Electronic Supplementary Material (ESI) for RSC Chemical Biology.
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Supporting information

Enhanced biophysical screening strategy to investigate the affinity of ASOs for their target RNA.

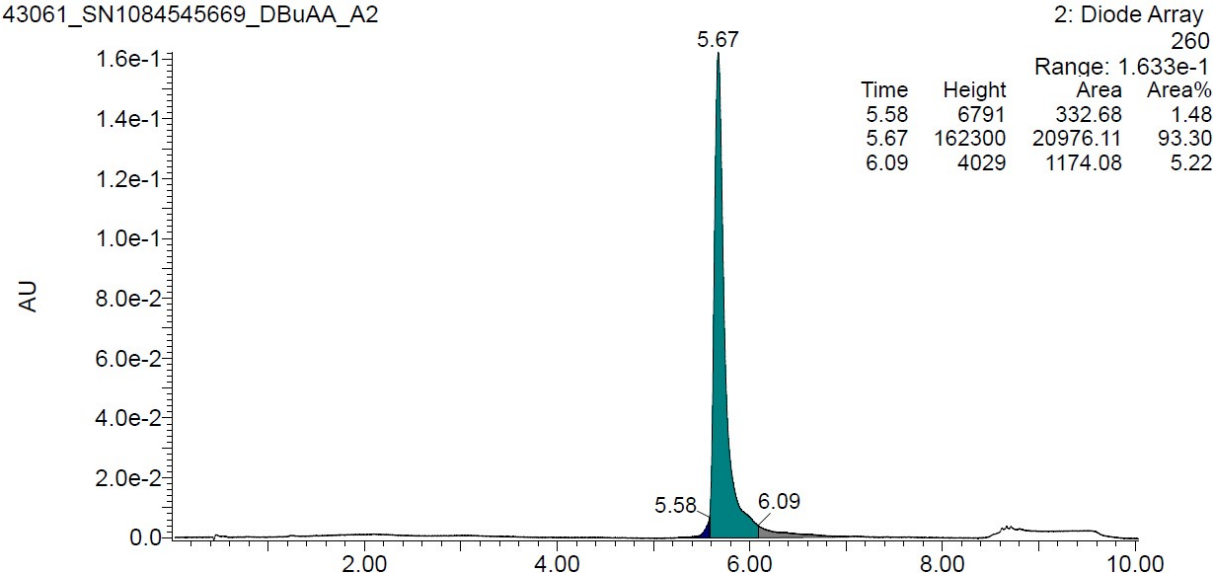
Table S1. Table of ASO sequences used in this study and correspondent properties. Bold letters denote LNA nucleotides, italic letters denote MOE nucleotides ^{m}C denotes 5-methylcytidine, † denotes 2'O-Methyl nucleotides. All sequences were fully phosphorothioate modified.

ASO	Sequence	Modifications included	Length	Average Molecular Weight (g/mol)	Molar Extinction Coefficient (L/mol·cm)	Calc. HRMS (3-)	Measured HRMS (3-)
1	**TG^{m}CTACAAAAC^{m}C^{m}CA**	LNA PS gapmers 3-8-3	14	4619.76	132100	1537.8213	1537.8464
2	*TG^{m}CTACAAAAC^{m}C^{m}CA*	MOE PS gapmers 3-8-3	14	4896.17	133200	1629.9050	1629.9308
3	†U†G†C†U†A†C†A†A†A†A†C†C†C†A	2'OMe PS	14	4801.93	138400	1598.5214	1598.5465
4	**TG^{m}CT†ACAAAAC^{m}C^{m}CA**	LNA PS gapmers 3-8-3, 2'-OMe in position 2	14	4649.79	132600	1547.8248	1547.8496
5	**TG^{m}CTA†CAAAAC^{m}C^{m}CA**	LNA PS gapmers 3-8-3, 2'-OMe in position 3	14	4649.79	132100	1547.8248	1547.8503
6	**AUU**AAUAAAAA**UGC**	LNA PS gapmers 3-8-3	14	4633.73	133800	1542.4694	1542.4938
7	**AAC**CCAGAAUA**AAU**	LNA PS gapmers 3-8-3	14	4651.70	131600	1548.4608	1548.4880
8	**TGA**TACAAAAC^{m}**C^{m}CA**	LNA PS gapmers 3-8-3	14	4629.76	141000	1541.1532	1541.1784
9	*TGA*TACAAAAC^{m}C^{m}CA	MOE PS gapmers 3-8-3	14	4906.17	142200	1633.2369	1633.2643
10	**TG^{m}CT**AA**AAAAC^{m}C^{m}CA**	LNA PS gapmers 3-8-3	14	4643.79	136500	1545.8251	1545.8483
11	*TG^{m}CTA*AA*AAAAC^{m}C^{m}CA*	MOE PS gapmers 3-8-3	14	4920.20	137600	1637.9088	1637.9323
12	**GT**CTGTGGAA**G^{m}CG**	LNA PS gapmers 2-8-3	13	4377.46	123100	1457.1327	1457.1570
13	**^{m}CGT**CAGTATGCG**AAT**C	LNA PS gapmers 3-8-3-1	16	5304.28	152200	1324.1164 (4-)	1324.1384 (4-)

ASO1

SN1084545669_DBuAA_5_50

43061_SN1084545669_DBuAA_A2



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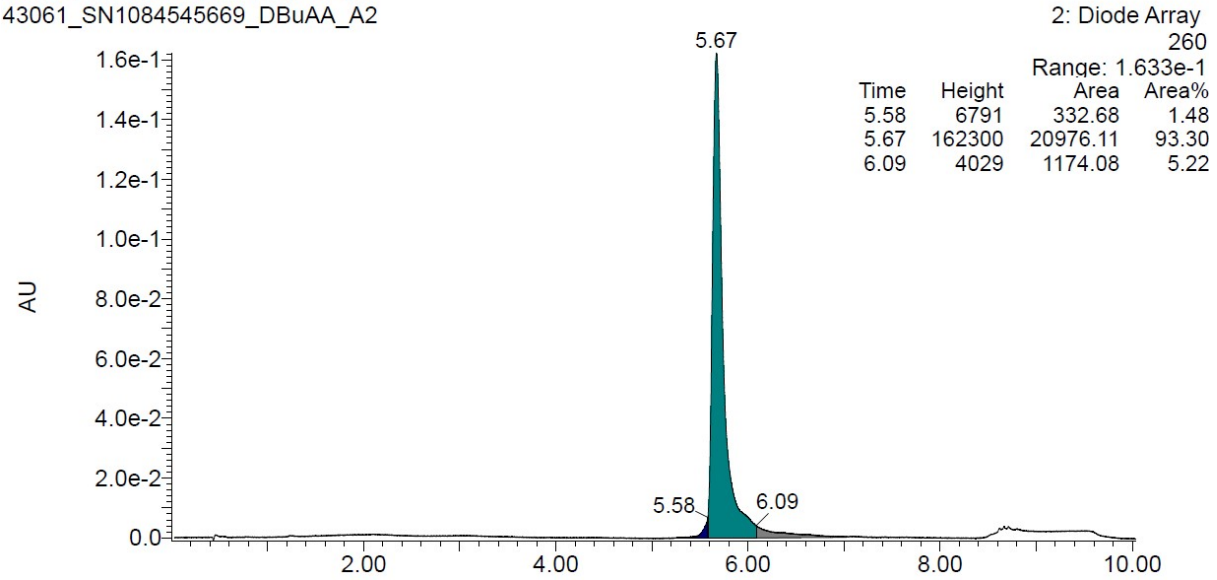
43061_SN1084545669_DBuAA_A2



