Electronic Supplementary Material (ESI) for Chemical Communications.

DNA micelles as nanoreactors: efficient DNA functionalization with hydrophobic organic molecules

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Supporting Information

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SI-I General

Unless otherwise stated, all commercial reagents and solvents were used without additional purification. Magnesium sulfate hexahydrate ($MgSO_4 \cdot 6H_2O$), tris(hydroxymethyl)aminomethane (Tris), urea, palmitic acid *N*-hydroxysuccinimide (C_{16}-NHS), chloroform ($CHCl_3$), hexane (Hex), tetrahydrofurane (THF), dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), dichloromethane (DCM), ethyl acetate (EA), decanoic acid, docosahexaenoic acid, *N*-hydroxysuccinimide (NHS), *N*,*N*'-Dicyclohexylcarbodiimide (DCC), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and succinic anhydride were used as purchased from Sigma-Aldrich. Carboxy terminated polystyrene (PS-COOH) with M_n of 900 (PDI = 1.5) was purchased from Polymer Source. Acetic acid and boric acid were purchased from Fisher Scientific and used without further purification. GelRed™ nucleic acid stain was purchased from Biotium Inc. Acetone ACS reagent grade was purchased from Fisher. Acrylamide/Bis-acrylamide (40% 19:1 solution), ammonium persulfate and tetramethylenediamine were obtained from Bioshop Canada Inc. and used as supplied. 1 μmol Universal 1000Å LCAACPG supports and standard reagents used for automated DNA synthesis were purchased through Bioautomation. Sephadex G-25 (super fine, DNA grade) was purchased from Glen Research. Analytical thin layer chromatography (TLC) was performed on TLC plates purchased from Sigma-Aldrich. TAMg buffer is composed of 45 mM Tris and 12.5 mM $MgCl_2.6H_2O$ with the pH adjusted to 8.0 using glacial acetic acid. TBE buffer is 90 mM Tris, 90 mM boric acid and 2 mM EDTA with a pH of 8.0. TEAA mobile phase is 50 mM triethylammonium acetate with the pH adjusted to 8.0 using glacial acetic acid.

SI-II Instrumentation

Standard automated oligonucleotide solid-phase synthesis was performed on a Mermade MM6 Synthesizer from Bioautomation. HPLC purification was carried out on an Agilent Infinity 1260. DNA quantification measurements were performed by UV absorbance with a NanoDrop Lite spectrophotometer from Thermo Scientific. Gel electrophoresis experiments were carried out on a 20 X 20 cm vertical Hoefer 600 electrophoresis unit. Gel images were captured using a ChemiDocTM MP System from Bio-Rad Laboratories. Thermal annealing of all DNA micelles was conducted using an Eppendorf Mastercycler® 96 well thermocycler. Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI-MS) was carried out using Dionex Ultimate 3000 coupled to a Bruker MaXis Impact™ QTOF. Column chromatography to purity organic compounds was performed on a CombiFlash® R_f^+ system with RediSep® Silica columns (230-400 mesh) using a proper eluent system. ^{1}H NMR was recorded on 500 MHz AV500 equipped with a 60 position SampleXpress sample changer (Bruker) and 300 MHz Varian Mercury equipped with an SMS-100 sample changer (Agilent). DynaPro (model MS) molecular-sizing instrument was used to measure the particle size distributions. Visualization of TLC was achieved by UV light (254 nm). Chemical shifts were quoted in parts per million (ppM) referenced to the appropriate residual solvent peak or 0.0 ppm for tetramethylsilane. Abbreviations for ^{1}H NMR: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet. High-resolution mass spectra were obtained from Exactive Plus Orbitrap Mass Spectrometer (Thermo Scientific)**.**

SI-III Synthesis, Purification and Characterization of DNA strands

IIIa. Solid-phase synthesis

DNA synthesis was performed on a 1 μmole scale, starting from the required nucleotide modified 1000 Å LCAA-CPG solid-support. Coupling efficiency was monitored after removal of the dimethoxytrityl (DMT) 5-OH protecting groups. DMT-dodecane-diol (cat.# CLP-1114), Fmoc-Amino-DMT C-3 CED phosphoramidites (cat.# CLP-1661) were purchased from ChemGenes. MMT protected 5'-amino-modifier C6 (cat.# 10-1906-90) and TFA protected 5'-amino-modifier C3 (cat# 10-1923-90) were purchased from Glen Research. Coupling efficiency was monitored by the removal of DMT group on 5'-OH groups. In a glove box under nitrogen atmosphere, DMT-dodecane-diol and Fmoc-Amino-DMT C-3 CED were dissolved in acetonitrile and shaken for 10 mins to achieve final concentration of 0.1 M. The DMT-dodecane-diol amidite was activated with 0.25M 5-(ethylthio)tetrazole in anhydrous acetonitrile and the extended coupling times of 5 minutes were used. The amino modifier amidite was activated by 0.25M 5-(ethylthio)tetrazole in anhydrous acetonitrile but the coupling was performed manually inside the glove box. 3% dichloroacetic acid in dichloromethane was used to remove DMT protecting group on the DNA synthesizer. After the synthesis was completed, CPG was treated with 28% aqueous ammonium hydroxide solution for 16-18 hours at 60^{o}C in water bath. The crude mixture then was concentrated under reduced pressure at 60^{o}C and filtered by 0.22μm centrifugal filter before purifying by RP-HPLC.

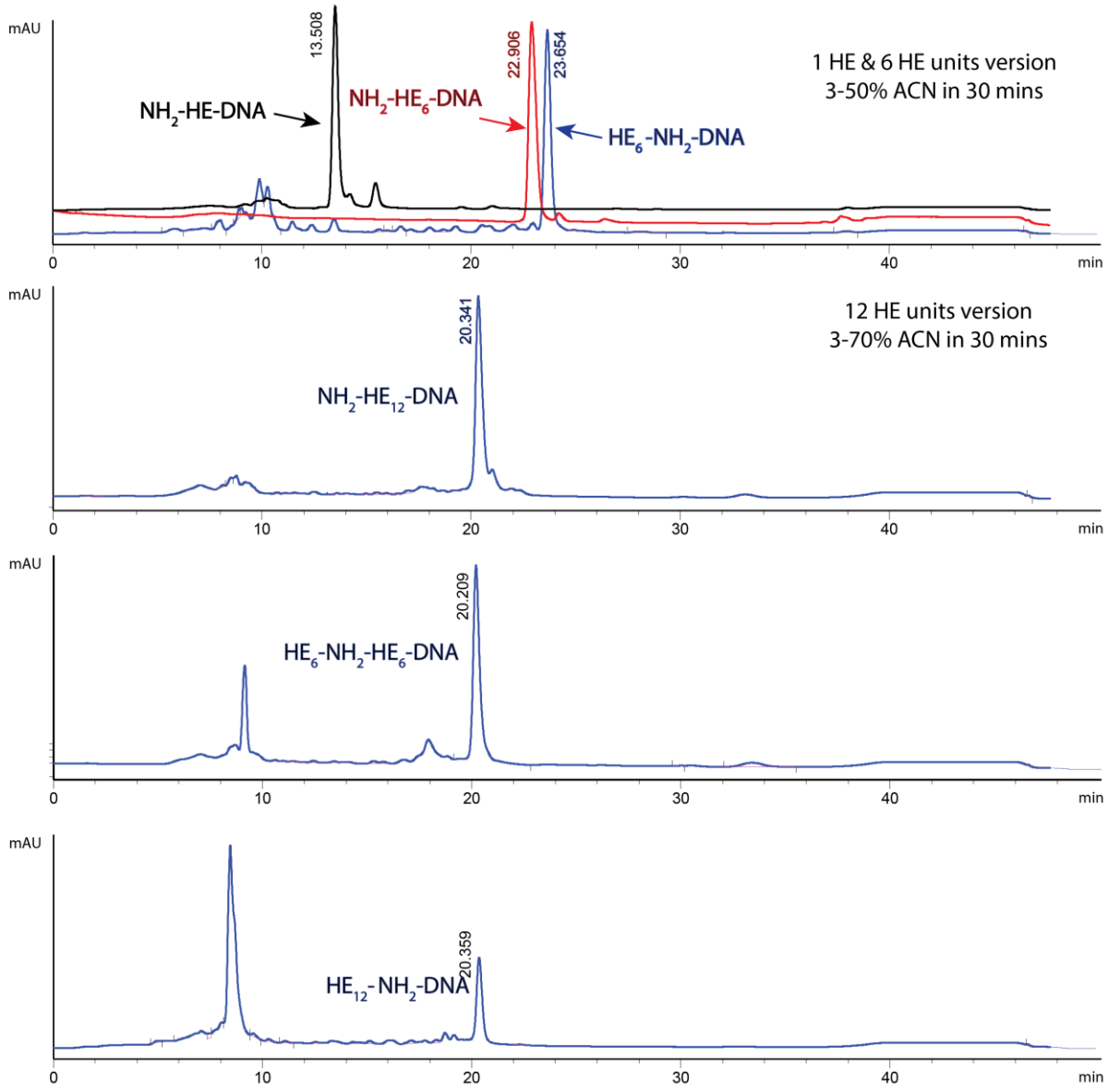
Supporting Table ST1 | DNA amphiphiles used for reactions inside the micellar core (D = dodecane diol, **NH_2** = Amino C-3 CED).

Molecules	Sequences (from 5' to 3')
NH_2-DNA	**NH_2**-TTTTTCAGTTGACCATATA
NH_2-HE-DNA	**NH_2-D** TTTTTCAGTTGACCATATA
NH_2-HE_{12}-DNA	**NH_2-DDDDDDDDDDDD** TTTTTCAGTTGACCATATA
HE_{12}-NH_2-DNA	**DDDDDDDDDDDDNH_2**-TTTTTCAGTTGACCATATA
HE_6-NH_2-HE_6-DNA	**DDDDDD-NH_2-DDDDDD** TTTTTCAGTTGACCATATA
NH_2-HE_6-DNA	**NH_2-DDDDDD** TTTTTCAGTTGACCATATA
HE_6-NH_2-DNA	**DDDDDD-NH_2-** TTTTTCAGTTGACCATATA
(DNA)'-HE_{12}	TATATGGTCAACTGAAAAA **DDDDDDDDDDDD**
(DNA)'-HE_6	TATATGGTCAACTGAAAAA **DDDDDD**

IIIb. HPLC purification

All DNA strands with amino-modified monomer (except (DNA)'-HE_6 and (DNA)'-HE_{12}) were purified by RP-HPLC. Two mobile phases were TEAA and HPLC grade acetonitrile. Elution gradient used: amphiphiles with 12 HE units (3-70% acetonitrile over 30 minutes at 60^{o}C and with 0-6 HE units (3-50% acetonitrile over 30 minutes at 60^{o}C). Column used: Hamilton PRP 1 5 µm 2.1x150mm**.** Crude DNA amphiphiles (~0.5 OD) was injected as a 20-50µL solution in Millipore water and then detected using a diode array detector monitoring absorbance at 260nm.

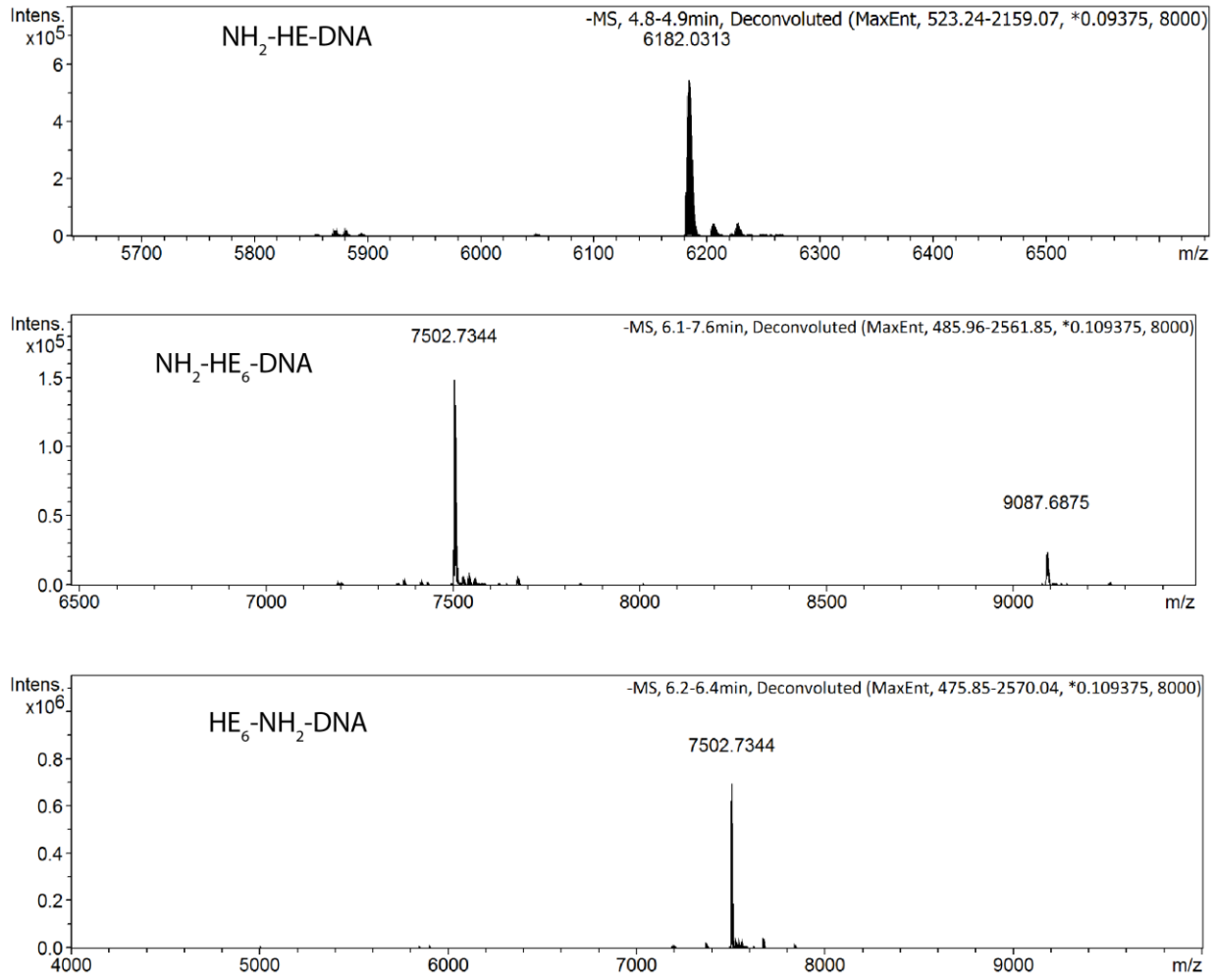
IIIc. HPLC traces of crude products

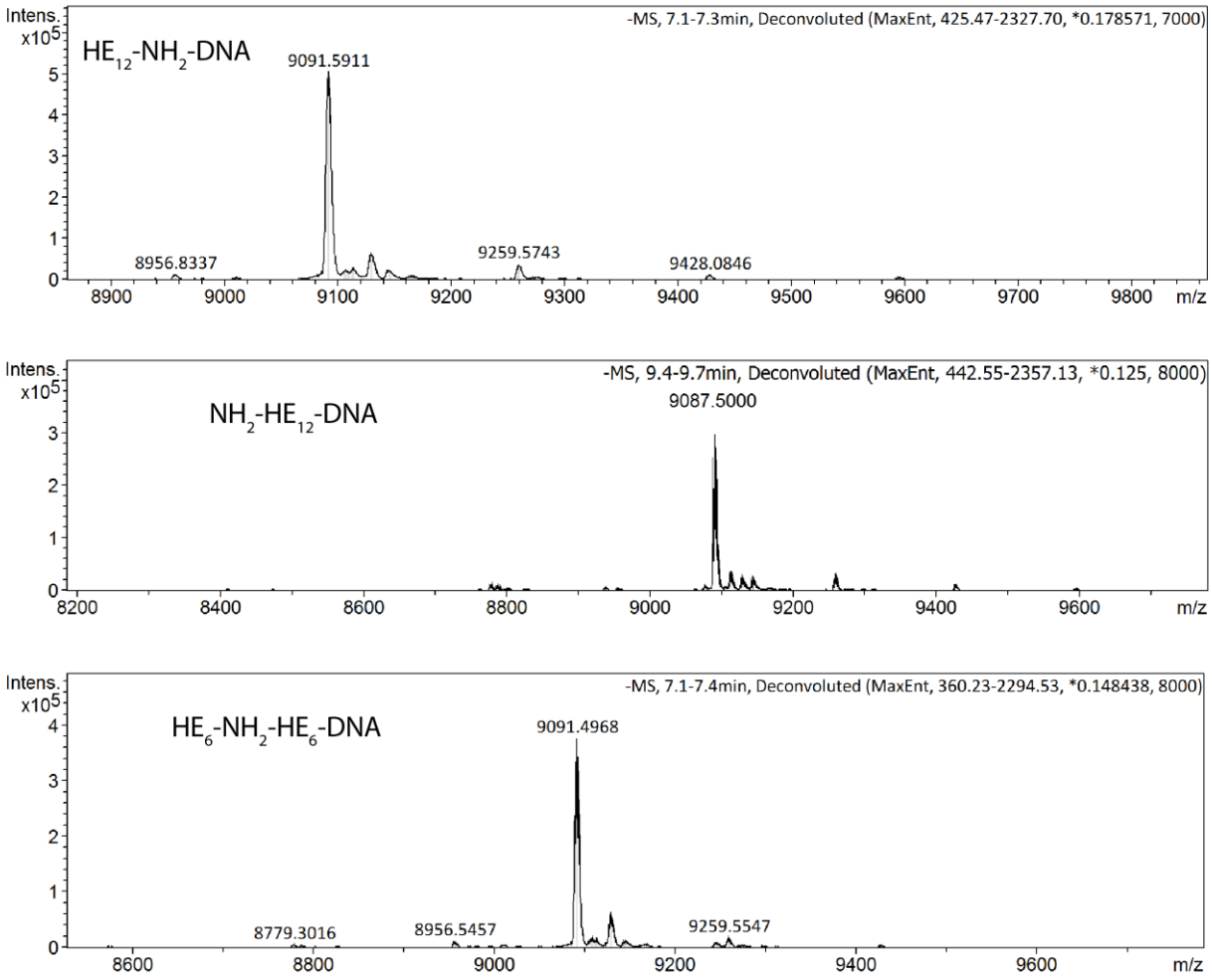


Supporting Figure SF1 | HPLC traces of crude products of the DNA amphiphiles

IIId. LC-MS characterization of DNA amphiphiles

The oligonucleotides were analyzed by LC-ESI-MS in negative ESI mode. Samples were run through an acclaim RSLC 120 C18 column (2.2µM 120Å 2.1 x 50mm) using a gradient of 98% mobile phase A (100mM 1,1,1,3,3,3-hexafluoro-2-propanol and 5mM triethylamine in water) and 2 % mobile phase B (Methanol) to 40 % mobile phase A and 60% mobile phase B in 8 minutes. The data was processed and deconvoluted using the Bruker DataAnalysis software version 4.1 (Supporting Figure SF2).

