Supporting Information

Performing DNA Nanotechnology Operations on a Zebrafish

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Method and Materials

Zebrafish husbandry, and egg collection

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Leiden University and approved by the Animal Ethics Committee of Leiden University. Zebrafish livestock was maintained and handled according to the guidelines from http://zfin.org. Fertilization was performed by natural spawning at the beginning of the light period, and eggs were raised at 28°C. All experimental procedures were conducted in compliance with the directives of the animal welfare committee of Leiden University.

Synthesis and characterization of amphiphilic oligonucleotides

5-(dode-1-cynyl) deoxyuracil phosphoramidite was synthesized as reported previously (Structure in **Scheme S2**).[1-2] The modified uracil phosphoramidite was dissolved in CH_3CN to a final concentration of 0.15 M, in the presence of 3 Å molecular sieves, and the prepared solution was directly connected to a DNA synthesizer (ÄKTA oligopilot plus, GE Healthcare (Uppsala, Sweden)).

Oligonucleotides were synthesized on a 10 μmol scale using standard β-cyanoethylphosphoramidite coupling chemistry. Deprotection and cleavage from the PS support were carried out by incubation in concentrated aqueous ammonium hydroxide solution for 5 h at 55 °C. Following deprotection, the oligonucleotides were purified by using reverse-phase chromatography, using a C15 RESOURCE RPCTM 3 mL reverse phase column (GE Healthcare) through a custom gradient elution (A: 100 mM triethylammonium acetate (TEAAc) and 2.5% acetonitrile, B: 100 mM TEAAc and 65% acetonitrile). Fractions were desalted using centrifugal dialysis membranes (MWCO 3000, Sartorius Stedim). Oligonucleotide concentrations were determined by UV absorbance using extinction coefficients. Finally, the identity and purity of the

oligonucleotides were confirmed by RPC-HPLC and MALDI-TOF mass spectrometry (**Figure S4 and Figure S5**).

Native polyacrylamide gel electrophoresis (PAGE) to detect DNA replacement

Native polyacrylamide gel electrophoresis was performed to demonstrate DNA replacement in buffer in the absence of liposomes by using a 15% gel (mono:bis ratio of acrylamide : 19/1) made with TBE buffer (90 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) and run at 120 V for 100 min in TBE. After electrophoresis, DNA was visualized under UV light without the usage of a staining solution. **Figure S6** showed that both C488 and C594 hybridize with U4T18 (lane 4 and lane 5, respectively) and that the 20-mer can efficiently peel off C594 from U4T18 (lane 6). After C594 was removedfrom U4T18 by the 20-mer, C488 hybridized with free U4T18 (lane 7).

DNA replacement on liposomes measured by Fluorescence Resonance Energy Transfer (FRET) assay

Rh-liposomes were mixed with CrATTO488 or CrATTO594 such that [U4T18] = [CrATTO488] = [CrATTO594] = 0.906 μM and with a final lipid (DOPC+DOPE) concentration of 0.45 mM. Rh-DHPE / (DOPC+DOPE+CHO)=3 / 97 molar ratio in PBS buffer at room temperature. Fluorescence emission spectra of donor/acceptor, CrATTO488/Rh-DHPE or Rh-DHPE/CrATTO594 in the 500–700 nm region were recorded with excitation at 470 nm using a SpectraMax M2 (Molecular Devices) fluorescence spectrophotometer. Measurements were carried out at constant temperature of 25°C.

After lipid-DNA (U4T18, 0.906 μM) was stably anchored into the membrane of vesicles (diameter 120 nm), two Fluorescence Resonance Energy Transfer (FRET) systems, Rhodamine (Rh)-DHPE/ATTO594 and ATTO488/Rh-DHPE, were used to demonstrate the availability of the anchor sequence for hybridization and DNA replacement. (**Figure S7a**)

In the Rh-DHPE/C594 system, ATTO594 was covalently attached to the 3' end of a 20-mer DNA (C594, 0.906 μM) to act as an acceptor. In parallel, rhodamine-functionalized phospholipid (Rh-DHPE) was incorporated into the liposomal bilayer to function as a donor. As demonstrated by a clear emission peak at 624 nm (Rh-DHPE, acceptor fluorescence peak), hybridization only occurred upon mixing of C594 with U4T18-grafted Rh-DHPE-containing vesicles, positioning both dyes sufficiently close to each other to achieve FRET (**Figure S7b**, dashed purple line). Then C594 was peeled off from U4T18 by hybridizing with a 20-mer DNA (1.812 μM) which resulted in a higher signal of the donor Rh-DHPE (592 nm) and lowering of the acceptor (ATTO594, 624 nm) signal (**Figure S7b**, black line). For proving replacement enabled by the C488/Rh system, ATTO488 was covalently attached to the 3' end of a 14-mer DNA that is complementary to U4T18 (C488, 0.906 μM) to act as a donor while rhodamine embedded in the membrane functioned as an acceptor. Disruption of liposomes by addition of Triton X-100 resulted in an increased donor peak (ATTO488, 520 nm) and slightly decreased acceptor peak (Rh-DHPE, 592 nm) (**Figure S7c**), confirming that replacement by hybridization on the surface of the liposomes was successfully achieved..

