Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION FOR

"Mismatch discrimination of lipidated DNA and LNA-probes (LiNAs) in hybridization-controlled liposome assembly"

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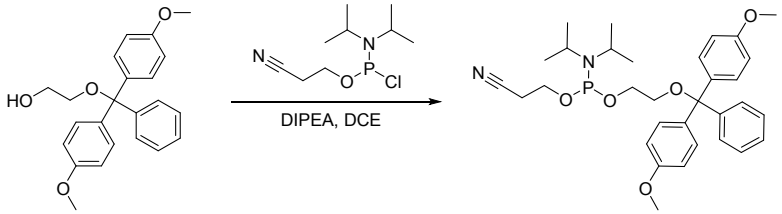
Instrumentation

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^{TM} 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 spectrophotometer with a Peltier controlled 6×6 sampler changer and Cary WinUV software.



Scheme S1. Synthesis of ethylene glycol linker phosphoramidite (2-(bis(4-methoxyphenyl)-(phenyl)-methoxy)-ethyl-(2-cyanoethyl) diisopropylphosphoramidite).

2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethan-1-ol[34] (307 mg, 0.84 mmol) was dissolved in anhydrous 1,2-dichloroethane (3 mL) and *N*,*N*-diisopropylethylamine (0.35 mL, 2.00 mmol) was added. 2-Cyanoethyl-*N*,*N*-diisopropylcholorophosphoramidite (0.25 mL, 1.12 mmol) was added at 0 °C (icebath) under a nitrogen atmosphere. After stirring at 1.5 h, the reaction mixture was washed with a saturated, aqueous solution of $NaHCO_3$ (5 mL) followed by extraction with $CHCl_3$ (3×5 mL). The combined organic phases were dried with $MgSO_4$ and the solvent removed under reduced pressure without heating. Purification by column chromatography using petroleum ether: EtOAc:NEt_3 5:1:0.1 → 2:1:0.1 followed by 3 times coevaporation with acetonitrile (without heating) afforded 2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl(2-cyanoethyl) diisopropylphosphoramidite (307 mg, 65%) as a colorless oil.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.17-1.21 (12H, m, C*H*$_3$CH), 1.99 (1H, s, MeCN), 2.57-2.62 (2H, m, C*H*$_2$CN), 3.21-3.26 (2H, m, OC*H*$_2$), 3.59-3.88 (12H, m, POC*H*$_2$, C*H*$_3$, C*H*CH$_3$, POC*H*$_2$), 6.79-6.84 (4H, m, CH$_{arom}$), 7.19-7.37 (7H, m, CH$_{arom}$), 7.45-7.48 (2H, m, CH$_{arom}$) ppm.

^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 20.46, 20.55 (*C*H$_2$CN), 24.73, 24.76, 24.82, 24.86 (*C*H$_3$CH), 43.16, 43.33 (*C*HCH$_3$), 55.33, 55.36 (OCH$_3$), 58.44, 58.69 (PO*C*H$_2$), 63.06, 63.29 (PO*C*H$_2$), 63.80, 63.90 (O*C*H$_2$), 85.98 (OCH), 113.15, 126.79, 127.85, 128.34, 130.20, 136.42, 136.46, 145.18, 158.53 (CH$_{arom}$) ppm.

^{31}P NMR ($CDCl_3$, 121.5 MHz): δ = 112.65 ppm.

HRMS (ESI): *m*/*z* calcd. for $C_{32}H_{41}N_2O_5P$ $[M+Na]^+$ 587.2645, found 587.2645.

Synthesis of lipid-modified oligonucleotides

The lipid modifications were synthesized and incorporated into oligonucleotides as previously described.[34,35] Briefly, oligonucleotides with lipid modifications was synthesized on a 0.2 µmol scale using standard conditions for automated synthesis for unmodified, standard phosphoramidites. For the lipid modified, non-nucleosidic phosphoramidites, the 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 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 was performed at least in duplicate.