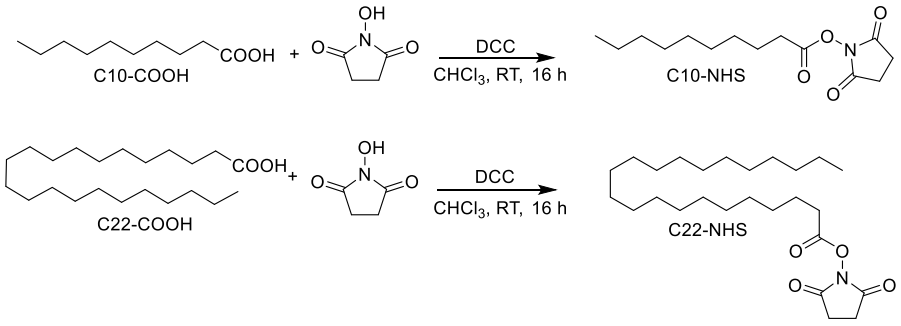




Supporting Figure SF2 | MS characterizations of HPLC purified DNA amphiphiles

SI-IV. Synthesis of activated NHS-ester molecules

IVa. Synthesis of decanoic acid- NHS ester (C_{10}-NHS) and docosahexaenoic acid (C_{22}-NHS)



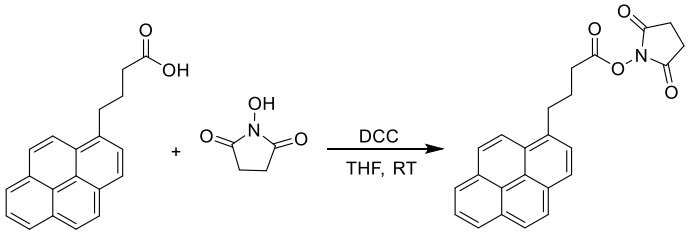
Synthesis of C_{10}-NHS: To a stirred solution of C_{10}-COOH (2 mmol) in $CHCl_3$ was added 2 mmol N-hydroxysuccinimide. Then the mixture was cooled down to 0^{o}C using water bath followed by adding DCC (4 mmol). After adding DCC, the water bath was removed, allowing the mixture to be back to room temperature (22^{o}C). The mixture was stirred overnight for 16 hours. After that, the crude mixture was checked by TLC before filtering to remove urea which formed as by-product, purified by CombiFlash (EA:Hex = 1:1) and concentrated under *vacuo* to give desired product as a white solid (C_{10}-NHS) with 80% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.85 (s, 4H), 2.54 (t, 2H), 1.54 (quint, 2H), 1.25 (m, 12H), 0.85 (t, 3H); HRMS EI m/z calculated for $C_{14}H_{23}NNaO_4$ $[M+Na]^+$: 292.1519, found: 292.1515.

Synthesis of C_{22}-NHS: Starting from C_{22}-COOH, the synthesis was performed similarly to the synthesis of C_{10}-NHS described above and a white solid was obtained as product with 70% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.82 (s, 4H), 2.59 (t, 2H), 1.70 (quint, 2H), 1.24 (m, 36H), 0.85 (t, 3H); HRMS EI m/z calculated for $C_{26}H_{47}NNaO_4$ $[M+Na]^+$: 460.3397, found: 460.3408.

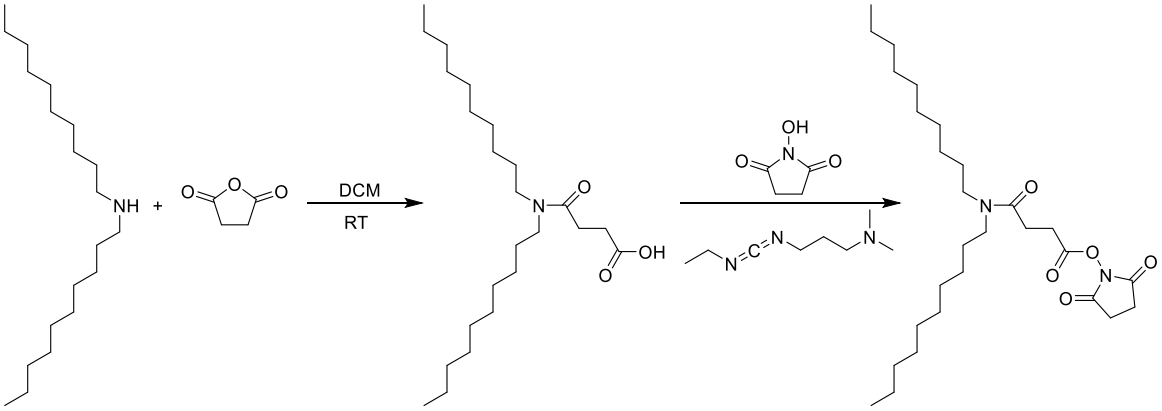
IVb. Synthesis of 1-pyrenebutyric acid-NHS ester (pyrene-NHS)



To a stirred solution of 1-pyrenebutyric acid and (1 mmol) in 25 mL THF was added 1 mmol N-hydroxysuccinimide. The mixture was cooled down to 0^{o}C using ice bath followed by dropwise addition of DCC (1 mmol, in 5 mL THF). The ice bath was then removed, allowing the mixture to be back to room temperature. The mixture was stirred overnight. After the reaction, the crude mixture was filtered to remove urea which formed as by-product. The yellow filtrate was collected and concentrated under reduced pressure. The product was purified by recrystallization from ethanol to give desired product as a yellow solid (pyrene-NHS) with 31% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88-8.51 (m, 9H), 3.49 (t, 2H), 2.88 (s, 4H), 2.74 (t, 2H), 2.31 (quint, 2H); HRMS EI m/z calculated for $C_{24}H_{19}NNaO_4$ $[M+Na]^+$: 408.1206, found: 408.1192; m/z calculated for $C_{24}H_{19}KNO_4$ $[M+K]^+$: 424.0946, found: 424.0930.

IVc. Synthesis of N,N-didecylsuccinamide NHS ester (NDS-NHS)

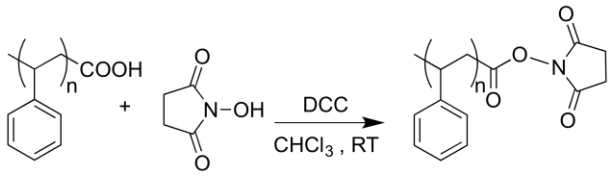


To a stirred solution of succinic anhydride in DCM, didecylamine **(A)** (4 mmol) and triethylamine (8 mmol) were added. The reaction mixture was stirred at 37ºC for 16 hours. Then, the crude mixture was added HCl 1M and extracted with diethyl ether followed by drying with $MgSO_4$ and concentrating under *vacuo* to obtain oily product **(B).**

The oily product **(B)** and N-hydroxysuccinimide were dissolved in $CHCl_3$. Then EDC was added to the mixture at 0ºC using ice bath. Removal of ice bath brought the mixture to room temperature and it was stirred for 16 hours. After that, the crude mixture was purified by CombiFlash using Hexane:Ethyl Acetate 1:1 and concentrated under *vacuo* to furnished transparent oily product with 25% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.21 (t, 4H), 2.85 (s, 4H), 3.01 (t, 2H), 2.74 (t, 2H), 1.26 (m, 28H), 0.85 (t, 6H). HRMS EI m/z calculated for $C_{28}H_{50}N_2NaO_5$ $[M+Na]^+$: 517.3612, found: 517.3627.

IVd. Synthesize polystyrene-NHS (PS-NHS)



The synthesis was performed similarly to the synthesis of C_{22}-NHS starting from PS-COOH (M_n = 900, PDI = 1.5). The crude mixture after 16 hours was precipitated using ethyl ether. The presence of singlet at 2.85 demonstrated forming of PS-NHS ester.

SI-V. General procedure for a conjugation reaction

Va. Single-stranded system

First, solution of DNA amphiphiles at 5μM concentration was prepared in 1x TAMg buffer. Then, the solution was thermally annealed (95 to 4^{o}C in 1 hour) in order to form micelles. Separately, 10 mM of chosen NHS ester molecule was prepared in organic solvent (DMSO or THF). Then, the reagent was added to micelles solution (1:10 ratio to total volume of micelle solution) and the mixture was shaken for 16 hours at room temperature. The crude mixture after reaction was dried and analyzed by RP-HPLC and LC-MS. Yield of conjugate reaction was calculated from the area under the curve ratio obtained from HPLC between the product peak and the sum of starting material and product peak.

Vb. Double-stranded system

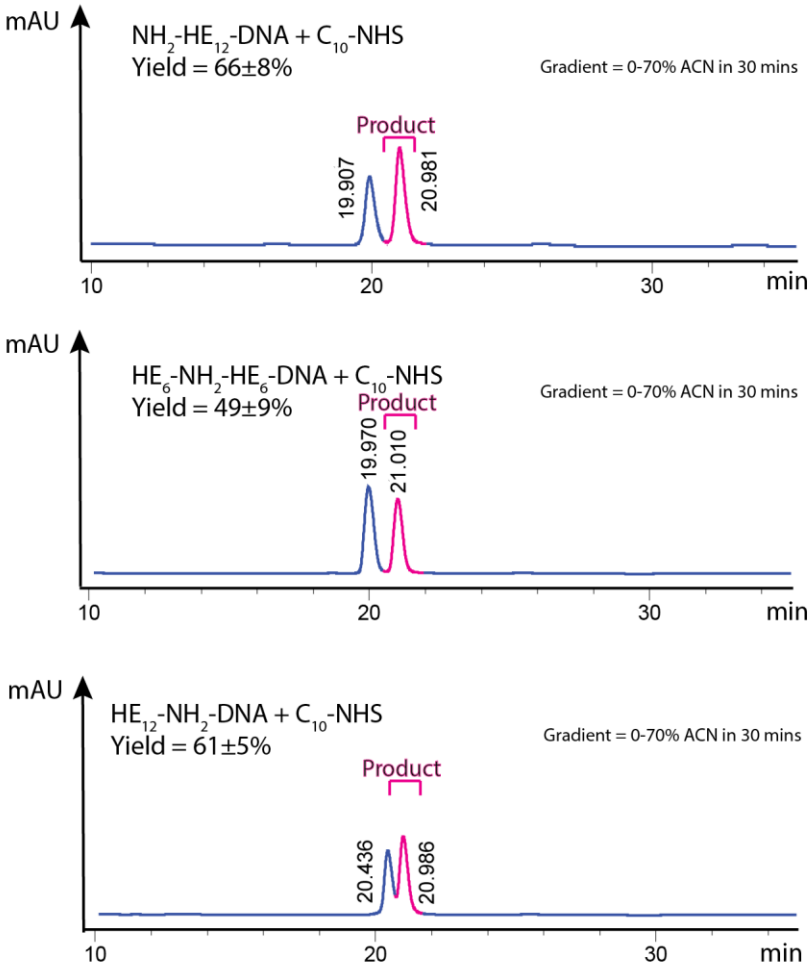
First, solution of DNA amphiphiles ((DNA)'-HE_6 or (DNA)'-HE_{12}) at 10μM concentration was prepared in 1x TAMg followed by thermally annealed (95 to 4^{o}C in 1 hour) in order to pre-form micelles. In another tube, solution of complementary strand with (DNA)' at 10μM in 1x TAMg and added to pre-form micelles. Separately, in a glass vial, 10 mM of chosen NHS ester molecule was prepared in organic solvent (DMSO or THF). The reagent was added to micelles solution (1:10 ratio to total volume of micelle solution) and the mixture was shaken for 16 hours at room temperature. The crude mixture after reaction was dried and analyzed by RP-HPLC and LC-MS as single-stranded system. Yield of conjugate reaction was calculated from the area under the curve ratio obtained from HPLC between the product peak and the sum of starting material and product peak.

SI-VI. HPLC analyses of conjugate reactions between DNA amphiphiles and NHS-ester molecules

VIa. Reaction of DNA amphiphiles with C_{10}-NHS

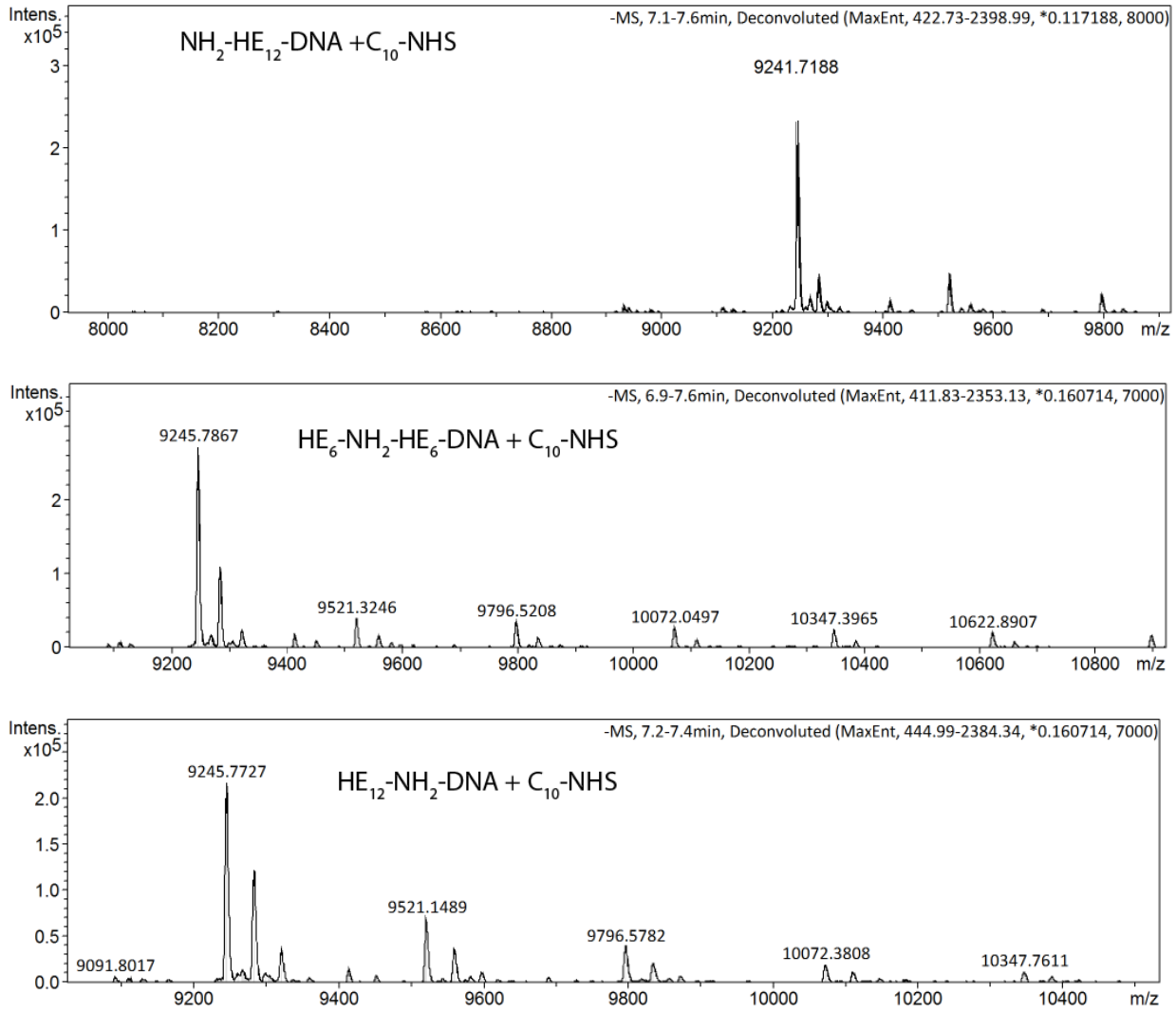
1. **Set 1: 12 HE units**

1.1. HPLC traces



Supporting Figure SF3 | HPLC traces of crude products of the DNA amphiphiles containing 12 HE units with C_{10}-NHS

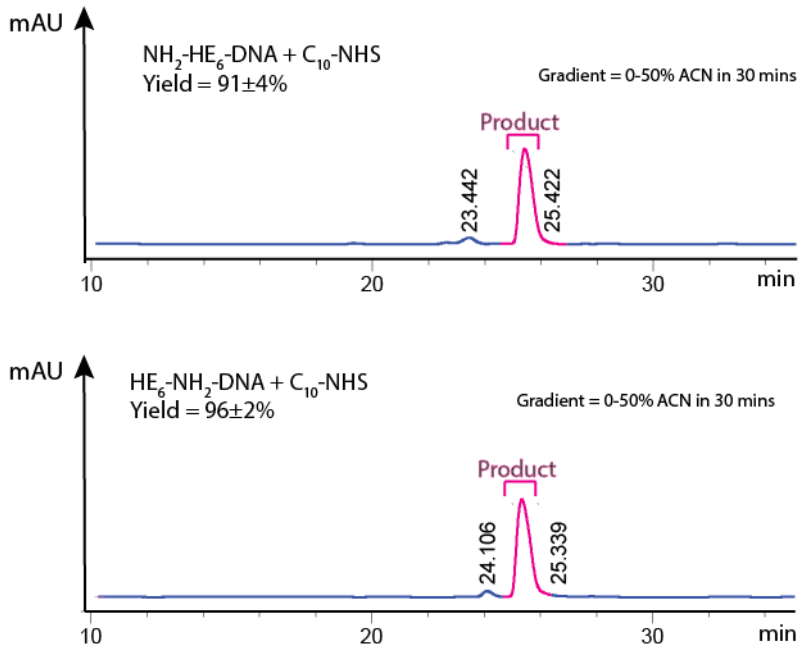
1.2.LC-MS characterization



Supporting Figure SF4 | MS characterizations of HPLC purified DNA amphiphiles containing 12 HE units conjugated with C_{10}-NHS

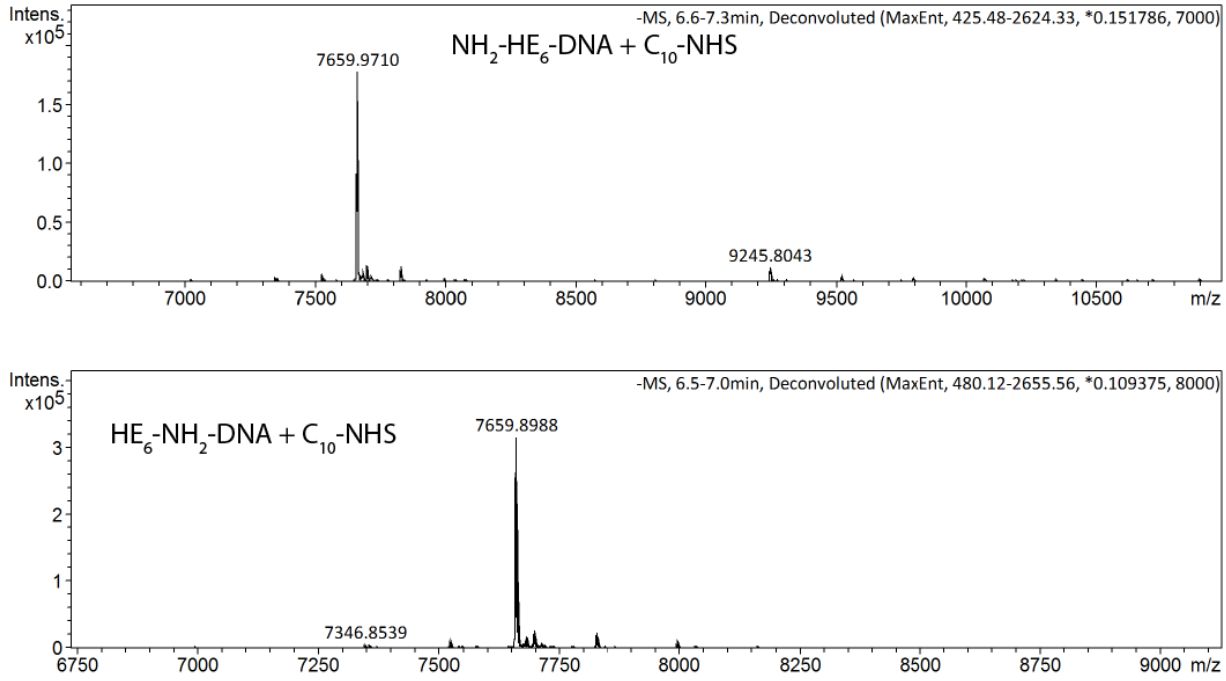
2. Set 2: 6 HE units

2.1.HPLC traces



Supporting Figure SF5 | HPLC traces of crude products of the DNA amphiphiles containing 6 HE units with C_{10}-NHS

