

## Supplementary File S1 for Pedersen et al. (2013)

Lykke Pedersen,      Peter H Hagedorn,      Marie Lindholm,  
Morten Lindow

This document is the supplementary file S1 for the manuscript entitled “A kinetic model of enzyme recruiting oligonucleotides predicts an optimal affinity and explains why shorter and less affine oligonucleotides may be more potent” and it is a vignette for the R-package ASOmodels.

The functions and data used to produce the figures in the main manuscript and this supplementary file are available after installing the ASOmodels package in R

```
> require(devtools)
> install_github('ASOmodel',username='lykkep')
> require(ASOmodels)
```

The ASOmodels package contains the following functions:

1. `Trel`
2. `TrelNO`
3. `Trelstoc`
4. `plot.doseresponse`
5. `EC50`
6. `EC50NO`
7. `EC50stoc`
8. `diffASO`
9. `pretty10expLP`

## Contents

S1 The rate-equations of the ASO model	3
S2 Supplementary Table S1	4
S3 Supplementary Figure S1	5
S4 Supplementary Figure S2	6
S5 Supplementary Figure S3	6
S6 Supplementary Figure S4	7
S7 Supplementary Figure S5	9
S8 Supplementary Figure S6	10
S9 Supplementary Figure S7	11

## S1 The rate-equations of the ASO model

The kinetic ASO model governs seven ODEs for the seven variables: free target ( $T$ ), free oligonucleotide ( $O$ ), free RNase H ( $E$ ), complex of oligonucleotide and target ( $OT$ ), complex of oligonucleotide, target and RNase H ( $OTE$ ), complex of cleaved target, oligonucleotide and RNase H ( $OCE$ ), and complex of cleaved target and oligonucleotide ( $OC$ ).

$$\frac{d[T]}{dt} = v_{\text{prod}} - k_{T \rightarrow \emptyset}[T] - k_{O+T \rightarrow OT}[T][O] + k_{OT \rightarrow O+T}[OT] \quad (1)$$

$$\begin{aligned} \frac{d[OT]}{dt} = & k_{O+T \rightarrow OT}[O][T] - k_{OT \rightarrow O+T}[OT] \\ & - k_{OT+E \rightarrow OTE}[OT][E] + k_{OTE \rightarrow OT+E}[OTE] - k_{T \rightarrow \emptyset}[OT] \end{aligned} \quad (2)$$

$$\begin{aligned} \frac{d[OTE]}{dt} = & k_{OT+E \rightarrow OTE}[E][OT] - k_{OTE \rightarrow OT+E}[OTE] \\ & - (k_{T \rightarrow \emptyset} + k_{OTE \rightarrow OCE})[OTE] \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{d[E]}{dt} = & -k_{OT+E \rightarrow OTE}[E][OT] + k_{OTE \rightarrow OT+E}([OTE] + [OCE]) \\ & + k_{T \rightarrow \emptyset}[OTE] \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{d[O]}{dt} = & k_{OT \rightarrow O+T}[OT] - k_{O+T \rightarrow OT}[O][T] \\ & + k_{OC \rightarrow O+C}[OC] + k_{T \rightarrow \emptyset}([OT] + [OTE]) \end{aligned} \quad (5)$$

$$\frac{d[OCE]}{dt} = k_{OTE \rightarrow OCE}[OTE] - k_{OTE \rightarrow OT+E}[OCE] \quad (6)$$

$$\frac{d[OC]}{dt} = k_{OTE \rightarrow OT+E}[OCE] - k_{OC \rightarrow O+C}[OC] \quad (7)$$

Complex formation and breaking are denoted by rate constants  $k$  with subscripts. The target production rate is denoted by  $v_{\text{prod}}$  and the degradation rate by  $k_{T \rightarrow \emptyset}$ .

Steady-state is reached when the Eqs. (1)-(7) are equated to zero. Using Maple16 the steady-state concentrations are found. They all depend on the roots to a fourth order polynomial with coefficients calculated within the R-function `Trel()`. The one root that ensures that all concentrations are non-negative and also fullfills that

$$\begin{aligned} [O] + [OTE] + [OT] + [OCE] + [OC] &= O_t \quad \text{and} \\ [OTE] + [OCE] + [E] &= E_t \quad , \end{aligned}$$

is chosen.