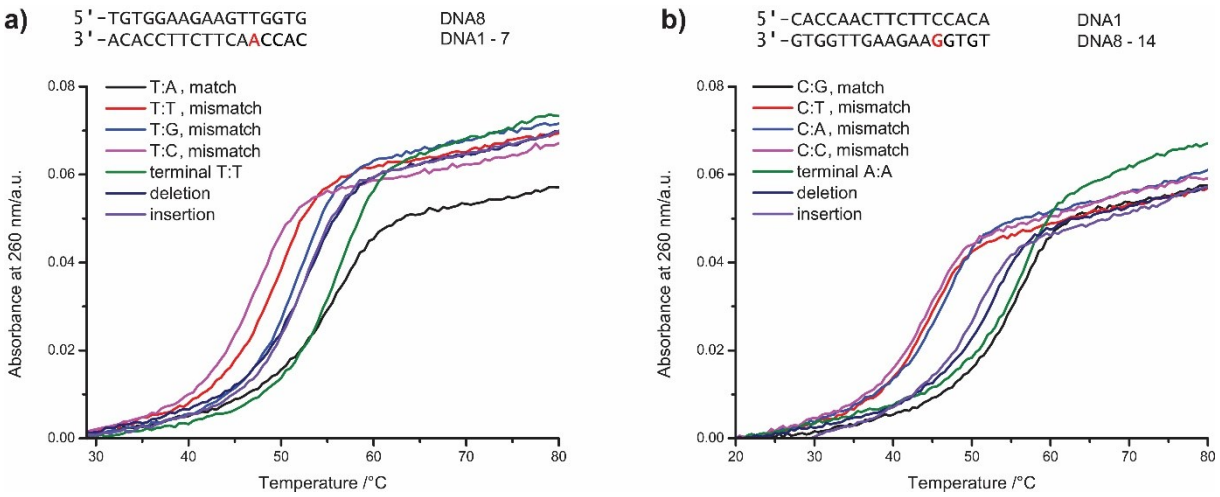


Figure S1. Thermal denaturation curves for unmodified DNA duplexes containing single nucleotide variations (mismatches, insertions and deletions) for two complementary sequences at an oligonucleotide concentration of 1.0 µM and without liposomes. The position of the internal mismatch is indicated in red.

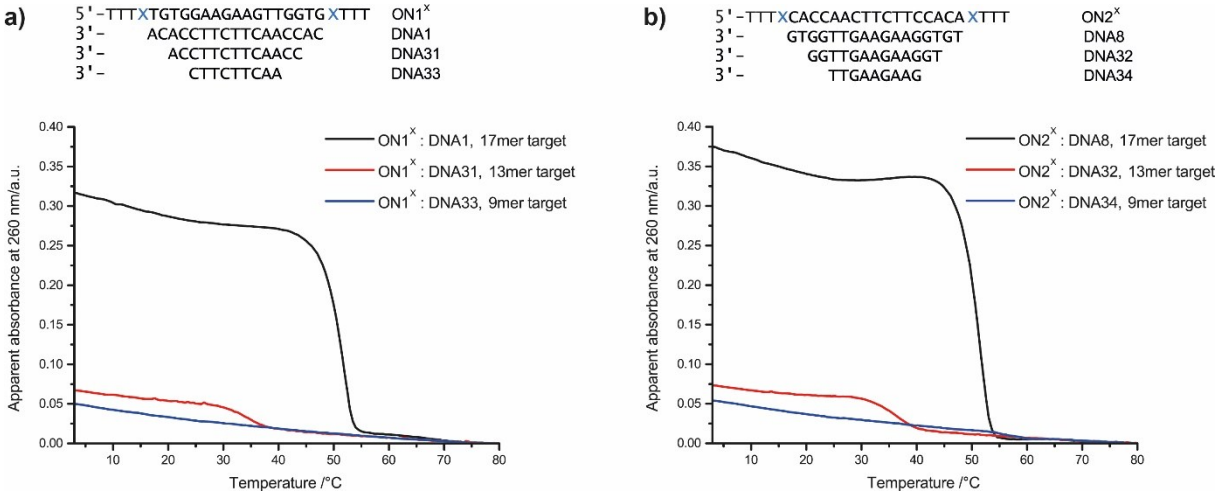


Figure S2. Thermal dissociation curves for LiNA probe strands (with 17 nucleotides in the base-pairing region) in the presence of complementary unmodified strands of 17, 13 and 9 base pairs in length.

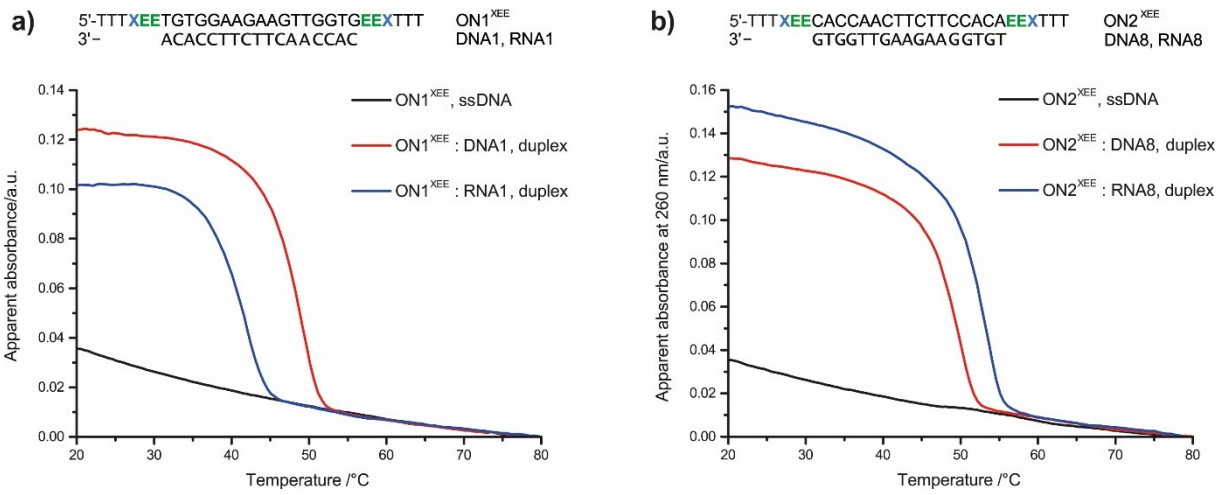


Figure S3. Thermal dissociation curves for liposomes functionalized with LiNA probe strands containing two ethylene glycol linkers (E) inserted between the base pairing region and the hydrophobic modification (probe strand alone (black curves), in a duplex with complementary DNA (red curve) and with complementary RNA (blue curve)).