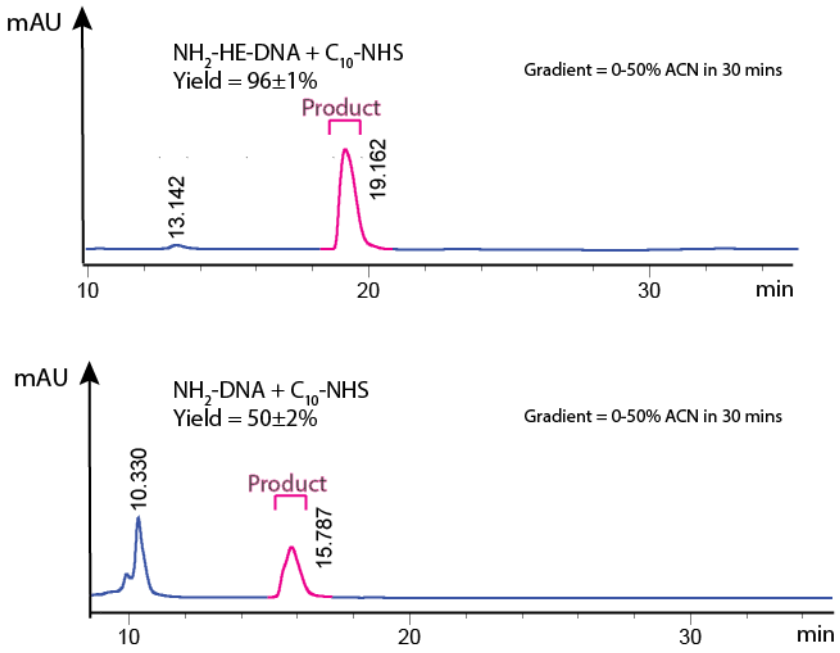
2.2.LC-MS characterization



Supporting Figure SF6 | MS characterizations of HPLC purified DNA amphiphiles containing 6 HE units conjugated with C_{10}-NHS

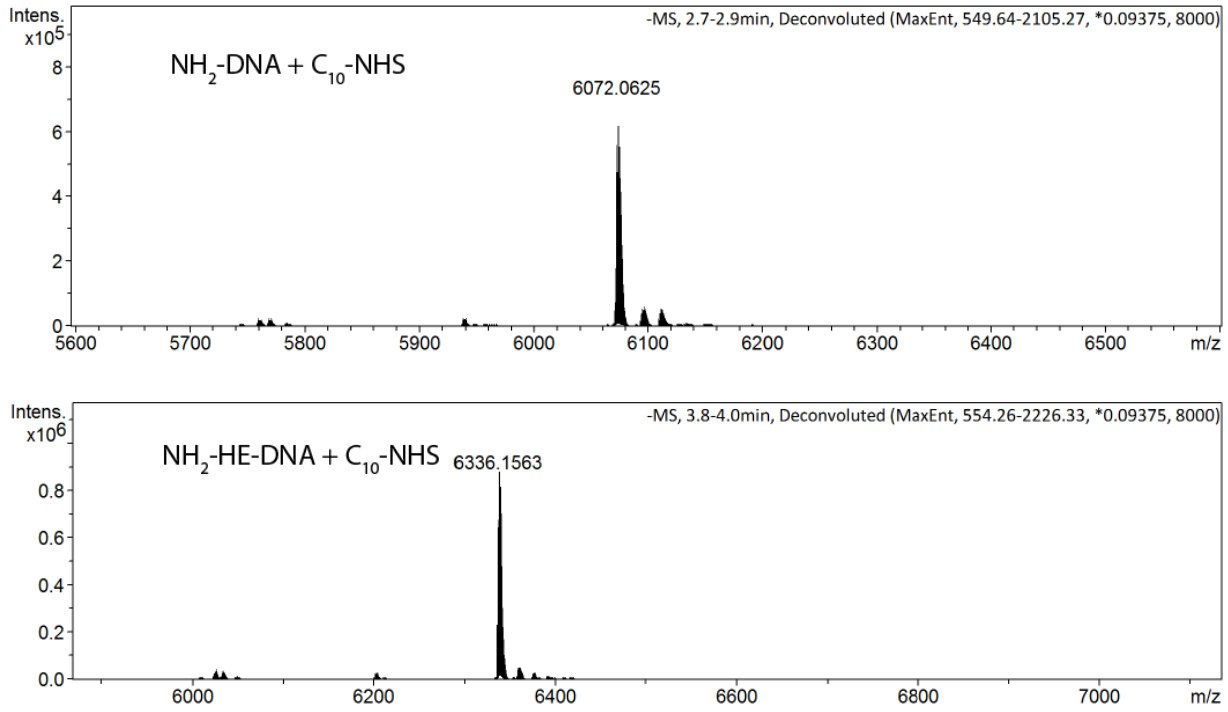
3. Set 3: 0-1 HE units (Non-micelle forming-control experiment)

3.1. HPLC traces



Supporting Figure SF7 | HPLC traces of crude products of the DNA amphiphiles with C_{10}-NHS

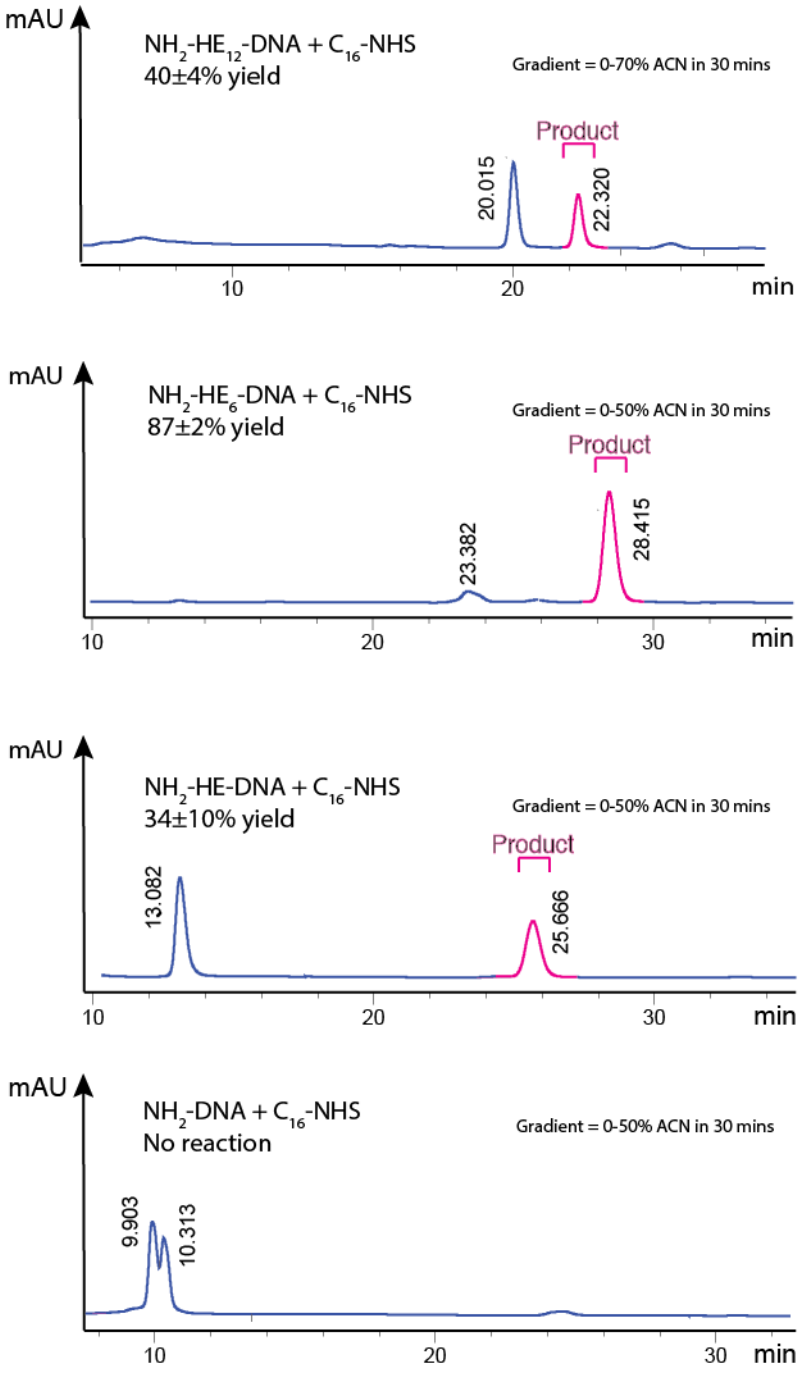
3.2. LC-MS characterization



Supporting Figure SF8 | MS characterizations of HPLC purified DNA amphiphiles conjugated with C_{10}-NHS

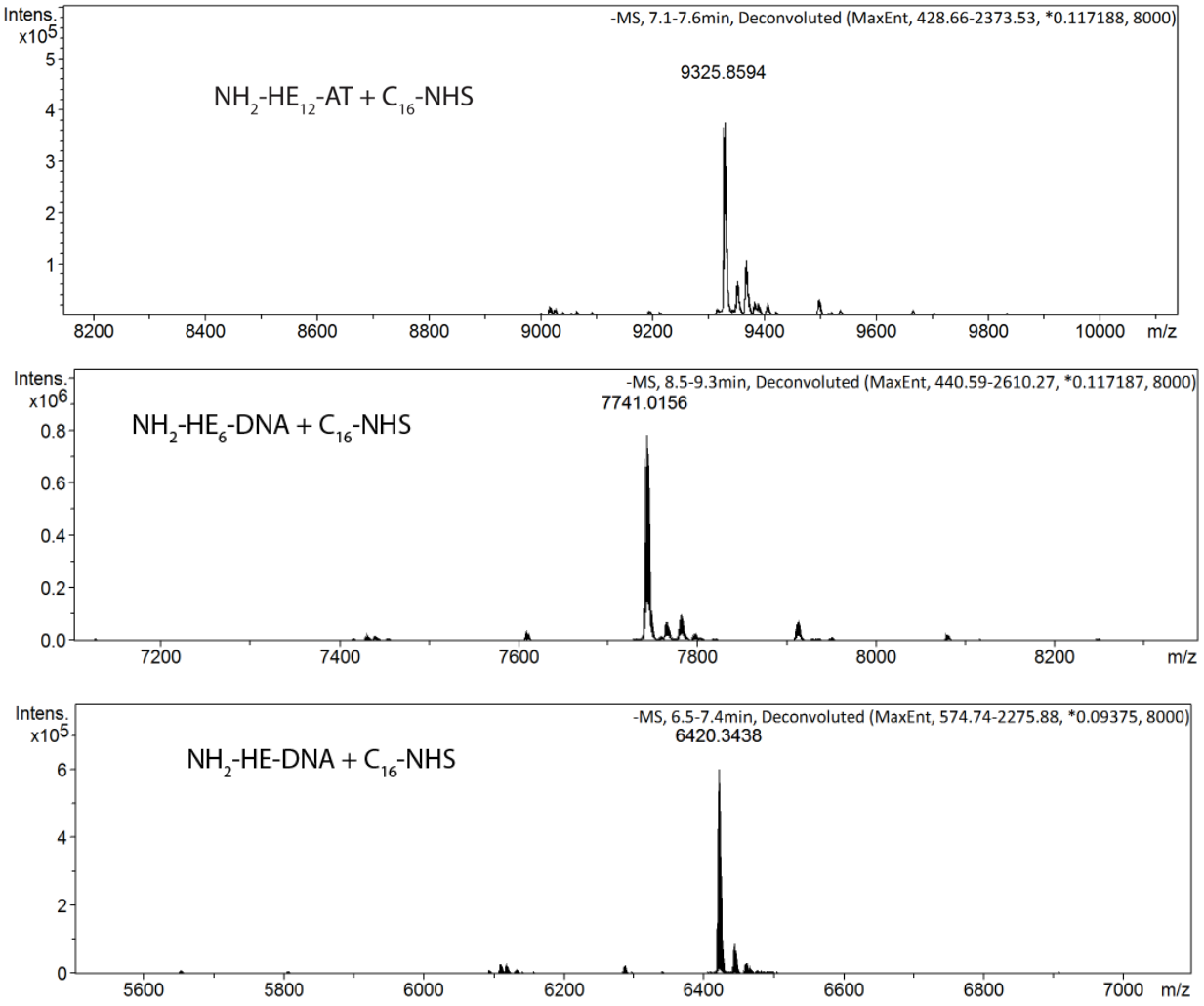
VIb. Reaction of DNA amphiphiles with C_{16}-NHS

1. HPLC traces



Supporting Figure SF9 | HPLC traces of crude products of the DNA amphiphiles with C_{16}-NHS

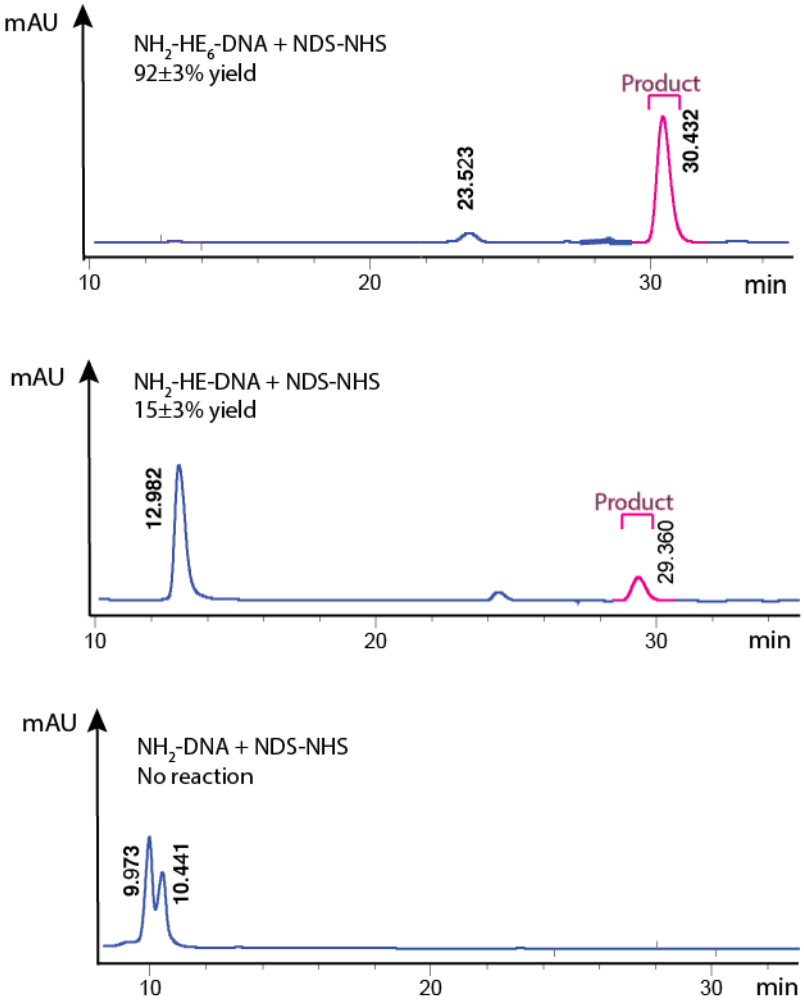
2. LC-MS characterization



Supporting Figure SF10 | MS characterizations of HPLC purified DNA amphiphiles conjugated with C_{16}-NHS

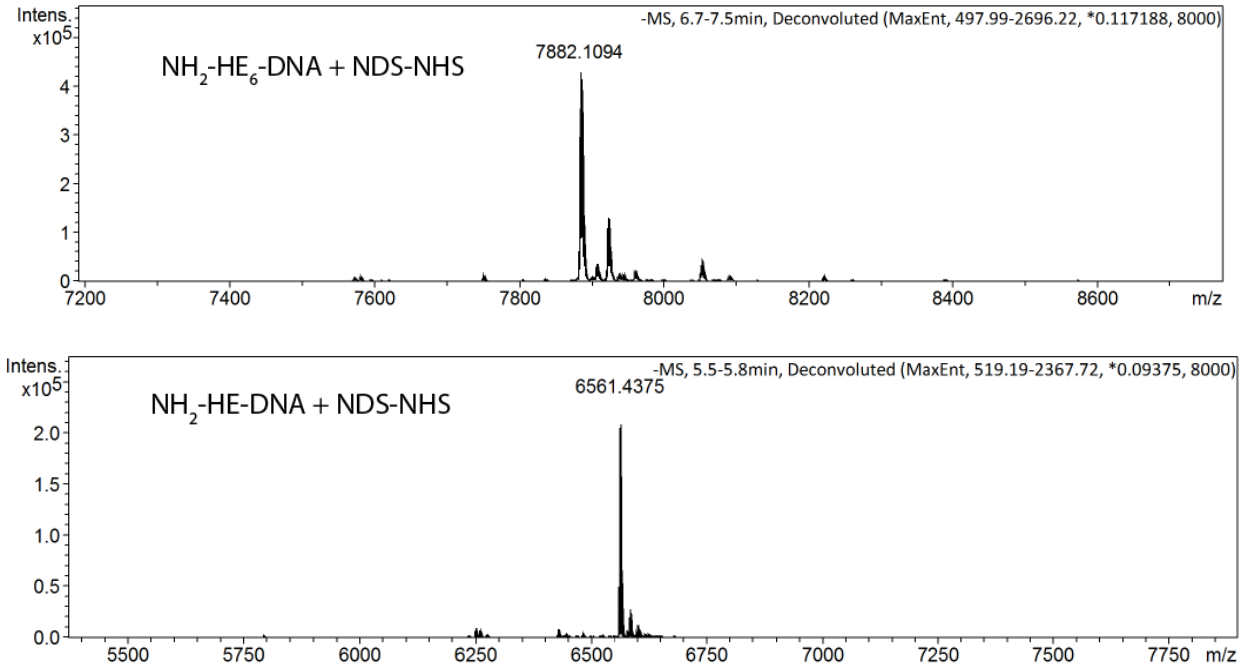
VIc. Reaction of DNA amphiphiles with (C_{20}) NDS-NHS

1. HPLC traces



Supporting Figure SF11 | HPLC traces of crude products of the DNA amphiphiles with NDS-NHS

