Supplementary File S1 for Pedersen et al. (2013)

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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

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).

$$\frac{\mathrm{d}[T]}{\mathrm{d}t} = v_{\mathrm{prod}} - k_{\mathrm{T}\to\emptyset}[T] - k_{\mathrm{O+T}\to\mathrm{OT}}[T][O] + k_{\mathrm{OT}\to\mathrm{O+T}}[OT] \tag{1}$$

$$\begin{aligned}\frac{\mathrm{d}[OT]}{\mathrm{d}t} &= k_{\mathrm{O+T}\to\mathrm{OT}}[O][T] - k_{\mathrm{OT}\to\mathrm{O+T}}[OT] \\ &\quad - k_{\mathrm{OT+E}\to\mathrm{OTE}}[OT][E] + k_{\mathrm{OTE}\to\mathrm{OT+E}}[OTE] - k_{\mathrm{T}\to\emptyset}[OT]\end{aligned} \tag{2}$$

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

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

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

$$\frac{\mathrm{d}[OCE]}{\mathrm{d}t} = k_{\mathrm{OTE}\to\mathrm{OCE}}[OTE] - k_{\mathrm{OTE}\to\mathrm{OT+E}}[OCE] \tag{6}$$

$$\frac{\mathrm{d}[OC]}{\mathrm{d}t} = k_{\mathrm{OTE}\to\mathrm{OT+E}}[OCE] - k_{\mathrm{OC}\to\mathrm{O+C}}[OC] \tag{7}$$

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

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

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

is chosen.

When there is no oligonucleotide added to the system, the steady-state concentration of target is $[T] = \frac{v_{\text{prod}}}{k_{\text{T}\to\emptyset}}$. 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

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

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

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

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

$$\text{Eff} = 1 - \lim_{O_t \to \infty} T_{\text{rel}} = 1 - T_{\text{rel,min}} \quad . \tag{10}$$

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_{prod}	Production of target	0.2 nM/min	[5]
$k_{\text{T}\to\emptyset}$	Degradation of target	0.04 min^{-1}	[7]
K_{dOT}	Dissociation constant of OT	0.3 nM	[2]
K_{dOTE}	Dissociation constant of OTE	70 nM	[1]
$k_{\text{O+T}\to\text{OT}}$	Rate of $O + T \to OT$	0.2 $(\text{nM}\,\text{min})^{-1}$	[2]
$k_{\text{OT+E}\to\text{OTE}}$	Rate of $OT + E \to OTE$	5 $(\text{nM}\,\text{min})^{-1}$	[1]
$k_{\text{OTE}\to\text{OCE}}$	Rate of cleavage	8 min^{-1}	[1]
α	Ratio of $\frac{k_{\text{OT}\to\text{O+T}}}{k_{\text{OC}\to\text{O+C}}} \leq 1$	0.1	

