Electronic Supplementary Information

Enzymatic Dephosphorylation-Triggered Self-Assembly of DNA Amphiphiles

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EXPERIMENTAL SECTION

Synthesis of linker phosphoramidite

The synthesis route of linker phosphoramidite refers to Figure S1. Briefly, two grams 6-bromohexan-1-ol (11 mmol), N,N-diisopropylethylamine (DIPEA) (2.85 g, 22 mmol) and 4-aminophenol (0.6 g, 5.5 mmol) were dissolved in anhydrous DMF (20 mL). The reaction was allowed to reflux under the nitrogen gas protection and monitored by thin-layer chromatography (TLC). When the reaction was completed, the mixture was diluted with dichloromethane (100 mL) and washed successively with saturated $NaHCO_3$ and brine. The organic layer was collected and dried by anhydrous Na_2SO_4. The solvent was removed by rotary evaporator. After purification through a silica gel column, 1.3 g hydroxylated compound was obtained as a colorless solid (76.3% yield).

The hydroxylated compound (1.3 g, 4.2 mmol) and 4,4'-dimethoxytrityl chloride (2.9 g, 8.4 mmol) were dissolved in anhydrous pyridine (25 mL). The reaction was allowed to stir overnight at room temperature under the protection of nitrogen gas. Then, the solvent was removed by rotary evaporator. After purification through a silica gel column, 1.1 g DMT-protected linker compound was obtained as a colorless foamed solid (28.6% yield).

DMT-protected linker (1.0 g, 1.1 mmol) and DIPEA (0.42 g, 3.3 mmol) were dissolved in anhydrous dichloromethane (20 mL). The reaction

flask was allowed to cool on ice bath under the protection of nitrogen gas. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.39 g, 1.7 mmol) was added dropwise. The ice bath was removed, and the reaction was stirred for an additional one hour. When the reaction was completed, dichloromethane (100 mL) was added, and the mixture was washed successively by saturated $NaHCO_3$, brine and water. The organic layer was collected and dried by anhydrous Na_2SO_4. The solvent was removed by rotary evaporator. After purification through a silica gel column, 1.0 g linker phosphoramidite was obtained as a colorless solid (81.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.7 Hz, 4H), 7.31 (d, *J* = 8.5 Hz, 8H), 7.25 (t, *J* = 4.9 Hz, 4H), 7.18 (t, *J* = 7.1 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 8H), 6.81 (d, *J* = 8.3 Hz, 2H), 6.53 (d, *J* = 8.5 Hz, 2H), 3.97-3.82 (m, 2H), 3.76 (s, *J* = 8.8 Hz, 12H), 3.75-3.62 (m, 2H), 3.14 (t, *J* = 7.2 Hz, 4H), 3.02 (t, 4H), 2.63 (t, *J* = 6.4 Hz, 2H), 1.65-1.56 (m, 4H), 1.50 (m, 4H), 1.43-1.31 (m, 4H), 1.26 (m, 4H), 1.20 (dd, *J* = 16.1, 6.7 Hz, 12H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 146.94. ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.44, 143.58, 142.56, 134.87, 128.15, 126.34, 125.82, 124.70, 119.00, 115.77, 114.54, 111.43, 111.10, 83.78, 61.48, 57.06, 53.33, 49.70, 41.66, 28.26, 25.27, 24.43, 22.77, 22.61, 18.44 (Figures S10-S12).

DNA synthesis

All oligonucleotides used in this work were synthesized on the PolyGen 12-column DNA/RNA synthesizer, using the corresponding controlled pore glass (CPG). Linker and lipid phosphoramidites and phosphorylation reagent were double coupled with CPG for 600 seconds on the DNA synthesizer. Terminal 4,4'-dimethoxytrityl group was removed from DNA on the DNA synthesizer. After synthesis, the obtained oligonucleotides were cleaved and deprotected, followed by purified by reversed-phase HPLC using a BioBasic 4 column. After drying in vacuum and desalting, the obtained oligonucleotides were quantified by measuring their absorbances at 260 nm.

Retention time analysis of oligonucleotides

Oligonucleotides were diluted with TBS buffer (10 mM Tris-HCl buffer, pH 7.4, 137 mM NaCl, 4.7 mM KCl and 5 mM $MgCl_2$) to a final concentration of 10 μM (100 μL). Then, the above solution was injected into the HPLC for retention time analysis.

Enzymatic dephosphorylation

DNA-lipid-P was diluted with TBS buffer to a final concentration of 10 μM (100 μL). Then, ALP was added to the above solution. After mixing, the solution was incubated at 37 °C for ten minutes and then 75 °C for five minutes to deactivate ALP.