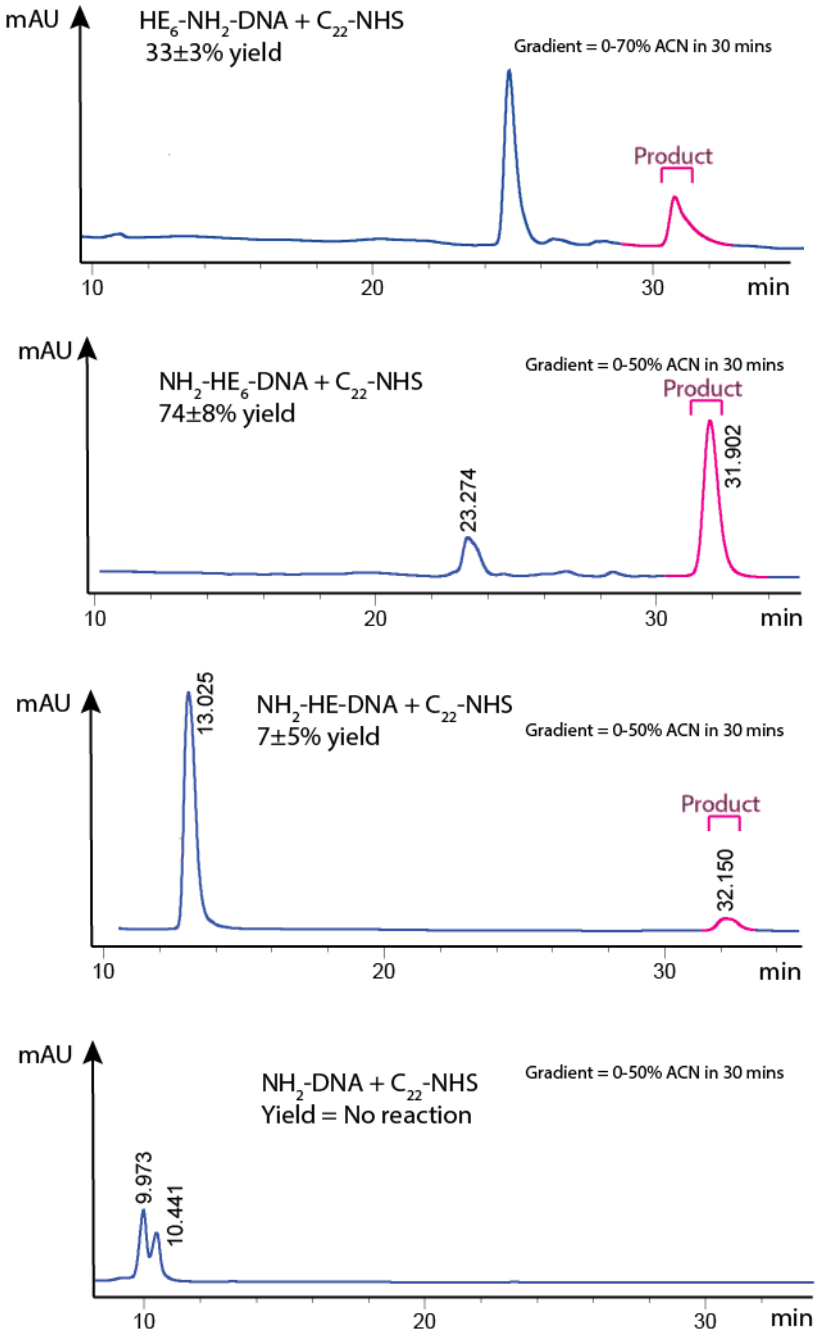
2. LC-MS characterization

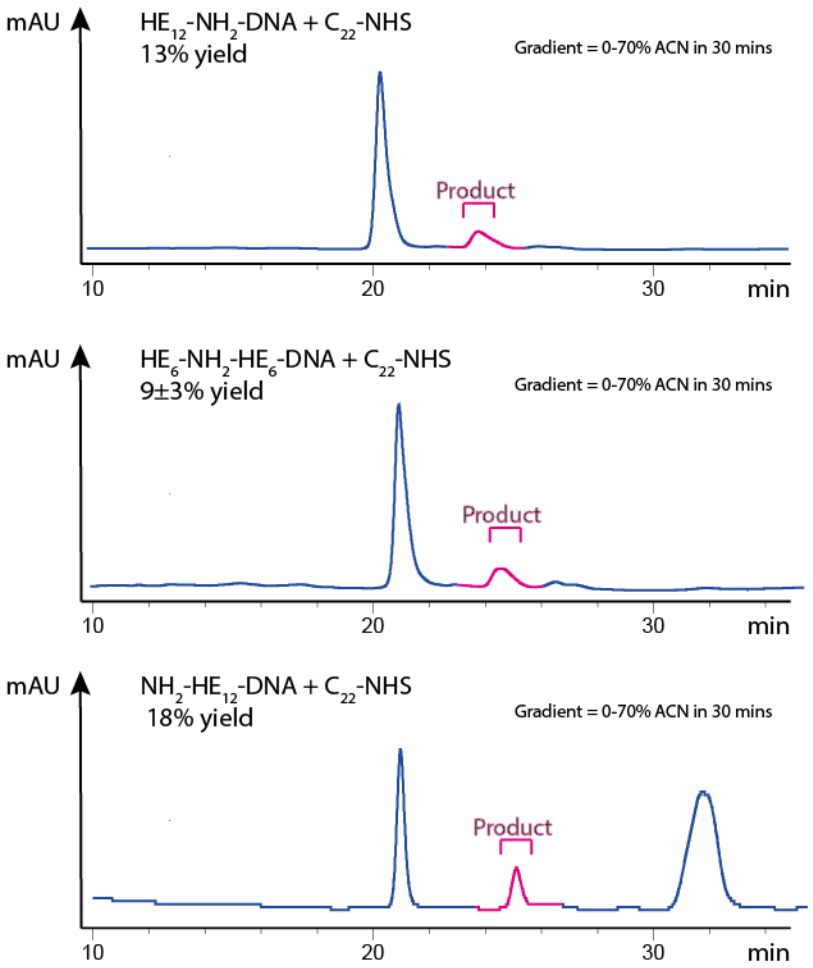


Supporting Figure SF12 | MS characterizations of HPLC purified DNA amphiphiles conjugated with NDS-NHS

VId. Reaction of DNA amphiphiles with C_{22}-NHS

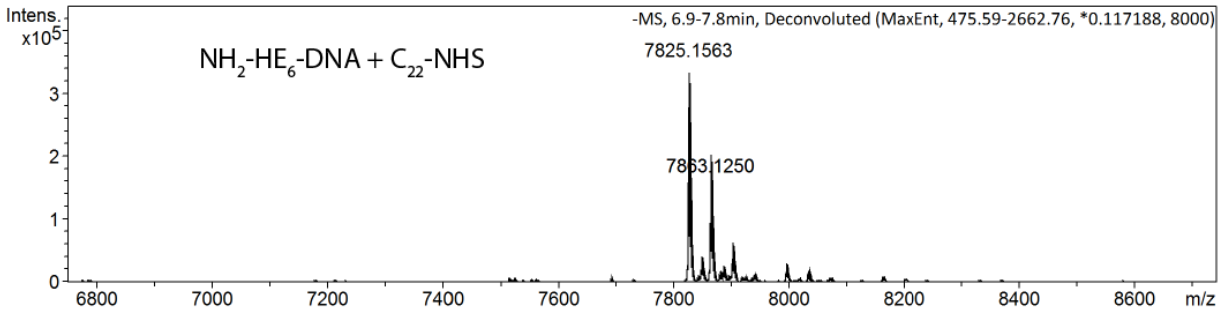
1. HPLC traces



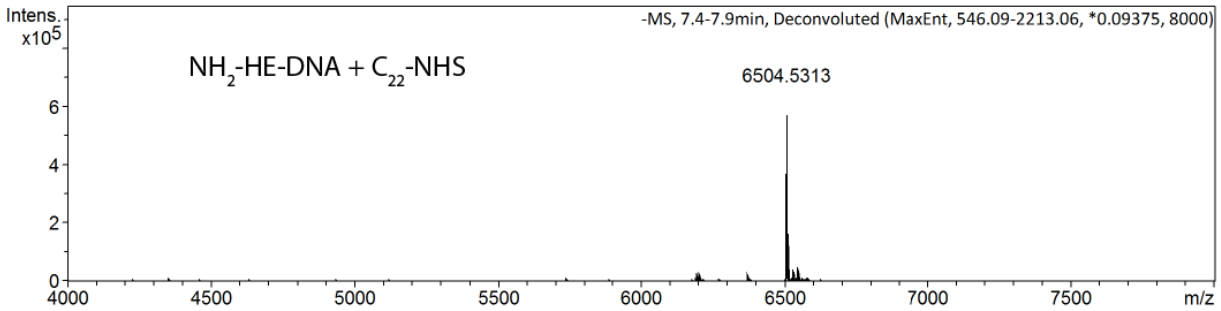


Supporting Figure SF13 | HPLC traces of crude products of the DNA amphiphiles with C_{22}-NHS

2. LC-MS characterization



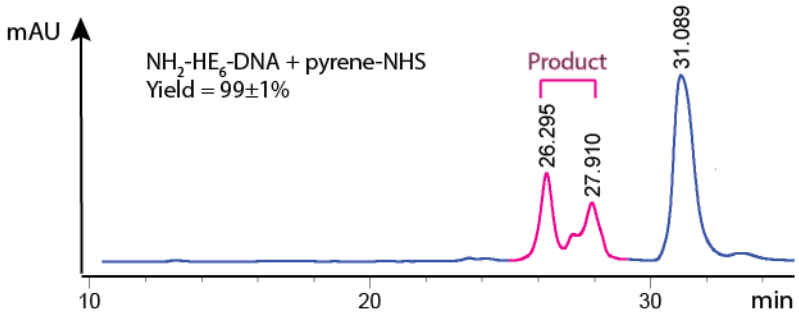
The mass 7863.1250 in this case is $[M+K]^+$



Supporting Figure SF14 | MS characterizations of HPLC purified DNA amphiphiles conjugated with C_{22}-NHS

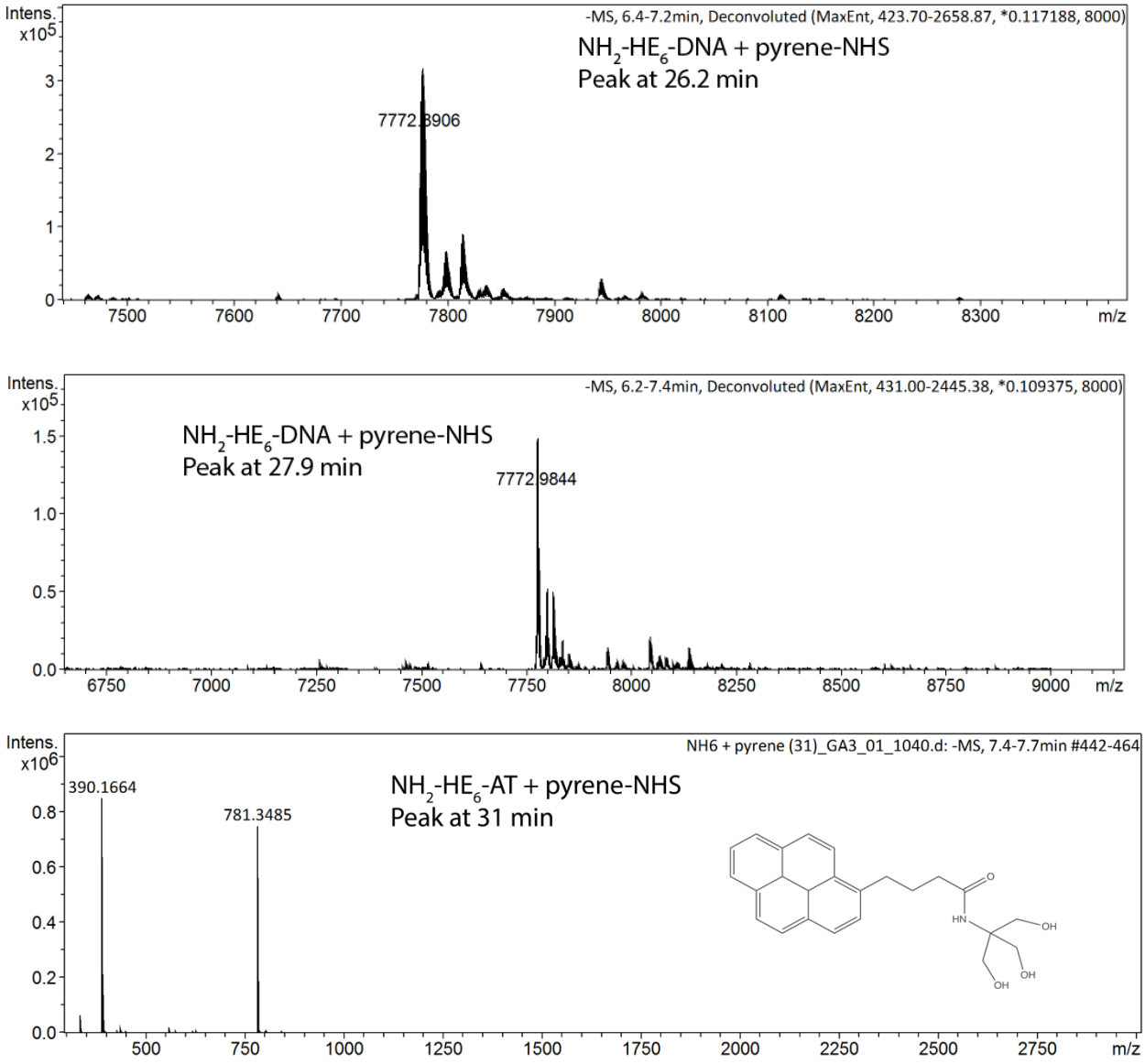
VIe. Reaction of NH_2-HE_6-AT with Pyrene-NHS

1. HPLC traces



Supporting Figure SF15 | HPLC trace of crude product of the DNA amphiphiles with pyrene-NHS

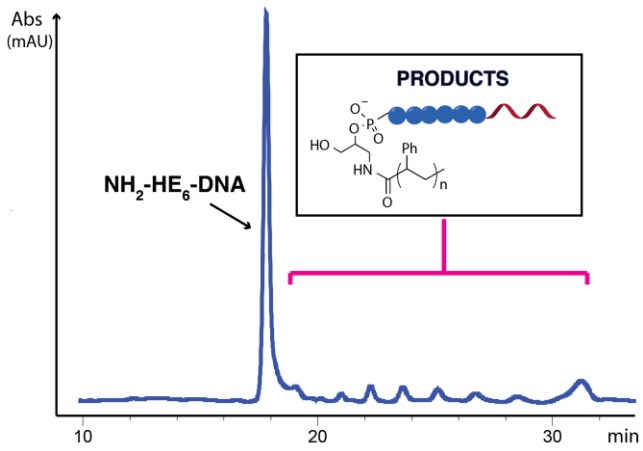
2. LC-MS characterization



Supporting Figure SF16 | MS characterizations of HPLC purified DNA amphiphiles conjugated with pyrene-NHS

VIf. Reaction of NH_2-HE_6-DNA with Polystyrene-NHS

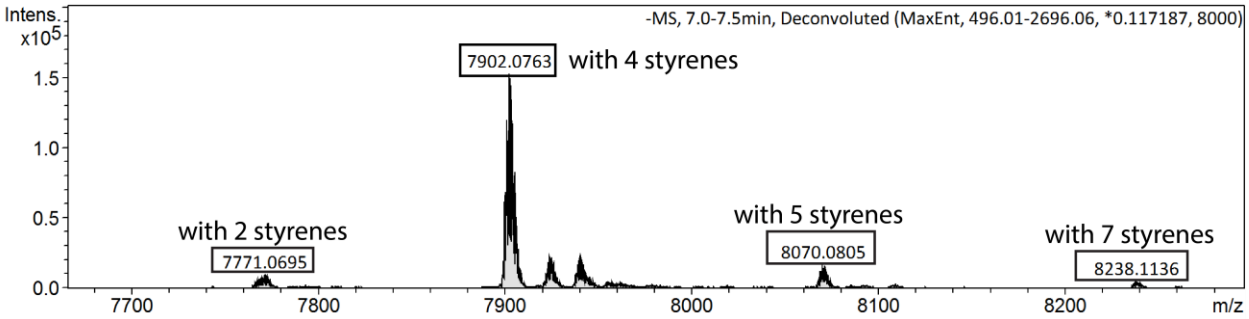
1. HPLC characterization



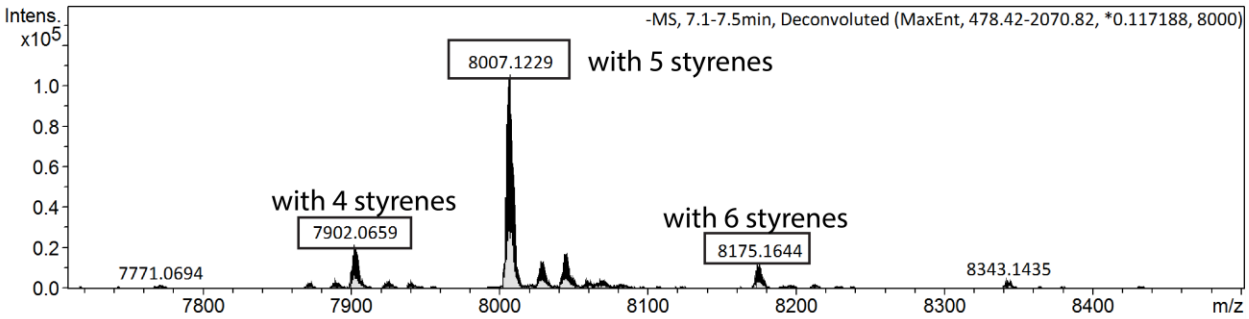
2. LC-MS characterization

The mass spectra results presented here indicated masses of NH_2-HE_6-DNA with polystyrene with different numbers of styrene monomers