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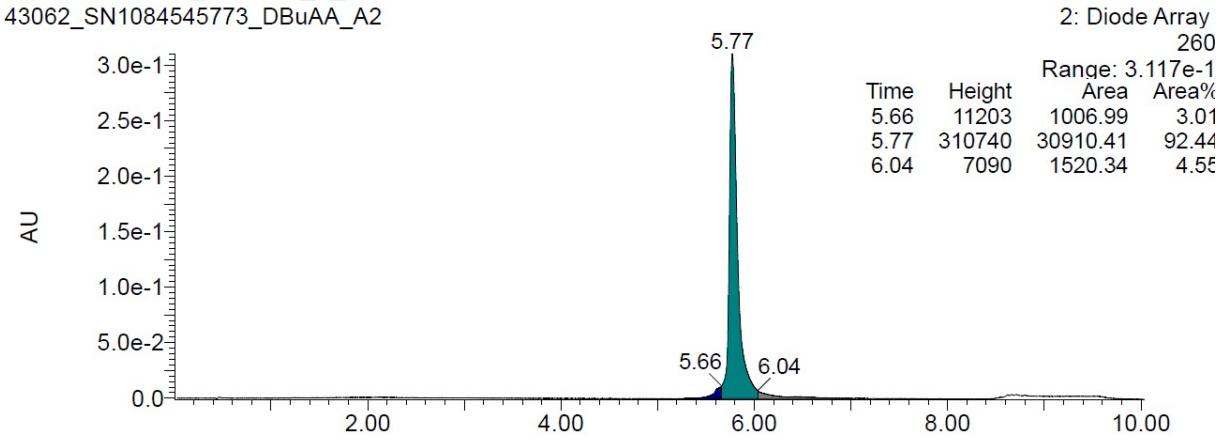
43062_SN1084545773_DBuAA_A2



ASO4

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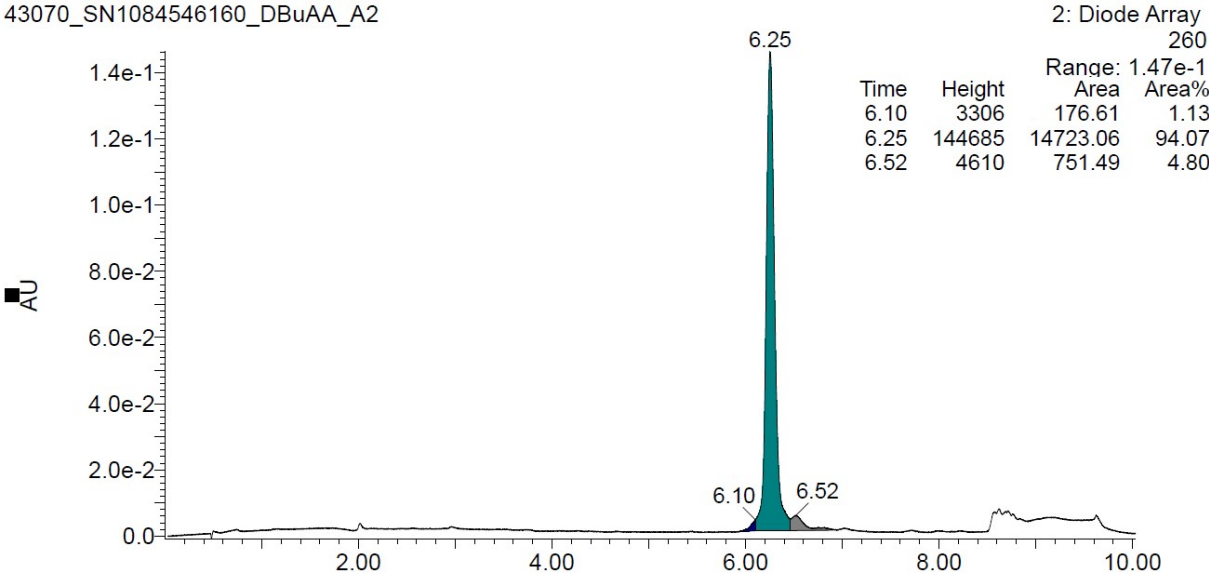
43070_SN1084546160_DBuAA_A2



ASO5

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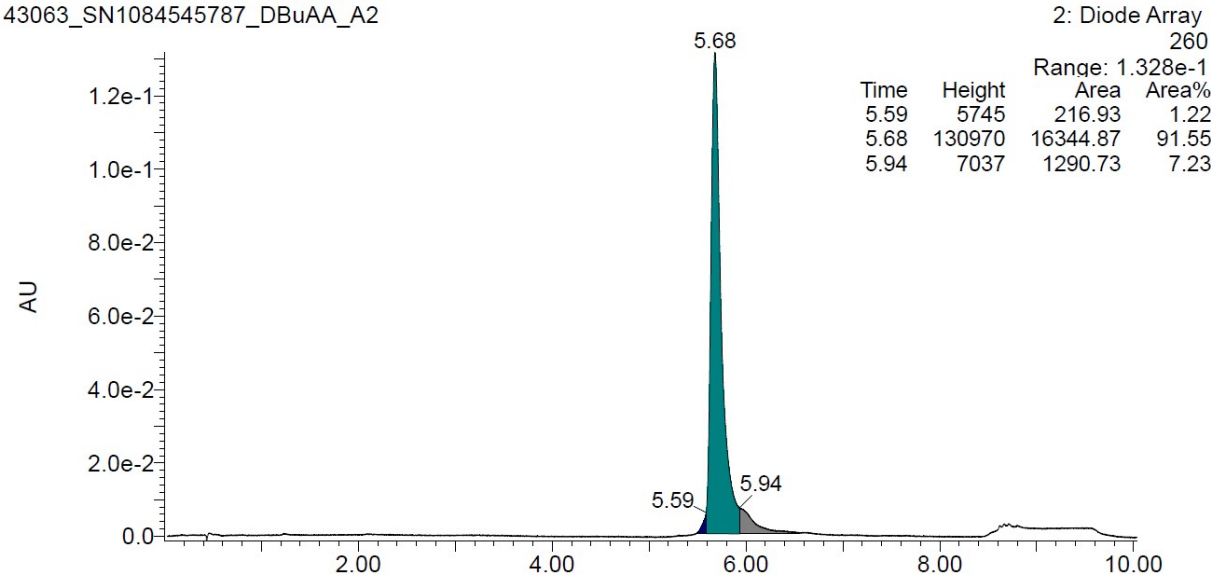
43063_SN1084545787_DBuAA_A2



