SUPPORTING INFORMATION FOR

Design, Synthesis and Membrane anchoring strength of Lipidated Polyaza crown ether DNA-conjugates (LiNAs) studied by DNA-controlled assembly of liposomes.

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Instrumentation

NMR spectra were recorded on a Varian Gemini 2000 Spectrometer. Chemical shifts are reported with TMS as an internal standard for ^{1}H NMR, relative to the deuterated solvent for ^{13}C NMR and with phosphoric acid as an external standard for ^{31}P NMR. Mass spectrum of the ethylene glycol linker phosphoramidite was recorded on a Sciex Q-Star Pulse instrument. Oligonucleotides were synthesized on an ExpediteTM 8900 nucleic acid synthesis system (Perceptive Biosystems Inc.) and purified by HPLC using a Waters 600 HPLC system (Waters 600 Controller, Waters 2996 PDA Detector, Waters 717Plus Autosampler, Waters Fraction Collector III) with a Xterra MS C18 10µm, 7.8 × 150 mm reversed-phase column or a Dionex Ultimate 3000 with a DIONEX Acclaim© C18 3 µm 300 Å reverse phase column. Mass spectra of oligonucleotides were recorded on a Voyager Elite Research Station (Perceptive Biosystems) or a Bruker Daltonics microflex LT MALDI-TOF. Water was purified by the Milli-Q® Advantage A10 Ultrapure Water Purification System (Merck Millipore). Liposomes were prepared by extrusion using a LIPEX™ Extruder (Northern Lipids). Thermal denaturation experiments were carried out on a Perkin Elmer Lambda 35 UV/VIS spectrometer with a PTP-6 (Peltier Temperature Programmer) and analysis of the data by the PETEMP v.5.1. software and PECSS software package v.4.3 or a Varian Cary 3E or 100 Bio UV-visible spectrophotometers with a Peltier controlled 6×6 sampler changer and Cary WinUV software.

Synthesis of lipid-modified oligonucleotides (LiNAs)

The lipid modifications were synthesized and incorporated into oligonucleotides (ONs) as previously described.[34,35] Briefly, oligonucleotides with lipid modifications were synthesized on a 0.2 µmol scale using standard conditions for automated synthesis for unmodified, standard phosphoramidites. For the lipid modified, non-nucleosidic phosphoramidites, amidites were dissolved in 1,2-dichloroethane at a concentration of 0.05 or 0.1 M, 1.8 M pyridine hydrochloride was used as activator, the coupling time was 15-30 minutes and the DMT protecting group on the last nucleotide was removed. For the ethylene glycol linker, tetrazole was used as an activator and the coupling time was 15 minutes. After deprotection and cleavage from the solid support using standard conditions (conc. NH_3(aq.) over night at 55 °C), the oligonucleotides were purified by HPLC using the following gradient program: 2 min. isocratic with 0.05 M triethylamine ammonium acetate, pH 7.4 (buffer A), followed by a 38 min. linear gradient to 70% 1:3 H_2O:MeCN (buffer B), which was increased to 100% over 7 min. and then continued for 30 min., with a flow rate of 2.5 mL/min. (Waters) or 2 min. isocratic with 0.05 M triethylamine ammonium acetate (TEAA) (buffer A) followed by a 8 min. linear gradient to 70% 1: 3 H_2O:MeCN (buffer B), which was increased to 100% over 20 min. and then continued for 30 min. with a flow rate of 1.0 mL/min. (Dionex). The identity of the purified oligonucleotides was verified by mass spectrometry.

Preparation of POPC liposomes

1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC) was suspended in 10 mM HEPES buffer, 110 mM Na^+, pH 7.0 at a concentration of 10 mM, and extruded 10 times through two stacked polycarbonate filters with a pore size of 50 nm using compressed N_2 (~20-40 bar).

Thermal denaturation (T_m) experiments

Melting temperatures were determined as the maximum of the first derivative of thermal melting curves obtained by recording the absorbance of a sample at 260 nm as a function of the temperature at rate of 1 °C/min. Prior to the melting experiments, the samples were heated to 90 °C and then gradually cooled to the starting temperature (usually 10 or 20 °C). 1.0 µM of each oligonucleotide strand was used. For thermal denaturation experiments with liposomes, 62 nM of each oligonucleotide strand (if not noted otherwise), liposomes corresponding to a POPC concentration of 0.5 mM, and a rate of 0.5 °C/min. were used, and the melting temperature was determined as the minimum of the first derivative. All melting temperatures are reported with an uncertainty of ±1 °C and were performed at least in duplicate experiments and multiple thermal cycles (at least three).

Table S1. LiNA sequences and MALDI-MS of synthesized LiNAs.