When there is no oligonucleotide added to the system, the steady-state concentration of target is  $[T] = \frac{v_{\text{prod}}}{k_{T \rightarrow \emptyset}}$ . When oligonucleotide is added to the system, the total concentration of target at steady-state is the sum of the concentrations  $[T]$ ,  $[OT]$ , and  $[OTE]$ . The relative total target concentration at steady-state is then calculated as

$$T_{\text{rel}} = \frac{[T] + [OT] + [OTE]}{\frac{v_{\text{prod}}}{k_{T \rightarrow \emptyset}}} . \quad (8)$$

The half maximal effect concentration ( $EC_{50}$ ) is the concentration of total nucleotide needed to reduce the target concentration by half. The  $EC_{50}$  is a measure of the potency of an oligonucleotide. A more potent oligonucleotide will have a lower  $EC_{50}$  value. In mathematical terms the  $EC_{50}$  value is defined as

$$EC_{50} = \left( O_t \left| T_{\text{rel}} = \frac{\text{Eff}}{2} + T_{\text{rel},\text{min}} \right. \right) , \quad (9)$$

where the efficacy (Eff, the maximum decrease in  $T_{\text{rel}}$ ), and the minimum value of  $T_{\text{rel}}$  ( $T_{\text{rel},\text{min}}$ ) are defined by

$$\text{Eff} = 1 - \lim_{O_t \rightarrow \infty} T_{\text{rel}} = 1 - T_{\text{rel},\text{min}} . \quad (10)$$

## S2 Supplementary Table S1

Supplementary Table S1: Default values for the parameter-space of the ASO model. Concentrations are measured in nM and time in min.

Parameter	Description	Default value	Ref
$E_t$	Total RNase H concentration	1 nM	[1]
$O_t$	Total oligonucleotide conc	$\mathcal{O}(\mu M)$	
$v_{\text{prod}}$	Production of target	0.2 nM/min	[5]
$k_{T \rightarrow \emptyset}$	Degradation of target	0.04 min <sup>-1</sup>	[7]
$K_{dOT}$	Dissociation constant of $OT$	0.3 nM	[2]
$K_{dOTE}$	Dissociation constant of $OTE$	70 nM	[1]
$k_{O+T \rightarrow OT}$	Rate of $O + T \rightarrow OT$	0.2 (nM min) <sup>-1</sup>	[2]
$k_{OT+E \rightarrow OTE}$	Rate of $OT + E \rightarrow OTE$	5 (nM min) <sup>-1</sup>	[1]
$k_{OTE \rightarrow OCE}$	Rate of cleavage	8 min <sup>-1</sup>	[1]
$\alpha$	Ratio of $\frac{k_{OT \rightarrow O+T}}{k_{OC \rightarrow O+C}} \leq 1$	0.1	