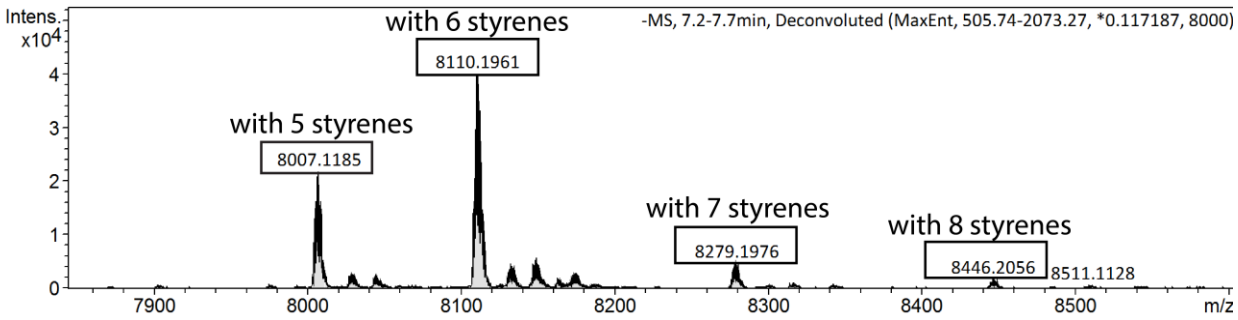
2.1. Peak at 22 min



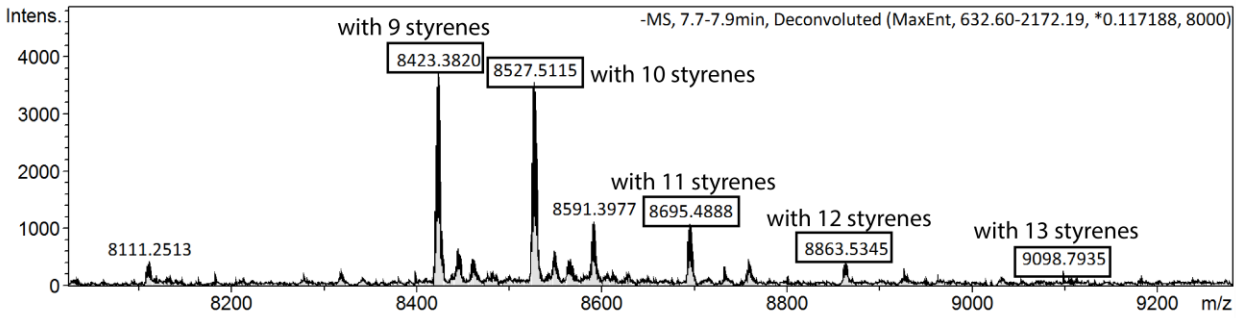
2.2. Peak at 23 min



2.3. Peak at 24 min



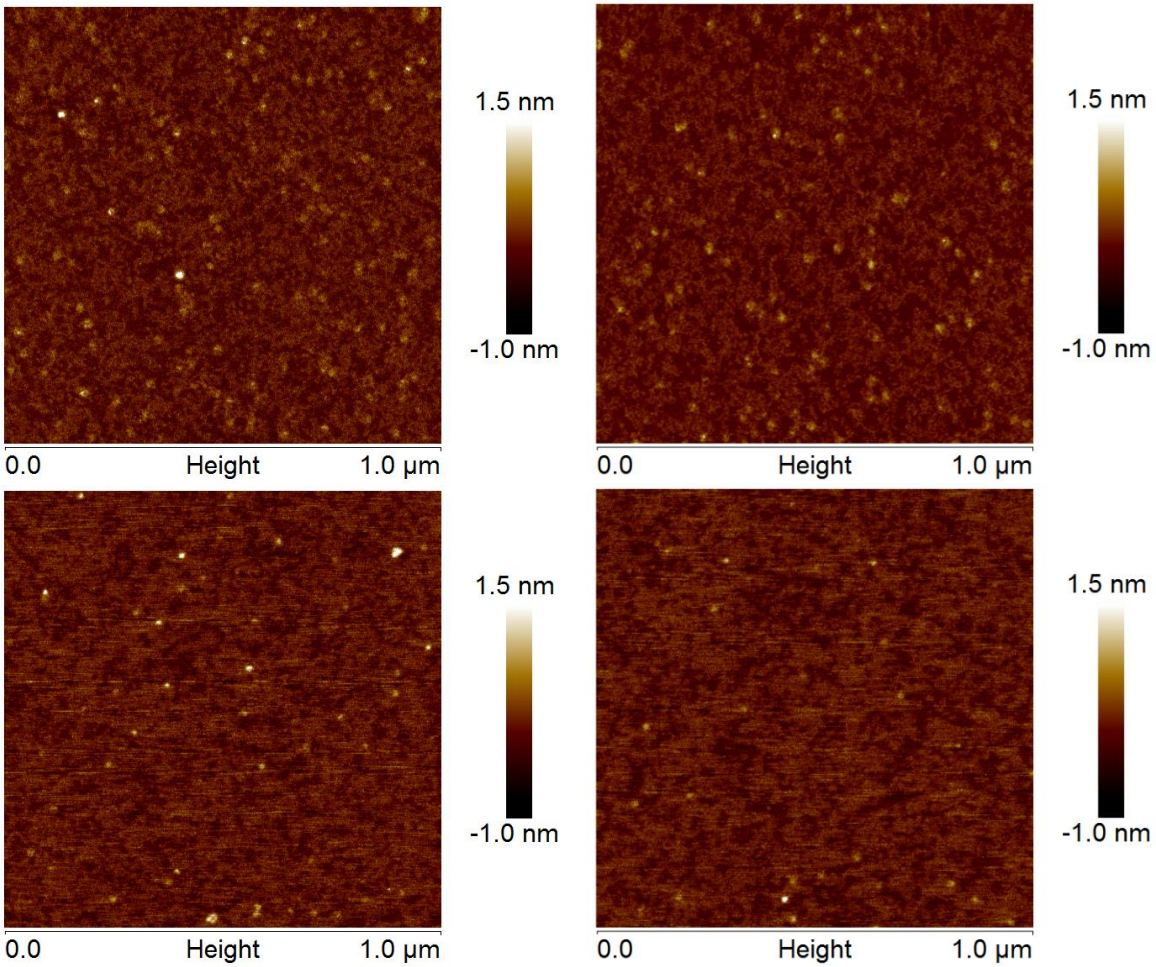
2.4.Peak at 31 min



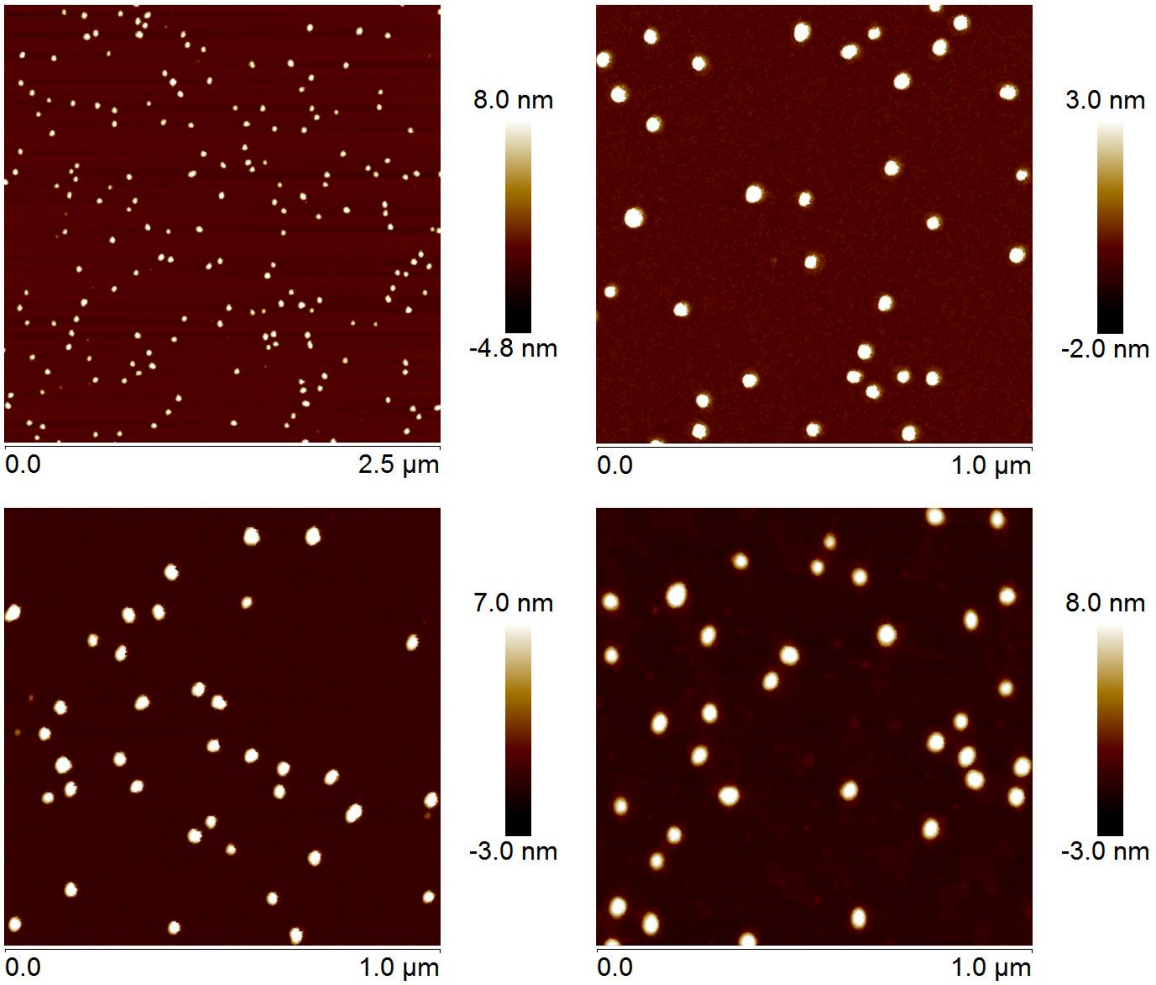
Supporting Figure SF17 | MS characterizations of HPLC purified NH_2-HE_6-DNA conjugated with Polystyrene-NHS

SI-VII AFM measurements of DNA micelles

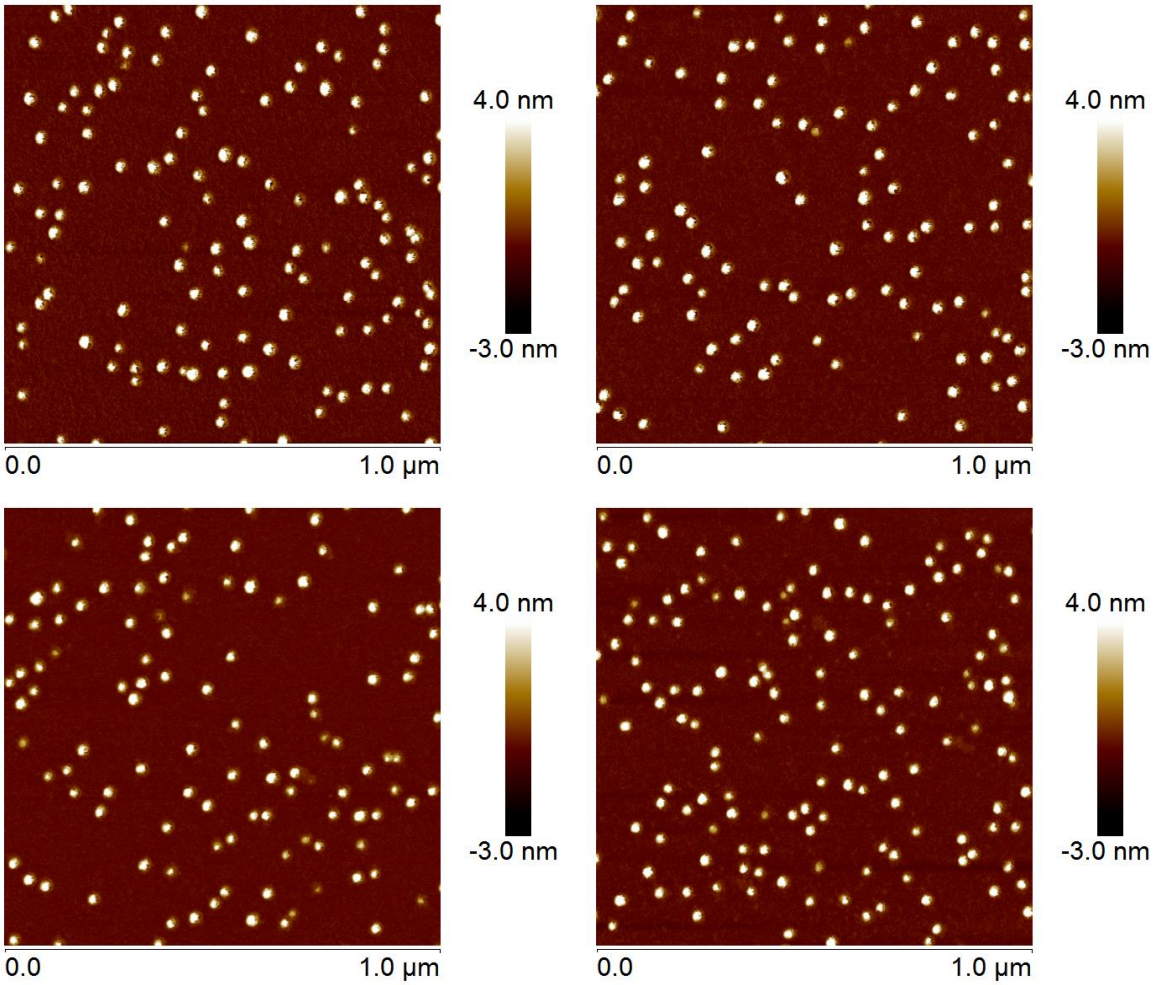
5 μM DNA amphiphiles in 1xTAMg was annealed from 95ºC to 4ºC for 1 hour. The sample was diluted with 1x TAMg to 1.67 μM. Then, 5 μL of sample was deposited on freshly cleaved mica for 5 seconds, and washed three times with 50 μL of H_2O. Excess liquid was brown off by the stream of nitrogen for 30 seconds. The sample was then dried under vacuum for at least 20 minutes prior to imaging. Measurement was acquired in ScanAsyst mode under dry condition using ScanAsyst-Air triangular silicon nitride probe (tip radius = 2 nm, k = 0.4 N/m, f_o = 70kHz; Bruker, Camarillo, CA).



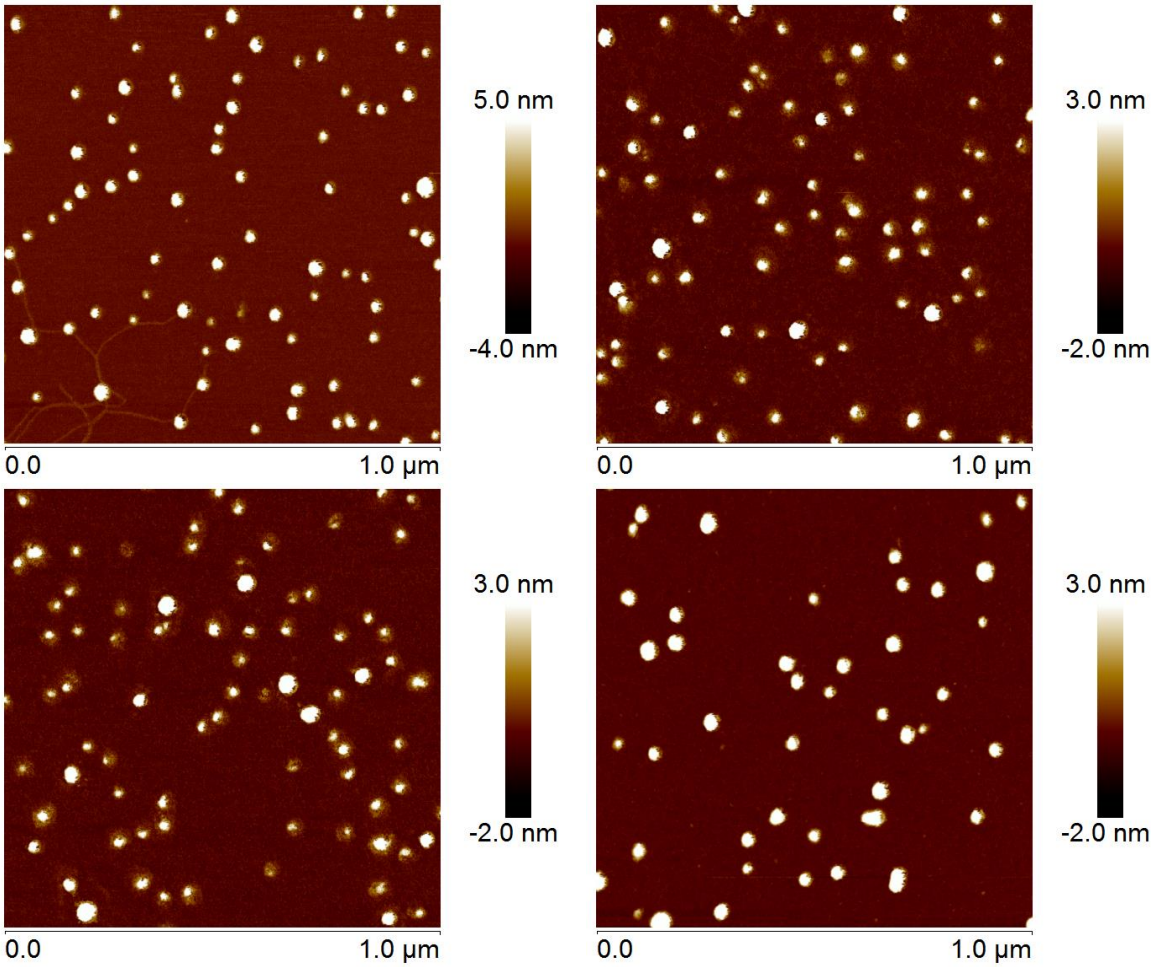
Supporting Figure SF18 | AFM images of NH_2-HE_6-DNA micelles. Spherical structures with the size of 15.5±3.4 nm in diameter were observed. The average height was 0.9±0.3 nm.



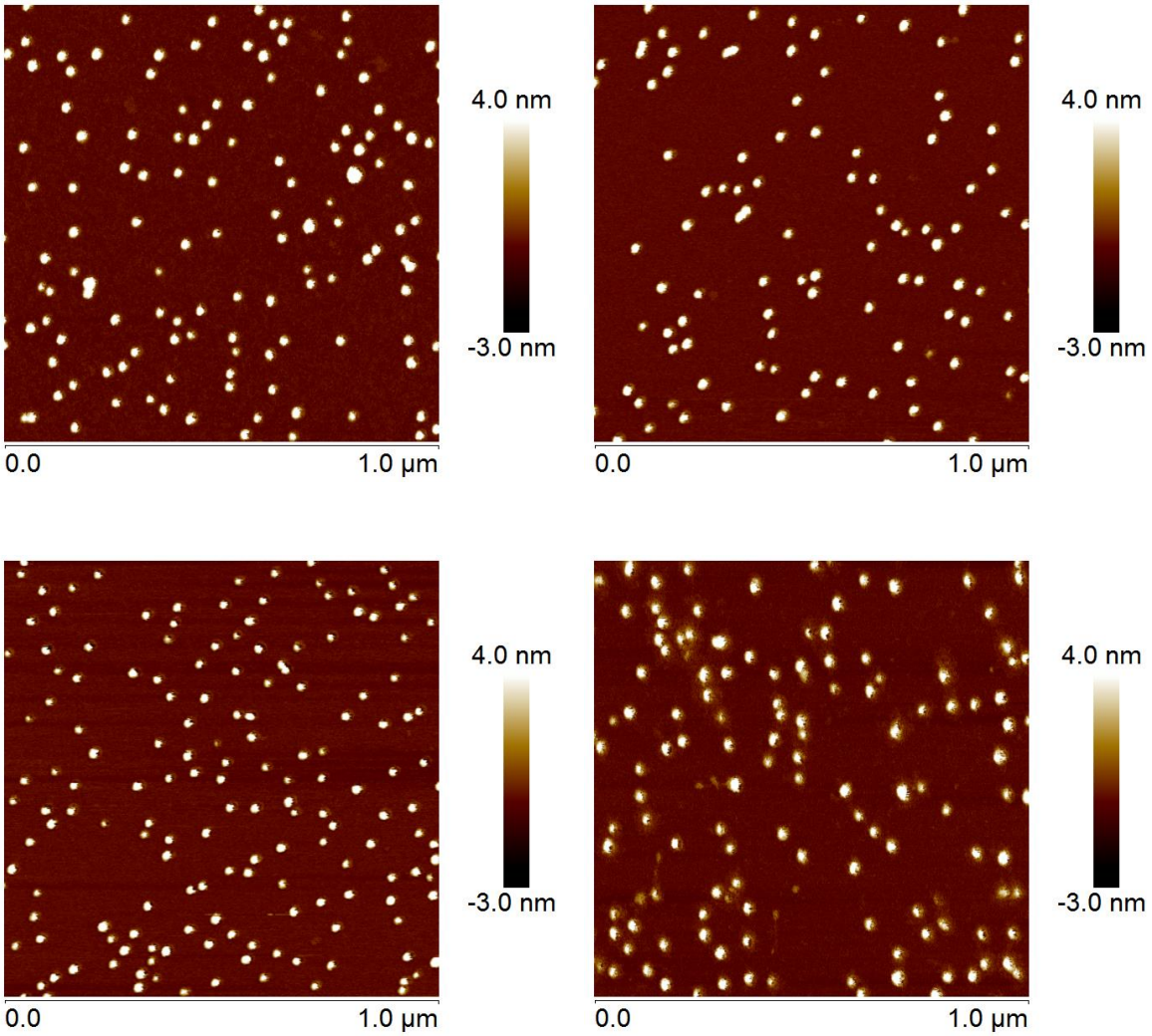
Supporting Figure SF19 | AFM images of HE_6-NH_2-DNA micelles. Spherical structures with the size of 37.0±5.2 nm in diameter were observed. The average height was 9.1±1.0 nm.