ASO6

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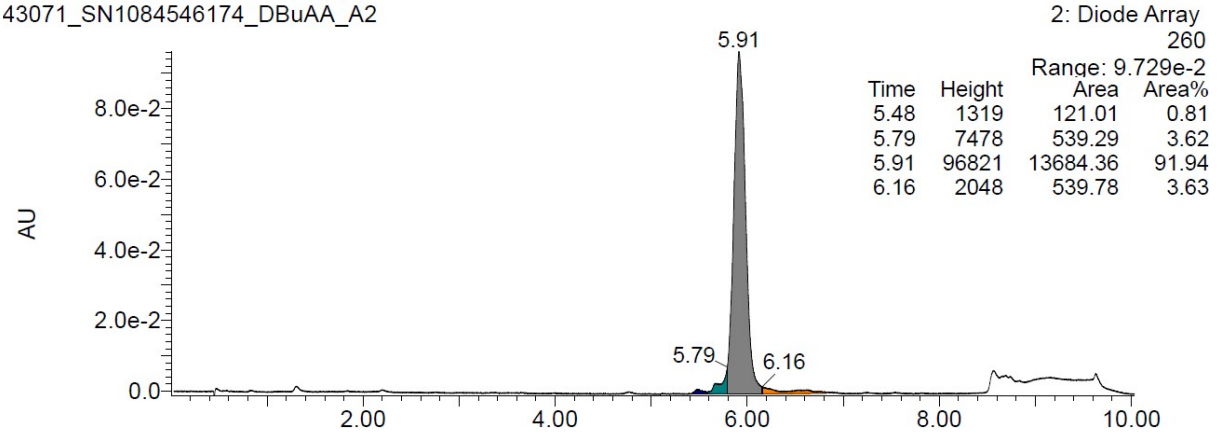
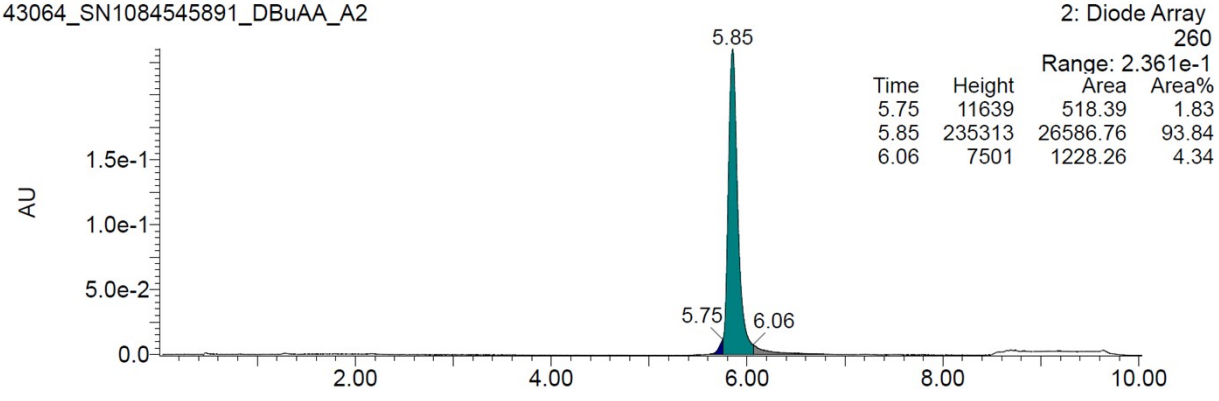
43071_SN1084546174_DBuAA_A2



ASO 7

SN1084545891_DBuAA_5_50

43064_SN1084545891_DBuAA_A2



ASO8

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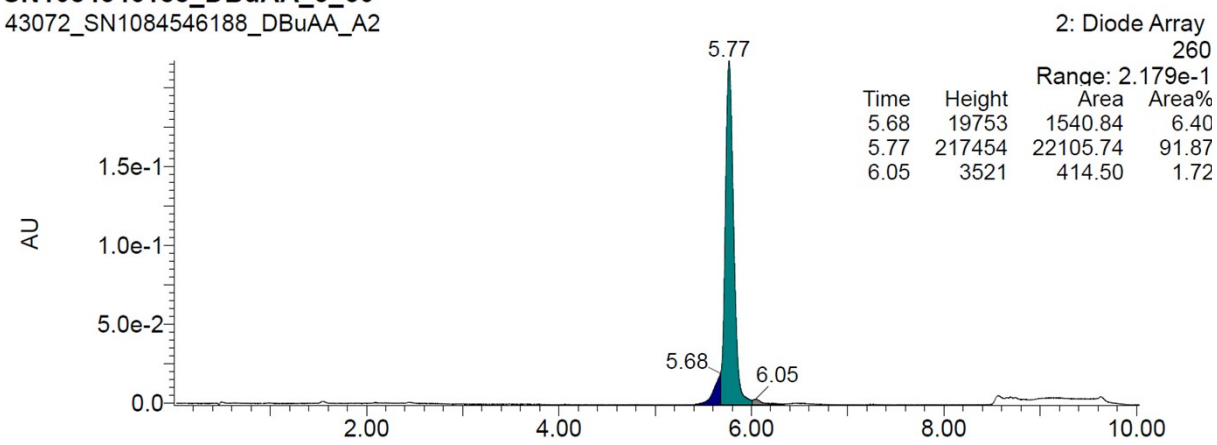
43072_SN1084546188_DBuAA_A2



ASO9

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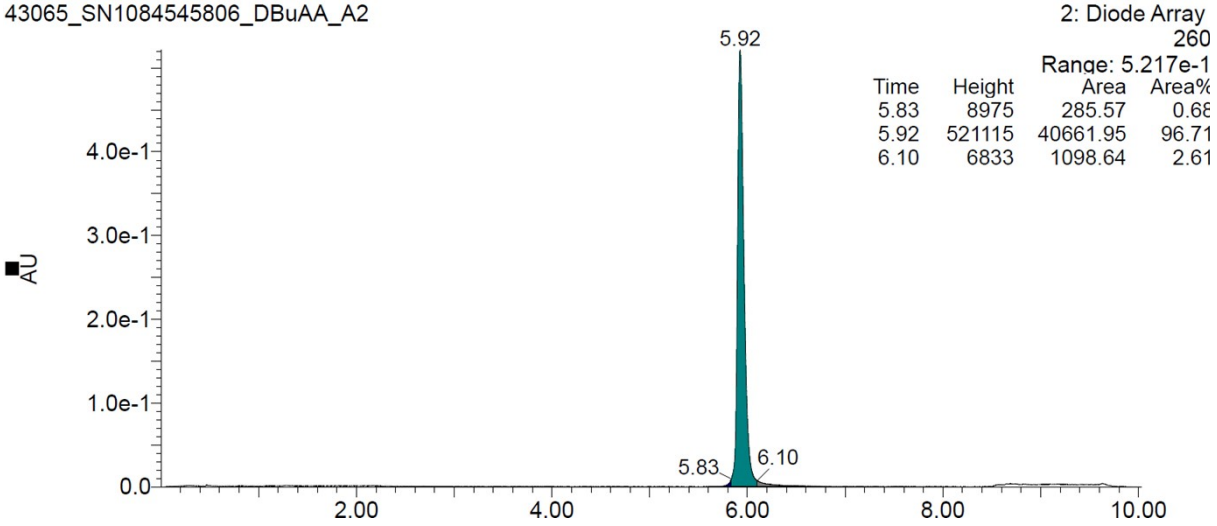
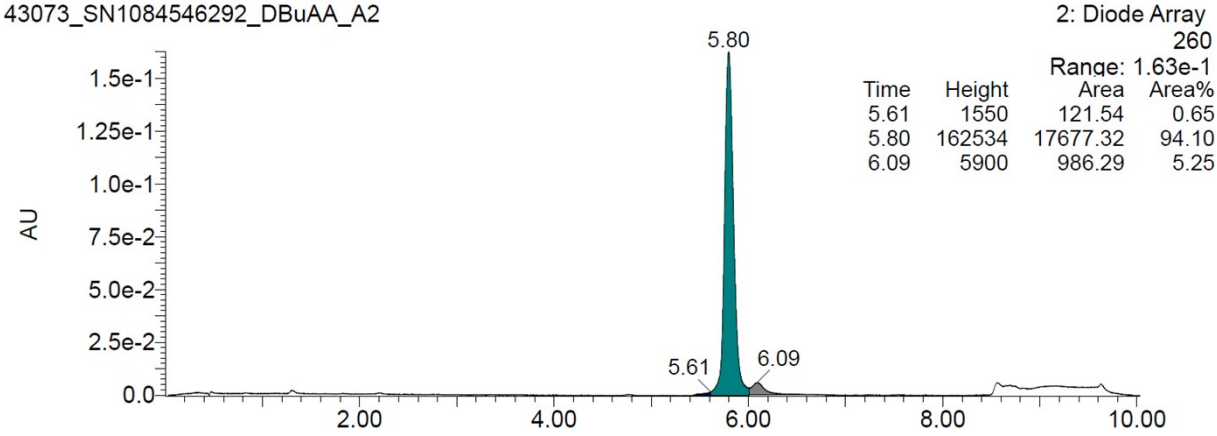
43065_SN1084545806_DBuAA_A2



