Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Amphiphilic DNA tiles for controlled insertion and 2D assembly on fluid lipid membranes: Effect on mechanical properties

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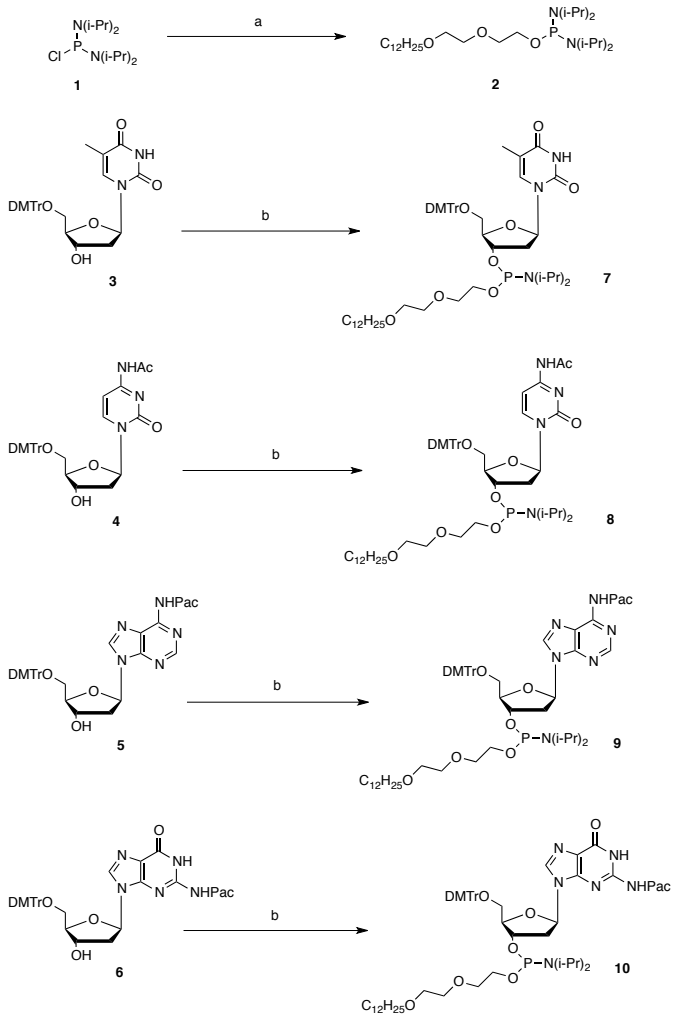
Synthesis of dod-DEG nucleoside phosphoramidites.

Scheme S1.[a]



[a] Reagents and conditions: (a) $C_{12}H_{25}(OCH_2CH_2)_2OH$, Et_3N, THF, 88 %; (b) **2**, 1*H*-tetrazole, CH_3CN, CH_2Cl_2, 76 % for dT, 64% for dC, 57% for dA, 35% for dG.

1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite (2): To a solution of bis(diisopropylamino)chlorophosphine (**1**) 1.54 g, 5.62 mmol) and triethylamine (1.05 mL, 7.50 mmol) in THF (6 mL) at 0 °C was added 2-(2-dodecyloxyethoxy)ethanol (1.50 g, 5.62 mmol) in THF (1.5 mL). After stirring for 1 h at room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The crude residue was dissolved in pentane and the insoluble matter was filtered off. The solvent was removed *in vacuo* to give **2** (2.50 g, 88 %) as a colorless oil, which was used in the next step without further purification. ^{31}P NMR

($CDCl_3$, 162 MHz): δ = 127.6 ppm. HRMS (ESI, positive mode, MeOH): calcd. for $C_{28}H_{61}N_2O_3P$ $[M + H]^+$ 505.4493; found 505.4492.

5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxythymidine 3'-*O*-(1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*-diisopropyl)phosphoramidite (7): A mixture of 5'-*O*-(4,4'-dimethoxytrityl)thymidine (**3**) (2.50 g, 4.95 mmol) and 1*H*-tetrazole (0.231 g, 3.30 mmol) was dried by repeated azeotropic distillation in acetonitrile. To the mixture dissolved in acetonitrile (25 mL) was added 1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite (**2**) (1.80 g, 3.30 mmol) in CH_2Cl_2 (5 mL). After stirring for 5 h, the reaction mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:5 to 1:1) to give **7** (2.50 g, 76% yield) as a white foam. The ^{31}P NMR was recorded for a mixture of two diastereoisomers: ^{31}P NMR ($CDCl_3$, 162 MHz) δ = 148.7, 148.4 ppm. HRMS (ESI, positive mode, MeOH): calcd. for $C_{53}H_{78}N_3O_{10}P$ $[M + Na]^+$ 970.5317; found 970.5309.