In the control experiments involving CrU4T18-liposomes, similar emission spectra were obtained before and after liposomal disruption, indicating that no FRET occurred in the absence of complementary anchoring units on the vesicle surface (**Figure S8**).

Agarose gel electrophoresis to monitor the hybridization chain reaction

Agarose gel electrophoresis analysis was used to prove U4T28 initiated HCR with M1 and M2 (**Figure S9**). 2% agarose gel was prepared by using SB buffer (10 mM NaOH, pH adjusted to 8.5 with boric acid) [3] and was run at 90 V for 3 h. After electrophoresis, the agarose gel was stained with ethidium bromide and DNA was visualized under UV light. The hairpin sequences M1 and M2 did not hybridize in the absence of U4T28

(**Figure S9, lane 3**). The chain length of the resulting duplex DNA is inversely related to the initiator concentration (**Figure S9, lane 6-8**).

Hybridization chain reaction on the surface of liposomes

To prove DNA extension on the surface of liposomes, M2 was labeled with a fluorophore (Cy3). U4T28 (0.906 μM) was incubated with liposomes (0.45mM) for 30min at 50 ^{o}C. PTHK polysulfone membrane filters (100.000 Da) were used to remove unincorporated U4T28 by centrifugation at 1000 rpm (rotor: FA-45-18-11) for 30 min. M1/M2-Cy3 (two equivalents in relation to U4T28) were added to the system at RT for 1 h, after which again a 100.000 Da molecular weight cut off filter was used in a centrifugation step to remove free M1 and M2-Cy3 (1000 rpm, 30 min). After re-suspension and centrifugation, the supernatant was washed twice with PBS buffer. Afterwards, fluorescence intensity of the supernatant containing liposomes was measured. The fluorescence spectra were recorded in the range of 530-620 nm with excitation at 520 nm for M2-Cy3, using a SpectraMax M2 (Molecular Devices) fluorescence spectrophotometer. Measurements were carried out at 25 °C.

As **Figure S10** shows, fluorescence intensity of M2-Cy3 was high only in the HCR experiment (**Figure S10, dashed red line**) while in the absence of M1 (**Figure S10, blue line**) or U4T28 (**Figure S10, dashed black line**) negligible Cy3 signals were observed.

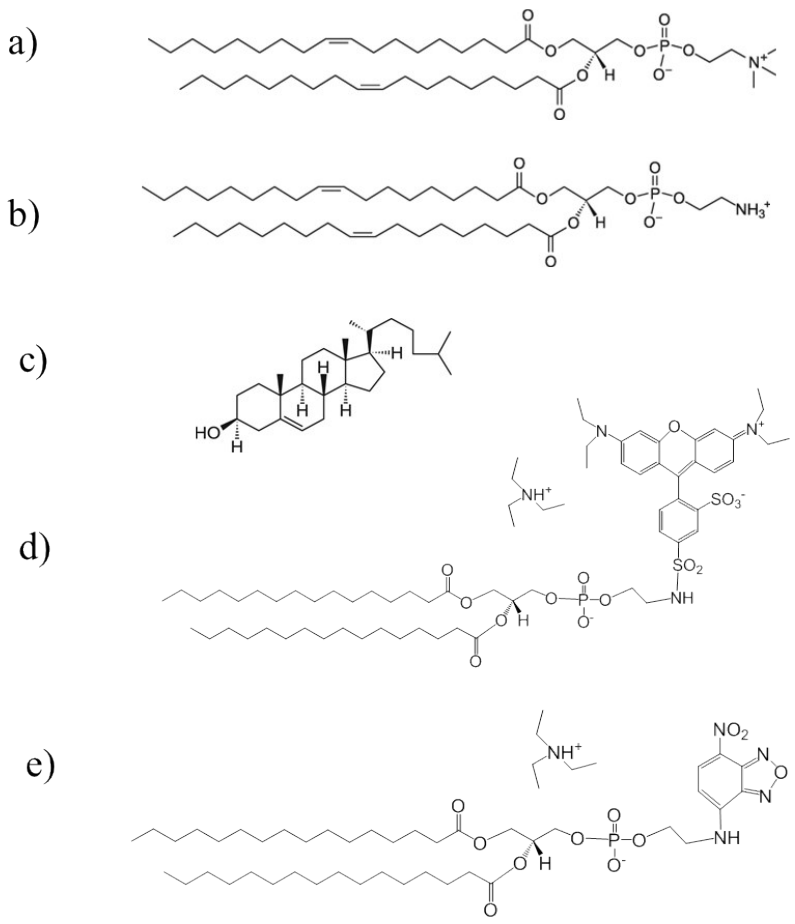
References

1. Anaya, M.; Kwak, M.; Musser, A. J.; Mullen, K.; Herrmann, A., *Chemistry* 2010, ***16,*** 12852-12859.

2. Kwak, M.; Minten, I. J.; Anaya, D. M.; Musser, A. J.; Brasch, M.; Nolte, R. J.; Mullen, K.; Cornelissen, J. J.; Herrmann, A., *J Am Chem Soc* 2010, ***132,*** 7834-7835.