Effect of partial target sequence complementarity on liposome assembly. To investigate the effect of target strands shorter than the probe strands on liposome assembly, we used target strands with mismatches in the terminal ends of the complementary region of the DNA probe form a duplex shorter than the maximum length. The LiNA probe strands ON1^{X} and ON2^{X} were used in combination with target strands of decreasing length, with similar results for both probe strands (Figure **S2** and Supporting Information Table **S17**). Liposome assembly was observed for 13-mer target strands, but not for a short 9-mer target strand (Figure **S2**). These results support the proposed mechanism for liposome assembly, shorter target strands leave flexible "overhangs/linkers" in the probe, with the probe strand hybridized to the short target strand and allow the probe to be anchored with both ends into the same liposome instead of hybridization-forced release of one of the membrane anchors. As expected the T_m of the DNA duplexes, both in the presence and absence of liposomes, decrease with decreasing target length (Supporting Information Table **S16-S17**). No T_m could be determined for 9-mer targets. We assume that strong interactions between the four palmityl chains, caused by the hydrophobic effect, are energetically more favorable than hybridization to the 9-mer target strand. However, a T_m can be found for a duplex consisting of an unmodified 17-mer and an unmodified 9-mer DNA strand with the same sequences (Supporting Information Table **S16**).

Influence of linker length between the DNA probe region and the membrane anchor on liposome assembly. The influence of a linker (spacer) inserted between the lipid-membrane anchor and the base pairing region in the probe strand was investigated by introduction of two ethylene glycol moieties (E) between the probe strand and the lipid membrane anchor. This relatively short linker did not impair the liposome assembly for either DNA or RNA target strands (Figure **S3** and Supporting Information Table S18) and the formed duplex was rigid enough to allow liposome aggregation. These results are in good agreement with the results obtained for the measurements with shorter target strands with sequences only a few base pairs shorter than the probe strand. Short ethylene glycol linker resemble the situation of a few unpaired nucleobases by retaining the relative stiffness of the dsDNA at the terminal ends, effectively enabling liposome aggregation by release of one of the lipid membrane anchors. For probes modified with a longer TATA oligonucleotide linker between the base-pairing region and the lipid membrane anchors, no transitions were seen with either unmodified DNA or RNA (for sequences see Supporting Information Table **S19**). The results are consistent with the proposed mechanism of liposome aggregation as well as the results seen for the use of target sequences much shorter than the probe strand. In both cases, the linker is flexible and long enough to allow the lipophilic substituents at both ends of the probe to be anchored into the same liposome, even if the probe is hybridized to a target sequence effectively preventing liposome aggregation.

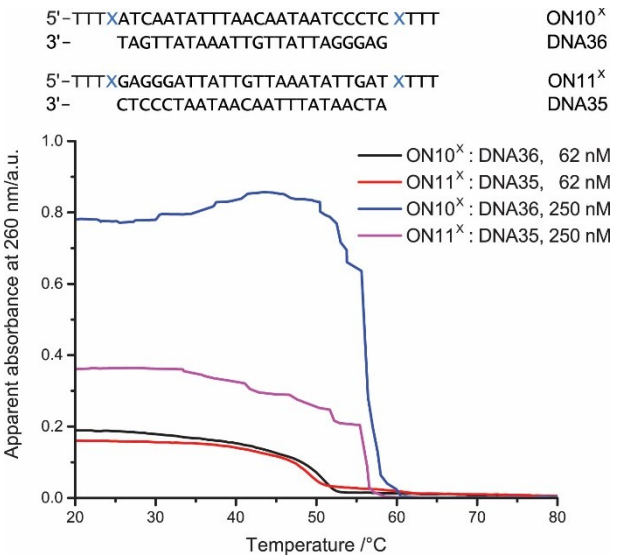


Figure **S4**. Thermal dissociation curves for liposomes functionalized with two 24-mer LiNA probe strands associated with the anthrax lethal factor at two different concentrations.