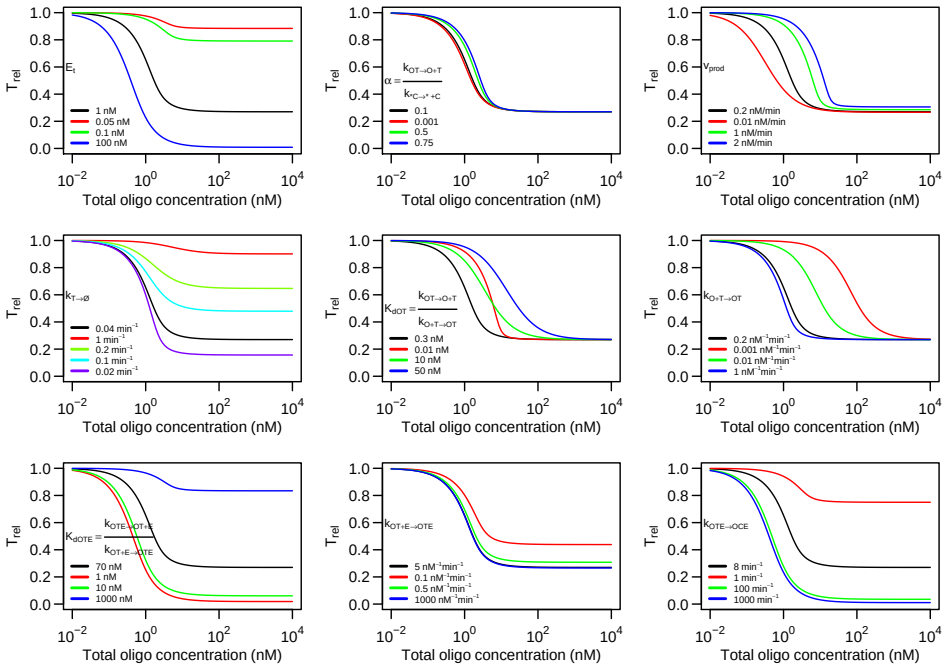
S3 Supplementary Figure S1

The R-function `Trel()` calculates T_{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_{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-reponse curves as the parameters vary. These plots are produced using `plot.doseresponse()`.