N^4-Acetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxycytidine 3'-*O*-(1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*-diisopropyl)phosphoramidite (8): A mixture of N^4-acetyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine (**4**) (2.00 g, 3.96 mmol) and 1*H*-tetrazole (0.185 g, 2.64 mmol) was dried by repeated azeotropic distillation in acetonitrile. To the mixture dissolved in acetonitrile (20 mL) was added 1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite (**2**) (1.51 g, 2.64 mmol) in CH_2Cl_2 (2 mL). After stirring for 5 h, the reaction mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:3 to 1:1) to give **8** (1.66 g, 64%) as a white solid. The ^{31}P NMR was recorded for a mixture of two diastereoisomers: ^{31}P NMR ($CDCl_3$, 162 MHz) δ = 149.1, 148.6 ppm. HRMS (ESI, positive mode, MeOH): calcd. for $C_{54}H_{79}N_4O_{10}P$ $[M + Na]^+$ 997.5426; found 997.5421.

N^6-Phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine 3'-*O*-(1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*-diisopropyl)phosphoramidite (9): A mixture of N^6-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (**5**) (3.03 g, 6.0 mmol) and 1*H*-tetrazole (0.280 g, 4.0 mmol) was dried by repeated azeotropic

distillation in acetonitrile. To the mixture dissolved in acetonitrile (30 mL) was added 1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite (**2**) (2.75 g, 4.0 mmol) in CH_2Cl_2 (2 mL). After stirring for 5 h, the reaction mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:5 to 1:1) to give **9** (3.14 g, 57%) as a white foam. The ^{31}P NMR was recorded for a mixture of two diastereoisomers: ^{31}P NMR ($CDCl_3$, 162 MHz) δ = 148.8, 148.6 ppm. HRMS (ESI, positive mode, MeOH): calcd. for $C_{57}H_{75}N_6O_8P$ $[M + Na]^+$ 1113.5801; found 1113.5795.

N^2-Phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine 3'-*O*-(1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*-diisopropyl)phosphoramidite (10): A mixture of N^2-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (**6**) (2.00 g, 3.96 mmol) and 1*H*-tetrazole (0.185 g, 2.64 mmol) was dried by repeated azeotropic distillation in acetonitrile. To the mixture dissolved in acetonitrile (20 mL) was added 1-(2-(2-(dodecyloxy)ethoxy)ethyl)-*N*,*N*,*N*',*N*'-tetraisopropylphosphorodiamidite (**2**) (1.86 g, 2.64 mmol) in CH_2Cl_2 (2 mL). After stirring for 5 h, the reaction mixture was filtered, and the filtrate was evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-hexane = 1:3 to 1:1) to give **10** (0.410 g, 35%) as a white solid. The ^{31}P NMR was recorded for a mixture of two diastereoisomers: ^{31}P NMR ($CDCl_3$, 162 MHz) δ = 149.0, 148.3 ppm. HRMS (ESI, positive mode, MeOH): calcd. for $C_{61}H_{83}N_6O_{11}P$ $[M + Na]^+$ 1129.5750; found 1129.5745.

Synthesis of dod-DNA: The synthesis of dod-DEG-DNA was carried with on Applied Biosystem 3400 DNA/RNA synthesizer using an ultramild phosphoramidite method. To apply the ultra-mild deprotection condition, phenoxyacetyl protected dA and 4-isopropyl-phenoxyacetyl protected dG (Glen Res) were used as monomers and phenoxyacetic anhydride was used in Cap A solution. The synthesized DNAs were cleaved form the resin and deprotected by the ultra-mild condition, *i.e.* treating with 28% NH_3 aqueous at room temperature for 2 h. The resulting mixtures were purified by reverse-phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm) eluting with 5–45 % (40 min) or 20–80 % (60 min) acetonitrile in 0.1 M triethylammonium acetate (TEAA), pH 7.0, at a flow rate 3.0 mL/min.

Sequence used in the assembly of DNA tile.

DNA sequences used for DNA tile were designed according to the reported sequences by Winfree *et al.*[1] dod-DEG modified sites were shown by boldface.