Agarose gel electrophoresis analysis

TAMRA-labeled oligonucleotides were diluted with TBS buffer to a

final concentration of 1 μM (20 μL). The above samples were stood still for 30 minutes at room temperature. Then, oligonucleotides were subjected to 1% agarose gel electrophoresis assays.

Size analysis of oligonucleotides

Dynamic light scattering (DLS) was used to measure the particle size of oligonucleotides. Oligonucleotides were diluted with TBS buffer to a final concentration of 10 μM (200 μL). After incubation at room temperature for 30 minutes, the above samples were subjected to DLS assays.

AFM analysis

10 μL of C15-DNA micelles (5 μM) in DPBS buffer solution were deposited on the freshly cleaved mica surface and allowed to absorb for 5 minutes, followed by removing with filter paper. The surface was washed twice with 20 μL of Millipore water and the water was removed with filter paper. Finally, the mica surface was dried by nitrogen gas prior to imaging.

Fluorescence spectroscopy of Nile Red-encapsulated oligonucleotides

Oligonucleotides were diluted with TBS to a final concentration of 1 μM (200 μL), and then 2 μL of Nile Red stock solution (0.1 M in acetone) was added. The samples were vortexed briefly, sealed, and incubated overnight at room temperature in the absence of light. Fluorescence spectra were recorded at room temperature using an excitation wavelength of 550 nm and monitoring emission between 590 and 750

nm, with excitation and emission slit widths both set at 5 nm. For enzymatic dephosphorylation, ALP (1 U) was added to the corresponding sample. The solution was incubated at 37 °C for ten minutes and then 75 °C for five minutes to deactivate ALP. All samples were cooled to room temperature before fluorescence assay.

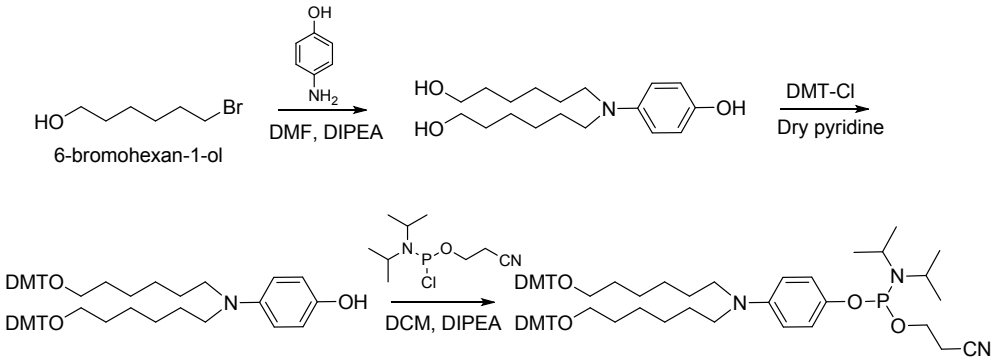

Figure S1 Synthesis route of linker phosphoramidite.

Table S1 DNA sequences designed in this work

Name	Sequence (5’ to 3’)
C6-DNA	C6-TTT TTT TTT TTT TTT TTT TT
C9-DNA	C9-TTT TTT TTT TTT TTT TTT TT
C12-DNA	C12-TTT TTT TTT TTT TTT TTT TT
C15-DNA	C15-TTT TTT TTT TTT TTT TTT TT
DNA	TTT TTT TTT TTT TTT TTT TT
DNA-lipid	$(lipid)_2$-linker-TTT TTT TTT TTT TTT TTT TT
DNA-lipid-P	P_4-$(lipid)_2$-linker-TTT TTT TTT TTT TTT TTT TT

Note: P indicates phosphate group. The synthesis details of C6-DNA, C9-DNA, C12-DNA and C15-DNA were reported in a previous literature[1]

Table S2. HPLC purification program

Time/min	A (0.1M TEAA)	B (acetonitrile)
0	95%	5%
4	95%	5%
4.01	90%	10%
40	5%	95%
50	5%	95%

Note: TEAA indicates triethylamine acetate solution (pH 7.0).