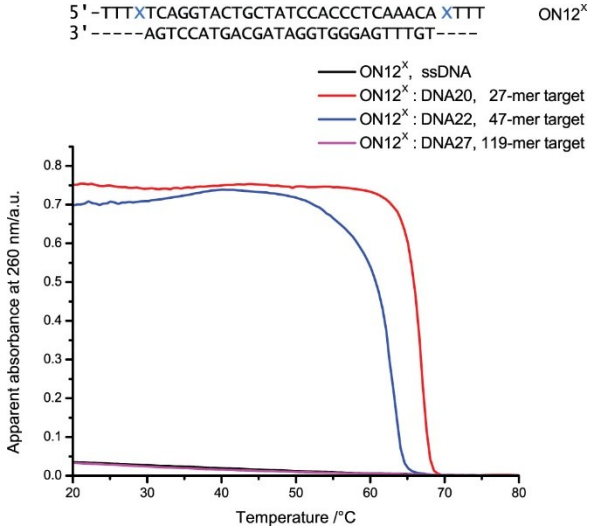


Figure **S5**. Thermal dissociation curves for liposomes functionalized with 27-mer LiNA probes associated with Staphylococcus aureus in the presence of unmodified DNA target strands (27-mer, 47-mer and 119-mer) at 62 nM DNA concentration.

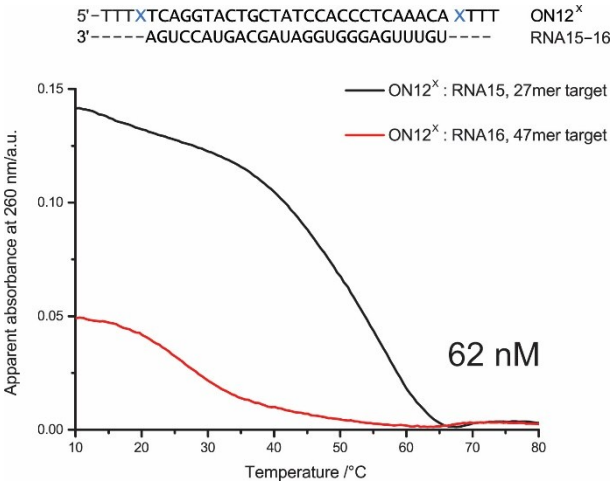


Figure **S6**. Thermal dissociation curves in the presence of liposomes for a 27-mer DNA probe strand associated with *Staphylococcus aureus* and 27- and 47-mer RNA target strands (62 nM oligonucleotides).

Table S1. DNA sequences of probes and targets and molecular weights of synthesized oligonucleotides.[1]

Oligonucleotide	Sequence	Mass of $[M+H]^+$	
		Calculated	Experimental(m/z)
ON1^{X}	5'-TTT **X** TGT GGA AGA AGT TGG TG **X** TTT	9013.7	9014.9
ON2^{X}	5'-TTT **X** CAC CAA CTT CTT CCA CA **X** TTT	8702.6	8699.1
ON1^{Y}	5'-TTT **Y** TGT GGA AGA AGT TGG TG **Y** TTT	9652.6	9650.4
ON1^{2X}	5'-TTT **XX** TGT GGA AGA AGT TGG TG **X** TTT	9936.4	9947.3
ON2^{2X}	5'-TTT **XX** CAC CAA CTT CTT CAC CA **X** TTT	9625.3	9629.3
ON1^{LNA}	5'-TTT **X** TGT^{L} GGA AGA AGT^{L} TGG T^{L}G **X** TTT	9097.6	9097.7
ON2^{LNA}	5'-TTT **X** CAC CAA CT^{L}T CTT^{L} CCA CA **X** TTT	8758.6	8759.0
ON4^{X}	5'-TTT **X** TGT GGA AGA AGT TGG TG	7178.8	7177.6
ON6^{X}	5'-TTT **X** CAC CAA CTT CTT CCA CA	6866.8	6869.0
ON3^{X}	5'-TGT GGA AGA AGT TGG TG **X** TTT	7178.8	7178.5
ON7^{X}	5'-CAC CAA CTT CTT CCA CA **X** TTT	6866.8	6872.1
ON3^{LNA}	5'-TGT^{L} GGA AGA AGT^{L} TGG T^{L}G **X** TTT	7262.8	7263.1
ON7^{LNA}	5'-CAC CAA CT^{L}T CTT^{L} CCA CA **X** TTT	6923.7	6918.7
ON1^{XEE}	5'-TTT **XEE** TGT GGA AGA AGT TGG TG **EEX** TTT	9509.6	9502.0
ON2^{XEE}	5'-TTT **XEE** CAC CAA CTT CTT CCA CA **EEX** TTT	9182.6	9205.6
ON1^{TATA}	5'-TTT **X** TATA TGT GGA AGA AGT TGG TG TATA **X** TTT	11496.1	11498.5
ON2^{TATA}	5'-TTT **X** TATA CAC CAA CTT CTT CCA CA TATA **X** TTT	11169.1	11179.2
ON10^{X}	5'-TTT **X** ATC AAT ATT TAA CAA TAA TCC CTC **X** TTT	10906.0	10911.8
ON11^{X}	5'-TTT **X** GAG GGA TTA TTG TTA AAT ATT GAT **X** TTT	11144.0	11132.8
ON12^{X}	5'-TTT **X** TCA GGT ACT GCT ATC CAC CCT CAA ACA **X** TTT	11815.1	11818.7
ON13^{X}	5'-TTT **X** CGA TGA TTT CAA CTT CTT CAC CAA CTT **X** TTT	11811.1	11827.4

[1]**X**: palmityl substituted macrocycle, **Y**: cholesteryl substituted macrocycle, **E**: ethylene glycol unit.