3. Brody, J. R.; Kern, S. E., *Biotechniques* 2004, ***36,*** 214-216.

Supplementary Tables



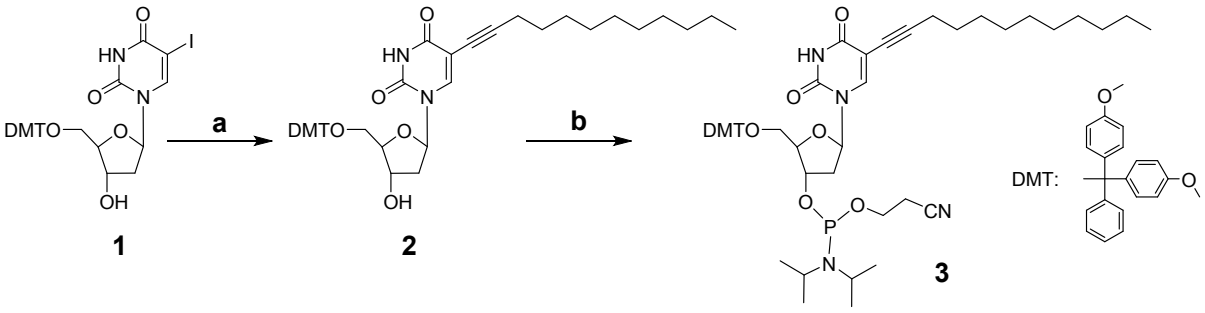
Scheme S1. Structures of lipids: DOPC(a), DOPE(b), Chol(c), fluorescent lipids: Rh-DHPE(d), NBD-DHPE(e).

DNA	Sequence (5'→3') *
U4T18	5'-UUUUGCGGATTCGTCTGC-3'
CrU4T18	5'-GCAGACGAATCCGCUUUU-3'
U4T28	5'-UUUUAATTGGGTGCGGCTTAGGATCTGA-3'
C 488	5'-GCAGACAGGTCCGC-3'-*ATTO488*
C 594	5'-GCAGACAGGTCCGCGTTTGT-3'-*ATTO594*
20-mer	5'-ACAAACGCGGATTCGTCTGC-3'
M1	5'-GTGCGGCTTAGGATCTGATGAAACTCAGATCCTAAGCCGCACCCAATT-3'
M1-FAM	*6-FAM*-5'-GTGCGGCTTAGGATCTGATGAAACTCAGATCCTAAGCCGCACCCAATT-3'
M2	5'-GTTTCATCAGATCCTAAGCCGCACAATTGGGTGCGGCTTAGGATCTGA-3'
M2-Cy3	*Cy3*-5'-GTTTCATCAGATCCTAAGCCGCACAATTGGGTGCGGCTTAGGATCTGA-3'

*: U represents the modified uracil base.

*: *ATTO594, ATTO488, FAM, Cy3* fluorescent dyes covalently bound to the DNA oligonucleotides.

Table S1. Sequences and modifications of DNAs.



Scheme S2. Synthesis of 5-(dode-1-cynyl) deoxyuracil **2** and 5-(dode-1-cynyl) deoxyuracil phosphoramidite **3**.

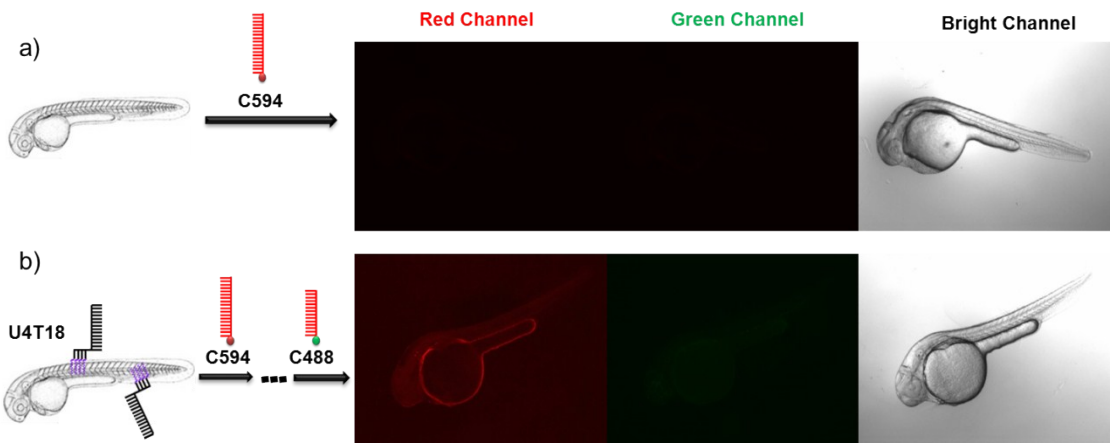


Figure S1: Fluorescence images of two control experiments: (a) untreated 2dpf zebrafish without DNA anchoring unit was incubated with 1 µM ATTO594 labeled complementary DNA (C594) for 1h. No fluorescence labelling was detected. (b) 2dpf zebrafish was decorated with U4T18, incubated with 1 µM C594 for 1h, washed three times with egg water, and subsequently exposed to solution containing 1 µM ATTO488 labeled complementary DNA (C488). Although the green labelled DNA was added to the fish, red fluorescence was detected on its surface. This is due to lack of the removal strand. After all treatments of the fish with DNA, washing three times with egg water was performed. Red channel = ATTO594; Green channel = ATTO488.