Supporting Figure SF20 | AFM images of NH_2-HE_{12}-DNA micelles. Spherical structures with the size of 26.0±3.4 nm in diameter were observed. The average height was 7.0±1.6 nm.



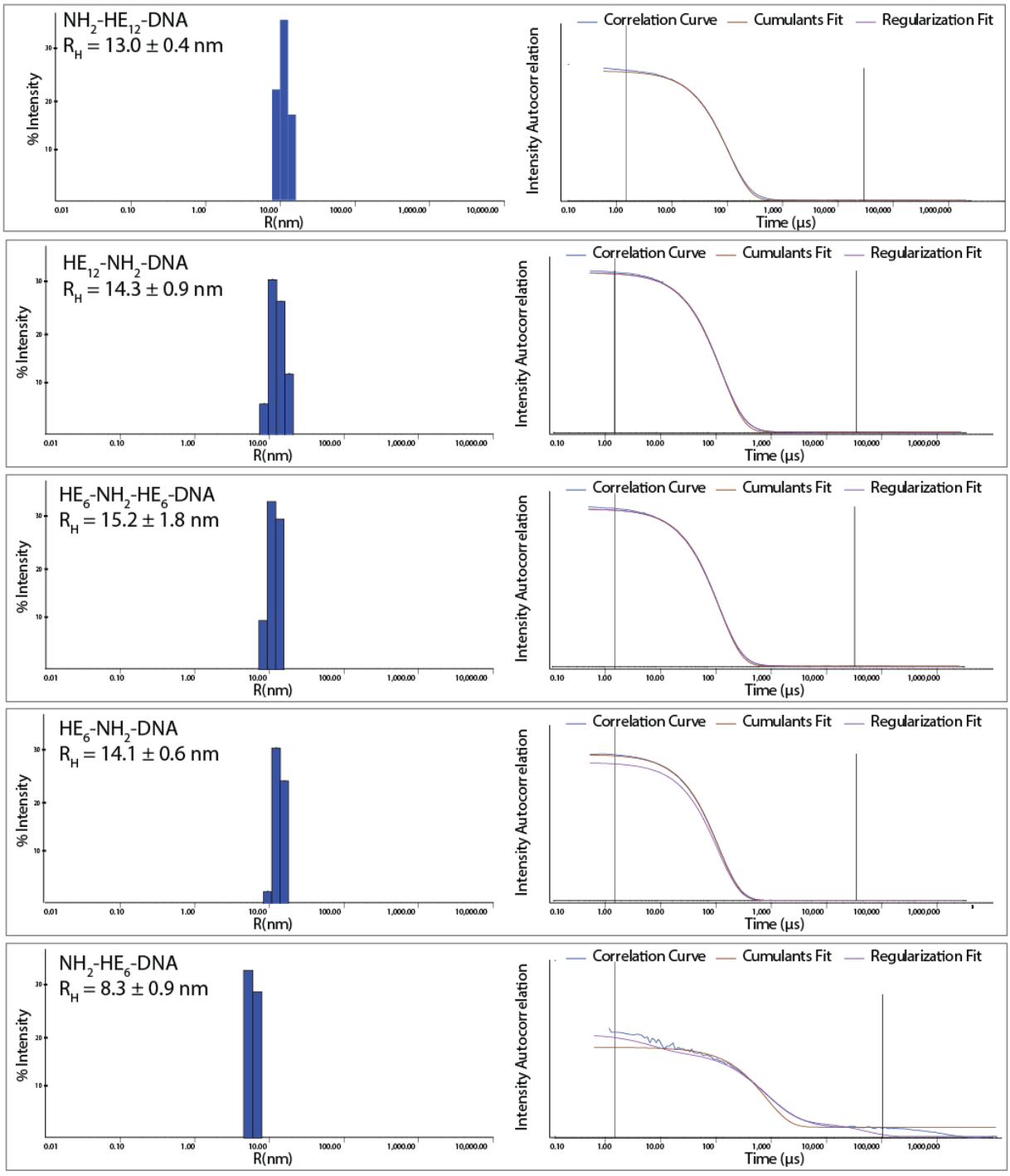
Supporting Figure SF21 | AFM images of HE_{12}-NH_2-DNA micelles. Spherical structures with the size of 27.2±6.5 nm in diameter were observed. The average height was 7.2±2.8 nm.



Supporting Figure SF22 | AFM images of HE_6-NH_2-HE_6-DNA micelles. Spherical structures with the size of 25.8±4.5 nm in diameter were observed. The average height was 6.7±1.7 nm.

SI-VIII DLS measurements of DNA micelles

20 µL of samples were analyzed on a DynaPro using a laser wavelength of 824 nm at 25ºC.

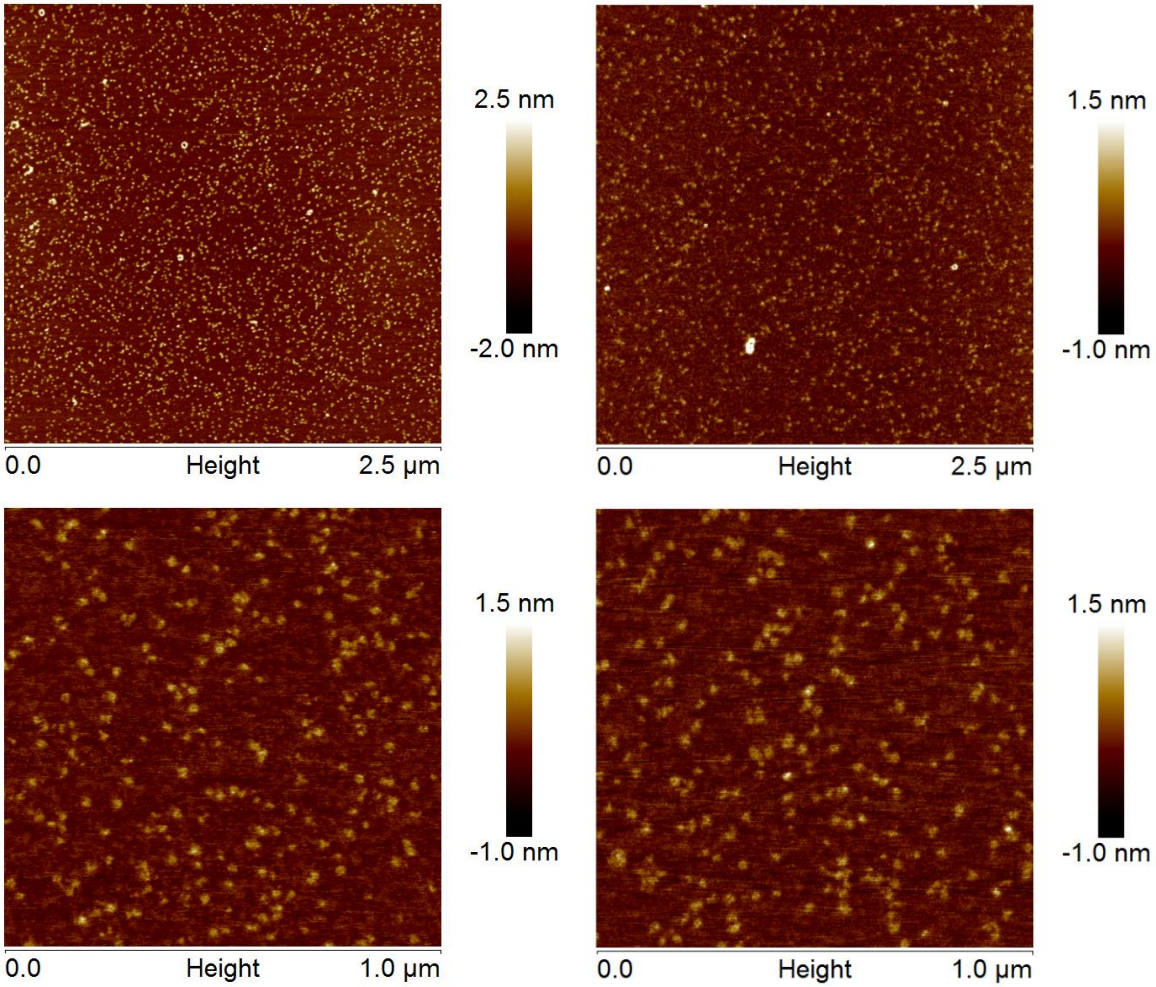


Right: autocorrelation curves of DNA amphiphiles micelles obtained from DLS

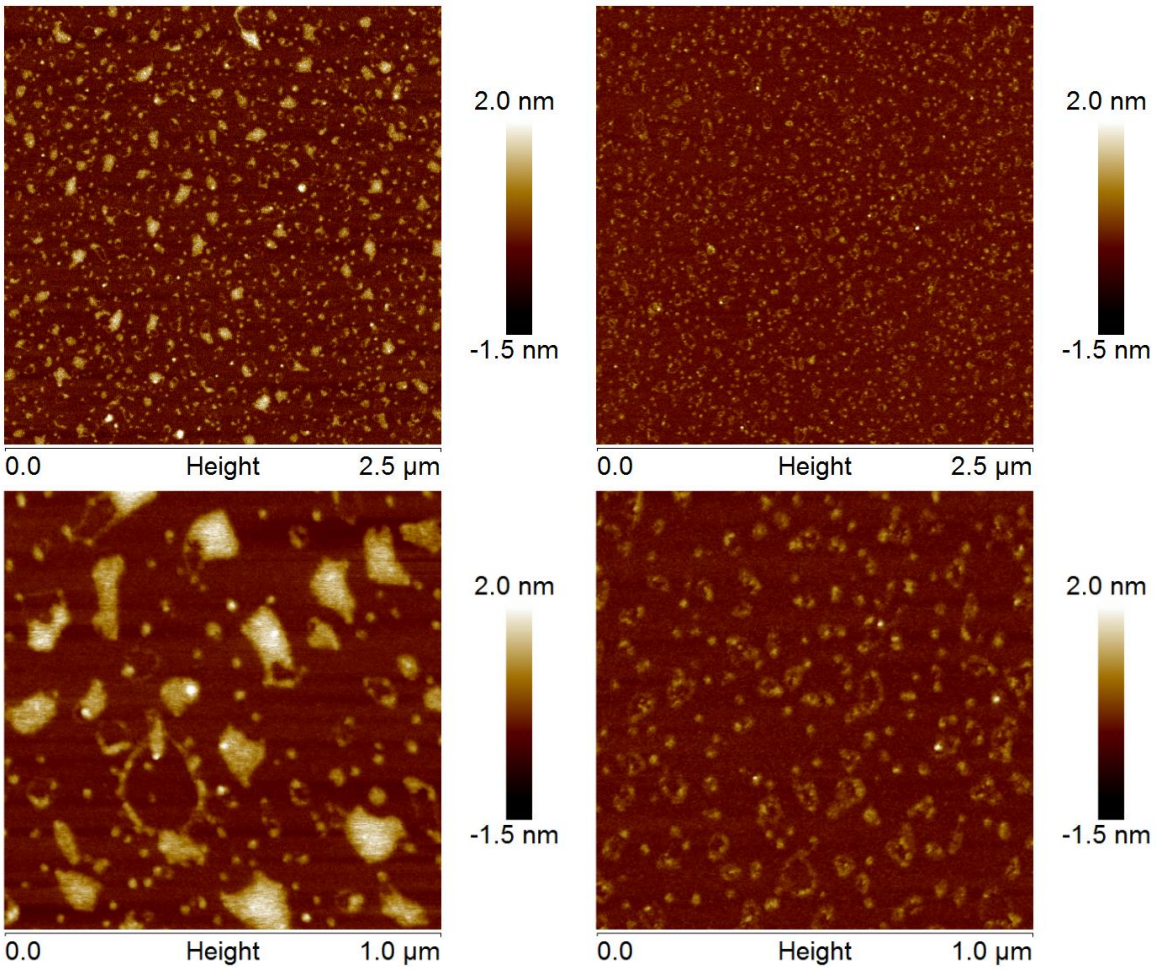
Supporting Figure SF23 | DLS measurements of amino-modified amphiphiles.

SI-IX Effect of organic solvent on the structures of NH_2-HE_6-DNA micelles

5 µM NH_2-HE_6-DNA in 1xTAMg was annealed from 95ºC to 4ºC for 1 hour. The sample was diluted with 1x TAMg to 1.67 µM then the organic solvents (DMSO and THF) was added in 1/10 volume ratio (i.e. 0.6 µL solvent and 6 µL samples). Then, 5 µL of sample was deposited on freshly cleaved mica for 5 seconds, and washed three times with 50 µL of H_2O. Excess liquid was brown off by the stream of nitrogen for 30 seconds. The sample was then dried under vacuum for at least 20 minutes prior to imaging.



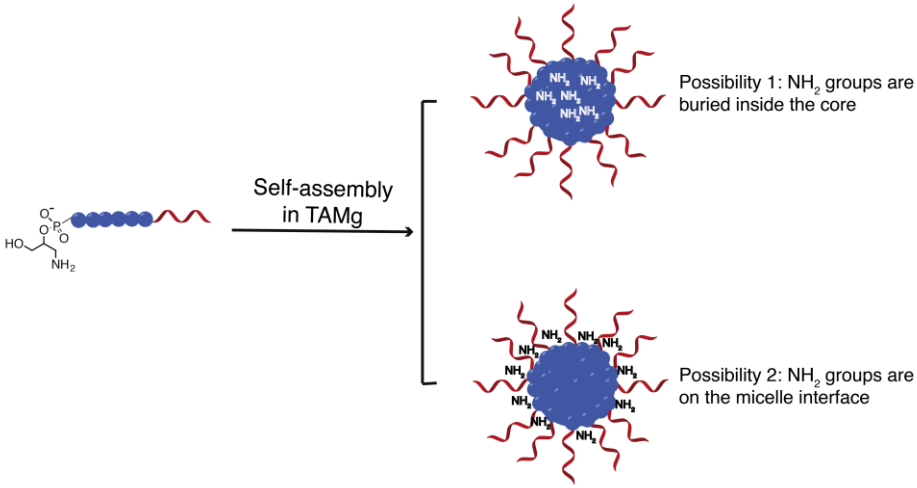
Supporting Figure SF24 | AFM images of NH_2-HE_6-DNA micelles in the presence of DMSO. The morphology of the structure was similar to the micelles without DMSO. The size of the structures was 17.1±4.2 nm and the average height was 1.4±0.5 nm. Although the micelles were relatively larger, the addition of DMSO does not disrupt the stability of preformed micelles.



Supporting Figure SF25 | AFM images of NH_2-HE_6-DNA micelles in the presence of THF. Large irregular aggregates and small spherical structures were clearly seen. The presence of the small structures with the size of 19.8±5.8 nm and the height of 0.9±0.2 nm) could suggest that the micelles were considerably stable against addition of THF. Large aggregates could be possibly due to the aggregation of these small structures.

SI-X Position of amino (NH_2) group.

One of the important design issues is that where the **NH_2** groups locate inside the micellar core. Depending on polyalkyl chain folding, the reactive **NH_2** groups within the **NH_2**-HE_6-AT micelles could either be buried inside the micellar core (if the alkyl chains are folded upon themselves in the core), or on the interface between the micelle core and corona (if the alkyl chains are unfolded).



If the **NH_2** group of **NH_2**-HE_6-DNA were on the micelle interface, then **NH_2**-HE_6-DNA would show similar reactivity to the conjugate HE_6-**NH_2**-DNA. In fact, the reaction of HE_6-**NH_2**-DNA with C_{22}-NHS under the same condition as **NH_2**-HE_6-DNA gave only 33±3% compared to 74±8% in case of NH_2-HE_6-DNA. In the conjugation reactions with C_{16}-NHS, **NH_2**-HE_6-DNA achieved 87±2% in yield, whereas the yield of **NH_2**-HE_{12}-DNA dropped to 40±4%.

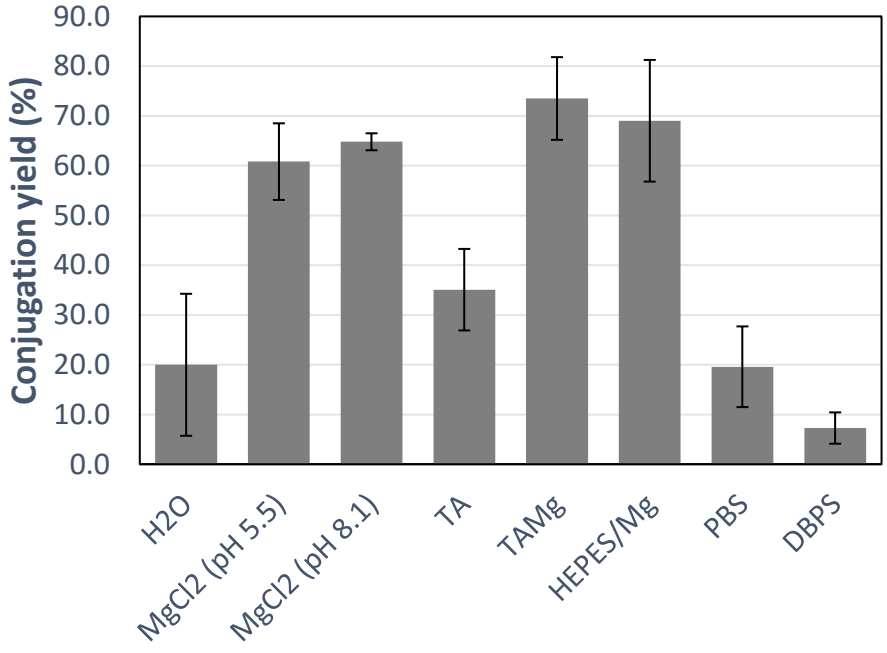
SI-XI Effect of the buffers on the conjugation efficiency of NH_2-HE_6-DNA with C_{22}-NHS

5μM of NH_2-HE_6-DNA was assembled in 1x buffer and annealed from 95 to 4°C in 1 hour. Separately, 10 mM of C_{22}-NHS in THF was prepared. To 10 volumes of NH_2-HE_6-DNA was quickly added 1 volume of C_{22}-NHS, and the mixture was gently shaken for 16 hours at room temperature. The crude mixture after reaction was dried and analyzed by RP-HPLC. Yield of conjugate reaction was calculated from the area under the curve ratio obtained from HPLC between the product peak and the sum of starting material and product peak. Supporting Table ST2 summarizes the buffers and their compositions used to compare the conjugation efficiency of NH_2-HE_6-DNA with C_{22}-NHS.