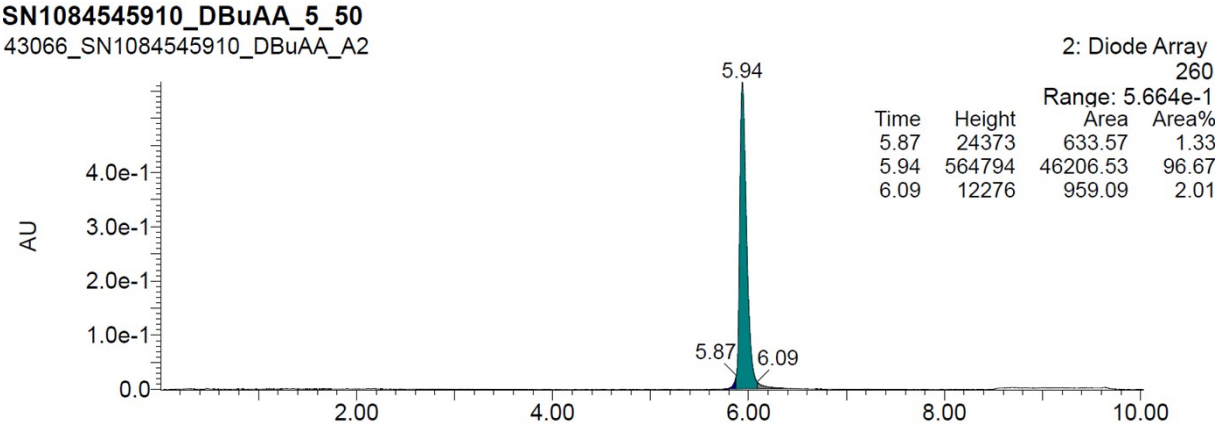
ASO10

SN1084546292_DBuAA_5_50

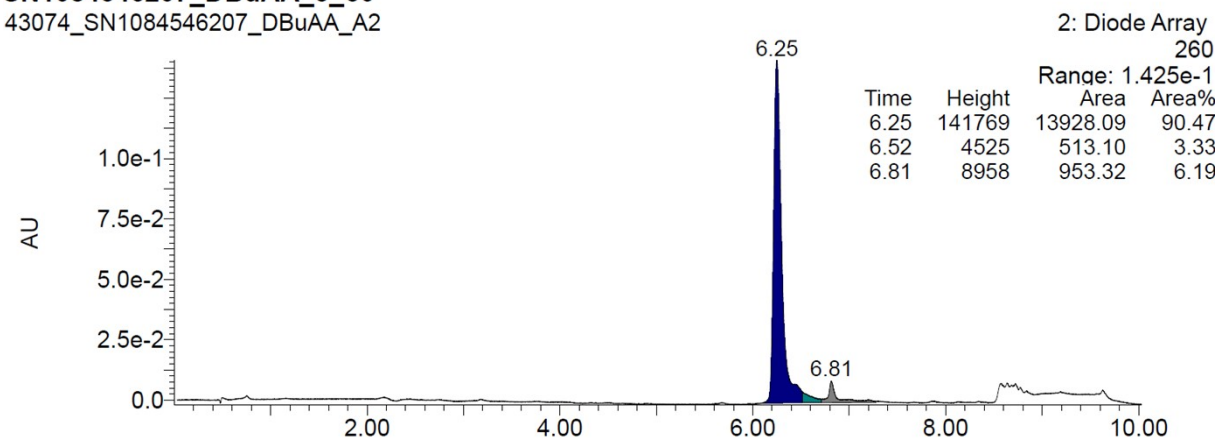
43073_SN1084546292_DBuAA_A2



ASO11



ASO12



ASO13

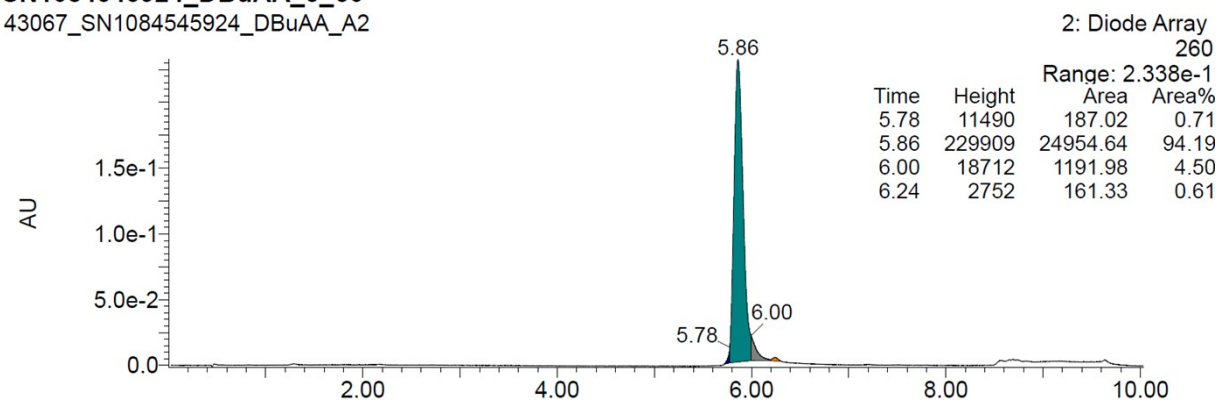


Figure S1. Oligonucleotide analytics for the sequences used in this study. Oligonucleotides were purified by preparative HPLC on aXBridge C18, 5µm 19x150mm column. A gradient from 15-50% solvent A (60mM dibutylammonium-acetate, pH 7, in acetonitrile:water=5:95) to solvent B (60mM dibutylammonium-acetate, pH 7 in acetonitrile) in 12 minutes using a flowrate of 20 mL/min. Analytical HPLC was performed on a Waters Acquity BEH C18, 1.7µm 2.1x100mm column using a gradient from 5-50% solvent A (60mM dibutylammonium-acetate, pH 7, in acetonitrile:water=5:95) to solvent B (60mM dibutylammonium-acetate, pH 7 in acetonitrile) at 60 °C and chromatograms were collected by UV detection at a wavelength of 260nM.