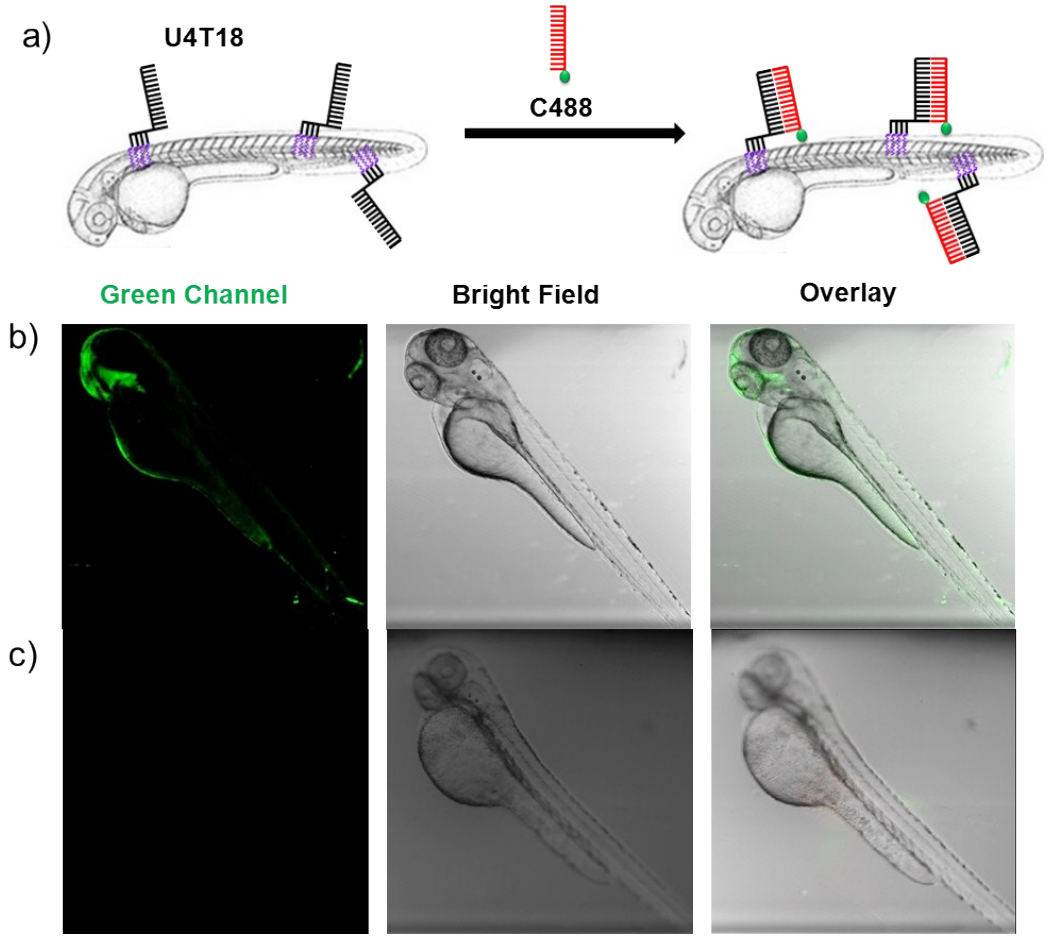


Figure S2. DNA docking on the surface of zebrafish embryos. a) Schematic representation of the addition of ATTO488 labeled complementary DNA (C488) to the U4T18 pretreated zebrafish embryos. Confocal fluorescence images of 2 dpf zebrafish embryos treated with b) 1 µM U4T18 for 1 h, after three times washing by egg water following exposure of the zebrafish with 1 µM C488 or c) only incubated with 1 µM C488 for 1 h and 3 times washing by egg water. Green channel: ATTO488.

