2.91368300	-0.91169700
H	-6.29185500	-1.10742000	0.56371900
H	-6.19435300	-2.22141400	1.92246300
H	-7.49434400	-3.95168500	0.46432500
H	-7.13585400	-2.83687300	-1.75913300
H	-8.86147100	-2.97756600	-1.40644800
H	-8.04819400	-1.41406600	-1.22986500
H	-8.94446400	-1.31803000	1.21715800

H	-9.68262500	-2.91449800	1.04413600
H	-8.53194300	-2.61662800	2.34826400
H	9.26933600	-1.56908300	-0.60900500

Coordinates of optimized dimer in gas phase:

C	4.01633400	3.98016400	-0.59453500
C	5.52929100	3.78420500	-0.69771100
C	6.10726900	3.39928100	0.66672200
C	5.42410400	2.15547500	1.25339400
C	3.92600700	2.30147800	1.27604300
C	3.23822300	1.99874200	2.38576000
C	1.74704400	2.04308400	2.48851500
C	1.06710600	2.09064700	1.10608900
C	1.77941100	3.15158300	0.22245100
C	3.26932100	2.73658600	-0.03297600
C	1.03049700	3.44375000	-1.09714000
C	-0.48345800	3.66587800	-0.94061900
C	-1.12460200	2.50600100	-0.15229900
C	-0.40983500	2.48131600	1.23933600
C	-1.32139000	1.61885100	2.12390600
C	-2.74356300	1.86663700	1.57502200
C	-2.61380400	2.72328000	0.28101200
C	-0.96450100	1.19585300	-0.93523000
C	3.37226100	1.56539000	-1.02587900
C	-3.69339600	2.36853900	-0.76753900
C	-3.54695900	3.23551400	-2.02595600
C	-5.11482100	2.54356700	-0.18129800
C	-6.16022500	1.73847600	-0.96633100
C	-7.56976900	1.94601300	-0.39717900
C	-8.62168100	1.04480300	-1.07650400
C	-8.84485700	1.43949200	-2.54105800
C	-9.94480600	1.10405400	-0.30050200
O	7.49381600	3.09569200	0.38731300
H	3.61231400	4.23100600	-1.59372300
H	3.80163500	4.85519000	0.04955000
H	6.02103100	4.70315500	-1.06928300
H	5.78030700	2.99815600	-1.43882200
H	6.07935300	4.24898700	1.38233800
H	5.82452000	1.94162700	2.26190200
H	5.68778900	1.26311200	0.64100400
H	3.74296700	1.69750700	3.30280200
H	1.45304700	2.92606500	3.09440800

H	1.37845600	1.16109700	3.05009800
H	1.15598900	1.08115300	0.63257300
H	1.79770600	4.10395500	0.81057500
H	1.19903900	2.60807100	-1.80957100
H	1.47564000	4.33788400	-1.57411600
H	-0.95304000	3.75335700	-1.94061700
H	-0.67729400	4.62779700	-0.43281700
H	-0.44228600	3.52214600	1.65549500
H	-1.24001400	1.89918800	3.18490500
H	-1.05612100	0.54499700	2.06694700
H	-3.36770000	2.38662000	2.31846800
H	-3.24254800	0.90552000	1.36290300
H	-2.74155700	3.79682000	0.55154900
H	0.09003800	0.97586800	-1.14101300
H	-1.36953100	0.33679100	-0.37098400
H	-1.48327200	1.23660600	-1.89730700
H	4.40784500	1.41063300	-1.35063200
H	3.03031500	0.61633100	-0.57496100
H	2.76599600	1.74148100	-1.92211100
H	-3.56601200	1.29655000	-1.05805000
H	-2.54635000	3.13927800	-2.46854300
H	-4.27729700	2.94852400	-2.78976800
H	-3.70392600	4.29682300	-1.80612600
H	-5.38837700	3.61516200	-0.17291900
H	-5.14019800	2.22849300	0.87839600
H	-5.89875600	0.66233300	-0.94421600
H	-6.13937900	2.03239800	-2.03378800
H	-7.86380400	3.00754400	-0.49918900
H	-7.56369500	1.74160200	0.69104000
H	-8.24975200	-0.02098700	-1.04961600
H	-7.91916000	1.36237100	-3.12118900
H	-9.58855300	0.79348200	-3.01886900
H	-9.20140900	2.47127200	-2.63048400
H	-10.35701400	2.11857100	-0.28179800
H	-10.70013600	0.45413800	-0.75500300
H	-9.81716400	0.78089300	0.73775000
H	7.97521100	2.89108600	1.20627000
C	4.99283200	-1.94399500	-2.20885000
C	6.48673300	-2.10629600	-2.48586500
C	7.29841100	-1.46957400	-1.35493300
C	6.94029700	-2.07756200	0.00964500
C	5.45902300	-2.03036600	0.27431400
C	5.00536500	-1.63224000	1.47078300
C	3.56227000	-1.58107300	1.85599800