Oligonucleotide	Sequence	Mass of $[M+H]^+$	
		Calculated	Experimental(m/z)
ON3.1	5'-TTT **X^{16}** TGT GGA AGA AGT TGG TG **X^{16}** TTT	9013.7	9014.9
ON3.2	5'-TTT **X^{16}** CAC CAA CTT CTT CCA CA **X^{16}** TTT	8702.6	8699.1
ON4.1	5'-TTT **X^{10}** TGT GGA AGA AGT TGG TG **X^{10}** TTT	8677.3	8674.4
ON4.2	5'-TTT **X^{10}** CAC CAA CTT CTT CCA CA **X^{10}** TTT	8366.2	8358.5
ON4.3	5'-TTT **X^{12}** TGT GGA AGA AGT TGG TG **X^{12}** TTT	8789.4	8788.1
ON4.4	5'-TTT **X^{12}** CAC CAA CTT CTT CCA CA **X^{12}** TTT	8478.4	8475.3
ON4.5	5'-TTT **X^{20}** TGT GGA AGA AGT TGG TG **X^{20}** TTT	9237.9	9239.7
ON4.6	5'-TTT **X^{20}** CAC CAA CTT CTT CCA CA **X^{20}** TTT	8926.9	8926.1
ON4.7	5'-T **X^{10}** TGT GGA AGA AGT TGG TG **X^{10}** T	7461.1	7456.8
ON4.8	5'-T **X^{10}** CAC CAA CTT CTT CCA CA **X^{10}** T	7150.1	7153.9
ON4.9	5'-T **X^{12}** TGT GGA AGA AGT TGG TG **X^{12}** T	7573.2	7572.6
ON4.10	5'-T **X^{12}** CAC CAA CTT CTT CCA CA **X^{12}** T	7262.2	7263.2
ON4.11	5'-T **X^{16}** TGT GGA AGA AGT TGG TG **X^{16}** T	7797.5	7799.2
ON4.12	5'-T **X^{16}** CAC CAA CTT CTT CCA CA **X^{16}** T	7486.4	7485.7
ON4.13	5'-T **X^{20}** TGT GGA AGA AGT TGG TG **X^{20}** T	8021.7	8016.7
ON4.14	5'-T **X^{20}** CAC CAA CTT CTT CCA CA **X^{20}** T	7710.7	7703.2
ON4.17	5'-TTT **X^{16}** TATA TGT GGA AGA AGT TGG TG TATA **X^{16}** TTT	11496.1	11498.5
ON4.18	5'-TTT **X^{16}** TATA CAC CAA CTT CTT CCA CA TATA **X^{16}** TTT	11169.1	11179.2
ON4.19	5'-TTT **X^{16} EE** TGT GGA AGA AGT TGG TG **EE X^{16}** TTT	9509.6	9502.0
ON4.20	5'-TTT **X^{16} EE** CAC CAA CTT CTT CCA CA **EE X^{16}** TTT	9182.6	9205.6
ON4.21	5'-T **X^{16} EE** TGT GGA AGA AGT TGG TG **EE X^{16}** T	8293.4	8296.1
ON4.22	5'-T **X^{16} EE** CAC CAA CTT CTT CCA CA **EE X^{16}** T	7966.4	7986.0
ON4.23	5'-TTT **X^{16}** TGT^{L} GGA AGA AGT^{L} TGG T^{L}G **X^{16}** TTT	9097.6	9097.7
ON4.24	5'-TTT **X^{16}** CAC CAA CT^{L}T CTT^{L} CCA CA **X^{16}** TTT	8758.6	8759.0

[1]**X**: lipid substituted macrocycle, **E**: ethylene glycol unit, T^{L} - LNA thymidine unit.

X = X^{10} - 2 x C_{10}, X^{12} - 2 x C_{12}, X^{16} - 2 x C_{16}, X^{20} - 2 x C_{20}, (C = number of carbons, saturated chains)

Table S2. T_m data for unmodified DNA targets

	Duplex	**without liposomes**	
Entry			
	5’-TGT GGA AGA AGT TGG TG	T_m/°C	ΔT_m/°C
1.a	3’-ACA CCT TCT TCA ACC AC	55.5	-
2.a	3’-ACA CCT TCT TCA TCC AC	49.0	-6.5
3.a	3’-ACA CCT TCT TCA GCC AC	51.5	-4.0
4.a	3’-ACA CCT TCT TCA CCC AC	48.0	-7.5
5.a	3’-TCA CCT TCT TCA ACC AC	57.0	1.5
6.a	3’-A_A CCT TCT TCA ACC AC	52.0	-3.5
7.a	3’-ACT ACC TTC TTC AAC CAC	52.0	-3.5
	5’-CAC CAA CTT CTT CCA CA		
1.b	3’-GTG GTT GAA GAA GGT GT	55.5	-
2.b	3’-GTG GTT GGA GAA TGT GT	44.5	-11.0
3.b	3’-GTG GTT GAA GAA AGT GT	46.0	-9.5
4.b	3’-GTG GTT GAA GAA CGT GT	44.0	-11.5
5.b	3’-GTG GTT GAA GAA GGT GA	56.0	0.5
6.b	3’-G_G GTT GAA GAA GGT GT	52.5	-3.0
7.b	3’-GTA GGT TGA AGA AGG TGT	50.5	-5.0

Table S3. T_m data for unmodified RNA targets

	Unmodified DNA/RNA		
Entry	Duplex	without liposomes	
	5’-TGT GGA AGA AGT TGG TG	T_m/°C	ΔT_m/°C
1.a	3’-ACA CCU UCU UCA ACC AC	51.0	-
2.a	3’-ACA CCU UCU UCA UCC AC	42.0	-9.0
3.a	3’-ACA CCU UCU UCA GCC AC	46.0	-5.0
4.a	3’-ACA CCU UCU UCA CCC AC	41.0	-10.0
5.a	3’-UCA CCU UCU UCA ACC AC	50.0	-1.0
6.a	3’-A_A CCU UCU UCA ACC AC	46.0	-5.0
7.a	3’-ACU ACC UUC UUC AAC CAC	46.0	-5.0
	5’-CAC CAA CTT CTT CCA CA		
1.b	3’-GUG GUU GAA GAA GGU GU	55.0	-
2.b	3’-GUG GUU GGA GAA UGU GU	41.5	-13.5
3.b	3’-GUG GUU GAA GAA AGU GU	46.0	-9.0
4.b	3’-GUG GUU GAA GAA CGU GU	41.5	-13.5
5.b	3’-GUG GUU GAA GAA GGU GA	58.0	3.0
6.b	3’-G_G GTUU GAA GAA GGU GU	56.5	1.5
7.b	3’-GUA GGU UGA AGA AGG UGU	55.5	0.5

Table S4. T_m mismatch data for X^{16}-LiNAs and DNA targets

	Modified DNA				
	Duplex	without liposomes		with liposomes	
Entry	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC		-	-	-
ON1^X	5'-TTT **X^{16}** TGT GGA AGA AGT TGG TG **X^{16}** TTT	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1.a	3'-ACA CCT TCT TCA ACC AC	46.0	-	47.0	-
2.a	3'-ACA CCT TCT TCA TCC AC	41.0	-5.0	40.0	-7.0
3.a	3'-ACA CCT TCT TCA GCC AC	43.5	-2.5	43.5	-3.5
4.a	3'-ACA CCT TCT TCA CCC AC	38.0	-8.0	38.5	-8.5
5.a	3'-TCA CCT TCT TCA ACC AC	47.5	1.5	47.0	0.0
6.a	3'-A_A CCT TCT TCA ACC AC	44.5	-1.5	42.5	-4.5
7.a	3'-ACT ACC TTC TTC AAC CAC	41.5	-4.5	43.0	-4.0
ON2^X	5'-TTT **X^{16}** CAC CAA CTT CTT CCA CA **X^{16}** TTT				
1.b	3'-GTG GTT GAA GAA GGT GT	48.0	-	49.5	-
2.b	3'-GTG GTT GGA GAA TGT GT	38.5[a]	-9.5	36.5	-13.0
3.b	3'-GTG GTT GAA GAA AGT GT	39.0[a]	-9.0	38.5	-11.0
4.b	3'-GTG GTT GAA GAA CGT GT	38.5[a]	-9.5	35.5	-14.0
5.b	3'-GTG GTT GAA GAA GGT GA	48.5	0.5	49.0	-0.5
6.b	3'-G_G GTT GAA GAA GGT GT	49.0[a]	1.0	44.5	-5.0
7.b	3'-GTA GGT TGA AGA AGG TGT	42.5[a]	-5.5	42.0	-7.5