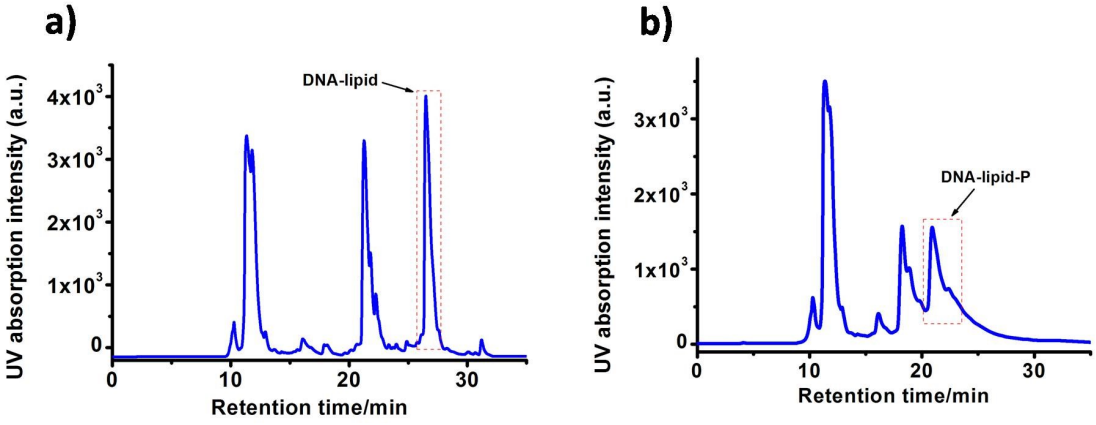

Figure S2 HPLC purification profiles of the synthesized oligonucleotides. (**a**) HPLC purification profile of DNA-lipid. DNA peak in 26.5 minutes is DNA-lipid. (**b**) HPLC purification profile of DNA-lipid-P. DNA peak in 20.9 minutes is DNA-lipid-P.

Table S3 Retention time of C6-DNA, C9-DNA, C12-DNA and C15-DNA

Name	Retention time (min)
DNA	10.0
C6-DNA	19.1
C9-DNA	23.8
C12-DNA	27.3
C15-DNA	30.8

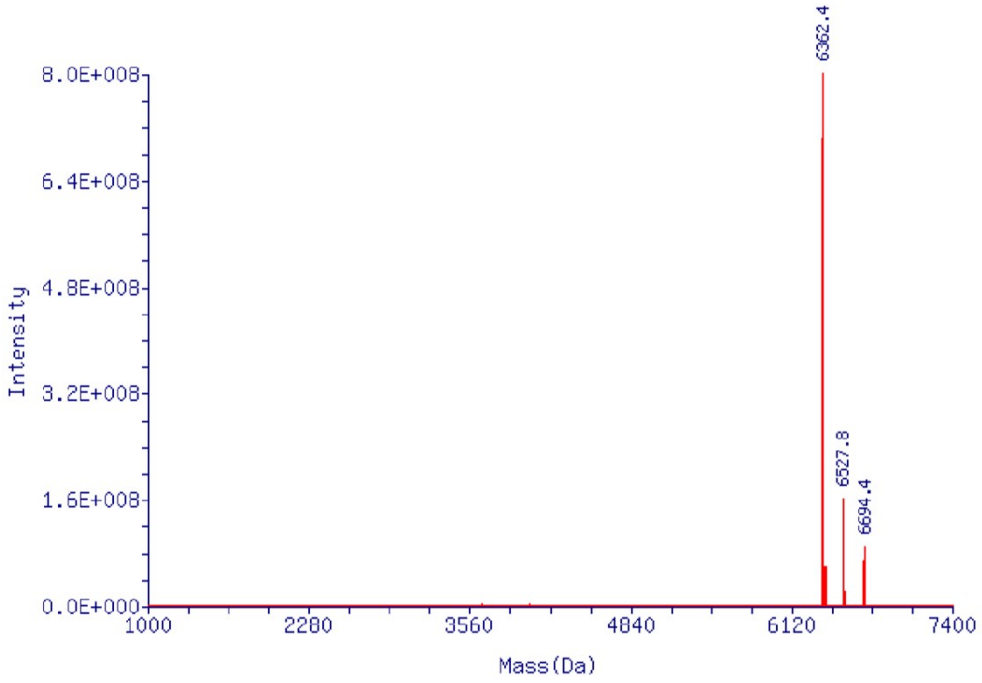

Figure S3 Mass spectrometry analysis of C6-DNA. Calculated molecular weight is 6360.9 Da, and the observed molecular weight is 6362.4 Da.