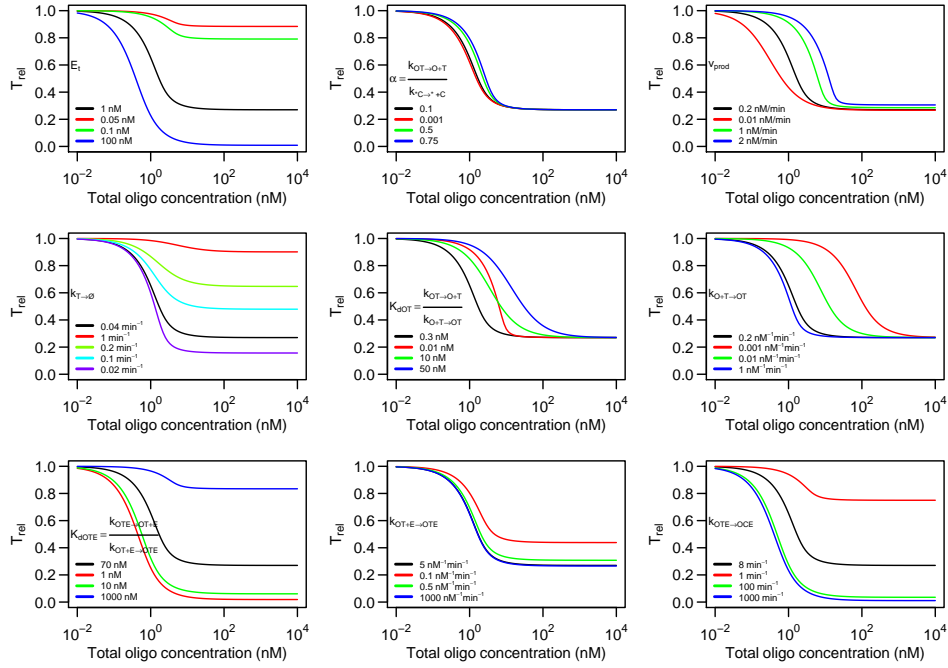
### S3 Supplementary Figure S1

The R-function `Trel()` calculates  $T_{\text{rel}}$  and takes  $O_t$  and the set of parameters as input:

```
> #The parameters are in vector-format
> parms <- c(Et = 1, KdOT = 0.3, kOpT = 0.2, KdOTE = 70, kOTpE = 5,
+           vprod = 0.2, kdegrad = 0.04, alpha=0.1, kcleav = 8)
> Trel(Ot=1, param=parms)
```

```
[1] 0.6538694
```

$T_{\text{rel}}$  can be calculated for a range of different oligonucleotide concentrations ( $O_t$ ) and from this a dose-response curve is obtained. Supplementary Figure S1 shows the change in the dose-response curves as the parameters vary. These plots are produced using `plot.doseresponse()`.



Supplementary Figure S1: Dose-response curves for different values of  $E_t$ ,  $\alpha$ ,  $v_{\text{prod}}$ ,  $k_{T \rightarrow \emptyset}$ ,  $K_{dOT}$ ,  $k_{O+T \rightarrow OT}$ ,  $K_{dOTE}$ ,  $k_{OT+E \rightarrow OTE}$ , and  $k_{OTE \rightarrow OCE}$  (top, left to bottom, right). Black lines correspond to the parameter values listed in Supplementary Table S1.

## S4 Supplementary Figure S2

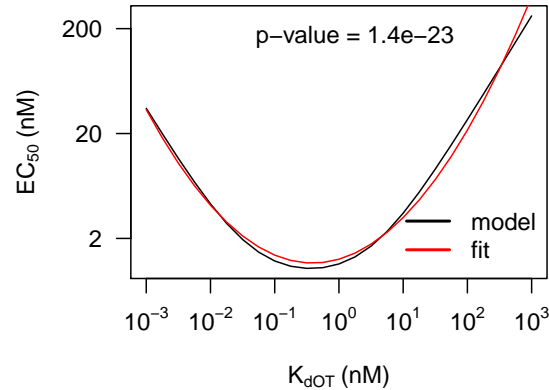
Using the R-function `drm()` from the `drc` package (v2.3-0) a dose-response curve is fitted to  $T_{\text{rel}}$  as a function of  $O_t$  to obtain an  $EC_{50}$ -value. This is calculated through the R-function `EC50()` that takes  $K_{dOT}$  and the set of parameters as input:

```
> EC50(KdOT=0.1,param=parms)
```

```
EC50  
1.218908
```

For a range of  $K_{dOT}$  values, the corresponding  $EC_{50}$ -values can be calculated. These can be fitted to a second order polynomial by using the R-function `lm()`, see Supplementary Figure S2.