Table S4 [a]) Thermal denaturation curve has extra features around T_m for which reason the T_m is determined with uncertainty.

Table S5. T_m data for cholesteryl-modified LiNAs - ON1^Y

	Cholesteryl-modified DNA				
Entry	Duplex	without liposomes		with liposomes	
ON1^Y	5'-TTT**Y**TGT GGA AGA AGT TGG TG**Y**TTT	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1	3'-ACA CCT TCT TCA ACC AC	46.0	-	46.0	-
2	3'-ACA CCT TCT TCA TCC AC	n.d.	n.d.	39.0	-7.0
3	3'-ACA CCT TCT TCA GCC AC	n.d.	n.d.	42.5	-3.5
4	3'-ACA CCT TCT TCA CCC AC	n.d.	n.d.	38.5	-7.5
5	3'-TCA CCT TCT TCA ACC AC	n.d.	n.d.	46.5	0.5
6	3'-A_A CCT TCT TCA ACC AC	n.d.	n.d.	41.5	-4.5
7	3'-ACT ACC TTC TTC AAC CAC	n.d.	n.d.	42.5	-3.5

Table S5. n.d.: not determined.

Table S6. T_m data for X^{16}-LiNAs and RNA targets

	Modified DNA/RNA Duplex	without liposomes		with liposomes	
Entry	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC		-	-	-
ON1[X]	5'-TTT **X[16]** TGT GGA AGA AGT TGG TG **X[16]** TTT	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1.a	3'-ACA CCU UCU UCA ACC AC	48.5[a]	-	42.0	-
2.a	3'-ACA CCU UCU UCA UCC AC	27.5	-21.0	34.5	-7.5
3.a	3'-ACA CCU UCU UCA GCC AC	43.0[a]	-5.5	40.0	-2.0
4.a	3'-ACA CCU UCU UCA CCC AC	24.0	-24.5	31.0	-11.0
5.a	3'-UCA CCU UCU UCA ACC AC	38.0[a]	-10.5	40.0	-2.0
6.a	3'-A_A CCU UCU UCA ACC AC	30.5[a]	-18.0	32.5	-9.5
7.a	3'-ACU ACC UUC UUC AAC CAC	28.5[a]	-20.0	37.5	-2.5
ON2[X]	5'-TTT **X[16]** CAC CAA CTT CTT CCA CA **X[16]** TTT				
1.b	3'-GUG GUU GAA GAA GGU GU	48.5[a]	-	51.5	-
2.b	3'-GUG GUU GAA GAA UGU GU	34.0[a]	-14.5	35.0	-16.5
3.b	3'-GUG GUU GAA GAA AGU GU	36.5[a]	-12.0	42.5	-9.0
4.b	3'-GUG GUU GAA GAA CGU GU	37.0	-11.5	35.5	-16.0
5.b	3'-GUG GUU GAA GAA GGU GA	28.5[a]	-20.0	52.0	0.5
6.b	3'-G_G GUU GAA GAA GGU GU	<30[a]	>18.5	48.5	-3.0
7.b	3'-GUA GGU UGA AGA AGG UGU	<30[a]	>18.5	43.0	-8.5

Table S6 [a]) Thermal denaturation curve has extra transitions which results in T_m values with more uncertainty.

Table S7. T_m data for double lipid modified X^{16}-LiNAs and DNA targets

	Triple-modified DNA				
	Duplex	without liposomes		with liposomes	
Entry	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC		-	-	-
	5'-TTT **X**^{16} **X**^{16} TGT GGA AGA AGT TGG TG **X**^{16} TTT	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1.a	3'-ACA CCT TCT TCA ACC AC	n.d	-	47.0	-
2.a	3'-ACA CCT TCT TCA TCC AC	n.d.	n.d.	40.5	-6.5
3.a	3'-ACA CCT TCT TCA GCC AC	n.d.	n.d.	43.5	-3.5
4.a	3'-ACA CCT TCT TCA CCC AC	n.d.	n.d.	39.5	-7.5
5.a	3'-TCA CCT TCT TCA ACC AC	n.d.	n.d.	47.0	0
6.a	3'-A_A CCT TCT TCA ACC AC	n.d.	n.d.	43.0	-4.0
7.a	3'-ACT ACC TTC TTC AAC CAC	n.d.	n.d.	42.5	-4.5
	5'-TTT **X**^{16} **X**^{16} CAC CAA CTT CTT CCA CA **X**^{16} TTT				
1.b	3'-GTG GTT GAA GAA GGT GT	n.d.	n.d.	49.0	-
2.b	3'-GTG GTT GGA GAA TGT GT	n.d.	n.d.	37.0	-12.0
3.b	3'-GTG GTT GAA GAA AGT GT	n.d.	n.d.	38.5	-10.5
4.b	3'-GTG GTT GAA GAA CGT GT	n.d.	n.d.	37.5	-11.5
5.b	3'-GTG GTT GAA GAA GGT GA	n.d.	n.d.	49.5	0.5
6.b	3'-G_G GTT GAA GAA GGT GT	n.d.	n.d.	45.5	-3.5
7.b	3'-GTA GGT TGA AGA AGG TGT	n.d.	n.d.	43.0	-6.0

Table S7. n.d.: not determined.