Table S2. Thermal denaturation data for unmodified DNA targets

Entry	Duplex	without liposomes	
	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. Thermal denaturation data for lipid-modified DNA probes ON1^X and ON2^X 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		-	-	-
	5’-TTT X TGT GGA AGA AGT TGG TG X 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
	5’-TTT X CAC CAA CTT CTT CCA CA X 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 S3 [a]) Thermal denaturation curve has extra features around T_m for which reason the T_m is determined with uncertainty.

Table S4. Thermal denaturation data for cholesteryl-modified DNA probes - ON1^{Y}

	Cholesteryl-modified DNA				
Entry	Duplex	without liposomes		with liposomes	
	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 S4. n.d.: not determined.

Table S5. Thermal denaturation 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 S6. Thermal denaturation data for lipid-modified DNA probes ON1^{X} and ON2^{X} 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		-	-	-
	5'-TTT X TGT GGA AGA AGT TGG TG X 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
	5'-TTT X CAC CAA CTT CTT CCA CA X 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 for which reason the T_m is determined with uncertainty.

Table S7. Thermal denaturation data for double lipid-modified DNA probes ON1^{2X} and ON2^{2X} 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 XX TGT GGA AGA AGT TGG TG X 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 XX CAC CAA CTT CTT CCA CA X 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. Thermal denaturation data for LNA modified LiNA probes and DNA targets

	Modified DNA with LNA		
	Duplex	without	with

		liposomes		liposomes	
Entry	5’-TGT GGA AGA AGT TGG TG 3’-ACA CCT TCT TCA ACC AC		-	-	-
	5’-TTT X TGT^{L} GGA AGA AGT^{L} TGG T^{L}G X 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 CAC CAA CT^{L}T CTT^{L} CCA CA X 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 S9. Concentration dependent thermal denaturation data for LNA modified LiNA probes and DNA targets

Comparison of modified 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 TGT^{L} GGA AGA AGT^{L} TGG T^{L}G X TTT 3’-ACA CCT TCT TCA ACC AC	53.0	50.5	49.5
2	5’-TTT X TGT GGA AGA AGT TGG TG X TTT 3’-ACA CCT TCT TCA ACC AC	48.0	46.0	44.5
1	5’-TTT X CAC CAA CT^{L}T CTT^{L} CCA CA X TTT 3’-GTG GTT GAA GAA GGT GT	51.5	49.0	47.5
2	5’-TTT X CAC CAA CTT CTT CCA CA X TTT 3’-GTG GTT GAA GAA GGT GT	48.0	46.0	45.0

Table S10. Concentration dependent thermal denaturation data for single lipid-modified DNA probes and DNA targets

Single-modified DNA			
Entry	Duplex	without liposomes	with liposomes

1	5’-TGT GGA AGA AGT TGG TG 3’-ACA CCT TCT TCA ACC AC	55.0			
			62.5 nM	125 nM	250 nM
		T_m/°C	T_m/°C	T_m/°C	T_m/°C
2	5’-TTT X TGT GGA AGA AGT TGG TG 3’-ACA CCT TCT TCA ACC AC X TTT	62.5	n.t.	n.t.	n.t.
3	5’-TGT GGA AGA AGT TGG TG X TTT 3’-TTT X ACA CCT TCT TCA ACC AC	60.0	(40)[a]	n.d	n.d.
4	5’-TTT X TGT GGA AGA AGT TGG TG 3’-TTT X ACA CCT TCT TCA ACC AC	76.0	n.t.	n.d.	n.d.
5	5’-TGT GGA AGA AGT TGG TG X TTT 3’-ACA CCT TCT TCA ACC AC X TTT	74.5	n.t.	n.d.	n.d.
6	5’-TTT X TGT GGA AGA AGT TGG TG	n.d.	n.t.	n.t.	n.t.
7	5’-TTT X CAC CAA CTT CTT CCA CA	n.d.	n.t.	n.t.	n.t.
8	5’-TGT GGA AGA AGT TGG TG X TTT	n.d.	n.t.	n.d.	n.d.
9	5’-CAC CAA CTT CTT CCA CA X TTT	n.d.	n.t.	n.d.	n.d.
10	5’-TTT X 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 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 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 TTT 3’-GTG GTT GAA GAA GGT GT	59.5	n.t.	n.d.	n.d.

Table S10 n.d.: not determined, n.t.; no transition, a) broad transition.