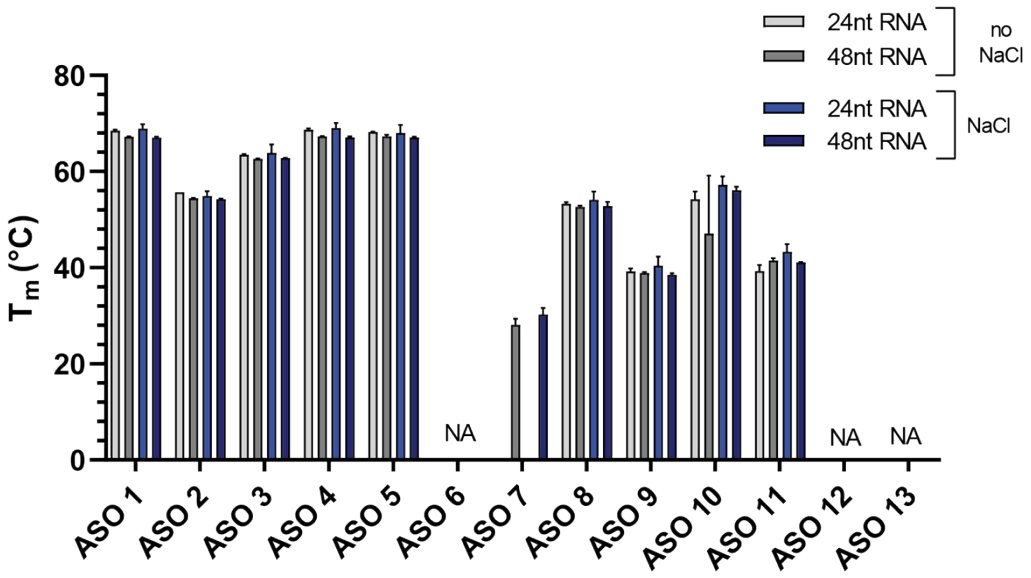


Figure S2. Differential scanning fluorimetry data for all ASOs and RNAs in two different buffer conditions, with salts and without salts.

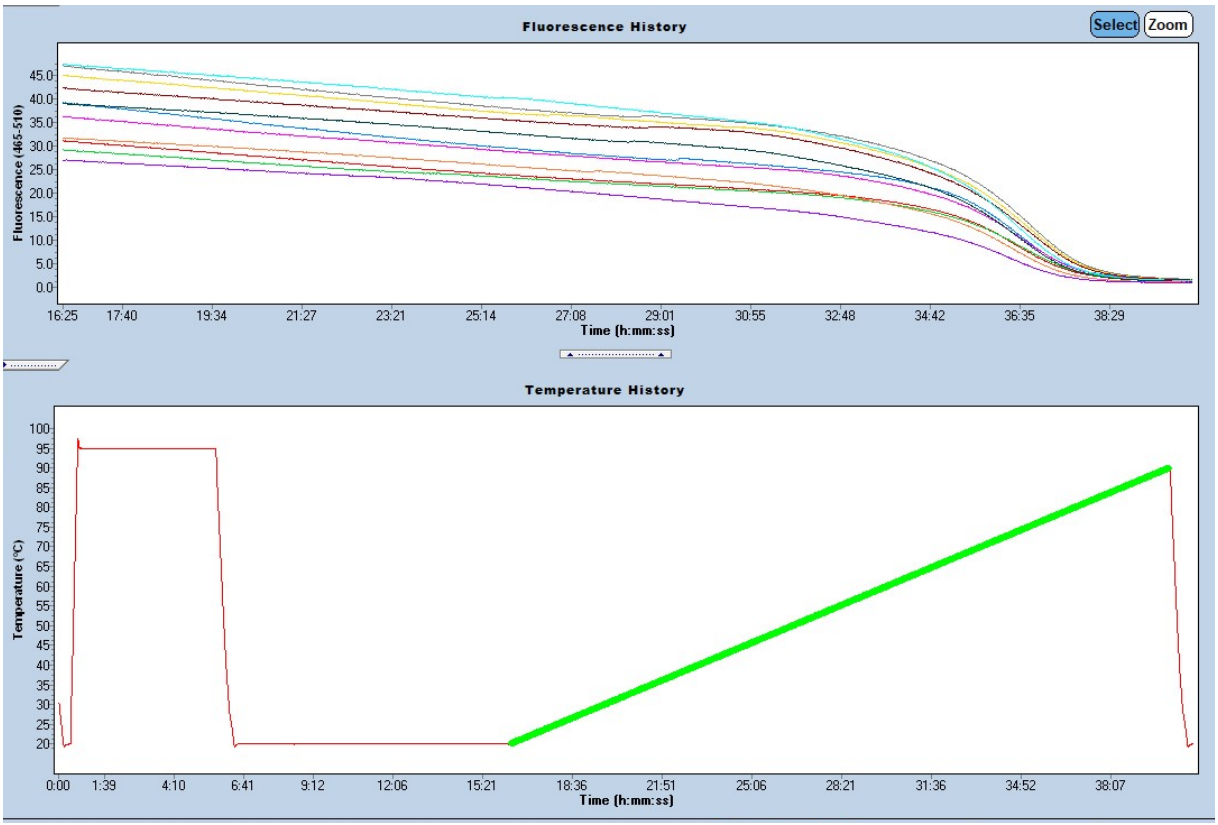


Figure S3. Fluorescence curves produced from the Roche 480 Lightcycler using RIBOgreen reporter dye. The fluorescence intensity curves for of ASOs/RNA hybridized duplexes and RNA used in this study where measures at 4 µM in 10 µl total volume and 50 nl of Quant-iT™ RiboGreen® (RIBO, Life Technologies, Ex max=~500 nm, Em max=~525 nm) dye stock solution in DMSO. The fluorescence intensity was monitored using the SYBR Green I/HRM dye filter combination (465–510 nm) from 20 to 95 °C at a ramp rate of 0.03 °C/s with 36 acquisitions per °C.