TA1: GTAGCGCCGTTAGTGGATGTC

TA2: GACTGCGTGTCAATGCTCACCGATCAACCAG

TA3: TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA

dod-DEG-TA4:

GATGGCGACATCCTGCCGCT**A**$_{DR}$**T**$_{DR}$GATTACACAGCCTGAGCATTGACAC

TA5: CTGACGCTGGTTGATCGGACGATACTACATGCCAGTTGGACTAACGG

TB1: CGTCAGGCTGCTGTGGTCGTGC

TB2: GCCATCCGTCGATACGGCACCATGATGCACG

TB3: CGCTACCGTGCATCATGGACTAACCAGTGACCGCATCGGACAGCAGC

dod-DEG-TB3:

CGCTACCGTGCATCATGGACTAACCAGTGA**C**$_{DR}$**C**$_{DR}$GCATCGGACAGCAGC

TB4: GCAGTCGCACGACCTGGCGTTGTACTACGCAATCCTGCCGTATCGACG

TB5:

ACTGGTTAGTGGATTGCGCGAGTAGTTTTCTACTCGCTTGTAGTACAACGCCACCGATG
CGGTC

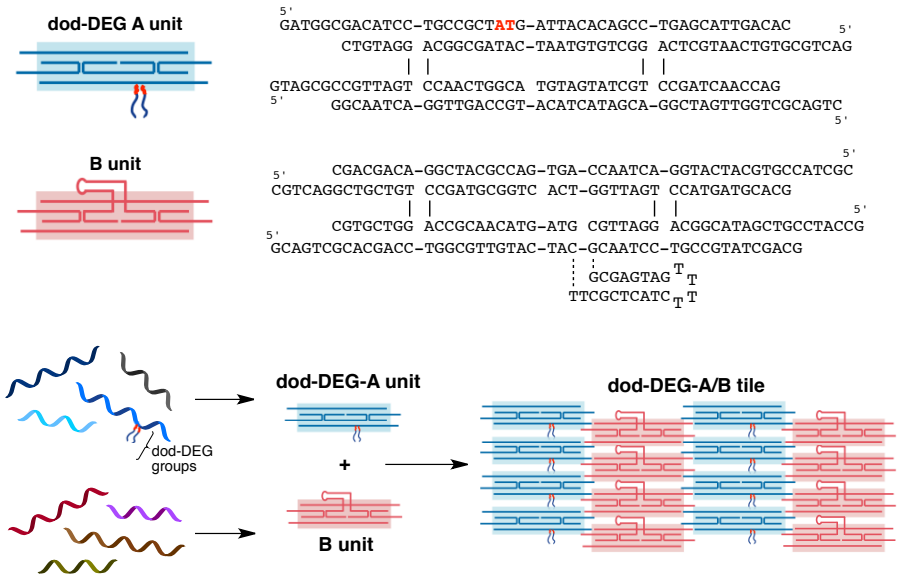


Figure S1. Design of amphiphilic DNA tile. dod-DEG modified sites were shown by boldface (red color) in the sequences.

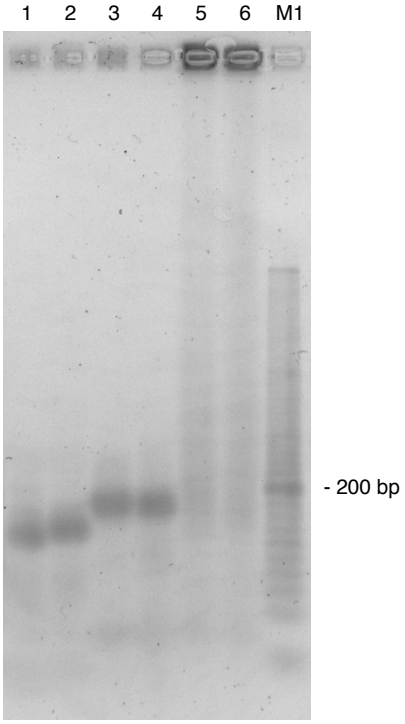


Figure S2. Gel mobility shift assay for detecting the formation of DNA tile (each 1 μM). Appropriate combination of single stranded DNAs self-assembled into the DNA tiles by annealing. The agarose gel (2%) in 1 × TBE buffer was run at 100 V for 150 min at 4 °C. Lane M1; 20 bp maker. Lane 1; A unit, lane 2; dod-DEG-A unit, lane 3; B unit, lane 4; dod-DEG-B unit, lane 5; A/B tiles, lane 6; dod-DEG-A/B tiles.