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

S4 Supplementary Figure S2

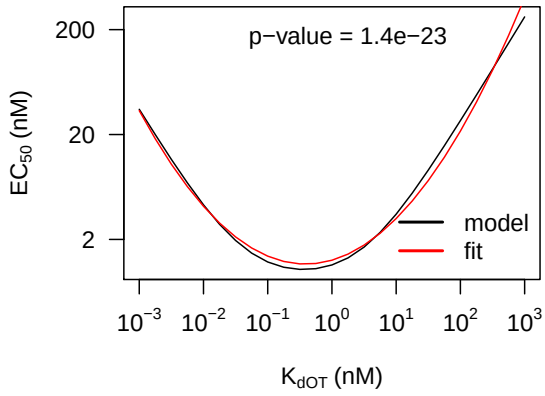
Using the R-function `drm()` from the drc package (v2.3-0) a dose-response curve is fitted to T_{rel} as a function of O_t to obtain an EC_{50}-value. This is calculated through the R-function `EC50()` that takes K_{dOT} and the set of parameters as input:

```
> 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.

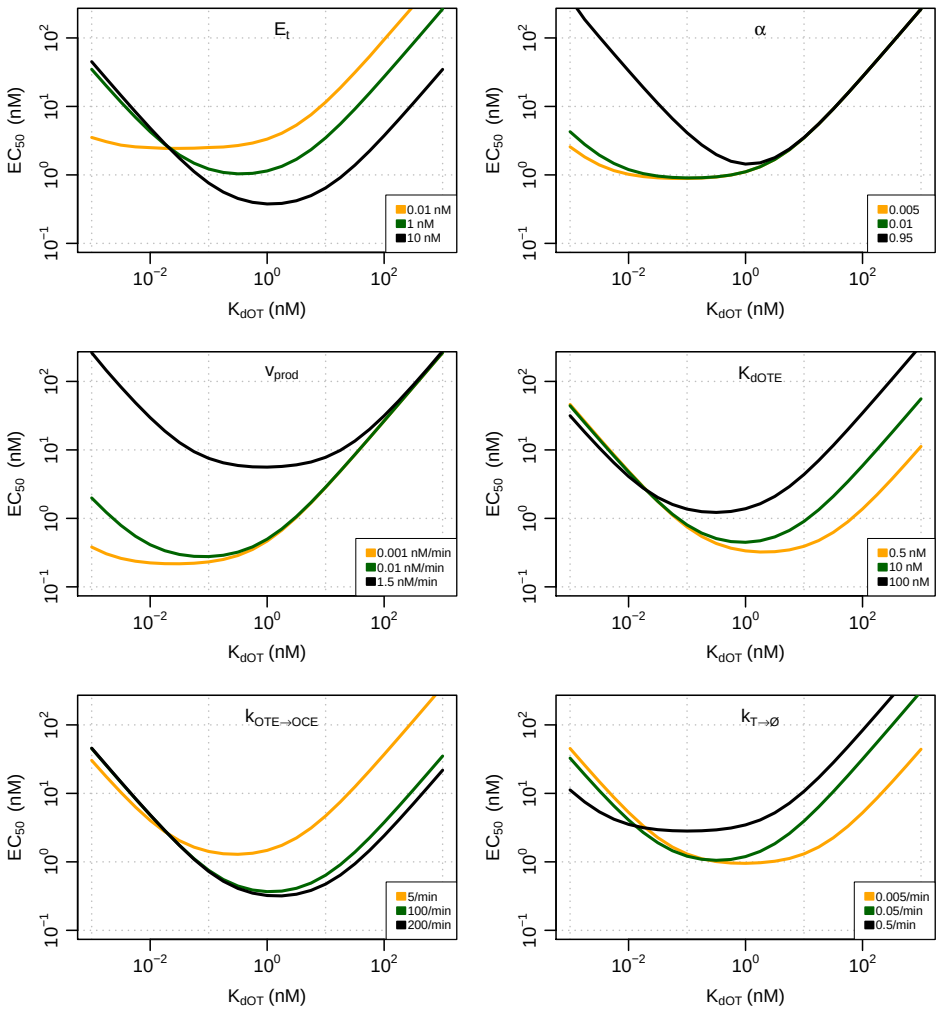
```
> D1_seq <- 10^seq(-3,3.2,by=0.25)
> ECseq <- sapply(D1_seq,EC50)
> FitPar <- lm(log10(ECseq) ~ log10(D1_seq) + I(log10(D1_seq)^2))
```



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), α, the rate of target cleavage ($k_{\mathrm{OTE}\rightarrow\mathrm{OCE}}$), the target production (v_{prod}) and degradation ($k_{\mathrm{T}\rightarrow\emptyset}$), and the dissociation constant for the OTE complex (K_{dOTE}).

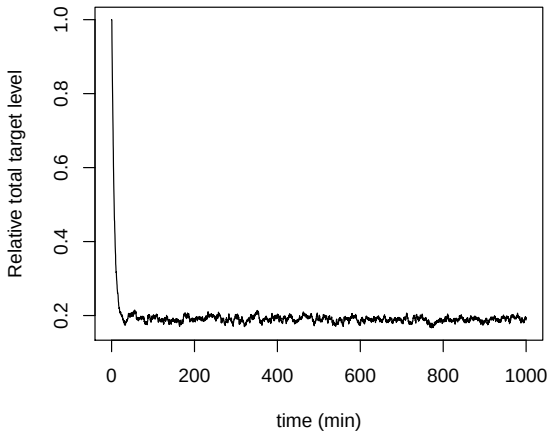
S6 Supplementary Figure S4

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,  kdegrad = 0.04,
+               kcleav = 2, kOT =0.06, kOTE=2, kC = 0.1)
> #Initital state vector
> x0 <- c(Tt=parms1["vprod"]/parms1["kdegrad"],
+         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","kdegrad*Tt","kOT*OT","kOTE*OTE","kdegrad*OT",