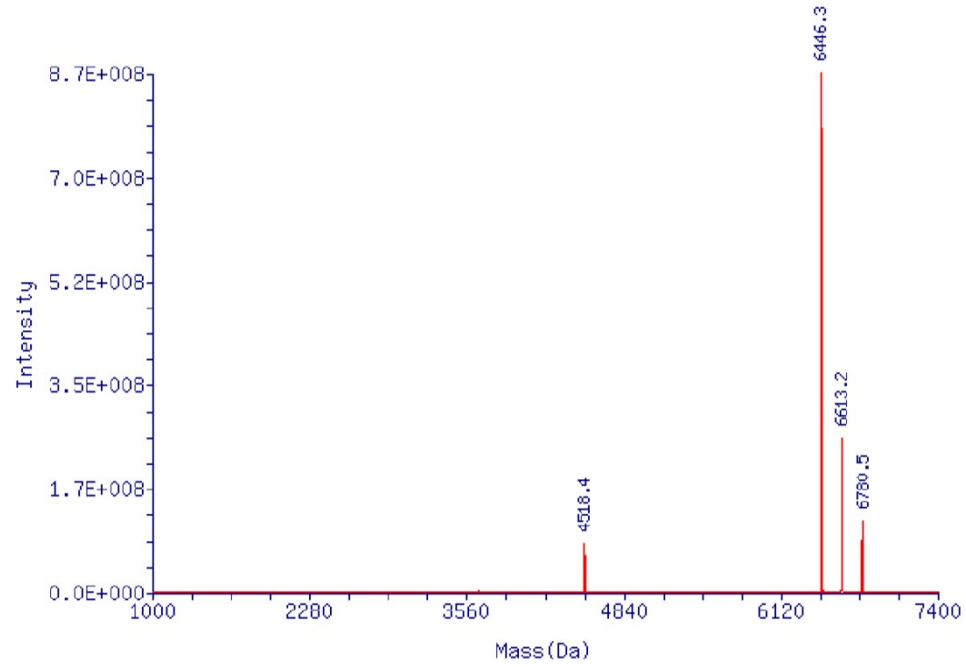

Figure S4 Mass spectrometry analysis of C9-DNA. Calculated molecular weight is 6445.3 Da, and the observed molecular weight is 6446.3 Da.

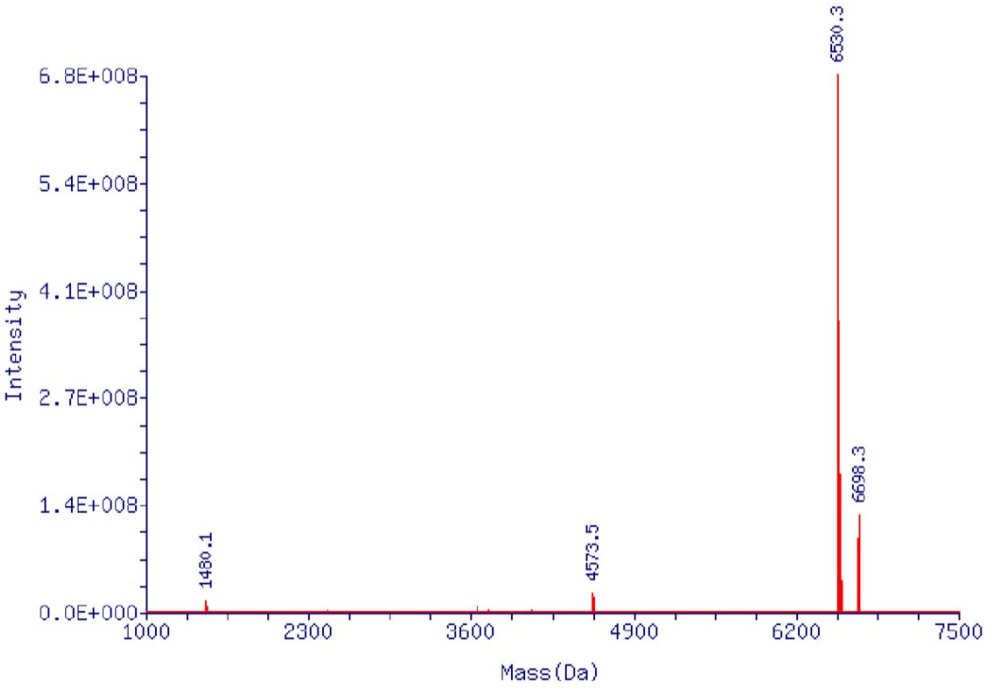

Figure S5 Mass spectrometry analysis of C12-DNA. Calculated molecular weight is 6529.3 Da, and the observed molecular weight is 6530.3 Da.