Table S8. Tm data for X[10]-LiNAs with DNA targets

Unmodified DNA/RNA			
Entry	Duplex	without liposomes	with liposomes
		T_m/°C	ΔT_m/°C
1	5'-TTT**X[10]**TGTGGAAGAAGTTGGTG**X[10]**TTT	n.d.	-
2	5'-TTT**X[10]**CACCAACTTCTTCCACA**X[10]**TTT	n.d.	-
3	5'-TTT**X[10]**TGTGGAAGAAGTTGGTG**X[10]**TTT 3'-TTT**X[10]**ACACCTTCTTCAACCAC**X[10]**TTT	70.5	-
4	5'-TTT**X[10]**TGTGGAAGAAGTTGGTG**X[10]**TTT 3'-ACACCTTCTTCAACCAC	61.5	-
5	5'-TTT**X[10]**CACCAACTTCTTCCACA**X[10]**TTT 3'-GTGGTTGAAGAAGGTGT	60.5	-
6	5'-TTT**X[10]**TGTGGAAGAAGTTGGTGTG**X[10]**TTT 3'- ACGGCCATATATGCTACACCTTCTTCAACCACCCAGAACACGTGGTT	57.5	-
7	5'-TTT**X[10]**CACCAACTTCTTCCACA**X[10]**TTT 3'-AACCACGTGTTCTAAGTGGTTGAAGAAGGTGTTGCATATATGGCCGT	58.0	-
8	5'-TTT**X[10]**TGTGGAAGAAGTTGGTG**X[10]**TTT 3'-ACACCUUCUUCAACCAC	52.5	-
9	5'-TTT**X[10]**CACCAACTTCTTCCACA**X[10]**TTT 3'-GUGGUUGAAGAAGGUGU	61.0	-
10	5'-TTT**X[10]**TGTGGAAGAAGTTGGTG**X[10]**TTT 3'-ACGGCCAUAUAUGCUACACCUUCUUCAACCACCCAGAACACGUGGUU	25.0[b]	-
11	5-TTT**X[10]**CACCAACTTCTTCCACA**X[10]**TTT 3'-AACCACGUGUUCUAAGUGGUUGAAGAAGGUGUUGCAUAUAUGGCCGU	46.5[c]	-

Table S8 Thermal denaturation experiments in 10 mM HEPES, 110 mM Na^+, pH 7.0. 1.0 µM DNA was used for measurements without liposomes and 62 nM DNA for measurements with liposomes. A "-" indicates that no transition was observed. a) Very broad transition of low intensity, b) thermal denaturation curve has extra features around the melting temperature for which reason it is determined with uncertainty, c) not reproducible transition observed at 32-37 °C, d) broad transitions in the stated interval.

Table S9. Tm data for X[12]-LiNAs with DNA targets

Unmodified DNA/RNA			
Entry	Duplex	without liposomes	with liposomes
		T_m/°C	ΔT_m/°C
1	5'-T**X[12]**TGTGGAAGAAGTTGGTG**X[12]**T	n.d.	-
2	5'-T**X[12]**CACCAACTTCTTCCACA**X[12]**T	n.d.	-
3	5'-T**X[12]**TGTGGAAGAAGTTGGTG**X[12]**T 3'-T**X[12]**ACACCTTCTTCAACCAC**X[12]**T	78.5[a]	69.5
4	5'-T**X[12]**TGTGGAAGAAGTTGGTG**X[12]**T 3'-ACACCTTCTTCAACCAC	53.5	52.5
5	5'-T**X[12]**CACCAACTTCTTCCACA**X[12]**T 3'-GTGGTTGAAGAAGGTGT	54.5	53.0
6	5'-T**X[12]**TGTGGAAGAAGTTGGTGTG**X[12]**T 3'-ACGGCCATATATGCTACACCTTCTTCAACCACCCAGAACACGTGGTT	55.0	42.5
7	5'-T**X[12]**CACCAACTTCTTCCACA**X[12]**T 3'-AACCACGTGTTCTAAGTGGTTGAAGAAGGTGTTGCATATATGGCCGT	-[b]	-
8	5'-T**X[12]**TGTGGAAGAAGTTGGTG**X[12]**T 3'-ACACCUUCUUCAACCAC	43.5	45.5
9	5'-T**X[12]**CACCAACTTCTTCCACA**X[12]**T 3'-GUGGUUGAAGAAGGUGU	54.0	56.0
10	5'-T**X[12]**TGTGGAAGAAGTTGGTG**X[12]**T 3'-ACGGCCAUAUAUGCUACACCUUCUUCAACCACCCAGAACACGUGGUU	n.d.	-
11	5'-T**X[12]**CACCAACTTCTTCCACA**X[12]**T 3'-AACCACGUGUUCUAAGUGGUUGAAGAAGGUGUUGCAUAUAUGGCCGU	n.d	44.5

Table S9 Thermal denaturation experiments in 10 mM HEPES, 110 mM Na^+, pH 7.0. 1.0 µM DNA was used for measurements without liposomes and 62 nM DNA for measurements with liposomes. A "-" indicates that no transition was observed. a) thermal denaturation curves show a smaller transition at a lower temperature (a feature often observed, b) despite an increase in the absorbance, no evident thermal transition can be determined. n.d.: not determined.

Table S10. Tm data for X^{14}-LiNAs with DNA targets

Unmodified DNA/RNA			
Entry	Duplex	without liposomes	with liposomes
		T_m/°C	ΔT_m/°C
1	5'-TTT**X**^{14}TGTGGAAGAAGTTGGTG**X**^{14}TTT	n.d.	n.d.
2	5'-TTT**X**^{14}CACCAACTTCTTCCACA**X**^{14}TTT	n.d.	n.d.
3	5'-TTT**X**^{14}TGTGGAAGAAGTTGGTG**X**^{14}TTT 3'-TTT**X**^{14}ACACCTTCTTCAACCAC**X**^{14}TTT	73.0[a]	65.0
4	5'-TTT**X**^{14}TGTGGAAGAAGTTGGTG**X**^{14}TTT 3'-ACACCTTCTTCAACCAC	53.0	52.0
5	5'-5'-TTT**X**^{14}CACCAACTTCTTCCACA**X**^{14}TTT 3'- GTGGTTGAAGAAGGTGT	53.5	51.0
3	5'-T**X**^{14}TGTGGAAGAAGTTGGTG**X**^{14}T 3'-T**X**^{14}ACACCTTCTTCAACCAC**X**^{14}T	85.0	n.d.
4	5'-T**X**^{14}TGTGGAAGAAGTTGGTG**X**^{14}T 3'-ACACCTTCTTCAACCAC	46.0	53.0
5	5'-T**X**^{14}CACCAACTTCTTCCACA**X**^{14}T 3'- GTGGTTGAAGAAGGTGT	47.0	51.0