Table S11. Thermal denaturation data for LNA single lipid-modified LiNA probes and DNA targets

	Single-modified DNA 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 S11 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 S12. Thermal denaturation data for unmodified split-probes DNA

Unmodified split-probes DNA		
Entry	Duplex	without liposomes Tm/°C
1	3’-ACA-CCT-TCT-TCA-ACC-ACG-TGG-TTG-AAG-AAG-GTG-T	77.5
2	3’-GTG-GTT-GAA-GAA-GGT-GTA-CAC-CTT-CTT-CAA-CCA-C	76.5
3	5’-TGT-GGA-AGA-AGT-TGG-TG CAC-CAA-CTT-CTT-CCA-CA-3’ 3’-ACA-CCT-TCT-TCA-ACC-AC-GTG-GTT-GAA-GAA-GGT-GT	55.0 + 76.5
4	5’-CAC-CAA-CTT-CTT-CCA-CA TGT-GGA-AGA-AGT-TGG-TG-3’ 3’-GTG-GTT-GAA-GAA-GGT-GT-ACA-CCT-TCT-TCA-ACC-AC	57.5 + 75.5
5	5’-TGT-GGA-AGA-AGT-TGG-TG TGT-GGA-AGA-AGT-TGG-TG-3’ 3’-ACA-CCT-TCT-TCA-ACC-AC-ACA-CCT-TCT-TCA-ACC-AC	58.0
6	5’-CAC-CAA-CTT-CTT-CCA-CA CAC-CAA-CTT-CTT-CCA-CA-3’ 3’-GTG-GTT-GAA-GAA-GGT-GT-GTG-GTT-GAA-GAA-GGT-GT	56.0

Table S12 The melting temperature seen for the oligonucleotides in entry 3.a and 4.a are consistent with the melting temperature of the 17-mer duplex (entry 1, Table S10) and the melting temperature of the 34-mer hairpin (entry 1 and 2, respectively).

Table S13. Thermal denaturation data for modified split-probe DNA

Modified split-probes DNA			
	Duplex	without liposomes	with liposomes
Entry		T_m/°C	T_m/°C
1	5’-TTT-**X**-TGT-GGA-AGA-AGT-TGG-TG I CAC-CAA-CTT-CTT-CCA-CA-**X**-TTT-3’ 3’-ACA-CCT-TCT-TCA-ACC-AC-GTG-GTT-GAA-GAA-GGT-GT	76.5	n.t.

2	5’-TTT-**X**-CAC-CAA-CTT-CTT-CCA-CA **I** TGT-GGA-AGA-AGT-TGG-TG-**X**-TTT-3’ 3’-GTG-GTT-GAA-GAA-GGT-GT-ACA-CCT-TCT-TCA-ACC-AC	76.5	n.t.
3	5’-TTT-**X**-TGT-GGA-AGA-AGT-TGG-TG **I** TGT-GGA-AGA-AGT-TGG-TG-**X**-TTT-3’ 3’-ACA-CCT-TCT-TCA-ACC-AC-ACA-CCT-TCT-TCA-ACC-AC	63.5	(40)[a]
4	5’-TTT-**X**-CAC-CAA-CTT-CTT-CCA-CA **I** CAC-CAA-CTT-CTT-CCA-CA-**X**-TTT-3’ 3’-GTG-GTT-GAA-GAA-GGT-GT-GTG-GTT-GAA-GAA-GGT-GT	63.5	56.0
5	5’-TTT-**X**-CAC-CAA-CTT-CTT-CCA-CA **I** TTT-**X**-TGT-GGA-AGA-AGT-TGG-TG-3’ 3’-GTG-GTT-GAA-GAA-GGT-GT-ACA-CCT-TCT-TCA-ACC-AC	n.d.	n.t.
6	5’-TTT-**X**-TGT-GGA-AGA-AGT-TGG-TG **I** TTT-**X**-TGT-GGA-AGA-AGT-TGG-TG-3’ 3’-ACA-CCT-TCT-TCA-ACC-AC-ACA-CCT-TCT-TCA-ACC-AC	n.d.	n.t.

Tabel S13 n.d.: not determined, n.t.: no transition, a) very broad transition.

The melting temperature in entry 1.a and 2.a are consistent with the melting temperature of both the 17-mer duplex (entry 4 and 5, Table S10) and the 34-mer hairpin (entry 1.a and 1.2, Table S11).

Table S14. Thermal denaturation data for unmodified split-probe DNA including mismatches

Unmodified split probe with mismatches

	Duplex	without liposomes	
Entry		T_m/°C	Δ T_m/°C
	5’-CAC-CAA-CTT-CTT-CCA-CA CAC-CAA-CTT-CTT-CCA-CA-3’		
1	3’-GTG-GTT-GAA-GAA-GGT-GTG-TGG-TTG-AAG-AAG-GTG-T	59.0	-
2	3’-GTG-GTT-GAA-GAA-TGT-GTG-TGG-TTG-AAG-AAG-GTG-T	52.5	-6.5
3	3’-GTG-GTT-GAA-GAA-AGT-GTG-TGG-TTG-AAG-AAG-GTG-T	54.0	-5.0
4	3’-GTG-GTT-GAA-GAA-CGT-GTG-TGG-TTG-AAG-AAG-GTG-T	53.0	-6.0

Table S15. Thermal denaturation data for modified split-probe DNA including mismatches