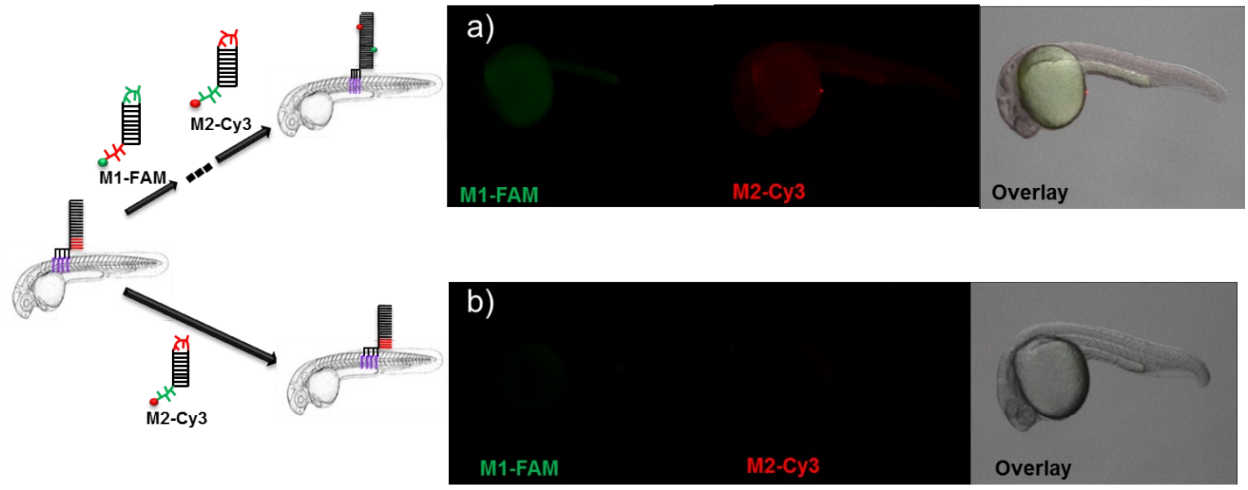
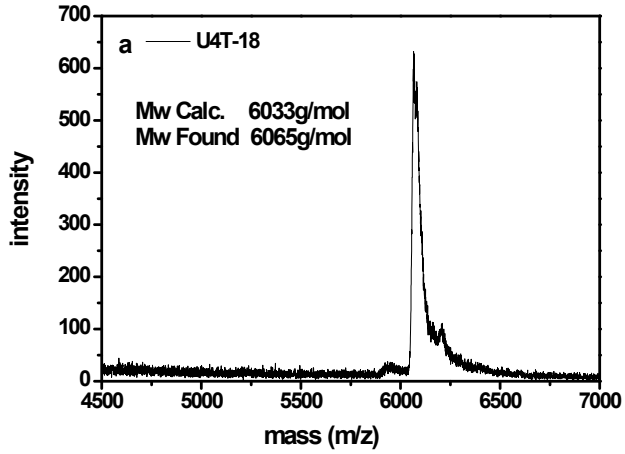
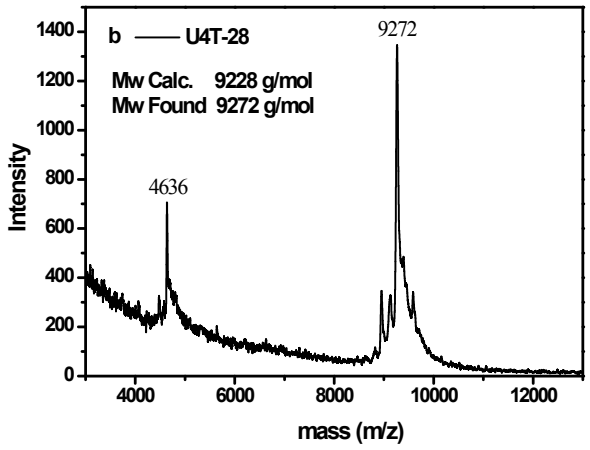


Figure S3. HCR on zebrafish skin depends on DNA hybridization between U4T28, M1 and M2. Fluorescence images of 1 dpf zebrafish a) that were first incubated with U4T28 and FAM fluorescently labeled M1 (M1-FAM) for 2 hours, subsequently washed 3 times with egg water before the addition of Cy3 fluorescently labeled M2 (M2-Cy3), or b) similar to a) but without M1-FAM. Green channel: M1-FAM. Red channel: M2-Cy3.