Table 10 Thermal denaturation experiments in 10 mM HEPES, 110 mM Na^+, pH 7.0. 1.0 µM DNA was used for measurements without liposomes and 62 nM DNA for measurements with liposomes. A "-" indicates that no transition was observed. a) thermal denaturation curves show a smaller transition at a lower temperature (a feature often observed, b) despite an increase in the absorbance, no evident thermal transition can be determined. n.d.: not determined.

Table S11. Tm data for X[16]-LiNAs with DNA targets

Unmodified DNA/RNA			
Entry	Duplex	without liposomes	with liposomes
		T_m/°C	ΔT_m/°C
1	5'-T**X[16]**TGTGGAAGAAGTTGGTG **X[16]**T	n.d.	-
2	5'-T **X[16]**CACCAACTTCTTCCACA **X[16]**T	n.d.	-
3	5'-T **X[16]**TGTGGAAGAAGTTGGTG **X[16]**T 3'-T **X[16]**ACACCTTCTTCAACCAC **X[16]**T	>80	74.0
4	5'-T **X[16]**TGTGGAAGAAGTTGGTG **X[16]**T 3'-ACACCTTCTTCAACCAC	54.0[a]	53.0
5	5'-T **X[16]**CACCAACTTCTTCCACA **X[16]**T 3'-GTGGTTGAAGAAGGTGT	51.0[a]	52.5
6	5'-T **X[16]**TGTGGAAGAAGTTGGTGTG **X[16]**T 3'-ACGGCCATATATGCTACACCTTCTTCAACCACCCAGAACACGTGGTT	-[b]	-
7	5'-T **X[16]**CACCAACTTCTTCCACA **X[16]**T 3'-AACCACGTGTTCTAAGTGGTTGAAGAAGGTGTTGCATATATGGCCGT	-[b]	40.0[d]
8	5'-T **X[16]**TGTGGAAGAAGTTGGTG **X[16]**T 3'-ACACCUUCUUCAACCAC	43.5[a]	49.0
9	5'-T **X[16]**CACCAACTTCTTCCACA **X[16]**T 3'-GUGGUUGAAGAAGGUGU	51.0[c]	56.5
10	5'-T **X[16]**TGTGGAAGAAGTTGGTG **X[16]**T 3'-ACGGCCAUAUAUGCUACACCUUCUUCAACCACCCAGAACACGUGGUU	-[b]	40.0[d]
11	5-T **X[16]**CACCAACTTCTTCCACA **X[16]**T 3'-AACCACGUGUUCUAAGUGGUUGAAGAAGGUGUUGCAUAUAUGGCCGU	42.5	51.5

Table S11 Thermal denaturation experiments in 10 mM HEPES, 110 mM Na^+, pH 7.0. 1.0 µM DNA was used for measurements without liposomes and 62 nM DNA measurements with liposomes. A "-" indicates that no transition was observed. a) thermal denaturation curve has extra features around the melting temperature for which reason it is determined with higher uncertainty, b) despite an increase in the absorbance, no evident thermal transition can be determined, c) thermal denaturation curve has a smaller transition at a lower temperature (a feature often observed), d) transition of very low intensity. n.d.: not determined.

Table S12. Tm data for X^{20}-LiNAs with DNA targets

Unmodified DNA/RNA			
Entry	Duplex	without liposomes	with liposomes
		T_m/°C	ΔT_m/°C
1	5'-T**X^{20}**TGTGGAAGAAGTTGGTG**X^{20}**T	n.d.	-
2	5'-T**X^{20}**CACCAACTTCTTCCACA**X^{20}**T	n.d.	-
3	5'-T**X^{20}**TGTGGAAGAAGTTGGTG**X^{20}**T 3'-T**X^{20}**ACACCTTCTTCAACCAC**X^{20}**T	71.0[a]	-
4	5'-T**X^{20}**TGTGGAAGAAGTTGGTG**X^{20}**T 3'-ACACCTTCTTCAACCAC	48.0[b]	-
5	5'-T**X^{20}**CACCAACTTCTTCCACA**X^{20}**T 3'-GTGGTTGAAGAAGGTGT	50.5[b]	-
6	5'-T**X^{20}**TGTGGAAGAAGTTGGTGTG**X^{20}**T 3'-ACGGCCATATATGCTACACCTTCTTCAACCACCCAGAACACGTGGTT	41.0[c]	-
7	5'-T**X^{20}**CACCAACTTCTTCCACA**X^{20}**T 3'-AACCACGTGTTCTAAGTGGTTGAAGAAGGTGTTGCATATATGGCCGT	51.0[d]	-
8	5'-T**X^{20}**TGTGGAAGAAGTTGGTG**X^{20}**T 3'-ACACCUUCUUCAACCAC	23.5[e]	-
9	5'-T**X^{20}**CACCAACTTCTTCCACA**X^{20}**T 3'-GUGGUUGAAGAAGGUGU	50.0[f]	-
10	5'-T**X^{20}**TGTGGAAGAAGTTGGTG**X^{20}**T 3'-ACGGCCAUAUAUGCUACACCUUCUUCAACCACCCAGAACACGUGGUU	-[g]	-
11	5-T**X^{20}**CACCAACTTCTTCCACA**X^{20}**T 3'-AACCACGUGUUCUAAGUGGUUGAAGAAGGUGUUGCAUAUAUGGCCGU	43.0[e]	-

Table S12 Thermal denaturation experiments in 10 mM HEPES, 110 mM Na^+, pH 7.0. 1.0 µM DNA was used for measurements without liposomes and 62 nM DNA measurements with liposomes. A "-" indicates that no transition was observed. a) Very broad transition, b) broad thermal denaturation curve with a smaller transition at a lower temperature (a feature often observed), c) very broad thermal denaturation curve with a smaller transition at a lower temperature (a feature often observed) d) Very broad transition with extra features, temperature determined with great uncertainty, e) vary broad and flat transition, f) thermal denaturation curve has extra features around the melting temperature for which reason it is determined with uncertainty.