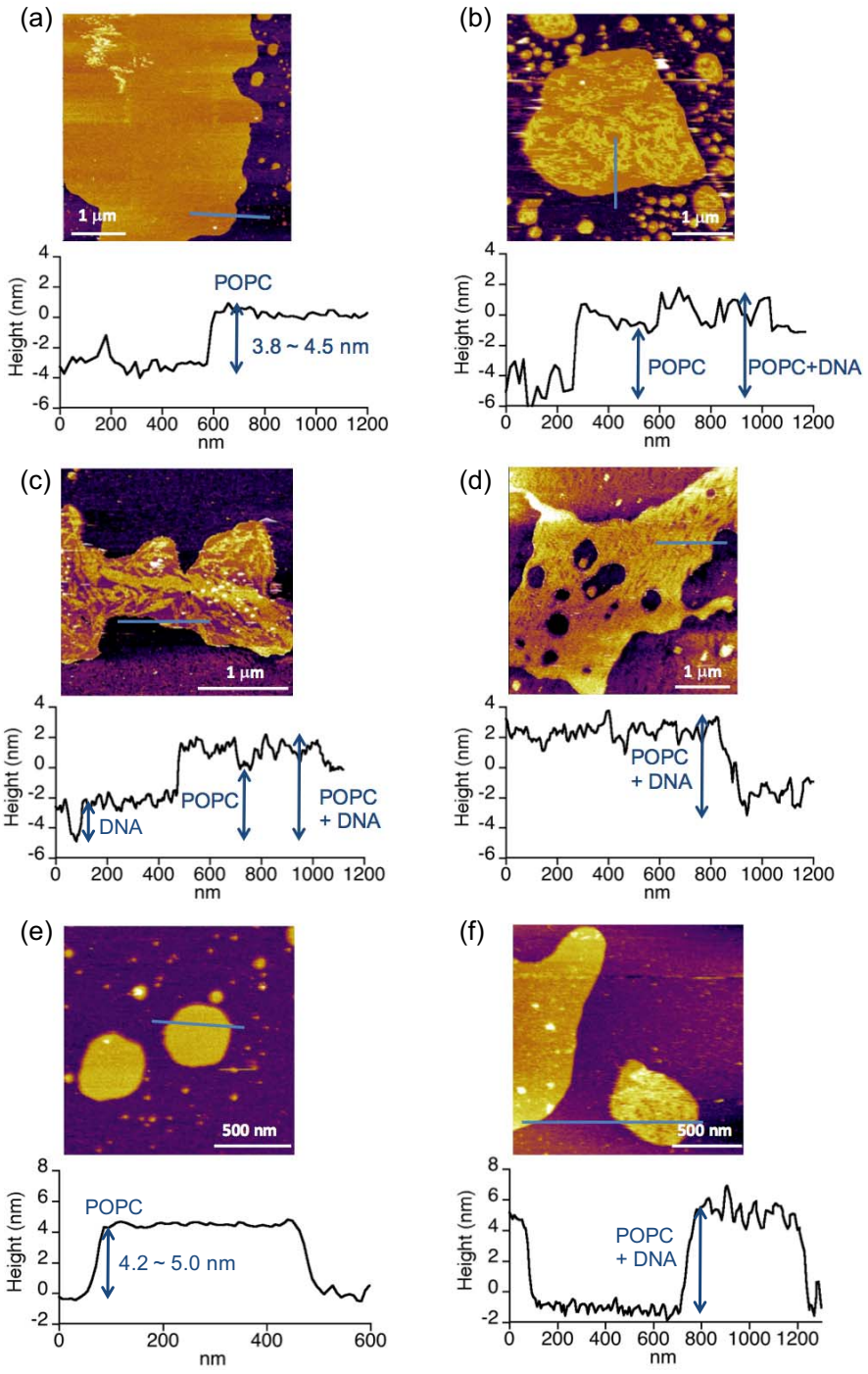


Figure S3. Height cross section analysis for AFM images in Fig. 3. (a) Unmodified A/B tiles (6 mM Mg^{2+}). (b) dod-DEG-A/B tiles (6 mM Mg^{2+}). (c) Unmodified A/B tiles (12.5 mM Mg^{2+}). (d) dod-DEG-A/B tiles (12.5 mM Mg^{2+}). (e) dod-DEG-A/B tiles (3 mM Mg^{2+}). (f) dod-DEG-A/dod-DEG-B tiles (3 mM Mg^{2+}). Detailed assignment was done using the high resolution image in Fig. 4 in the main text.