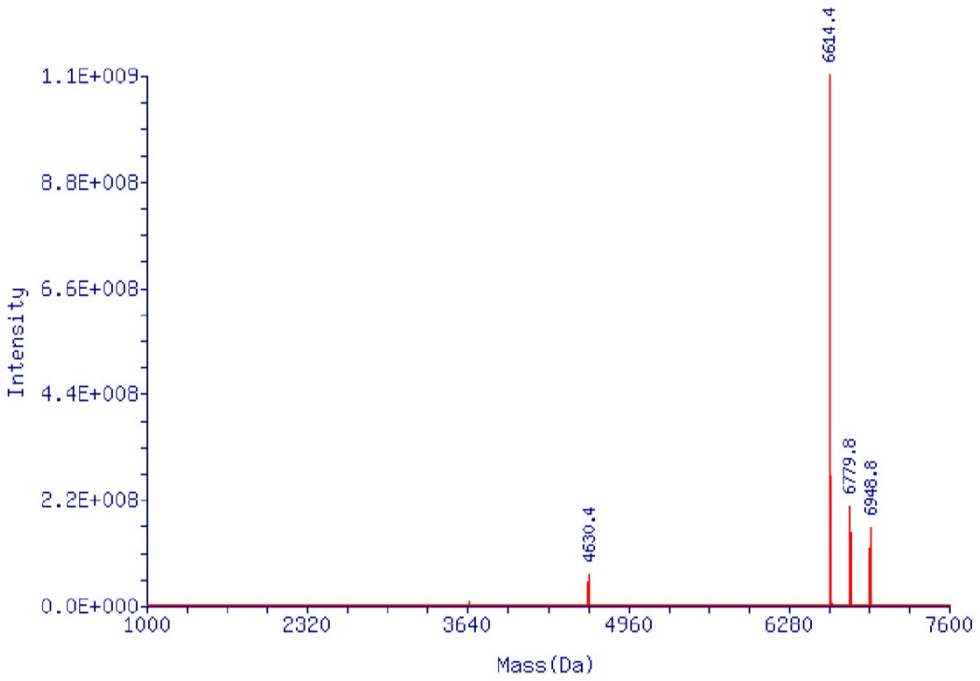

Figure S6 Mass spectrometry analysis of C15-DNA. Calculated molecular weight is 6613.7 Da, and the observed molecular weight is 6614.4 Da.

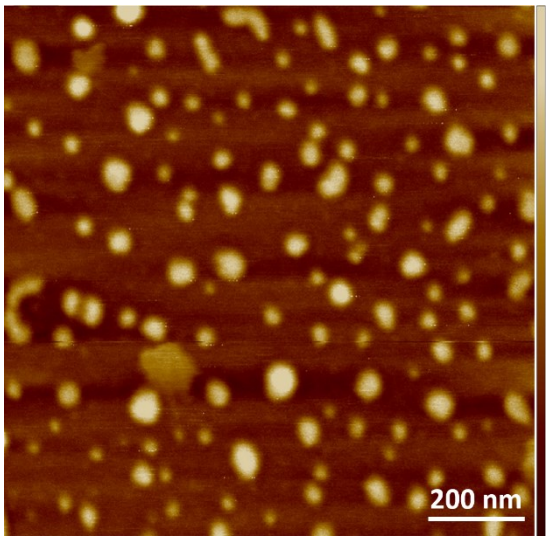

Figure S7 AFM topography image of the self-assembled C15-DNA micelles deposited on a mica surface.

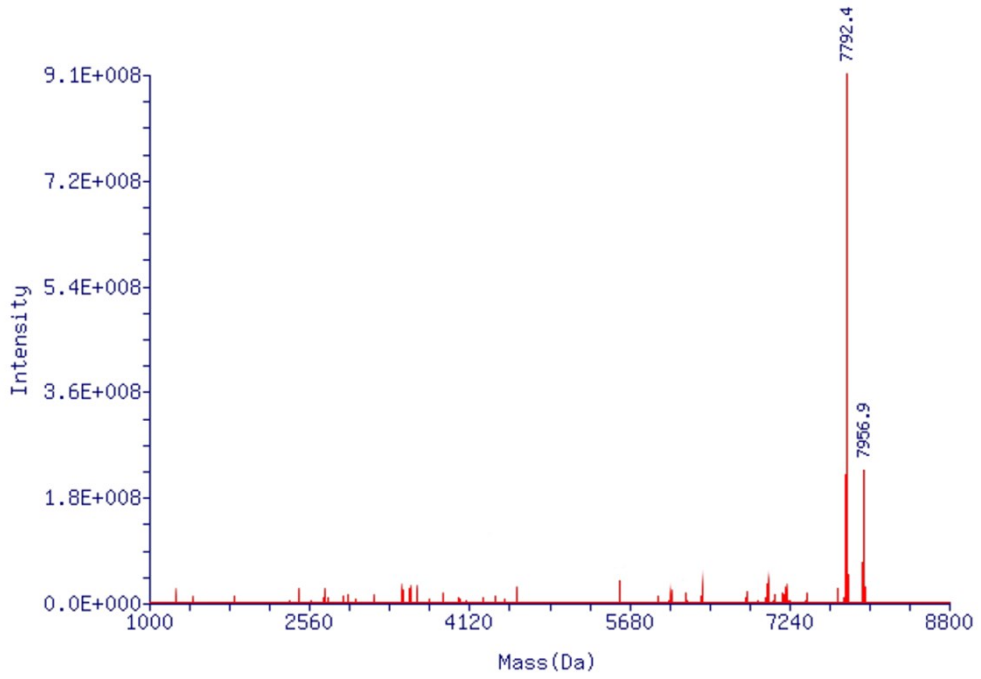

Figure S8 Mass spectrometry analysis of DNA-lipid-P, calculated molecular weight is 7789.8 Da, observed molecular weight is 7792.4 Da.