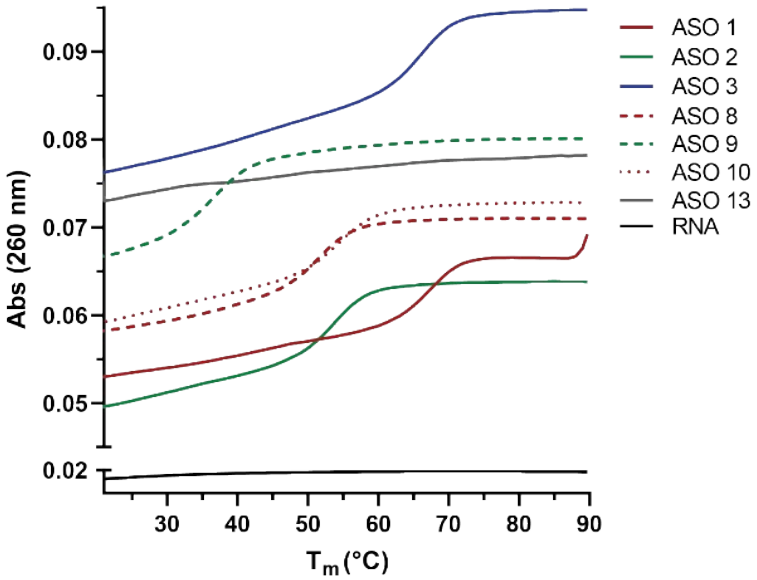
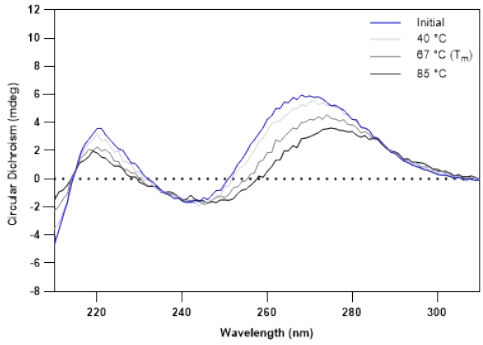
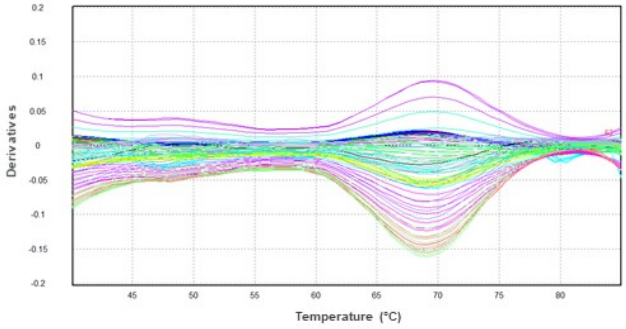


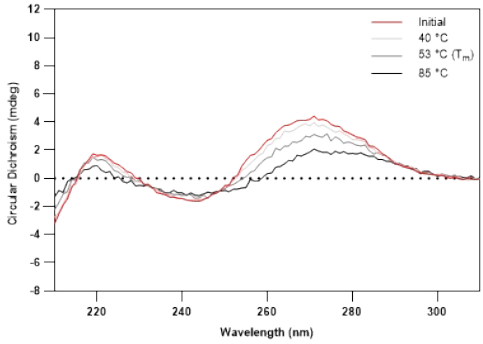
Figure S4. Raw denaturation melting temperature curves measured by absorbance at 260nm through UV spectroscopy.

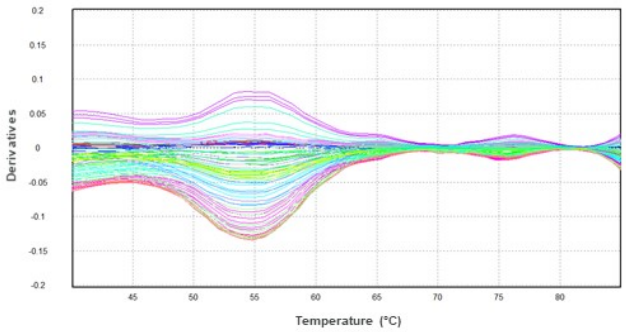
A)


Initial
40 °C
67 °C (Tm)
85 °C
Circular Dichroism (mdeg)
Wavelength (nm)

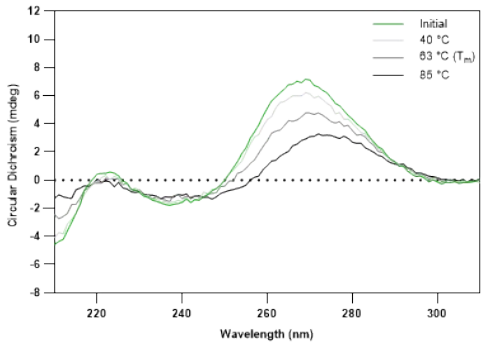

Derivatives
Temperature (°C)

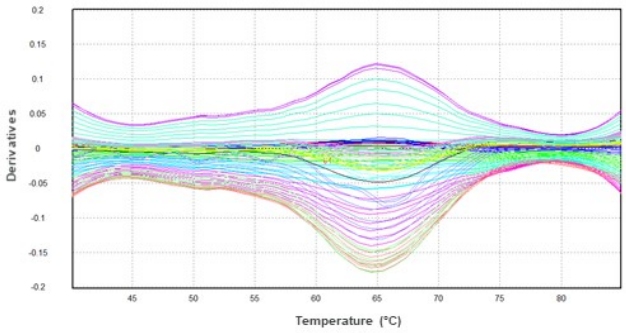
B)


Initial
40 °C
53 °C (Tm)
85 °C
Circular Dichroism (mdeg)
Wavelength (nm)

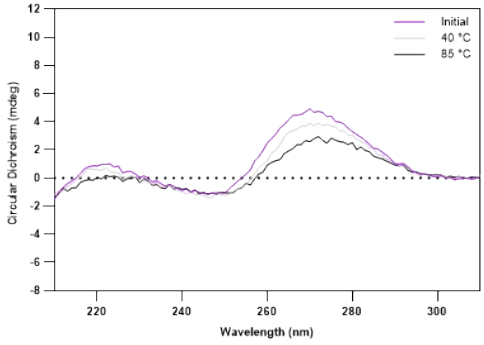

Derivatives
Temperature (°C)

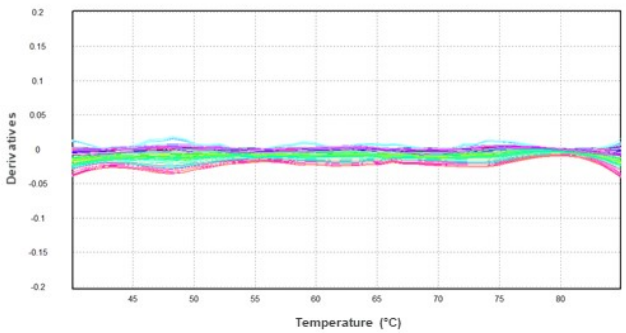
C)


Initial
40 °C
63 °C (Tm)
85 °C
Circular Dichroism (mdeg)
Wavelength (nm)