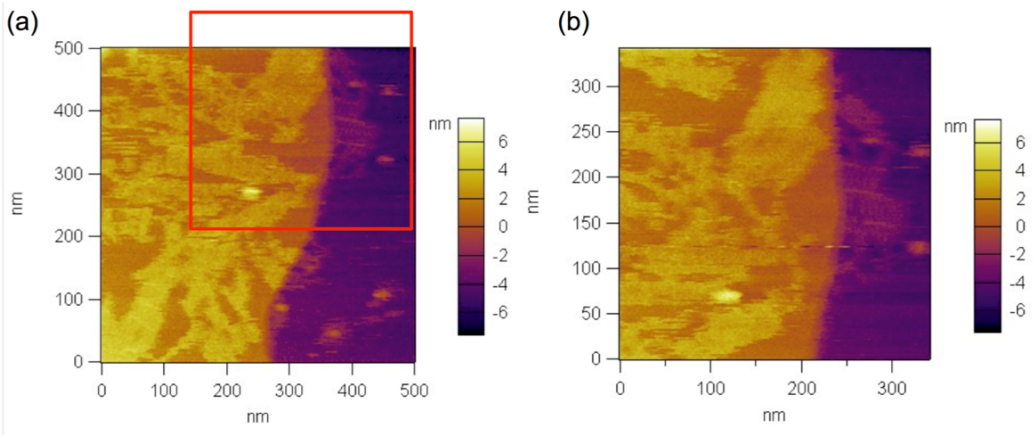


Figure S4. The bound DNA tiles diffuse and disassemble during scanning. (a) AFM image of dod-DEG-A/B tiles on POPC lipid membrane in the presence of 6 mM Mg^{2+}. (b) Zoomed-in AFM image in the region highlighted by the red square in (a) obtained by subsequent scanning after 9 minutes. The shape of the 2D-DNA structure on the membrane is different from that observed in the first scan.

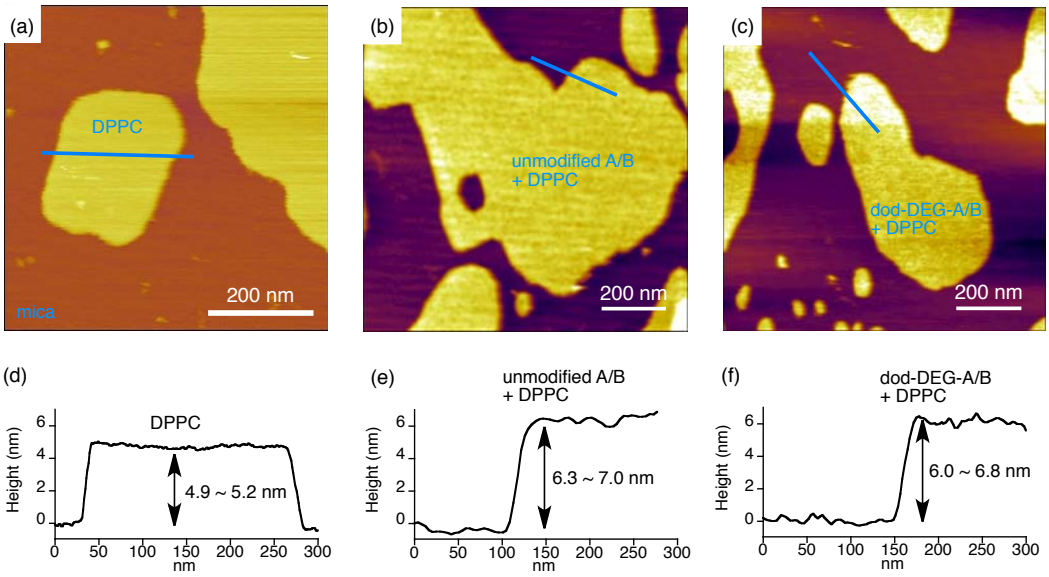


Figure S5. DNA tiles bound to pure DPPC solid phase at 10 mM Tris-HCl (pH 8.0) buffer containing 6 mM Mg^{2+}. (a) DPPC membrane only. Thickness is about 5 nm.[2–4] (b) With unmodified A/B tiles. (c) With dod-DEG-A/B tiles. The scale bar is 200 nm. (d-e) Height profile measured along the blue line in (a), (b) and (c), respectively. Under these conditions, both unmodified A/B and modified dod-DEG-A/B tiles effectively bound to the DPPC membrane.