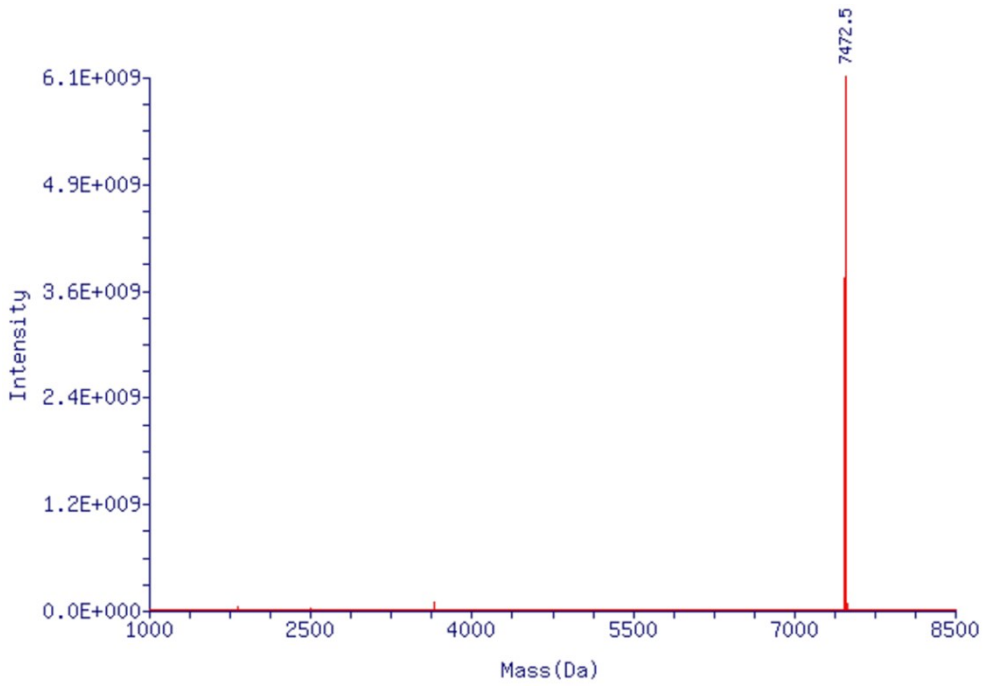

Figure S9 Mass spectrometry analysis of DNA-lipid, calculated molecular weight is 7470.9 Da, observed molecular weight is 7472.5 Da.

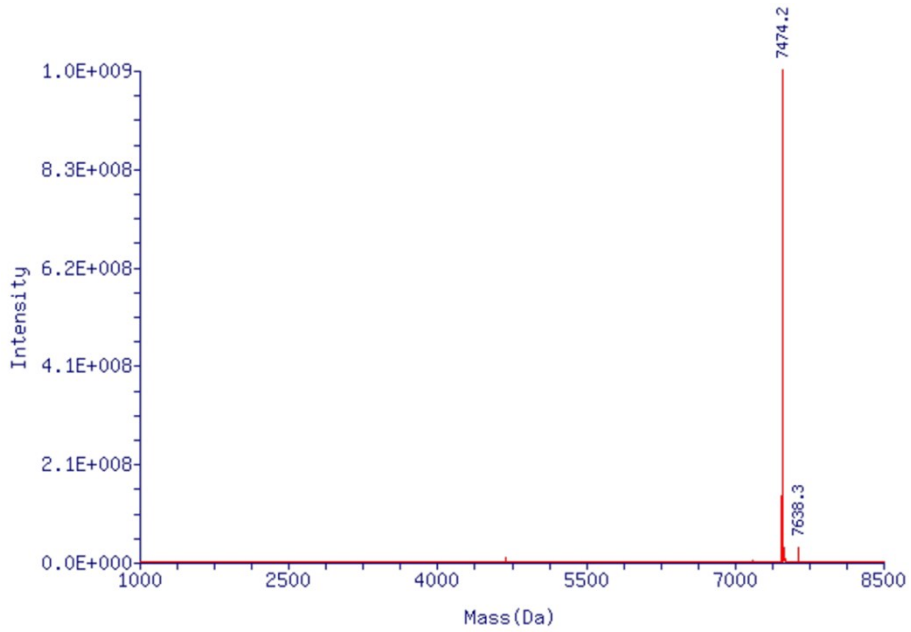

Figure S10 Mass spectrometry analysis of DNA-lipid-P treated with ALP (2 U), observed molecular weight is 7474.2 Da which is consistent with the molecular weight of DNA-lipid.