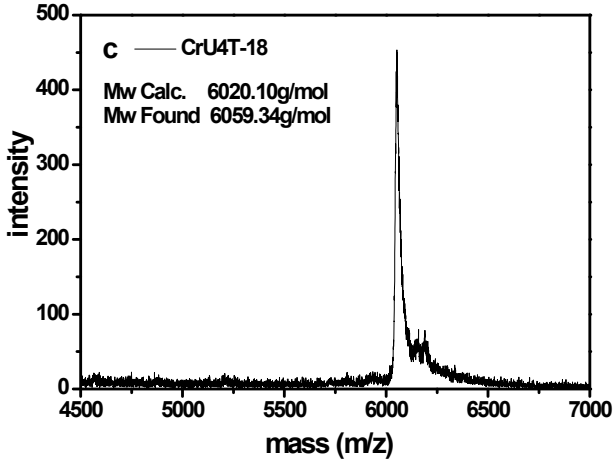


Figure S4. MALDI-TOF mass spectra of a) U4T28, b) U4T28 and c) CrU4T18

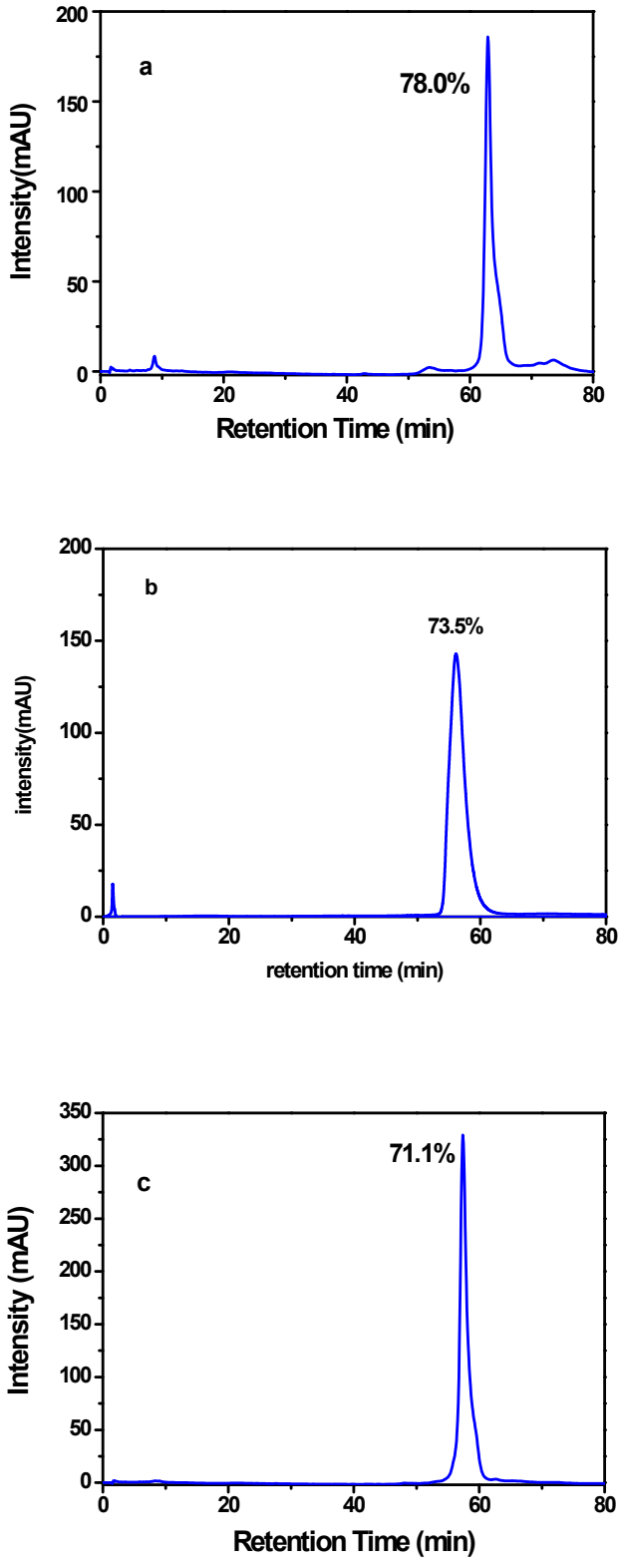


Figure S5. RPC HPLC analysis of purified lipid-DNAs: a. U4T; b. U4T28; c. CrU4T. Numbers beside the elution peaks represent the buffer B contents when lipid-DNAs were eluted.

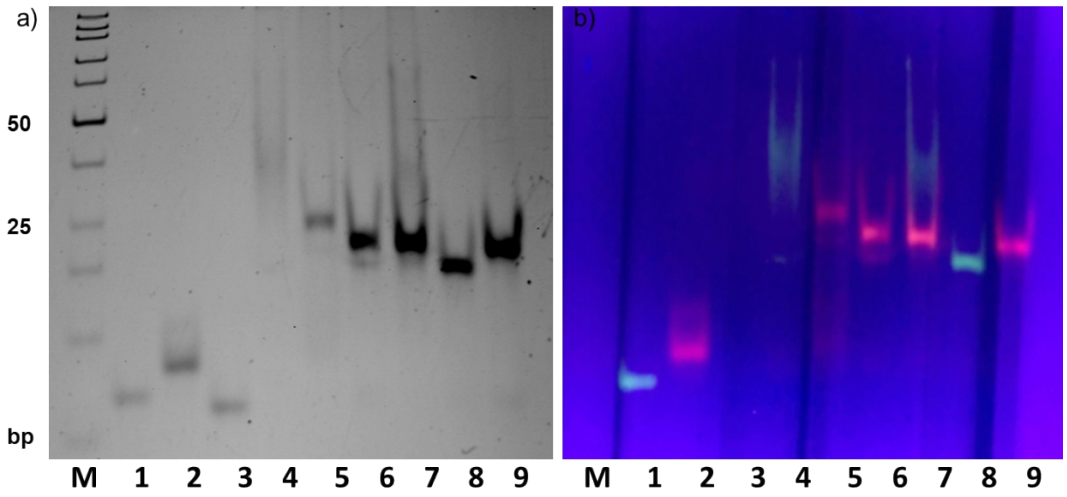


Figure S6. PAGE of DNA replacement in buffer (M is maker).. The sample run from left to right, M: Marker; Lane 1: C488; Lane 2: C594; Lane 3: 20-mer; Lane 4: U4T18 + C488; Lane 5: U4T18 + C594; Lane 6: C594 was peeled off from U4T18 by 20-mer; Lane 7: C488 hybridized with free U4T18 from Lane 6; Lane 8: C488 + 20-mer; Lane 9: C594 + 20-mer. The excitation with UV was at 366 nm.