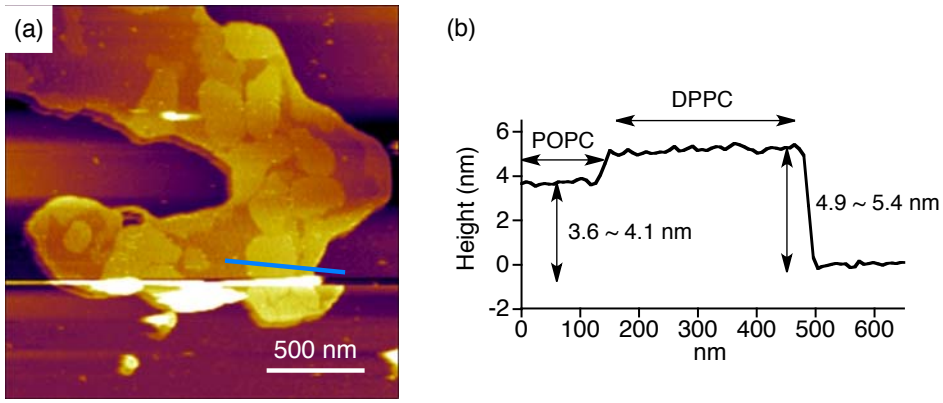


Figure S6. POPC/DPPC (1:1 molar ratio) membrane without DNA tiles at 10 mM Tris-HCl (pH 8.0) buffer containing 6 mM Mg^{2+}. The scale bar is 500 nm. (b) Height profile measured along the blue line showing the step height of POPC and DPPC patches. DPPC patches whose thickness is about 5 nm are thicker by about 1 nm than the POPC fluid phase.[4]

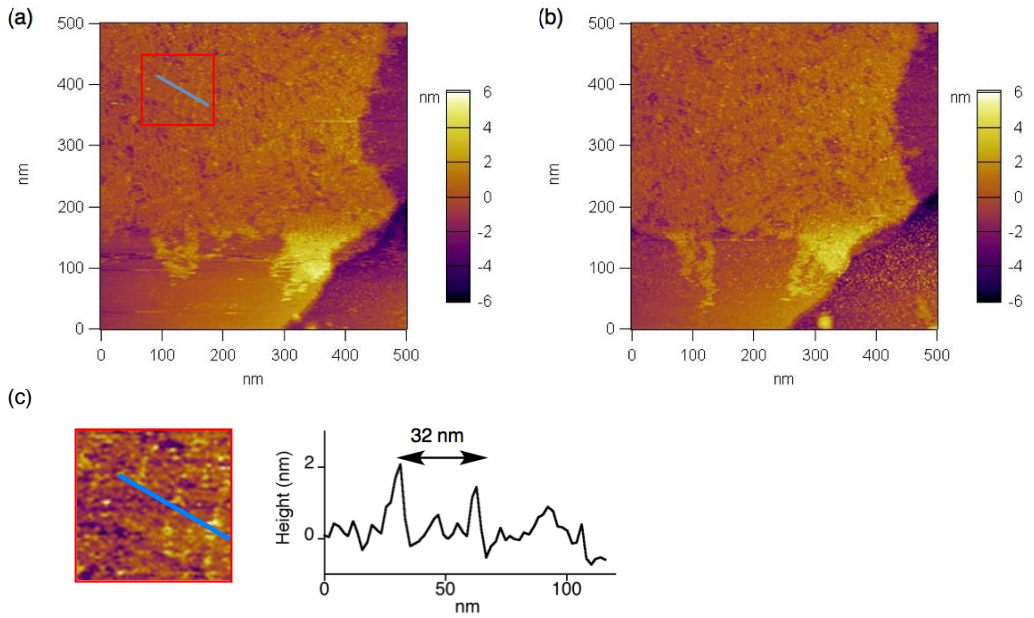


Figure S7. Continuous measurements of AFM images of dod-DEG-A/B tiles on POPC/DPPC lipid membrane. (a) AFM image of the same sample in Figure 5 in the subsequent scanning. (b) AFM image obtained in the next scan to (a). The DNA tiles on the POPC patch diffuse more than on the DPPC patch. (c) Height cross section analysis along the line in (a) shows a characteristic stripe pattern.

References

(1) E. Winfree, F. R. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539-544.

(2) R. Ziblat, L. Leiserowitz and L. Addadi, *J. Am. Chem. Soc.*, 2010, **132**, 9920-9927.

(3) J. Mou, J. Yang and Z. Shao, *J. Mol. Biol.*, 1995, **248**, 507-512.

(4) H. A. Rinia and B. de Kruijff, *FEBS Lett.*, 2001, **504**, 194-199.