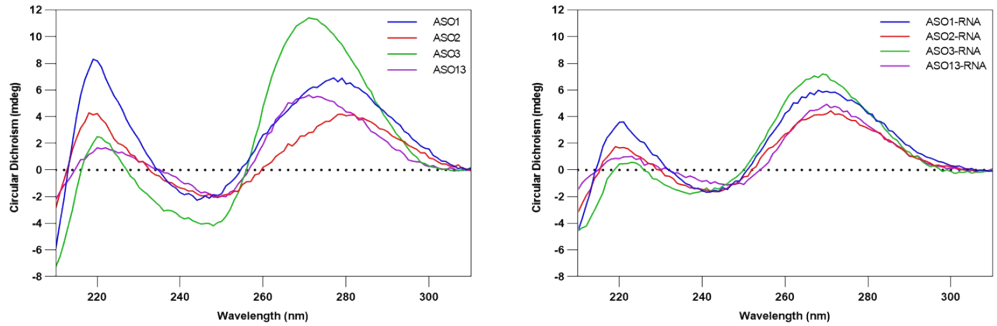

Derivatives
Temperature (°C)

D)


Initial
40 °C
85 °C
Circular Dichroism (mdeg)
Wavelength (nm)


Derivatives
Temperature (°C)

E)



F)

G)

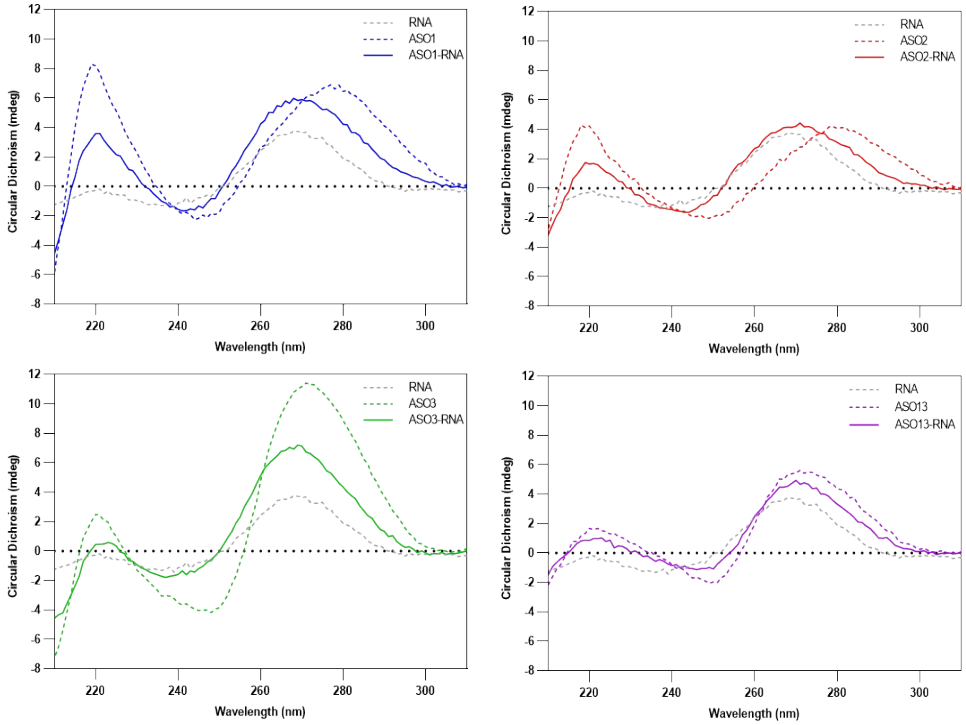


Figure S5. Circular Dichroism Spectroscopy. The CD spectra at 20°C (initial), 40°C, melting temperature (T_m) and 85 °C (left) and the derivative plots including all wavelengths (right) for A) ASO 1/RNA B) ASO 2/RNA C) ASO 3-RNA D) ASO 13/RNA. The CD derivative plots for E) ASO 1-RNA F) ASO 2-RNA G) ASO 3-RNA D) ASO 13-RNA at 85°C

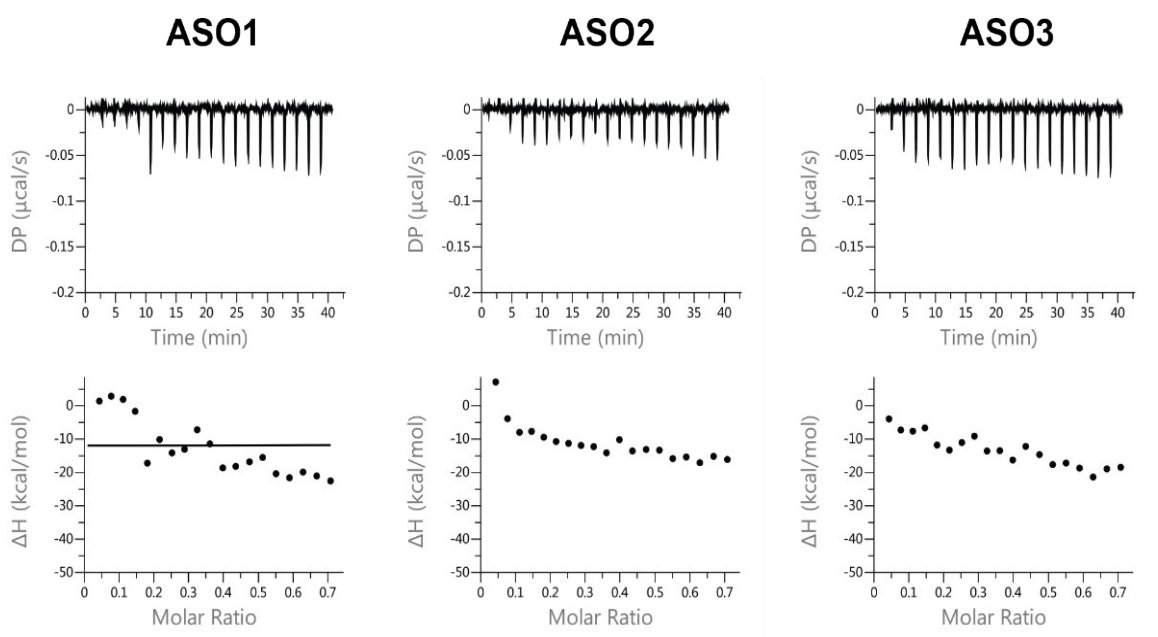


Figure S6. Isothermal calorimetry. Controls binding isotherms for ASO 1, 2 and 3 AT 37°C.

Table S2. SPR kinetics values measured at 25°C through a 1:1 binding model

ASO	AZ Sequence	Sugar Composition	Sugar Pattern	ka (1/Ms)	kd (1/s)	KD (M)	St dev KD
1	TGmCTACAAAACmCmCA	LNA-DNA-LNA	3-8-3	5.99E+06	2.59E-03	4.28E-10	1.71E-10
2	TGmCTACAAAACmCmCA	MOE-DNA-MOE	3-8-3	2.33E+05	5.38E-05	2.34E-10	9.06E-11
3	UGCUACAAAACCCA	OMe	14	3.02E+05	2.81E-05	7.81E-11	7.46E-11
8	TG**A**TACAAAACmCmCA	LNA-DNA-LNA	3-8-3	4.77E+05	2.75E-04	3.87E-10	9.06E-11
9	TG**A**TACAAAACmCmCA	MOE-DNA-MOE	3-8-3	4.61E+08	1.32E+01	2.83E-08	3.97E-10
10	TGmCT**A**AAAAACmCmCA	LNA-DNA-LNA	3-8-3	3.52E+08	9.73E+00	1.81E-08	7.79E-11
11	TGmCT**A**AAAAACmCmCA	MOE-DNA-MOE	3-8-3	9.95E+05	7.79E-03	7.92E-09	1.12E-09
13	mCGTCAGTATGCGAATC	LNA-DNA-LNA	3-8-3	NA	NA	NA	NA

Table S3. . SPR kinetics values measured at 37°C through a 1:1 binding model.