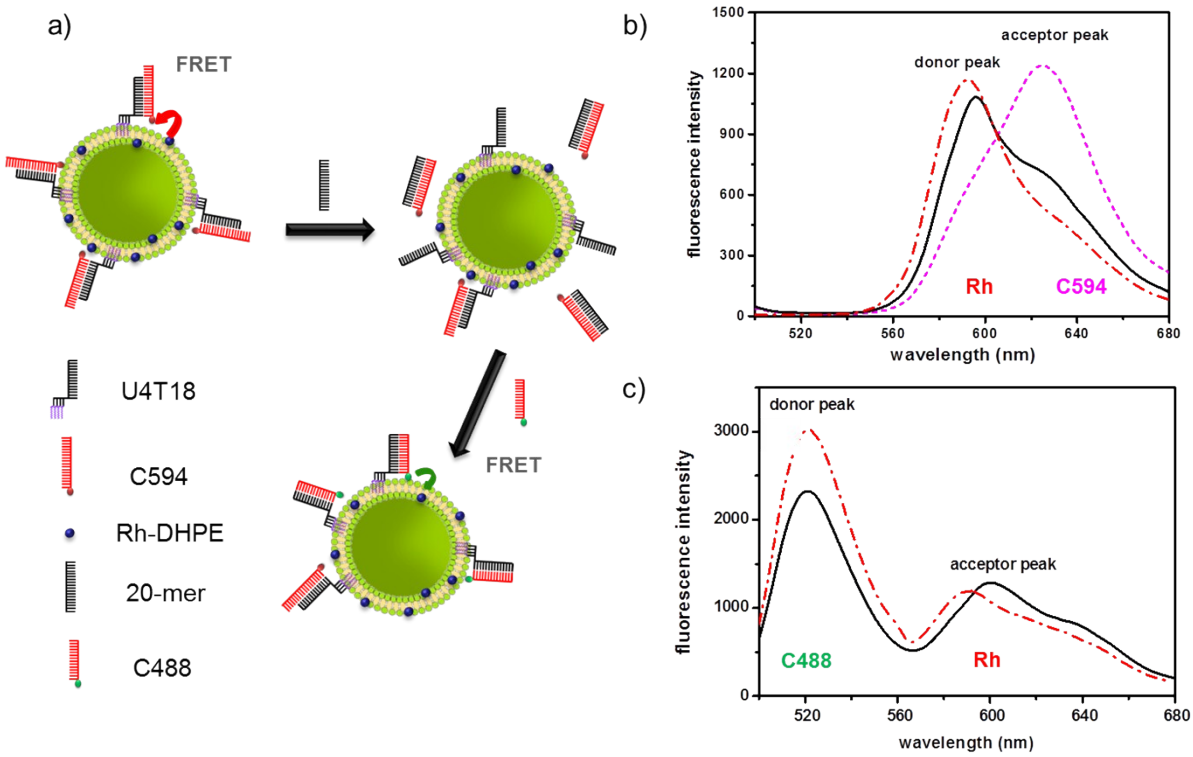


Figure S7. DNA hybridization and replacement at the surface of liposomes is demonstrated by FRET. a) Schematic representation of DNA replacement on the surface of liposomes. b) Fluorescence spectra (λ_{EX} = 470 nm) of FRET system with C594/Rh/20-mer. FRET is achieved when C594 hybridizes with U4T18 to bring the donor Rh closer to the acceptor C594 (dashed purple line). Afterwards, C594 was peeled off from U4T18 by hybridizing with 20-mer (black line). Disruption of liposomes by addition of Triton X-100 (0.3% (v/v)) results in termination of FRET (dashed red line). c) After C594 was removed (black line Fig. S7b), U4T18 remained on the liposome and maintained the ability to hybridize with C488, which leads to FRET between donor C488, and acceptor Rh (black line). Liposome disassembly after the addition of 0.3% (v/v) Triton X-100 results in an increase of C488 donor emission (dashed red line).