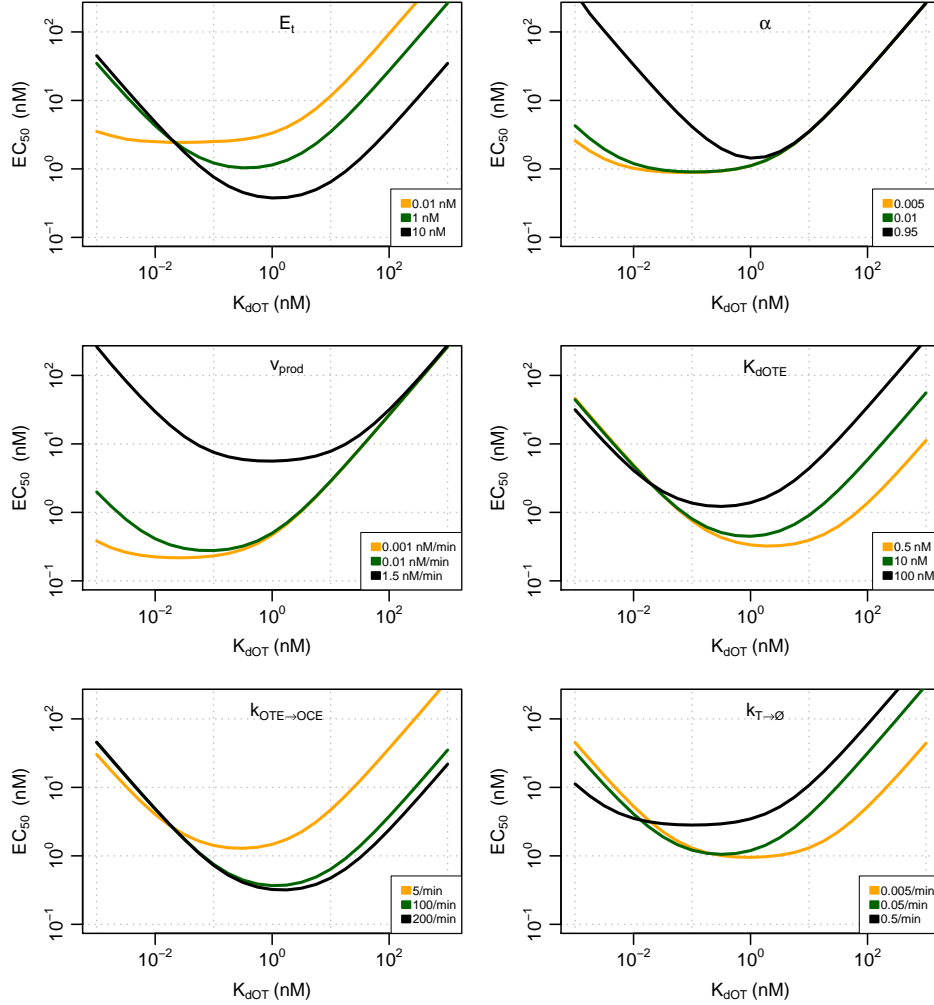
```
> D1_seq <- 10^seq(-3,3.2,by=0.25)  
> ECseq <- sapply(D1_seq,EC50)  
> FitPar <- lm(log10(ECseq) ~ log10(D1_seq) + I(log10(D1_seq)^2))
```



Supplementary Figure S2:  $EC_{50}$  as a function of  $K_{dOT}$  is fitted on a log-log scale to a second order polynomial.

## S5 Supplementary Figure S3

Supplementary Figure S3 shows  $EC_{50}$  as a function of  $K_{dOT}$  for various parameter values. It can be seen that the optimum affinity, quantified by  $K_{dOT}$ , changes as parameters are changed. A lower value of  $K_{dOT}$  corresponds to a better affinity for the oligonucleotide.



Supplementary Figure S3: The optimum affinity is dependent on the parameter settings. In the panels the  $EC_{50}$  concentration is plotted against the binding affinity quantified by  $K_{dOT}$  for various parameters. We have varied the total RNase H concentration ( $E_t$ ),  $\alpha$ , the rate of target cleavage ( $k_{OTE \rightarrow OCE}$ ), the target production ( $v_{prod}$ ) and degradation ( $k_{T \rightarrow \emptyset}$ ), and the dissociation constant for the OTE complex ( $K_{dOTE}$ ).