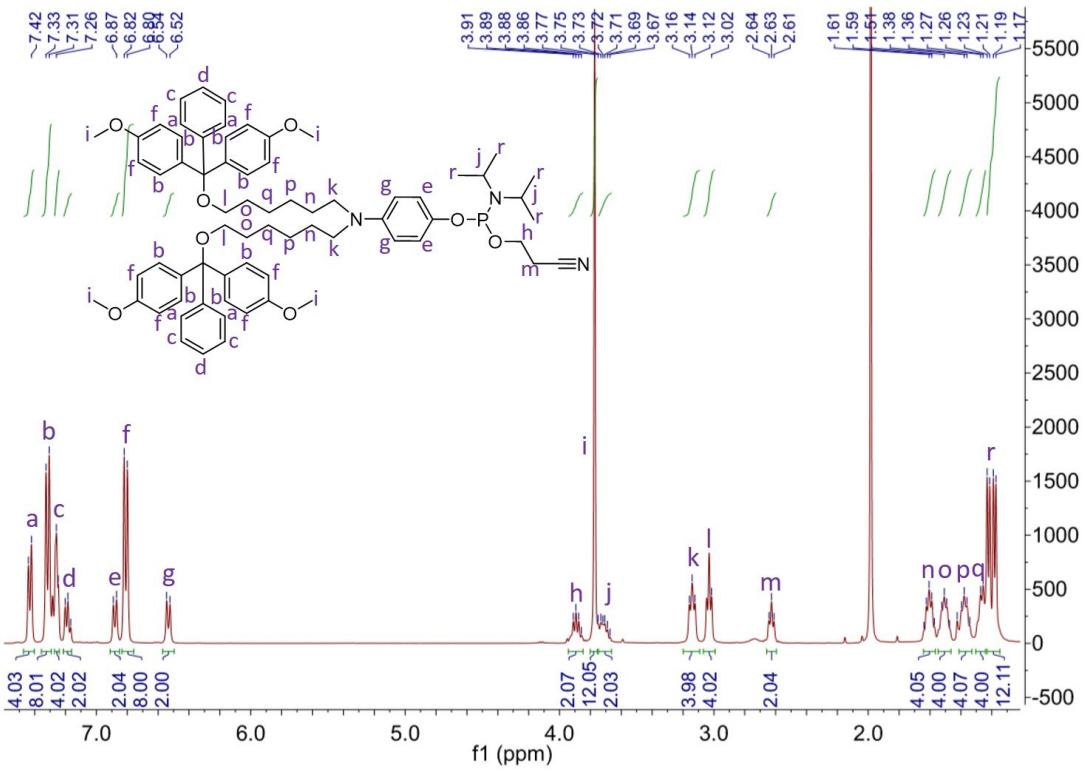

Figure S11 ^{1}H NMR spectra of linker phosphoramidite.