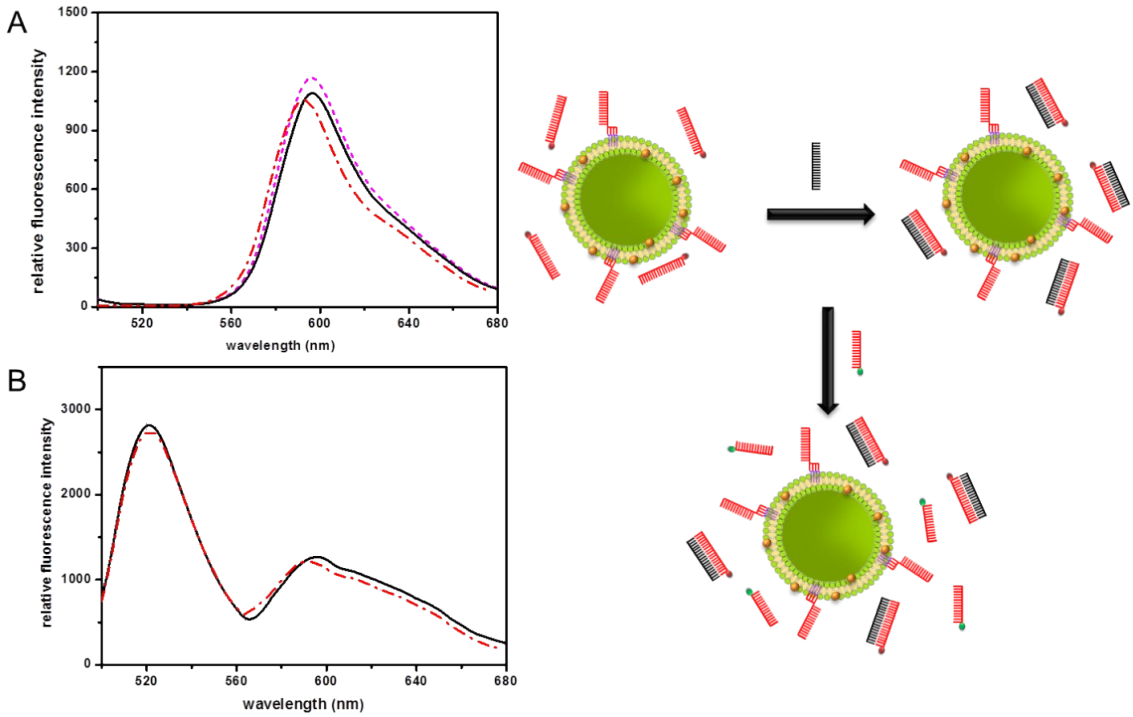


Figure S8. Absence of FRET response when liposomes are decorated with CrU4T18. a) Fluorescence spectra (λ_{EX} = 470 nm) of non-FRET system (CrU4T18/Rh-DHPE/C594) (dashed purple line). After 1h incubation, 20-mer was added to the system (black line). Disruption of liposomes by addition of Triton X-100 (0.3% (v/v)) results in termination of FRET (dashed red line). b) Fluorescence spectra of non-FRET system (CrU4T18/C488/Rh-DHPE) before (black line) and after (dashed red line) adding Triton X-100.

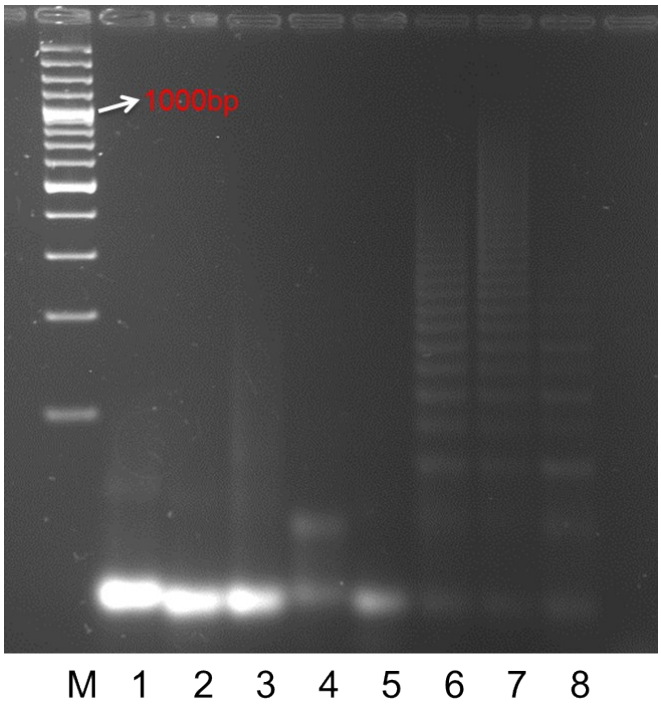


Figure S9. Agarose gel electrophoresis analysis of DNA HCR. Lane 1: M1; lane 2: M2; lane 3: M1+M2; lane 4: M1+U4T28; lane 5: M1+U4T28; lanes 6–8: three different molar ratios of initiator (1/1/1, 1/1/0.5, 1/1/2, M1/M2/U4T28).

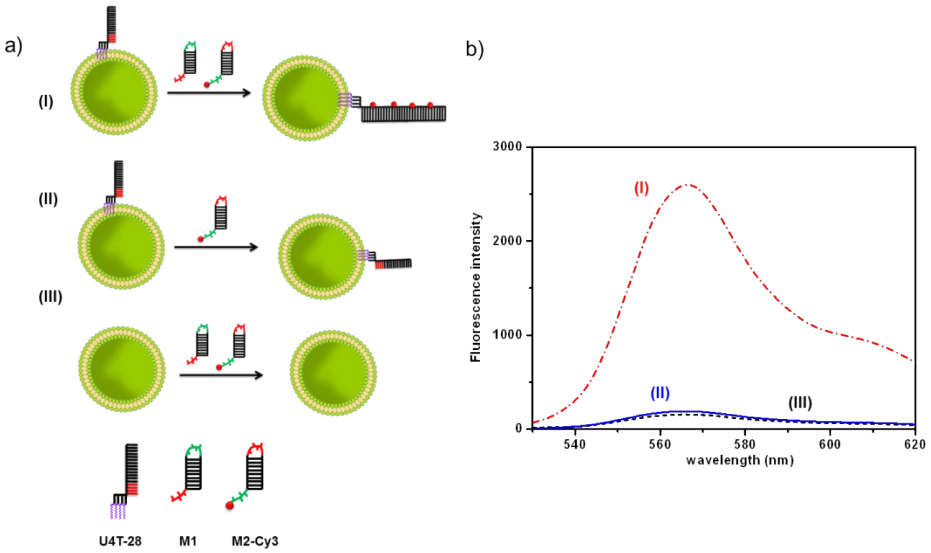


Figure S10. DNA hybridization chain reaction on the surface of liposomes. a) Schematic representation of HCR for decorating the outer surface of liposomes with a DNA shell. Liposomes (DOPC/DOPE/Chol = 2:1:1 mol%) decorated with U4T28 (I) (II), and incubated with M1 and Cy3 fluorescent labeled M2 (M2-Cy3) for 1 h, lead to hybridization chain reaction and concomitant fluorescence increase (I), In the absence of M1 (II) or plain liposomes were treated with M1 and M2-Cy3 (III) no fluorescence is observed. After centrifugation and filtration, the fluorescence of supernatant containing liposomes was measured. b) Fluorescence of polymerized M1/M2-Cy3 (I) at the liposomal membranes (dashed red line), and controls: HCR systems in absence of M1 (II, blue line) and U4T28 (III, dashed black line).