ASO	AZ Sequence	Sugar Composition	Sugar Pattern	ka (1/Ms)	kd (1/s)	KD (M)	St dev KD
1	TGmCTACAAAACmCmCA	LNA-DNA-LNA	3-8-3	1.50E+06	2.15E-04	1.41E-10	3.11E-11
2	TGmCTACAAAACmCmCA	MOE-DNA-MOE	3-8-3	1.11E+06	1.32E-03	2.79E-09	2.31E-09
3	UGCUACAAAACCCA	OMe	14	6.73E+05	1.11E-03	1.88E-09	2.15E-09
8	TG**A**TACAAAACmCmCA	LNA-DNA-LNA	3-8-3	8.86E+05	4.23E-03	5.50E-09	2.31E-09
9	TG**A**TACAAAACmCmCA	MOE-DNA-MOE	3-8-3	NA	NA	NA	NA
10	TGmCT**A**AAAAACmCmCA	LNA-DNA-LNA	3-8-3	5.80E+05	5.31E-03	1.89E-08	2.47E-08
11	TGmCT**A**AAAAACmCmCA	MOE-DNA-MOE	3-8-3	NA	NA	NA	NA
13	mCGTCAGTATGCGAATC	LNA-DNA-LNA	3-8-3	NA	NA	NA	NA

Table S4. ITC values measured at 37°C and 45°C for ASO 1, 2, 3 and 24nt RNA duplexes.

Samples	T(°C)	N (sites)	KD (M)	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)	Offset (kcal/mol)	Red. Chi-Sqr. (kcal/mol)²
24nt RNA + ASO1	37	0.927	8.09E-09	-35.8	-11.6	24.2	-5.6	7.2
24nt RNA + ASO2	37	0.972	3.91E-09	-21.9	-11.9	9.9	-7.0	1.4
24nt RNA + ASO3	37	1.1	3.32E-09	-24.5	-12.4	12.1	-13.4	3.5
24nt RNA + ASO1	45	0.927	1.22E-08	-46.0	-11.5	34.5	-2.1	18.0
24nt RNA + ASO2	45	1	4.87E-08	-38.3	-10.6	27.6	0.1	5.2
24nt RNA + ASO3	45	1.02	8.52E-09	-43.8	-11.7	32.0	-1.8	3.7

Table S5. Invariant parameters of ASO 2 in the presence and absence of RNA

Sample	Maximum Dimension (Å)	Radius of gyration (Å)	Molecular weight estimate (Da)
ASO	43	14.2	4050-5450
ASO + 24ntRNA	63	20.5	9950-11650

Table S6. Replicates SPR assay at 25°C.

Sample	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)	Model
ASO 1	5.00E+06	1.74E-03	3.48E-10	65.8	1.57	1:1 w Drift
	6.64E+06	2.07E-03	3.12E-10	54.7	1.7	1:1 w Drift
	6.33E+06	3.96E-03	6.25E-10	75.6	1.8	1:1 w Drift
ASO 2	2.30E+05	4.20E-05	1.83E-10	110.2	0.526	1:1 w Drift
	2.52E+05	4.56E-05	1.81E-10	93	1.03	1:1 w Drift
	2.18E+05	7.37E-05	3.39E-10	116.4	1.21	1:1 w Drift
ASO 3	2.98E+05	2.53E-05	8.49E-11	136.4	0.848	1:1 w Drift
	3.94E+05	5.88E-05	1.49E-10	108.6	1.23	1:1 w Drift
	2.14E+05	5.84E-08	2.73E-13	168.1	1.41	1:1 w Drift
ASO 8	2.74E+05	5.02E-05	1.83E-10	141.1	2.24	1:1 w Drift
	2.86E+05	3.85E-05	1.34E-10	123.5	2.31	1:1 w Drift
	8.71E+05	7.35E-04	8.44E-10	102.4	2.22	1:1 w Drift
ASO 9	8.78E+08	2.47E+01	2.81E-08	89.3	1.05	1:1 w Drift
	1.76E+08	4.49E+00	2.55E-08	77.9	1.43	1:1 w Drift
	3.30E+08	1.03E+01	3.12E-08	94.1	1.53	1:1 w Drift
ASO 10	1.31E+06	2.62E-04	2.00E-10	122.3	2.6	1:1 w Drift
	1.38E+06	2.31E-04	1.68E-10	108	2.52	1:1 w Drift
	3.04E+06	9.63E-04	3.16E-10	129.9	3.39	1:1 w Drift
ASO 11	1.01E+06	7.85E-03	7.75E-09	80.1	0.548	1:1 w Drift
	1.11E+06	7.64E-03	6.89E-09	69.3	0.983	1:1 w Drift
	8.66E+05	7.89E-03	9.11E-09	82.2	1.11	1:1 w Drift
ASO 13	2.37E+10	2.22E+03	9.36E-08	7.2	0.0688	1:1 w Drift
	4.23E+07	5.06E+00	1.20E-07	8.6	0.596	1:1 w Drift
	4.67E+07	2.42E+00	5.18E-08	5.4	0.579	1:1 w Drift

Table S7. Replicates SPR assay at 37°C.

Sample	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)	Model
ASO 1	1.34E+06	1.60E-04	1.19E-10	187.7	2.31	1:1 w drift
	1.65E+06	2.69E-04	1.63E-10	189	4.16	1:1 w drift
	2.43E+06	3.25E-04	1.34E-10	164.4	2.93	1:1 w drift
ASO 2	4.34E+05	1.68E-03	3.88E-09	74.6	0.283	1:1 w drift
	4.51E+05	1.96E-03	4.35E-09	72.7	0.923	1:1 w drift
	5.47E+05	2.38E-03	4.36E-09	52.2	0.782	1:1 w drift
ASO 3	6.45E+05	3.53E-04	5.48E-10	109.8	0.762	1:1 w drift
	8.28E+05	6.03E-04	7.28E-10	102.7	1.59	1:1 w drift
	1.16E+06	8.02E-04	6.93E-10	80.1	1.25	1:1 w drift
ASO 8	7.02E+05	5.37E-03	7.65E-09	74	0.369	1:1 w drift
	7.97E+05	6.51E-03	8.16E-09	75	1.02	1:1 w drift
	8.91E+05	7.29E-03	8.18E-09	56.6	0.803	1:1 w drift
ASO 9	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift
ASO 10	2.16E+06	5.27E-03	2.44E-09	95.4	0.604	1:1 w drift
	2.23E+06	5.71E-03	2.57E-09	96.3	1.05	1:1 w drift
	2.53E+06	6.29E-03	2.48E-09	83.5	0.779	1:1 w drift
ASO 11	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift
ASO 13	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift
	NA	NA	NA	NA	NA	1:1 w drift