Modified split probe with mismatches

	Duplex	without liposomes		with liposomes	
Entry		T_m/°C	Δ T_m/°C	T_m/°C	Δ T_m/°C
	5’-TTT-**X**-CAC-CAA-CTT-CTT-CCA-CA **I** CAC-CAA-CTT-CTT-CCA-CA-**X**-TTT-3’				
1	3’-GTG-GTT-GAA-GAA-GGT-GTG-TGG-TTG-AAG-AAG-GTG-T	63.5	-	56.0	-
2	3’-GTG-GTT-GAA-GAA-TGT-GTG-TGG-TTG-AAG-AAG-GTG-T	59.0	-4.5	49.5	-6.5
3	3’-GTG-GTT-GAA-GAA-AGT-GTG-TGG-TTG-AAG-AAG-GTG-T	60.0	-3.5	51.0	-5.0
4	3’-GTG-GTT-GAA-GAA-CGT-GTG-TGG-TTG-AAG-AAG-GTG-T	58.5	-5.0	48.5	-7.5

Table S16. Thermal denaturation data for unmodified DNA with short target strands

Unmodified DNA with short target strands		
	Duplex	
Entry		T_m/°C
	5'-TGT GGA AGA AGT TGG TG	
1.a	3'-ACA CCT TCT TCA ACC AC	57.0
1.b	3'-A CCT TCT TCA ACC	46.5
1.c	3'-CT TCT TCC A	24.5
	5'-CAC CAA CTT CTT CCA CA	
2.a	3'-GTG GTT GGA GAA GGT GT	57.0
2.b	3'-G GTT GAA GAA GGT	48.5
2.c	3'-TT GAA GAA G	21.5

Table S17. Thermal denaturation data for modified DNA with short target strands

Modified DNA 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. Thermal denaturation data for modified DNA with ethylene glycol linker

Modified DNA 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

Tabel 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 uncertainty.

Table S19. Thermal denaturation data for modified DNA with a TATA linker

Modified DNA 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, underligned section are regions complementary to the corresponding DNA probe.

Table S20. Thermal denaturation data for modified DNA associated with anthrax lethal factor

Duplexes associated with anthrax lethal factor

Entry	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’-TTT**X**ATCAATATTTAACAATAATCCCTC**X**TTT 3’-TTT**X**TAGTTATAAATTGTTATTAGGGAG**X**TTT	75.0	61.5	n.d.	n.d.
2	5’-TTT**X**ATCAATATTTAACAATAATCCCTC**X**TTT	n.d.	n.d.	n.t.	n.d.
3	5’-TTT**X**GAGGGATTATTGTTAAATATTGAT**X**TTT	n.d.	n.d.	(60.5)[a]	n.d.
4	5’-TTT**X**ATCAATATTTAACAATAATCCCTC**X**TTT 3’-TAGTTATAAATTGTTATTAGGGAG	49.5	50.0	n.d.	57.0
5	5’-TTT**X**GAGGGATTATTGTTAAATATTGAT**X**TTT	49.0	49.0	n.d.	55.5

	3’-CTCCCTAATAACAATTTATAACTA				
6	5’-TTT**X**ATCAATATTTAACAATAATCCCTC**X**TTT 3’- ATAGAAGTTAAAGATGTTATATTGGGACCTATTATAAAAAGAA TATAGGA ATAGTTATAAATTGTTATTAGGGAGACAACTGCTTAATTAATG AAGAGATACTCGGAGGAATTGATGAC	n.d.	n.t.	n.t.	n.t.
7	5’-TTT**X**GAGGGATTATTGTTAAATATTGAT**X**TTT 3’-GTCATCAATTCCTCCGAGTATCTCTTCATTAATTAAGCAGTT GTCTCCCTAATAACAATTTATAACTATTCCTATATTCTTTTTAT AATAGGTCCCAATATAACATCTTTAACTTCTAT	i.a.	n.t.	(60.0)[a]	n.t.

Table S20 n.d.: not determined, n.t. ; no transition, i.a.: Despite an increase in absorbance, no evident thermal transition can be determined, a) very weak transition

Table S21. Thermal denaturation data for modified DNA associated with *S. aureus*

	Duplexes associated with *S. aureus*			
Entry	Duplex	without liposomes	with liposomes	
			62.5 nM	250 nM
		T_m/°C	T_m/°C	T_m/°C
1	5’-TTT**X**TCAGGTACTGCTATCCACCCTCAAACA**X**TTT	n.d.	n.t.	n.t.
2	5’-TTT**X**TCAGGTACTGCTATCCACCCTCAAACA**X**TTT 3’-AGTCCATGACGATAGGTGGGAGTTTGT	64.0	66.0	n.d.
3	5’-TTT**X**TCAGGTACTGCTATCCACCCTCAAACA**X**TTT 3’-ACTAATACCGAGTCCATGACGATAGGTGGGAGTTTGTCCA CTTAATA	65.0	62.0	n.d.
4	5’-TTT**X**TCAGGTACTGCTATCCACCCTCAAACA**X**TTT 3’-ATTTCAAGTTTTCTCATAAATATTGTTGTACTTTTTACTA ATACCGAGTCCATGACGATAGGTGGGAGTTTGTCCACTTAATAATCGTGAACAT TCGTGTGGAAGTATACTGCAGATAG	n.d	n.t.	(44-70)[a]