Supporting Table ST2 | Buffer compositions (10x) for the conjugation of NH_2-HE_6-DNA with C_{22}-NHS

Buffers	10x buffer compositions
H_2O	-
$MgCl_2$ (pH 5.5)	125 mM $MgCl_2 \cdot 6H_2O$ (pH 5.5)
$MgCl_2$ (pH 8)	125 mM $MgCl_2 \cdot 6H_2O$ (pH 8.1, adjusted with 1M HCl)
TA	450 mM Tris, 200 mM acetic acid (pH 8.0)
TAMg	450 mM Tris, 200 mM acetic acid, 125 mM $MgCl_2 \cdot 6H_2O$ (pH 8.0)
HEPES/Mg	400 mM HEPES, 125 mM mM $MgCl_2 \cdot 6H_2O$ (pH 8.0, adjusted with 1M HCl)
PBS	10.6 mM KH_2PO_4, 1.6 M NaCl, 29.7 mM $Na_2HPO_4 \cdot 7H_2O$ (pH 7.4)
DPBS	9.0 mM $CaCl_2$, 4.9 mM $MgCl_2 \cdot 6H_2O$, 26.7 mM KCl, 14.7 mM KH_2PO_4, 1.4 M NaCl, 80.6 mM $Na_2HPO_4 \cdot 7H_2O$

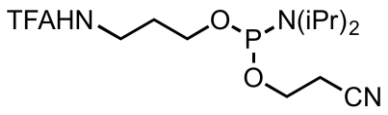
All conjugation reactions were carried out in TAMg buffer, and we were interested to probe whether the conjugation efficiency depends on the buffer choice. To examine the effect of each component of the buffer on the reaction efficiency, we compared the conjugation of NH_2-HE_6-AT with C_{22}-NHS in different buffer conditions (Supplementary Figure SF26) and the corresponding yields were highest with TAMg: TAMg (pH 8, 74±8%) ~ $MgCl_2$ (pH 8.1, 65±2%; pH 5.5, 61±8%) > TA (tris-acetate, pH 8, 35±8%) ~ H_2O (20±14%). Replacement of tris with non-nucleophilic (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) supplemented with $MgCl_2$ gave comparable yield (pH 8.1, 69±12%) to TAMg. These suggest that Mg^{2+} is important for efficient conjugation, most likely because it is required for micelle formation. Interestingly, good yields were obtained in tris buffer although tris is known to act as a competitor in NHS ester reactions. This suggests that the hydrophobic core of DNA-micelles prevents the interaction of NHS molecules with Tris. To our surprise, commercial non-amine-containing buffers were found to be less effective than amine-containing buffers: phosphate buffered saline (PBS, pH 7.4, 20±8%) and DPBS (Dulbecco's phosphate buffered saline supplemented with Ca^{2+} and Mg^{2+}, pH 7.4, 7±3%).

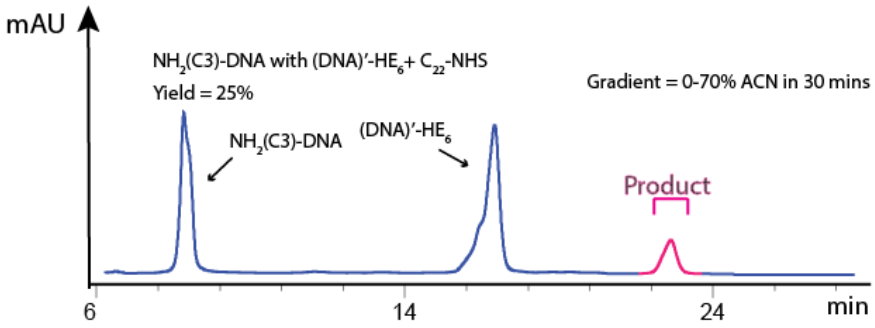


Supporting Figure SF26 | Conjugation yields of NH_2-HE_6-DNA with C_{22}-NHS in different buffer compositions

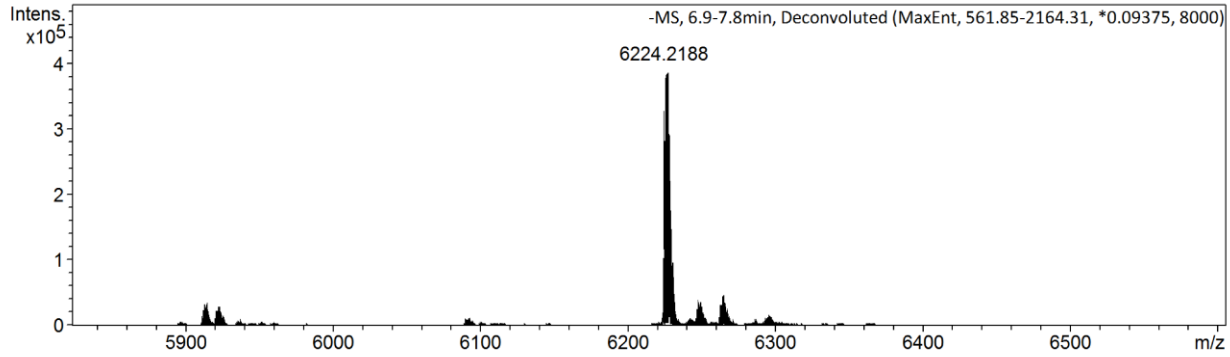
SI-XII Functionalized non-hydrophobically modified DNA using micelles system as auxiliary

XIIa. 5'-Amino-modifier C3-TFA (NH_2(C3)) phosphoramidite purchased from GlenResearch



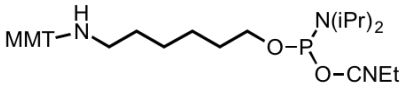


Supporting Figure SF27 | HPLC trace of crude products of the **NH_2(C3)-**DNA + C_{22}-NHS templated by (DNA)'HE_6 micelles

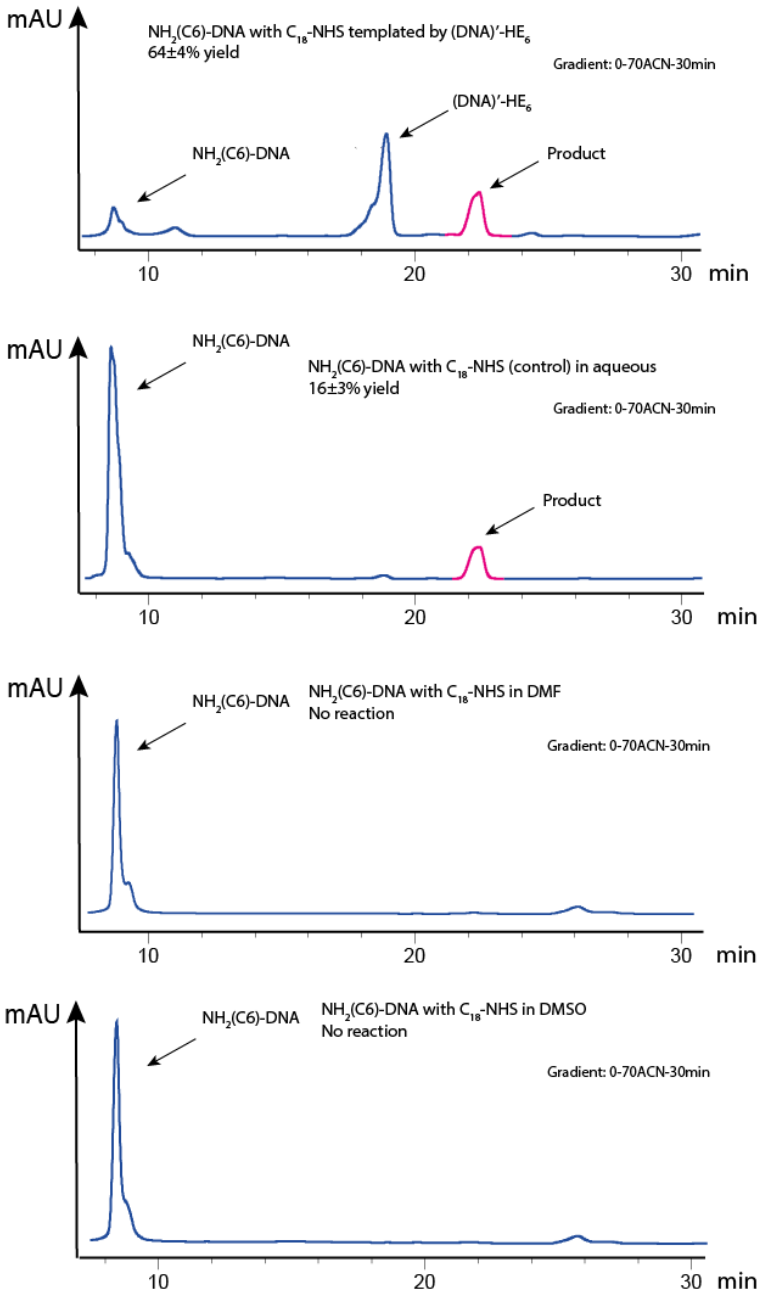


Supporting Figure SF28 | MS characterization of HPLC purified **NH_2(C3)-**DNA + C_{22}-NHS templated by (DNA)'HE_6 micelles

XIIb. 5'-Amino-modifier C6-MMT(NH_2(C6)) phosphoramidite purchased from GlenResearch



1. **Reaction of NH_2(C6)-DNA with C_{18}-NHS**

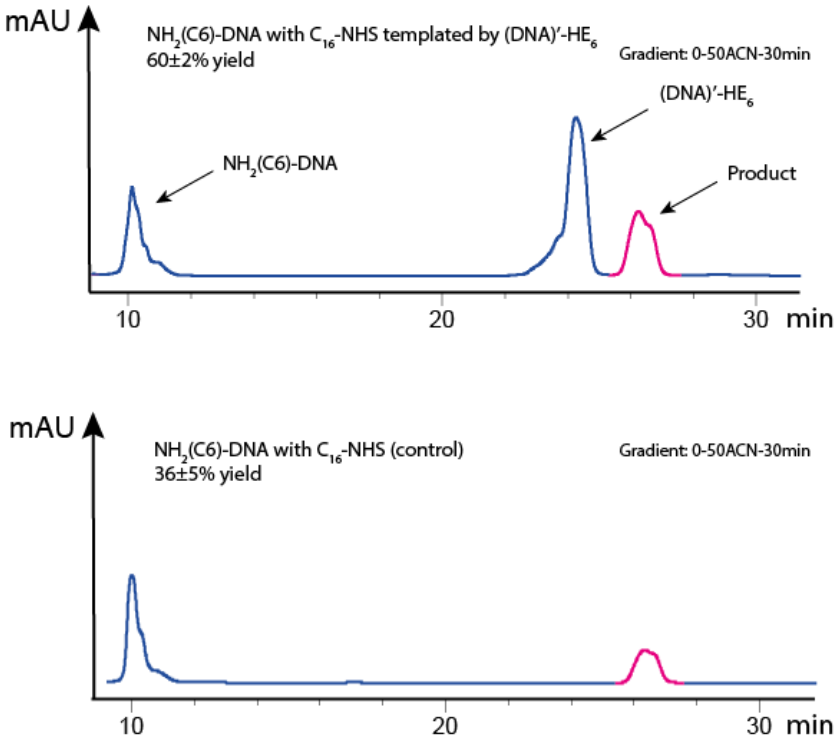


Supporting Figure SF29 | HPLC traces of reaction between NH_2(C6)-DNA with C_{18}-NHS with and without (DNA')-HE_6 template.

Recovery of template strand: With an appropriate HPLC gradient using triethylamine-acetic acid (TEAA) and acetonitrile (ACN), 3 peaks can be resolved clearly and collected including starting material (NH_2(C6)-DNA), template strand DNA'-HE_6 (retention time of 18 min) and product. After HPLC purification, DNA'-HE_6 was collected and recovered as described below:

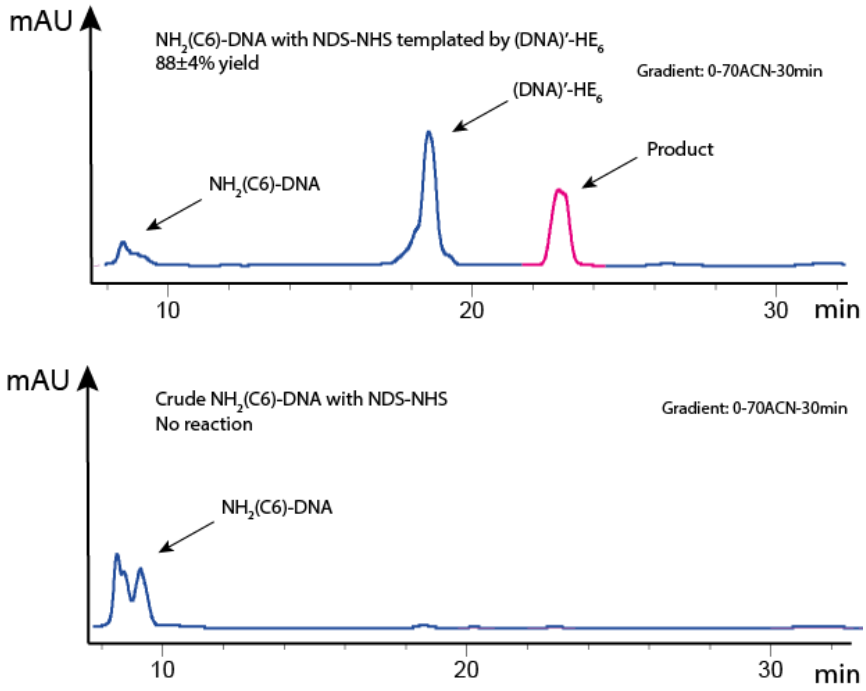
- Collect template strand after HPLC analysis in TEAA/ACN
- Evaporate TEAA buffer using Thermo Scientific SpeedVac at 60^{o}C
- Re-suspend template strand in water and quantify by NanoDrop machine.
- Compare the number of mole of template before and after the reaction and calculated the recovery efficiency to be about 85±5% after 1 cycle.

2. Reaction of NH_2(C6)-DNA with C_{16}-NHS



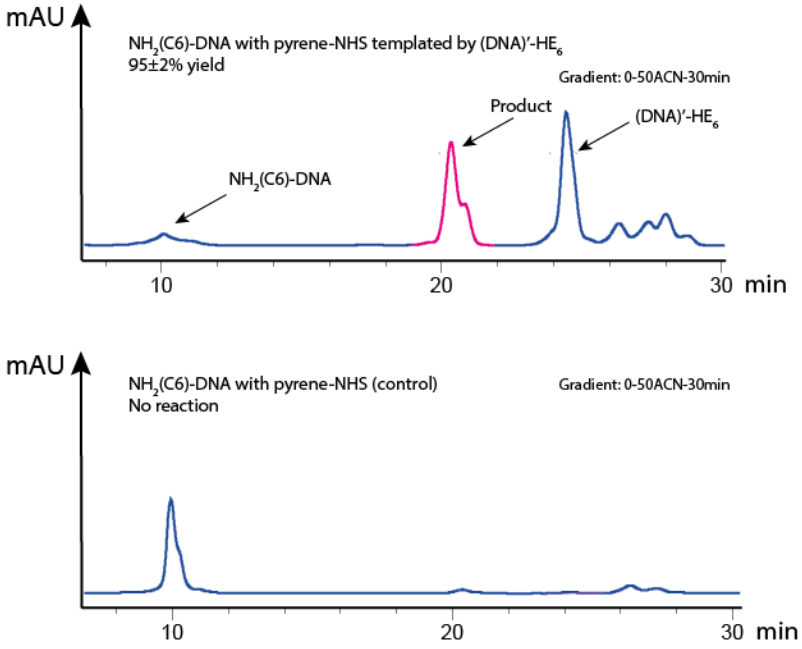
Supporting Figure SF30 | HPLC traces of reaction between NH_2(C6)-DNA with C_{16}-NHS with and without (DNA')-HE_6 template

3. Reaction of NH_2(C6)-DNA with NDS-NHS



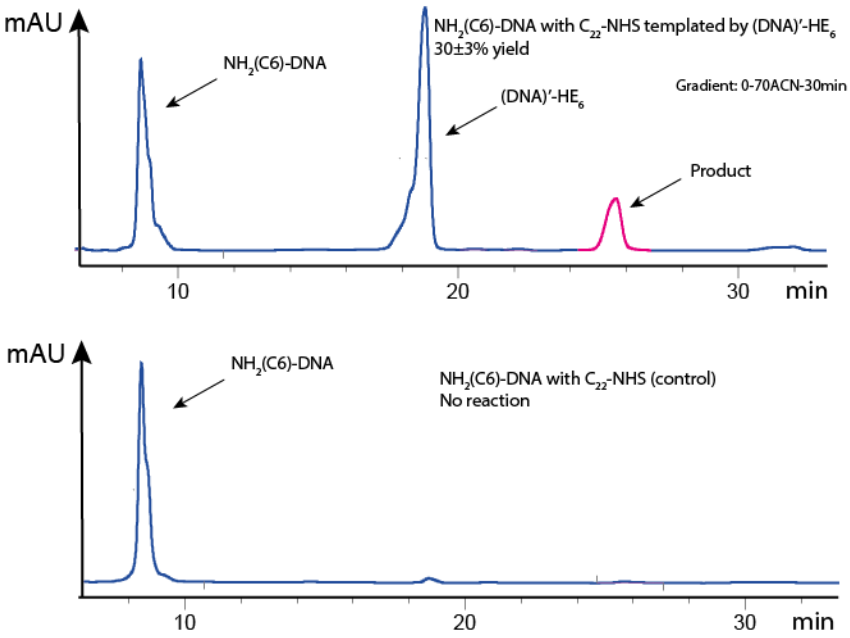
Supporting Figure SF31 | HPLC traces of reaction between **NH_2(C6)**-DNA with NDS-NHS with and without (DNA')-HE_6 template