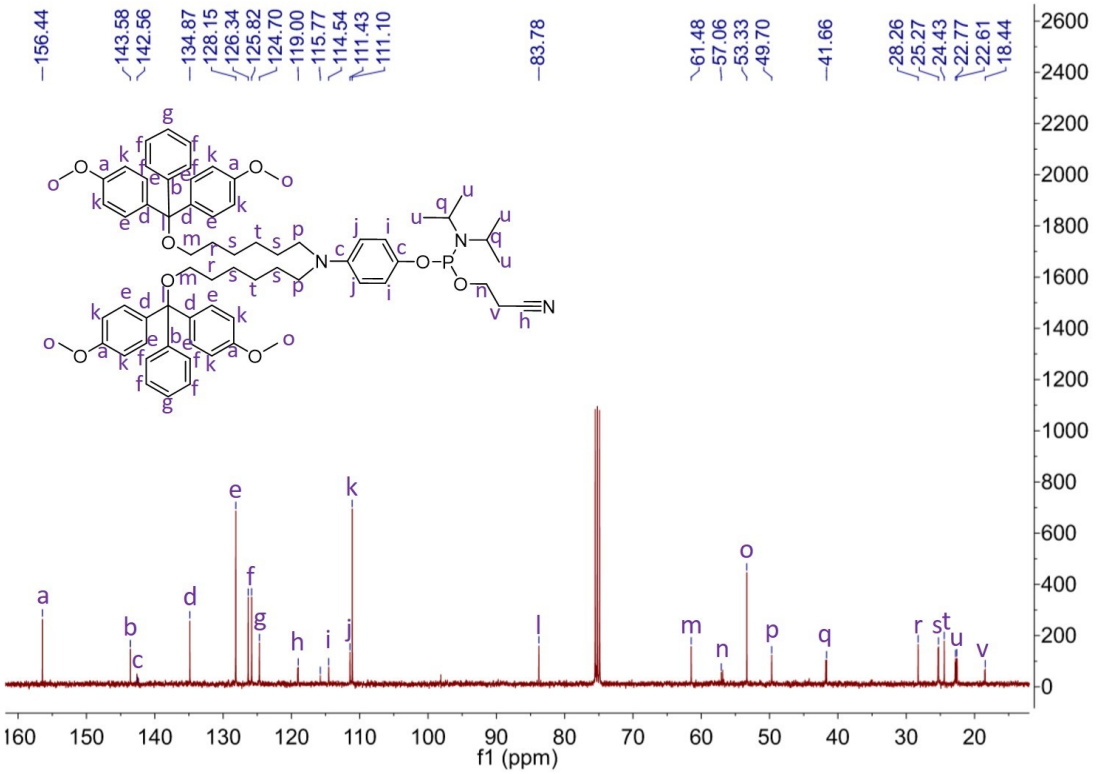

Figure S12 ^{13}C NMR spectra of linker phosphoramidite.

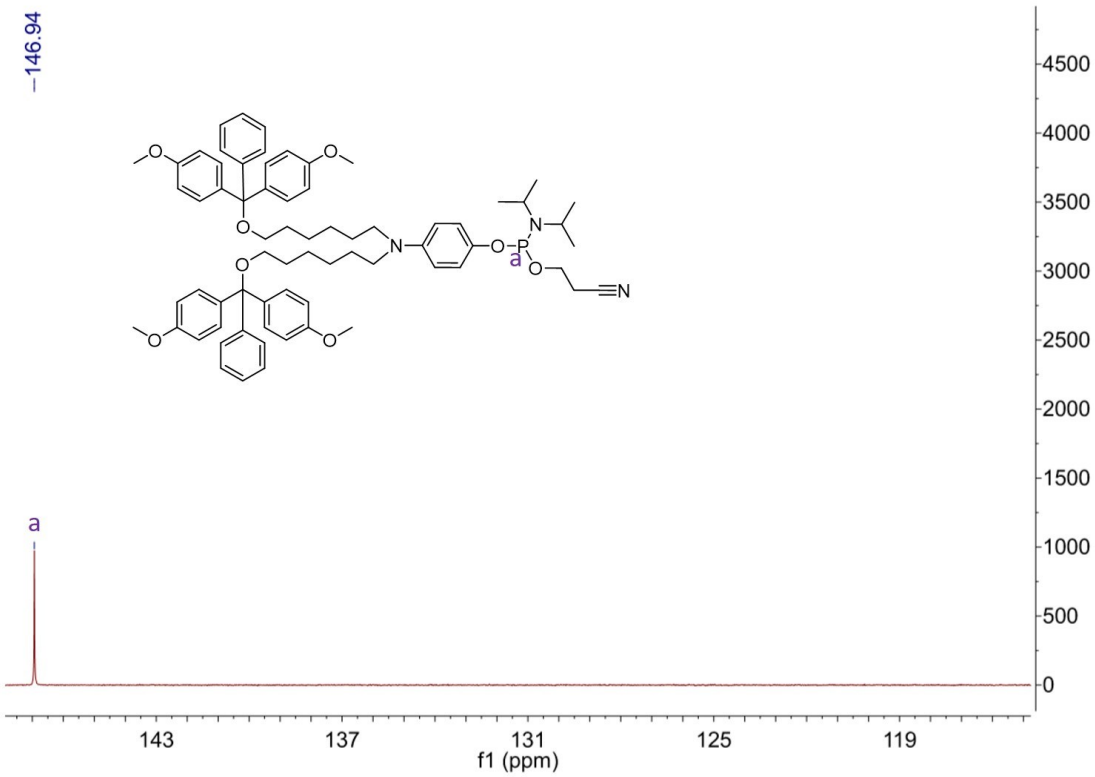

Figure S13 ^{31}P NMR spectra of linker phosphoramidite.

REFERENCES

1. C. Jin, J. He, J. Zou, W. Xuan, T. Fu, R. Wang, W. Tan, Phosphorylated Lipid-Conjugated Oligonucleotide Selectively Anchors on Cell Membranes with High Alkaline Phosphatase Expression. Nature communications 10 (2019) 2704.