5	5’-TTT**X**CGATGATTTCAACTTCTTCACCAACTT**X**TTT	n.d.	n.t.	n.t.
6	5’-TTT**X**CGATGATTTCAACTTCTTCACCAACTT**X**TTT 3’-GCTACTAAAGTTGAAGAAGTGGTTGAA	62.0	63.5	n.d.
7	5’-TTT**X**CGATGATTTCAACTTCTTCACCAACTT**X**TTT 3’-AGTACATTTGGCTACTAAAGTTGAAGAAGTGGTTGAAACT AAACTGG	62.5	60.5	n.d.
8	5’-TTT**X**CGATGATTTCAACTTCTTCACCAACTT**X**TTT 3’- TGTAAAGTTGTGGACATTGTCAACAAAATCTACACAGTACATTTGGCTACTAAA GTTGAAGAAGTGGTTGAAACTAAACTGGTGCAAGTTGTGCCGGACATCGTTGTC ATGGTGCTGG	n.d.	41.0	51.0

Table S21 n,d,: not determined, n.t.: no transition, a) transitions observed in the given interval.

Table S22. Thermal denaturation data for modified DNA associated with *S. aureus* and RNA targets

Duplexes associated with *S. aureus*			
Entry	Duplex	without liposomes	with liposomes
			62.5 nM
		T_m/°C	T_m/°C
1	5’-TTTXTCAGGTACTGCTATCCACCCTCAAACAXTTT 3’-AGUCCAUGACGAUAGGUGGGAGUUUGU	n.d.	49-65[a]
2	5’-TTTXTCAGGTACTGCTATCCACCCTCAAACAXTTT 3’-ACUAAUACCGAGUCCAUGACGAUAGGUGGGAGUUUGUCCA CUUAAUA	n.d.	26.5

Table S22 n.d.: not determined. a) Transitions were observed within the given interval.

Table S23. Thermal denaturation data for modified DNA and DNA targets associated with *S. aureus* including mismatches

Mismatch studies for duplexes associated with *S. aureus*							
	Duplex	without liposomes		with liposomes			
Entry			-	62.5 nM		250 nM	
	5’-TTT **X** TCA GGT ACT GCT ATC CAC CTT CAA ACA **X** TTT	T_m/°C	Δ T_m/°C	T_m/°C	Δ T_m/°C	T_m/°C	Δ T_m/°C
1	3’-ACT AAT ACC GAG TCC ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	65.0	-	58.5	-	64.0	-
2	3’-ACT AAT ACC GAG TCG ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	n.d.	-	56.5	-2.0	61.0	-3.0
3	3’-ACT AAT ACC GAG TCA ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	n.d.	-	51.5	-7.0	59.5	-4.5
4	3’-ACT AAT ACC GAG TCT ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TA	n.d.	-	43.5	-15.0	56.5	-7.5

Table S23 n.d.: not determined.

Table S24. Sequences and numbering of unmodified DNA oligonucleotides

Sequences and numbering of unmodified oligodeoxyribonucleic acids

ON	Sequence of oligodeoxyribonucleotide	#(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
DNA27	3’-ATT TCA AGT TTT CTC ATA AAT ATT GTT GTA CTT TTT ACT AAT ACC GAG TCC ATG ACG ATA GGT GGG AGT TTG TCC ACT TAA TAA TCG TGA ACA TTC GTG TGG AAG TAT ACT GCA GAT AG	119
DNA28	3’-TTG TAA AGT TGT GGA CAT TGT CAA CAA AAT CTA CAC AGT ACA TTT GGC TAC TAA AGT TGA AGA AGT GGT TGA AAC TAA ACT GGT GCA AGT TGT GCC GGA CAT CGT TGT CAT GGT GCT GG	119
DNA29 (anthrax)	3'- ATA GAA GTT AAA GAT GTT ATA TTG GGA CCT ATT ATA AAA AGA ATA TAG GAA TAG TTA TAA ATT GTT ATT AGG GAG ACA ACT GCT TAA TTA ATG AAG AGA TAC TCG GAG GAA TTG ATG AC	119
DNA30 (anthrax)	3'-GTCATCAATTCCTCCGAGTATCTCTTCATTAATTAAGCAGTTGTCTCCCTAATAACAATTTATAACTATTCCTATATTCTTTTTATAATAGGTCCCAATATAACATCTTTAACTTCTAT	119
DNA31	3’-ACC TTC TTC AAC C	13
DNA32	3’-GGT TGA AGA AGG T	13
DNA33	3’-CTT CTT CAA	9
DNA34	3’-TTG AAG AAG	9
DNA35	3'-CTC CCT AAT AAC AAT TTA TAA CTA	24
DNA36	3'-TAG TTA TAA ATT GTT ATT AGG GAG	24

Table S24 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 S25. Sequences and numbering of unmodified RNA oligonucleotides

Sequences and numbering of unmodified oligoribonucleic acids

ON	Sequence of oligoribonucleotide	#(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 S25 Numbering and sequences of unmodified RNA strands. For the 47-mer target strand (RNA16), the region complementary to the probe strand are underlined.

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ABBREVIATIONS

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

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