## S6 Supplementary Figure S4

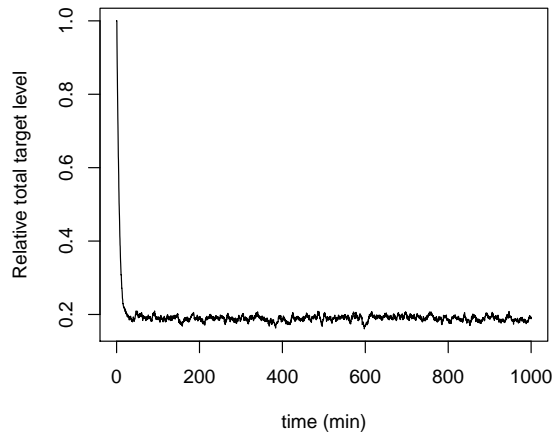
The stochastic simulation of the ASOmodel is carried out by use of the `ssa()` R-function from the GillespieSSA package (v.0.5-4). The inputs to `ssa` are an initial state vector (`x0`), which is the initial number of molecules, a propensity vector (`a`), which denotes the different states of the system, a state-change matrix (`nu`), which is the change in number of molecule (rows)

if a reaction occur (column), the model-parameters (parms) and the final time (tf).

```
> library(GillespieSSA)
> #Model parameters
> parms1 <- c(kOpT = 2E-5,kOTpE =50E-5 ,vprod = 150, kdeggrad = 0.04,
+           kcleav = 2, kOT =0.06, kOTE=2, kC = 0.1)
> #Initital state vector
> x0 <- c(Tt=parms1["vprod"]/parms1["kdeggrad"],
+         OT=0,OTE=0,E=1e3,O=1e5,OCE=0,OC=0)
> names(x0) <- c('Tt','OT','OTE','E','O','OCE','OC')
> #Propensity vector
> a <- c("vprod","kOpT*O*Tt","kdeggrad*Tt","kOT*OT","kOTE*OTE","kdeggrad*OT",
+        "kOTpE*OT*E","kdeggrad*OTE","kcleav*OTE","kC*OC","kOTE*OCE" )
> #State-change matrix
> nu <- matrix(0,7,length(a))
> dimnames(nu) <- list(names(x0),a)
> #T
> nu['Tt',c('vprod','kOT*OT')] <- 1
> nu['Tt',c('kOpT*O*Tt','kdeggrad*Tt')] <- -1
> #OT
> nu['OT',c('kOpT*O*Tt','kOTE*OTE')] <- 1
> nu['OT',c('kOT*OT','kOTpE*OT*E','kdeggrad*OT')] <- -1
> #OTE
> nu['OTE',c('kOTpE*OT*E')] <- 1
> nu['OTE',c('kOTE*OTE','kdeggrad*OTE','kcleav*OTE')] <- -1
> #E
> nu['E',c('kOTE*OTE','kdeggrad*OTE','kOTE*OCE')] <- 1
> nu['E',c('kOTpE*OT*E')] <- -1
> #O
> nu['O',c('kOT*OT','kdeggrad*OTE','kdeggrad*OT','kC*OC')] <- 1
> nu['O',c('kOpT*O*Tt')] <- -1
> #OCE
> nu['OCE',c('kcleav*OTE')] <- 1
> nu['OCE',c('kOTE*OCE')] <- -1
> #OC
> nu['OC',c('kOTE*OCE')] <- 1
> nu['OC',c('kC*OC')] <- -1
> #The Gillespie simulation
> Gillespie <- ssa( x0=x0,a=a,nu=nu,
+           parms = parms1,tf=1E3,method = "ETL")
```

Supplementary Figure S4 shows  $T_{\text{rel}}$  from the Gillespie simulation.