C	2.64527500	-2.34396700	0.88089600
C	3.07548300	-2.05130900	-0.58178000
C	4.54349000	-2.53045700	-0.84093200
C	2.08834700	-2.64164500	-1.61047000
C	0.61901700	-2.27959700	-1.34190200
C	0.21662400	-2.65977600	0.09631200
C	1.18912400	-1.89182800	1.05265900
C	0.52872200	-2.03875700	2.43232700
C	-0.98724400	-2.00663800	2.12723000
C	-1.15920700	-2.09628100	0.58229500
C	0.27743700	-4.18407000	0.27703900
C	4.63799000	-4.06773400	-0.86017300
C	-2.43289700	-2.86270000	0.16213500
C	-2.53984700	-2.96137900	-1.36584300
C	-3.68008100	-2.14504600	0.73319500
C	-4.98343600	-2.91023600	0.46944500
C	-6.18978900	-2.17673500	1.07104600
C	-7.52909300	-2.84514900	0.69285400
C	-7.87989800	-2.59183700	-0.77854300
C	-8.65252800	-2.32787000	1.60293300
O	8.66851300	-1.79821400	-1.67682300
H	4.41721600	-2.42714600	-3.02150800
H	4.72903200	-0.86851800	-2.24778600
H	6.76164600	-1.64417600	-3.45302800
H	6.76340700	-3.17517800	-2.58390100
H	7.19990900	-0.36256400	-1.34303900
H	7.51045900	-1.57113800	0.81067800
H	7.27965700	-3.13666200	0.03492000
H	5.68655400	-1.29990400	2.25332400
H	3.25268800	-0.51253700	1.91453200
H	3.42865800	-1.98606800	2.87861600
H	2.73380600	-3.43687200	1.08002400
H	3.06519200	-0.93047700	-0.70309200
H	2.19090200	-3.74599000	-1.63179400
H	2.36389000	-2.29515000	-2.62483300
H	-0.03316400	-2.80154400	-2.07093900
H	0.45599000	-1.19979100	-1.51321500
H	1.14713900	-0.79908800	0.78306400
H	0.82569300	-1.23159300	3.11832700
H	0.81794700	-2.98433900	2.91911000
H	-1.43649400	-1.06649600	2.50087200
H	-1.50342100	-2.83473400	2.63710300
H	-1.25789200	-1.04924600	0.18037100
H	1.29570000	-4.56434700	0.13613800

H	-0.04404600	-4.48245500	1.28014500
H	-0.36810200	-4.69653200	-0.44277500
H	5.65450600	-4.40108400	-1.09655800
H	4.37055000	-4.49019000	0.11495100
H	3.96687800	-4.50487700	-1.60709900
H	-2.39342000	-3.89550900	0.58385800
H	-1.64365000	-3.42100700	-1.80490900
H	-3.40039500	-3.56975000	-1.66397700
H	-2.66026900	-1.97487200	-1.82597400
H	-3.75225700	-1.12791800	0.30103100
H	-3.56187900	-1.99940200	1.82363600
H	-4.91475100	-3.93199800	0.88819300
H	-5.13009900	-3.04041400	-0.62135200
H	-6.19611700	-1.11974400	0.74164200
H	-6.08934000	-2.14323000	2.17250100
H	-7.43323400	-3.94851100	0.84854400
H	-7.08434300	-2.93938300	-1.44539900
H	-8.80286600	-3.10490700	-1.06413300
H	-8.02759800	-1.51383800	-0.97710200
H	-8.77938300	-1.24361300	1.50835800
H	-9.61196200	-2.79101700	1.34980500
H	-8.44965600	-2.54736800	2.65587700
H	9.28368400	-1.34654100	-1.07441600

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