Table S13. T_m data for LNA modified X^{16}-LiNAs and DNA targets

	Modified DNA with LNA				
	Duplex	without liposomes		with liposomes	
Entry	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC		-	-	-
	5'-TTT **X**[16] TG**T**[L] GGA AGA AG**T**[L] TGG **T**[L]G **X**[16] TTT	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
1.a	3'-ACA CCT TCT TCA ACC AC	54.0	-	57.0	-
2.a	3'-ACA CCT TCT TCA TCC AC	46.5	-7.5	47.5	-9.5
3.a	3'-ACA CCT TCT TCA GCC AC	50.0	-4.0	50.0	-7.0
4.a	3'-ACA CCT TCT TCA CCC AC	43.5	-10.5	45.0	-12.0
5.a	3'-TCA CCT TCT TCA ACC AC	53.0	-1.0	55.0	-2.0
6.a	3'-A_A CCT TCT TCA ACC AC	46.5	-7.5	48.0	-9.0
7.a	3'-ACT ACC TTC TTC AAC CAC	46.5	-7.5	48.5	-8.5
	5'-TTT **X**[16] CAC CAA C**T**[L]T CT**T**[L] CCA CA **X**[16] TTT				
1.b	3'-GTG GTT GAA GAA GGT GT	56.5	-	54.5	-
2.b	3'-GTG GTT GGA GAA TGT GT	40.0	-16.5	40.0	-14.5
3.b	3'-GTG GTT GAA GAA AGT GT	42.0	-14.5	41.5	-13.0
4.b	3'-GTG GTT GAA GAA CGT GT	40.5	-16.0	39.0	-15.5
5.b	3'-GTG GTT GAA GAA GGT GA	54.5	-2.0	54.0	-0.5
6.b	3'-G_G GTT GAA GAA GGT GT	51.5	-5.0	48.0	-6.5
7.b	3'-GTA GGT TGA AGA AGG TGT	48.5	-8.0	46.5	-8.0

Table S14. Concentration dependent T_m data for LNA modified X^{16}-LiNAs and DNA targets

	Comparison of modified LiNA strands with and without LNA at different concentrations			
		with liposomes		
	Duplex		T_m/°C	
Entry		50 nM	25 nM	12.5 nM
1	5'-TTT **X**[16] TG**T**[L] GGA AGA AG**T**[L] TGG **T**[L]G **X**[16] TTT 3'-ACA CCT TCT TCA ACC AC	53.0	50.5	49.5
2	5'-TTT **X**[16] TGT GGA AGA AGT TGG TG **X**[16] TTT 3'-ACA CCT TCT TCA ACC AC	48.0	46.0	44.5
1	5'-TTT **X**[16] CAC CAA C**T**[L]T CT**T**[L] CCA CA **X**[16] TTT 3'-GTG GTT GAA GAA GGT GT	51.5	49.0	47.5
2	5'-TTT **X**[16] CAC CAA CTT CTT CCA CA **X**[16] TTT 3'-GTG GTT GAA GAA GGT GT	48.0	46.0	45.0

Table S15. Concentration dependent T_m data for single lipid X^{16}-LiNAs and DNA targets

Entry	Single-modified DNA Duplex	without liposomes	with liposomes		
			62.5 nM	125 nM	250 nM
		T_m/°C	T_m/°C	T_m/°C	T_m/°C
1	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC	55.0			
2	5'-TTT **X**^{16} TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC **X**^{16} TTT	62.5	n.t.	n.t.	n.t.
3	5'-TGT GGA AGA AGT TGG TG **X**^{16} TTT 3'-TTT **X**^{16} ACA CCT TCT TCA ACC AC	60.0	(40)[a]	n.d	n.d.
4	5'-TTT **X**^{16} TGT GGA AGA AGT TGG TG 3'-TTT **X**^{16} ACA CCT TCT TCA ACC AC	76.0	n.t.	n.d.	n.d.
5	5'-TGT GGA AGA AGT TGG TG **X**^{16} TTT 3'-ACA CCT TCT TCA ACC AC **X**^{16} TTT	74.5	n.t.	n.d.	n.d.
6	5'-TTT **X**^{16} TGT GGA AGA AGT TGG TG	n.d.	n.t.	n.t.	n.t.
7	5'-TTT **X**^{16} CAC CAA CTT CTT CCA CA	n.d.	n.t.	n.t.	n.t.
8	5'-TGT GGA AGA AGT TGG TG **X**^{16} TTT	n.d.	n.t.	n.d.	n.d.
9	5'-CAC CAA CTT CTT CCA CA **X**^{16} TTT	n.d.	n.t.	n.d.	n.d.
10	5'-TTT **X**^{16} TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC	56.5	n.t.	n.d.	n.d.
11	5'-TTT **X**^{16} CAC CAA CTT CTT CCA CA 3'-GTG GTT GAA GAA GGT GT	58.5	n.t.	n.d.	n.d.
12	5'-TGT GGA AGA AGT TGG TG **X**^{16} TTT 3'-ACA CCT TCT TCA ACC AC	57.5	n.t.	n.d.	n.d.
13	5'-CAC CAA CTT CTT CCA CA **X**^{16} TTT 3'-GTG GTT GAA GAA GGT GT	59.5	n.t.	n.d.	n.d.

Table S15 n.d.: not determined, n.t.; no transition, a) broad transition.