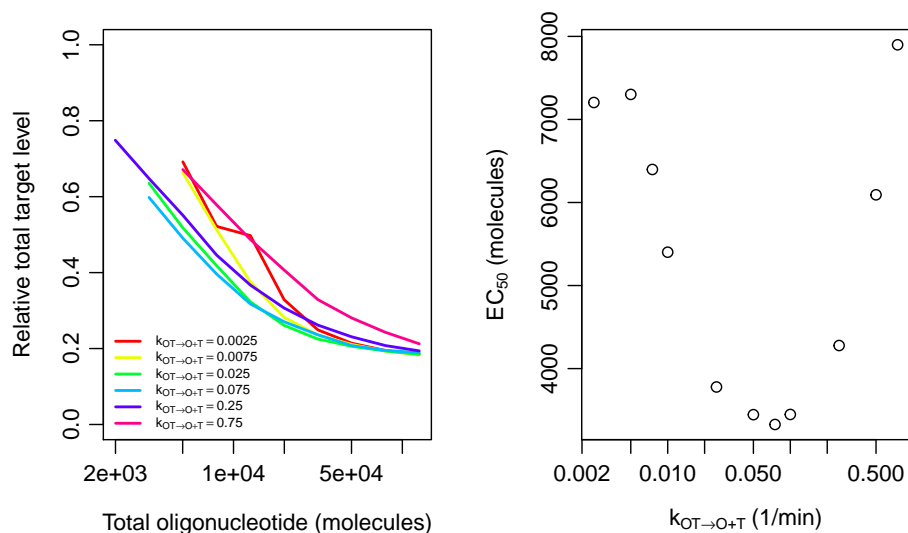


Supplementary Figure S4: The time-trace for the relative total target level when the ASO model is simulated stochastically.

## S7 Supplementary Figure S5

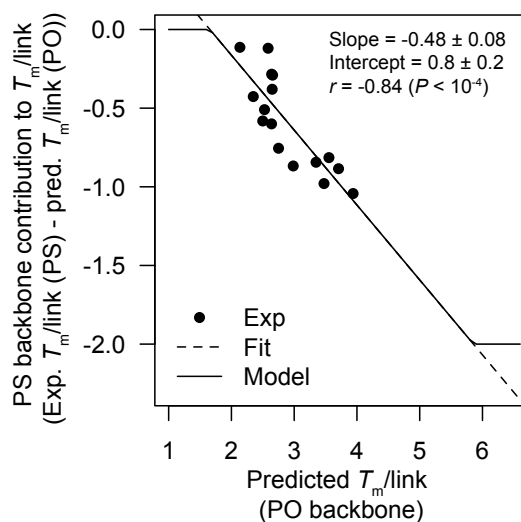
After a while the stochastic simulation reaches a plateau. In Supplementary Figure S4 the plateau starts around 50min. The mean of  $T_{\text{rel}}$  within the plateau is calculated through the R-function `Trelstoc()`. Using this function we can generate dose-response curves (Supplementary Figure S5, left). From these  $EC_{50}$ -values can be calculated using `EC50stoc()` and they are subsequently plotted as a function of  $k_{OT \rightarrow O+T}$  (Supplementary Figure S5, right):

```
> ##### Sequence of k(OT -> O+T) values
> lseq <- c(1,2.5,5,7.5)
> lKOT <- c(1E-3*lseq[-1],1E-2*lseq,1E-1*lseq)
> ##### Generation of dose-response curves
> DRcurve <- lapply(lKOT,function(ki){
+   sapply(10^seq(2.5,6,by=0.2),
+     function(i) Trelstoc(i,kOT=ki)$Tstat)})
> DRc <- lapply(DRcurve,function(x) x[,!is.na(x[3,])])
> ##### Calculation of EC50
> EC50_lKOT <- sapply(1:length(DRc),
+   function(x){EC50stoc(DRc[[x]][2,],DRc[[x]][1,])})
```