4. Reaction of NH_2(C6)-DNA with pyrene-NHS



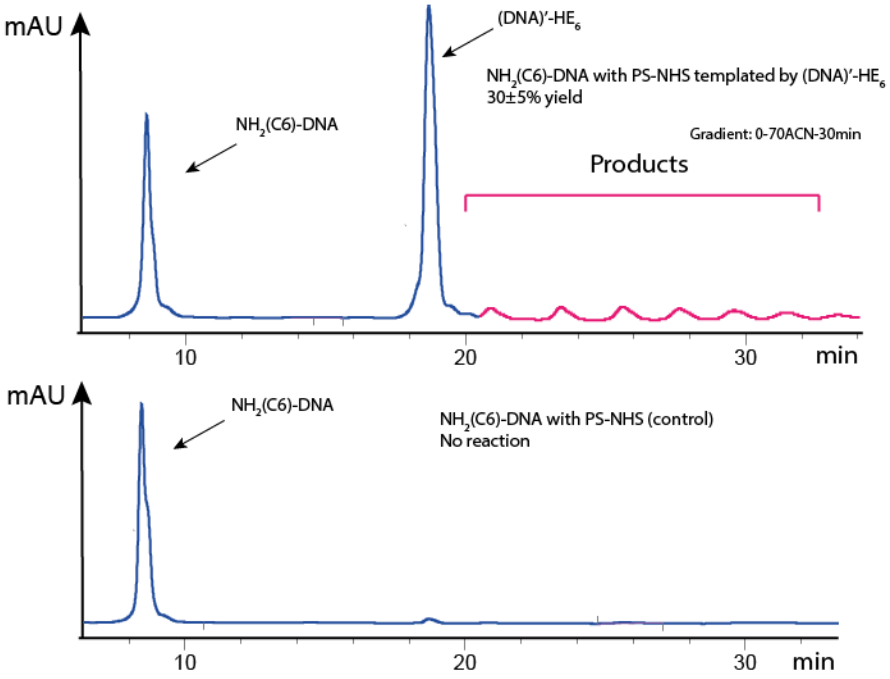
Supporting Figure SF32 | HPLC traces of reaction between **NH_2(C6)-**DNA with pyrene-NHS with and without (DNA')-HE_6 template

5. Reaction of NH_2(C6)-DNA with C_{22}-NHS



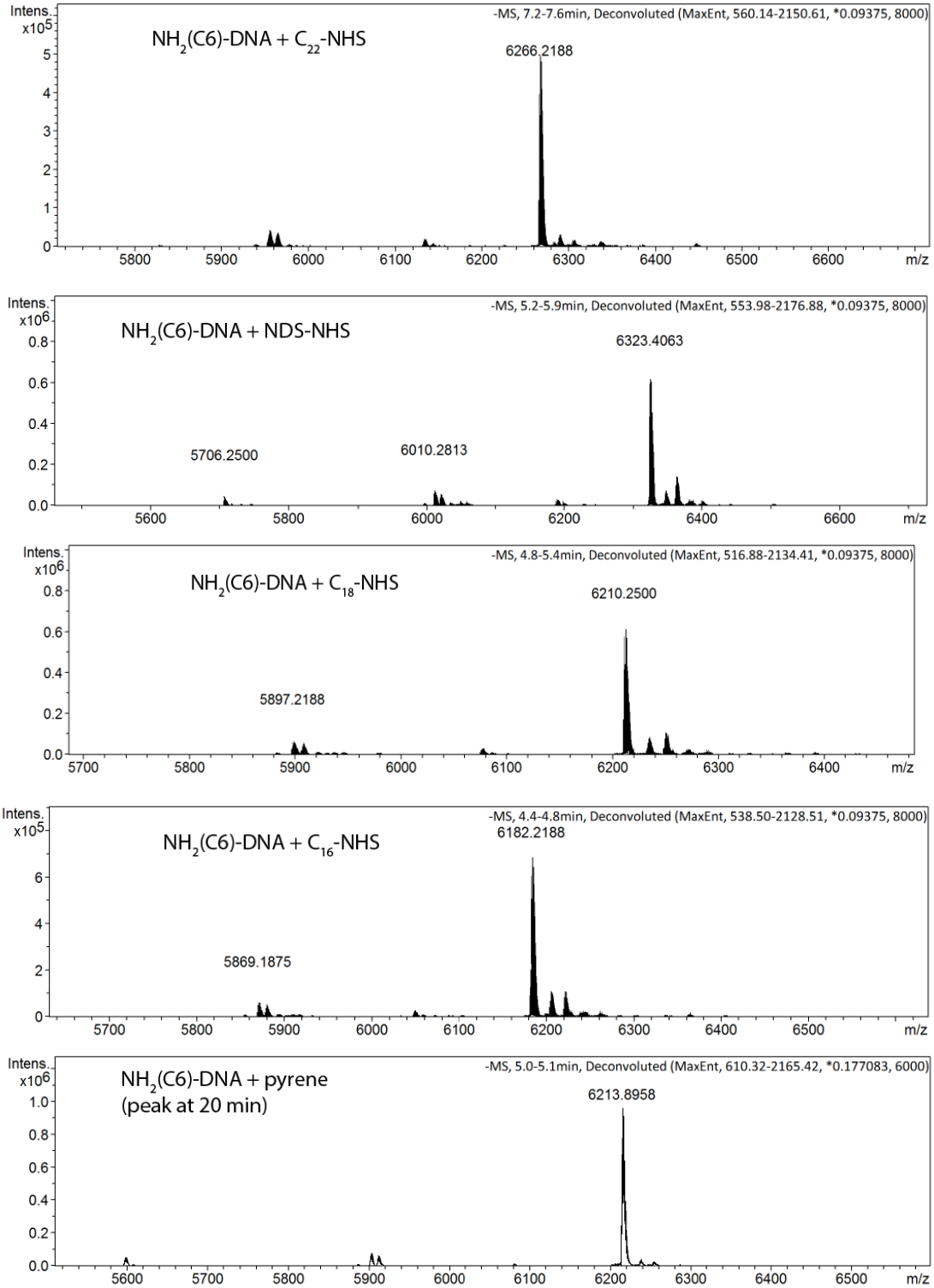
Supporting Figure SF33 | HPLC traces of reaction between NH_2(C6)-DNA with C_{22}-NHS with and without (DNA')-HE_6 template

6. Reaction of NH_2(C6)-DNA with Polystyrene-NHS (PS-NHS)



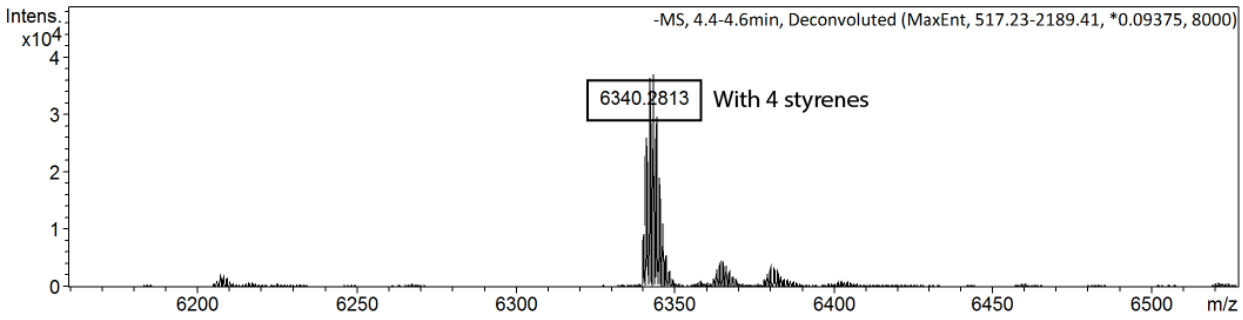
Supporting Figure SF34 | HPLC traces of reaction between **NH_2(C6)-**DNA with PS-NHS with and without (DNA')-HE_6 template

7. MS characterization of conjugate product of NH_2(C6)-DNA and hydrophobic molecules

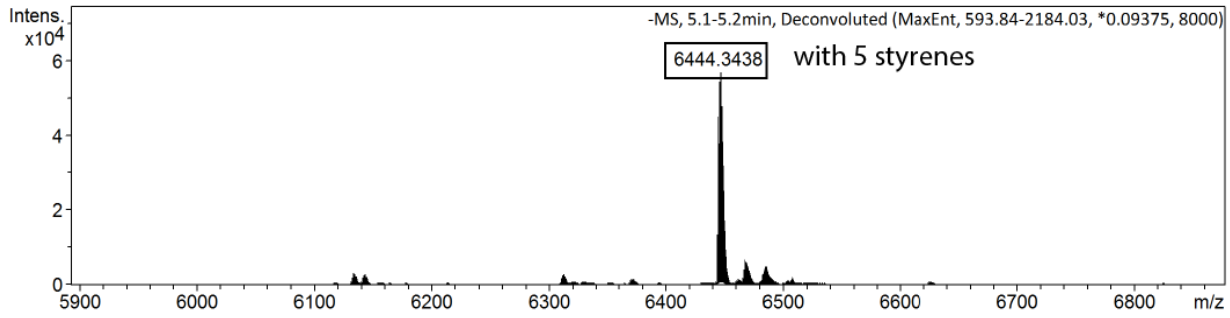


NH_2(C6)-DNA with PS-NHS:

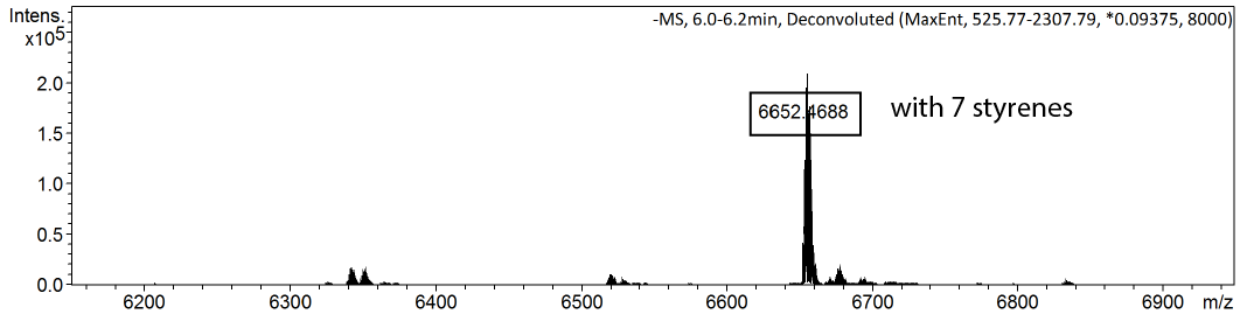
Peak at 21 mins



Peak at 23 mins



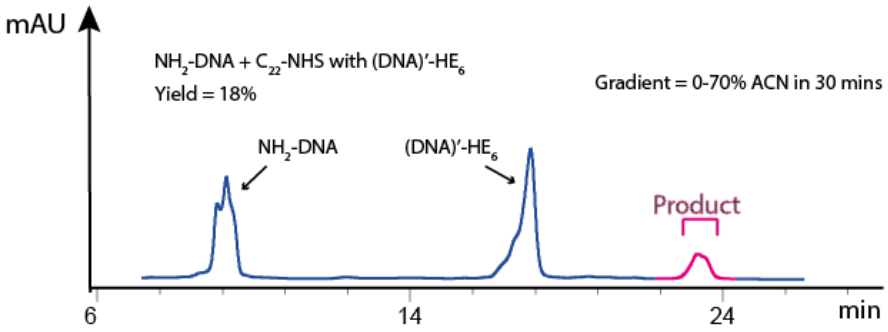
Peak at 27 mins



Supporting Figure SF35 | MS characterization of products between NH_2(C6)-DNA with hydrophobic organic molecules.

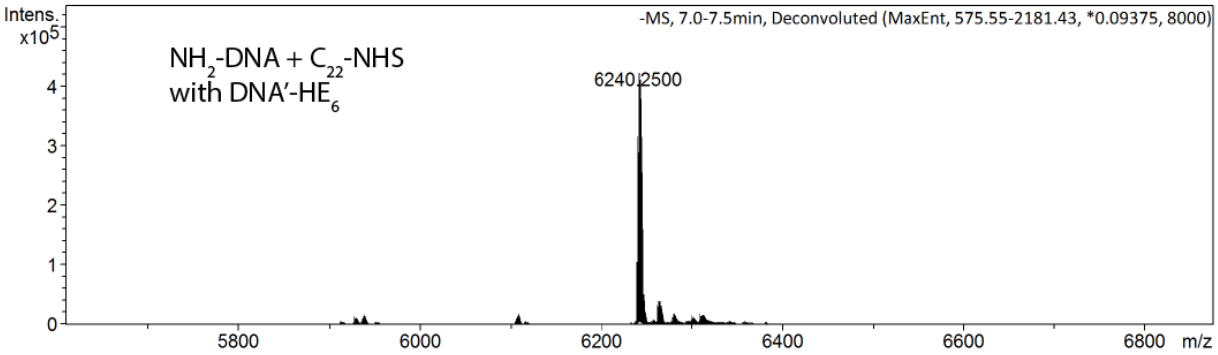
XIIc. Reaction of NH_2-DNA (Fmoc-C3-CED phosphoramidite) with C_{22}-NHS with DNA

1. HPLC characterization



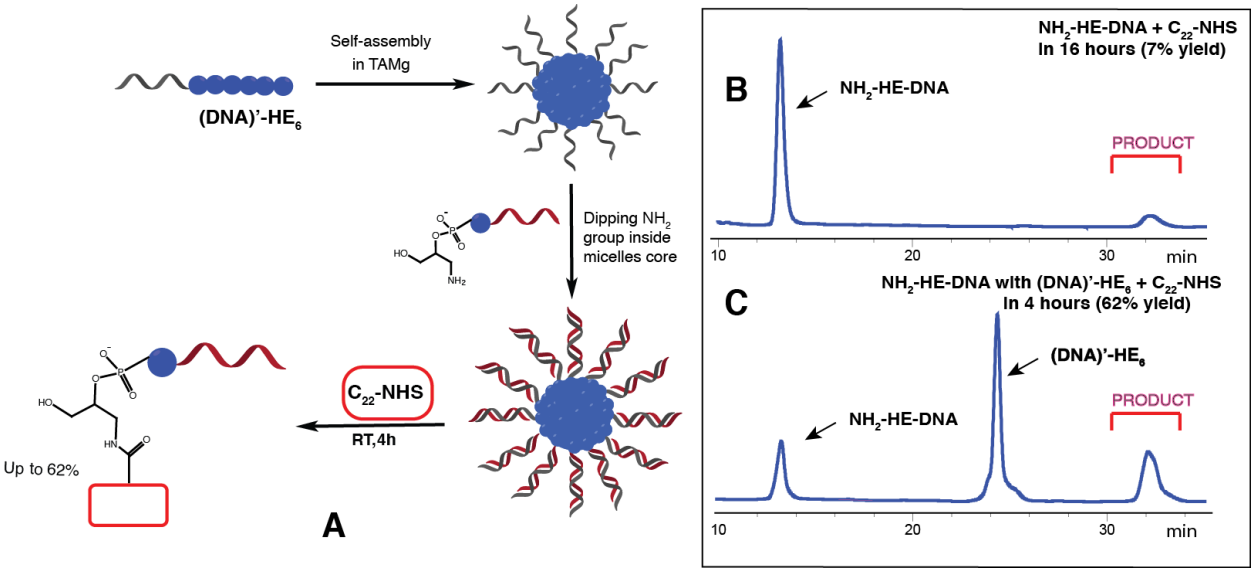
Supporting Figure SF36 | HPLC characterization of products between **NH_2**-DNA with C_{22}-NHS

2. LC-MS characterization



Supporting Figure SF37 | HPLC characterization of products between **NH_2**-DNA with C_{22}-NHS

SI-XIII Improving conjugation yield of NH_2-HE-DNA using DNA micelles template

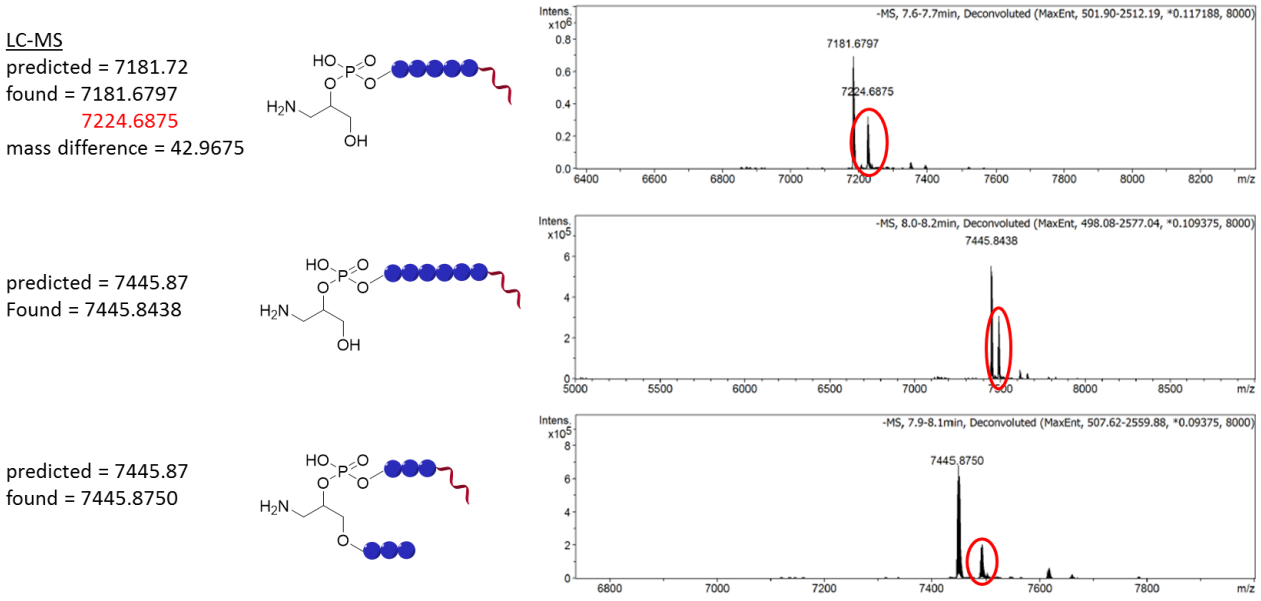


Supporting Figure SF38 | General methodology of double-stranded micelles system for improving coupling efficiency of set 3 micelles version. By hybridizing amphiphiles containing **DNA** sequence to its complementary strand **DNA'**, the **NH_2** was dipped inside hydrophobic core of DNA micelles. **B** and **C**. Reported yield of **NH_2**-HE-DNA with **C_{22}-NHS** without and with micelle template.

SI-XIV Note for the purification of NH_2 containing amphiphiles

We observed that a purification of NH_2-containing amphiphiles by the denaturing PAGE with urea generated the impurities which have additional mass of ~43 mass units. These impurities can be clearly observed by LC-MS (Supplementary Figure SF39). The products were further purified by RP-HPLC; however, the desired products and the impurities eluted at the same retention times, so it was difficult to remove the impurities by RP-HPLC.

We believe that the addition of mass is due to the reaction of amino group with the isocyanate, which can potentially form as the gel was heated during the run. The formation of isocyanate and ammonium at high temperature from the hydrolysis of urea is well-known in literature[1,2] and can induce chemical modification of protein during protein analysis which involves the use of urea as the denaturant[3,4]. Therefore, it is necessary to avoid the purification techniques involving urea, and we recommend to use RP-HPLC or anion-exchange HPLC to separate the desired products.



Supporting Figure SF39 | Representative examples of the NH_2-containing amphiphiles which contain adducts of ~43 mass units. The impurities (highlighted in red ovals) could be clearly observed in the mass spectra.

SI-XV References

1. J. R. Marier and D. Rose, *Anal. Biochem.*, 1964, **7**, 304-314.
2. P. Hagel, J. J. T. Gerding, W. Fieggen and H. Bloemendal, *Biochimica et Biophysica Acta (BBA) - Protein Structure*, 1971, **243**, 366-373.
3. G. R. Stark, W. H. Stein and S. Moore, *J. Biol. Chem.*, 1960, **235**, 3177-3181.
4. J. Čejka, Z. Vodrážka and J. Salák, *Biochimica et Biophysica Acta (BBA) - Protein Structure*, 1968, **154**, 589-591.