Table S16. T_m data for for single lipid modified LNA modified LiNAs and DNA targets

	LiNAs modified with LNA		
Entry	Duplex	without liposomes	with liposomes
1	5'-TGT GGA AGA AGT TGG TG 3'-ACA CCT TCT TCA ACC AC	58.0	-
		T_m/°C	T_m/°C
2	5'-TGT^L GGA AGA AGT^L TGG T^{L}G X TTT	n.d.	n.t
3	5'-CAC CAA CT^{L}T CTT^L CCA CA X TTT	n.d.	n.t
4	5'-TGT^L GGA AGA AGT^L TGG T^{L}G X TTT 3'-TTT X ACA CCT^L TCT T^{L}CA ACC AC	70.0	(33)[a]
5	5'-TGT GGA AGA AGT TGG TG 3'-TTT X ACA CCT^L TCT T^{L}CA ACC AC	63.5	-[b]
6	5'-TGT^L GGA AGA AGT^L TGG T^{L}G X TTT 3'-ACA CCT TCT TCA ACC AC	64.5	-[b]

Table S16 n.d.: not determined, n.t.: no transition, a) very broad transition, b) a very weak transition corresponding to the T_m for the unmodified duplex (entry 1) was observed.

Table S17. T_m data for X^{16}-LiNAs with short target strands

	LiNAs with short target strands		
	Duplex	without liposomes	with liposomes
Entry		T_m/°C	T_m/°C
	5'-TTT **X** TGT GGA AGA AGT TGG TG **X** TTT		
1.a	3'-ACA CCT TCT TCA ACC AC	47.0[a]	50.5
1.b	3'-A CCT TCT TCA ACC	30.0[a]	34.0
1.c	3'-CT TCT TCC A	i.a.	n.t.
	5'-TTT **X** CAC CAA CTT CTT CCA CA **X** TTT		
2.a	3'-GTG GTT GGA GAA GGT GT	49.0[a]	51.0
2.b	3'-G GTT GAA GAA GGT	31.0[a]	35.5
2.c	3'-TT GAA GAA G	i.a.	n.t.

Table S17 i.a.: Despite an increase in absorbance, no evident thermal transition can be determined (5-80 °C), n.t.: no transition, [a)] Thermal denaturation curve has extra features around T_m for which reason the melting temperature is determined with uncertainty.

Table S18. T_m data for LiNAs modified with ethylene glycol linkers

	Modified LiNAs with ethylene glycol linker		
	Duplex	without liposomes	with liposomes
		T_m/°C	T_m/°C
Entry			
1.a	5'-TTT **X EE** TGT GGA AGA AGT TGG TG **EE X** TTT	n.d.	n.t.
2.a	5'-TTT **X EE** TGT GGA AGA AGT TGG TG **EE X** TTT 3'-ACA CCT TCT TCA ACC AC	53.0[a]	47.0
3.a	5'-TTT **X EE** TGT GGA AGA AGT TGG TG **EE X** TTT 3'-ACA CCU UCU UCA ACC AC	47.5[a]	41.0
1.b	5'-TTT **X EE** CAC CAA CTT CTT CCA CA **EE X** TTT	n.d.	n.t.
2.b	5'-TTT **X EE** CAC CAA CTT CTT CCA CA **EE X** TTT 3'-GTG GTT GGA GAA GGT GT	49.5[a]	49.0
3.b	5'-TTT **X EE** CAC CAA CTT CTT CCA CA **EE X** TTT 3'-GUG GUU GGA GAA GGU GU	54.0	52.0

Table S18 n.d.: not determined, n.t.: no transition, [a]) Thermal denaturation curve has extra features around T_m for which reason the melting temperature is determined with higher uncertainty.

Table S19. T_m data for LiNAs with TATA linkers

LiNAs with nucleotide linker (TATA)

	Duplex	without liposomes	with liposomes	
		T_m/°C	T_m/°C	
Entry			62.5 nM	125 nM
1.a	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT	n.d.	n.d.	n.t.
2.a	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT 3'-ACA CCT TCT TCA ACC AC	47.5	n.t.	n.t.
3.a	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT 3'-ACA CCU UCU UCA ACC AC	45.0	n.t.	n.t.
4.a	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT 3'-ACGGCCATATATGCTACACCTTCTTCAACCACCCAGAACACGTGGTT	34.0[b]	n.t.	n.t.
5.a	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT 3'-ACGGCCAUAUAUGCUACACCUUCUUCAACCACCCAGAACACGUGGUU	46.5	n.t.	n.t.
1.b	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT	n.d.	n.t.	n.d.
2.b	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT 3'-GTG GTT GAA GAA GGT GT	59.0	n.t.	40.0[a]
3.b	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT 3'-GUG GUU GGA GAA GGU GU	55.0	n.t.	n.t.
4.b	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT 3'-AACCACGTGTTCTAAGTGGTTGAAGAAGGTGTTGCATATATGGCCGT	n.t.	n.t.	n.t.
5.b	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT 3'-AACCACGUGGUCUAAGUGGUUGAAGAAGGUGUUGCAUAUAUGGCCGU	44.0	n.t.	45.0

Table S19 n.d.: not determined, n.t.: no transition, a) very weak transition, b) broad transition, underlined section are regions complementary to the corresponding LiNA probe.

Table S20. Sequences of unmodified DNA oligonucleotides

Sequences and numbering of unmodified oligonucleotides

ON	Sequence of oligonucleotides	#(nucleotides)
DNA1	3'-ACA CCT TCT TCA ACC AC	17
DNA2	3'-ACA CCT TCT TCA TCC AC	17
DNA3	3'-ACA CCT TCT TCA GCC AC	17
DNA4	3'-ACA CCT TCT TCA CCC AC	17
DNA5	3'-TCA CCT TCT TCA ACC AC	17
DNA6	3'-A_A CCT TCT TCA ACC AC	16
DNA7	3'-ACAT CCT TCT TCA ACC AC	18
DNA8	3'-GTG GTT GAA GAA GGT GT	17
DNA9	3'-GTG GTT GAA GAA TGT GT	17
DNA10	3'-GTG GTT GAA GAA AGT GT	17
DNA11	3'-GTG GTT GAA GAA CGT GT	17
DNA12	3'-GTG GTT GAA GAA GGT GA	17
DNA13	3'-G_G GTT GAA GAA GGT GT	16
DNA14	3'-GTGA GTT GAA GAA GGT GT	18
DNA15	3'-ACA CCT TCT TCA ACC AC ACA CCT TCT TCA ACC AC	34
DNA16	3'-GTG GTT GAA GAA GGT GT GTG GTT GAA GAA GGT G T	34
DNA17	3'-GTG GTT GAA GAA TGT GT G TG GTT GAA GAA GGT G T	34
DNA18	3'-GTG GTT GAA GAA AGT GT GTG GTT GAA GAA GGT G T	34
DNA19	3'-GTG GTT GAA GAA CGT GT GTG GTT GAA GAA GGT G T	34
DNA20	3'-AGT CCA TGA CGA TAG GTG GGA GTT TGT	27
DNA21	3'-GCT ACT AAA GTT GAA GAA GTG GTT GAA	27
DNA22	3'-ACT AAT ACC GAG TCC ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	47
DNA23	3'-ACT AAT ACC GAG TCG ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	47
DNA24	3'-ACT AAT ACC GAG TCA ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	47
DNA25	3'-ACT AAT ACC GAG TCT ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	47
DNA26	3'-AGT ACA TTT GGC TAC TAA AGT TGA AGA AGT GGT TGA AAC TAA ACT GG	47