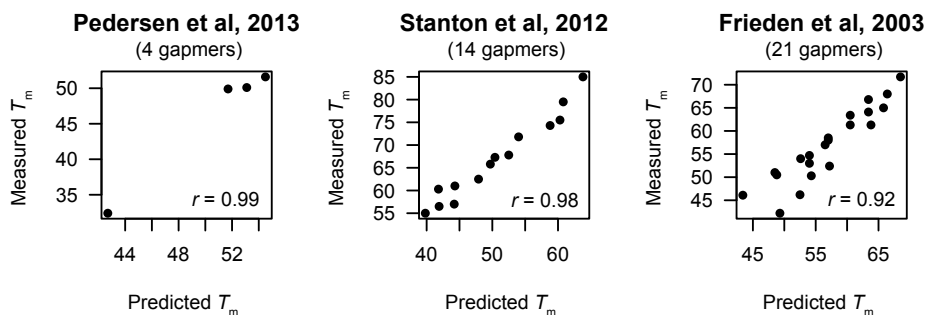
Supplementary Figure S5: Left: Dose-response curves for various values of  $k_{OT \rightarrow O+T}$  (compare to Supplementary Figure S1,middle). Right:  $EC_{50}$  as a function of  $k_{OT \rightarrow O+T}$ . A high value of  $k_{OT \rightarrow O+T}$  corresponds to a low affinity.

## S8 Supplementary Figure S6



Supplementary Figure S6: The effect on  $T_m$  of a phosphorothioate backbone was estimated using published data from Ref. [4].

## S9 Supplementary Figure S7



Supplementary Figure S7: Measured melting temperature versus predicted melting temperature. There are clear correlations ( $r > 0.92$ ,  $P < 0.01$ , Pearson's correlation) between predicted and measured  $T_m$ . Pedersen et al: 4 LNA-modified oligonucleotides targeting apolipoprotein B (this work), Stanton et al: 14 LNA-modified oligonucleotides targeting the glucocorticoid receptor [6]. Frieden et al: 21 LNA-modified oligonucleotides targeting the luciferase firefly gene [3].

Melting curves were recorded with a Perkin Elmer spectrophotometer. Oligonucleotide and its complementary RNA, both at  $1.5\mu M$ , were dissolved in buffer (20mM phosphate buffer, 100mM NaCl, 0.1nM EDTA, pH 7). Samples were denatured at  $95^\circ C$  for 3min and slowly cooled to  $20^\circ C$  prior to measurements. Melting curves were recorded at 260nm using a heating rate of  $1^\circ C/min$ , a slit of 2nm and a response of 0.2s. From this,  $T_m$  values were obtained from the maxima of the first derivatives of the melting curves.

## References

- [1] N. V. Amirkhanov and P. I. Pradeepkumar. Kinetic analysis of the RNA cleavage of the conformationally-constrained oxetane-modified antisense-RNA hybrid duplex by RNase H. *J Chem Soc*, 2002.
- [2] U. Christensen, N. Jacobsen, V. K. Rajwanshi, J. Wengel, and T. Koch. Stopped-flow kinetics of locked nucleic acid (LNA)-oligonucleotide duplex formation: studies of LNA-DNA and DNA-DNA interactions. *Biochem. J.*, 354(Pt 3):481–484, Mar. 2001.
- [3] M. Frieden, S. M. Christensen, N. D. Mikkelsen, C. Rosenbohm, C. A. Thru, M. Westergaard, H. F. Hansen, H. Ørum, and T. Koch. Expanding the design horizon of antisense oligonucleotides with alpha,ÎLNA. *Nucleic Acids Res.*, 31(21):6365–6372, 2003.
- [4] G. M. Hashem, L. Pham, M. R. Vaughan, and D. M. Gray. Hybrid oligomer duplexes formed with phosphorothioate DNAs: CD spectra and melting temperatures of S-DNA.RNA hybrids are sequence-dependent but consistent with similar heteronomous conformations. *Biochemistry*, 37(1):61–72, Jan. 1998.
- [5] H. Lodish. *Molecular Cell Biology*. W. H. Freeman, 2008.
- [6] R. Stanton, S. Sciabola, C. Salatto, Y. Weng, D. Moshinsky, J. Little, E. Walters, J. Kreeger, D. Dimattia, T. Chen, T. Clark, M. Liu, J. Qian, M. Roy, and R. Dullea. Chemical Modification Study of Antisense Gapmers. *Nucleic Acid Ther*, 22(5):344–359, Aug. 2012.
- [7] E. Yang, E. van Nimwegen, M. Zavolan, N. Rajewsky, M. Schroeder, M. Magnasco, and J. E. Darnell. Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. *Genome Res.*, 13(8):1863–1872, Aug. 2003.