Table S20 Numbering and sequences of unmodified DNA strands. For target strands longer than the probe strands, the regions of the targets strand complementary to the probe strands are underlined.

Table S21. Sequences of unmodified RNA oligonucleotides

Sequences and numbering of unmodified oligonucleotides

ON	Sequence of oligonucleotides	#(nucleotides)
RNA1	3’-ACA CCU UCU UCA ACC AC	17
RNA2	3’-ACA CCU UCU UCA UCC AC	17
RNA3	3’-ACA CCU UCU UCA GCC AC	17
RNA4	3’-ACA CCU UCU UCA CCC AC	17
RNA5	3’-UCA CCU UCU UCA ACC AC	17
RNA6	3’-A_A CCU UCU UCA ACC AC	16
RNA7	3’-ACAU CCU UCU UCA ACC AC	18
RNA8	3’-GUG GUU GAA GAA GGU GU	17
RNA9	3’-GUG GUU GAA GAA UGU GU	17
RNA10	3’-GUG GUU GAA GAA AGU GU	17
RNA11	3’-GUG GUU GAA GAA CGU GU	17
RNA12	3’-GUG GUU GAA GAA GGU GA	17
RNA13	3’-G_G GUU GAA GAA GGU GU	16
RNA14	3’-GUGA GUU GAA GAA GGU GU	18
RNA15	3'-AGU CCA UGA CGA UAG GUG GGA GUU UGU	27
RNA16	3'-ACU AAU ACC GAG UCC AUG ACG AUA GGU GGG AGU UUG UCC ACU UAA UA	47

Table S21 Numbering and sequences of unmodified RNA strands. For the 47-mer target strand (RNA16), the region complementary to the probe strand are underlined.

Figure S22. Tm curves for complementary LiNAs with TATA linkers

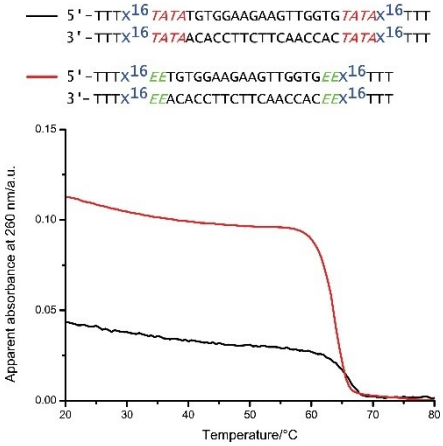

Figure S22 Thermal dissociation curves for liposomes functionalized with LiNAs containing three terminal thymidine and a polyethylene glycol linker (E) or TATA segment hybridized to a complementary LiNA of the same design. The position of the lipid membrane anchor is indicated in blue and polyethylene glycol linker (E) unit in green.

Preparation of freeze-dried POPC liposomes and polymer cartridge transfer

Preparation HEPES buffer: 0,953g HEPES, 0,234g HEPES sodium salt, 25g D-mannitol (5% [w:v]) and 3.164g NaCl is dissolved in 500mL MilliQ water. pH is adjusted to 7 by addition of sodium hydroxide or hydrochloric acid as needed.

Preparation of 100nm POPC liposomes: POPC (76mg) was suspended in 10mL D-mannitol containing HEPES buffer and stirred to a uniform milky solution. Liposomes were prepared by repeated extrusion (10 times) through double polycarbonate filters with a 100 nm pore size using compressed N2 (30 bar) and a LIPEX™ Extruder from Northern Lipids.

Preparation of samples for freeze-drying: 50µL of the 10mM POPC liposome solution extruded with 5%[w:v] D-mannitol is diluted with 900µL HEPES buffer containing 5% [w:v] D-mannitol. The LiNA strand is added to a final concentration of 50nM.

Protocol for freeze-drying: Samples are pre-frozen at -40°C for at least 1h before actual drying is begun, freeze-drying at -35°C and 0.100mbar pressure for 48h was successful in all cases with D-mannitol as the matrix. Final drying at 0°C and 0.010mbar for 2h is included to drive the final residual water, which is not locked in crystal structure, out of the matrix. Thaw conditioning at 10°C and 0.010 mbar is included to minimize the condensation of atmospheric water on the samples as they are removed from the freeze dryer. Once samples have been conditioned to room temperature they can be handled with little risk of rehydration. Samples with D-mannitol lower than 5%[w:v] were prepared but the matrix was generally to porous and the liposomes obtained after rehydration of the samples were generally very polydisperse. For samples containing 10%[w:v] D-mannitol and above, a longer drying period may be necessary as the increased ionic concentration in the liquid tends to retain water upon drying. Samples with very high LiNA concentrations similarly tended to increase the freeze-drying process due to retention of water.

Rehydration of freeze-dried samples: Samples are rehydrated in 1mL double filtrated water, gentle agitation of the sample may be necessary. These can immediately be added the complementary DNA and assembly measured by optical methods.

The transfer to a polymer cartridge has been performed on an existing reader system including a polymer cartridge with a sample and a reference chamber (Figure S23).

Figure S23. Optical reader system (400 nm) and polymer cartridge (top and buttom view)

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ABBREVIATIONS

LiNA, lipid-modified oligonucleotide; LNA, locked nucleic acids; T_m, thermal denaturation temperature; ON, oligonucleotide; SNP, single nucleotide polymorphism; PNA, peptide nucleic acids; DLS, dynamic light scattering