+          "kOTpE*OT*E","kdegrad*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','kdegrad*Tt')] <- -1
> #OT
> nu['OT',c('kOpT*O*Tt','kOTE*OTE')] <- 1
> nu['OT',c('kOT*OT','kOTpE*OT*E','kdegrad*OT')] <- -1
> #OTE
> nu['OTE',c('kOTpE*OT*E')] <- 1
> nu['OTE',c('kOTE*OTE','kdegrad*OTE','kcleav*OTE')] <- -1
> #E
> nu['E',c('kOTE*OTE','kdegrad*OTE','kOTE*OCE')] <- 1
> nu['E',c('kOTpE*OT*E')] <- -1
> #O
> nu['O',c('kOT*OT','kdegrad*OTE','kdegrad*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_{rel} from the Gillespie simulation.

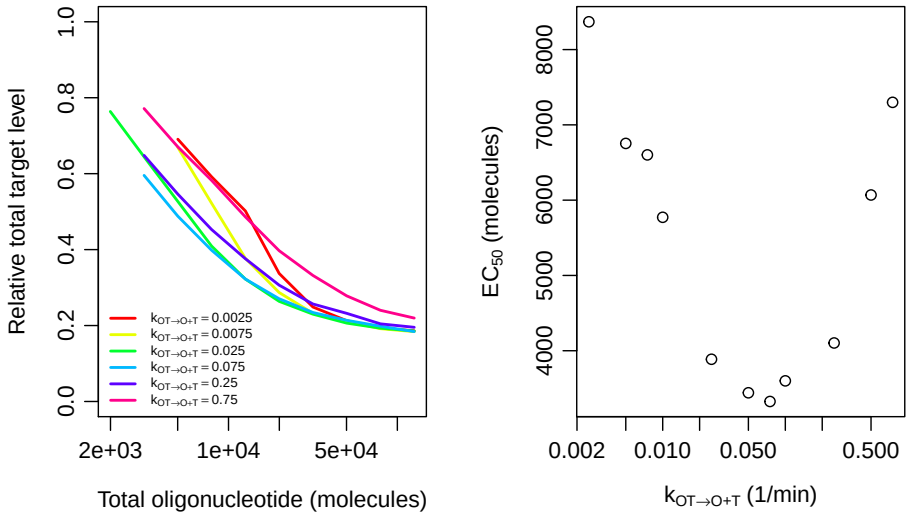


Supplementary Figure S4: The time-trace for the relative total target level when the ASO model is simulated stochastically.

S7 Supplementary Figure S5

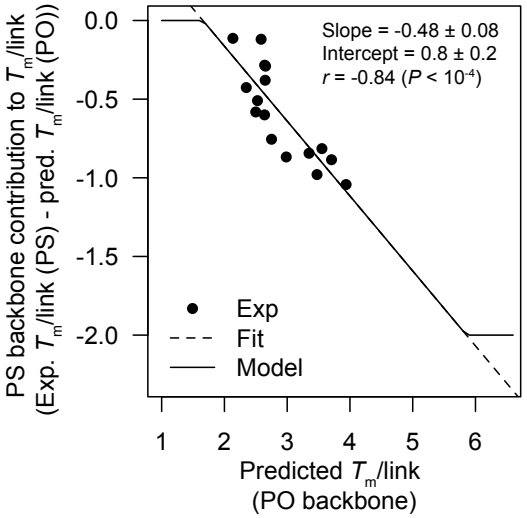
After a while the stochastic simulation reaches a plateau. In Supplementary Figure S4 the plateu starts around 50min. The mean of T_{rel} within the plateu is calculated through the R-function `Trelstoc()`. Using this function we can generate dose-response curves (Supplementary Figure S5,left). From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



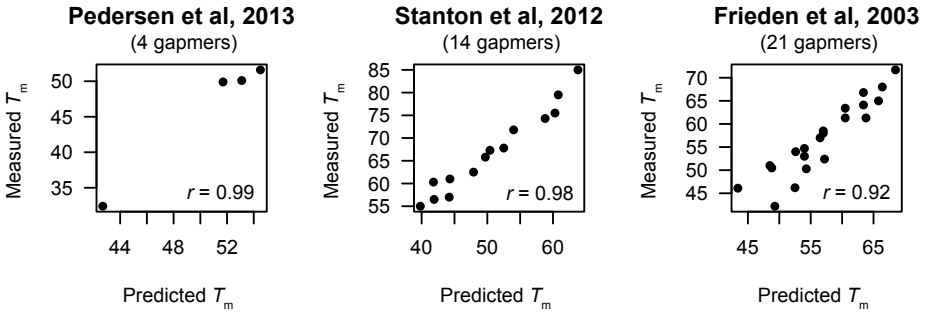
Supplementary Figure S5: Left: Dose-response curves for various values of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$ (compare to Supplementary Figure S1,middle). Right: EC_{50} as a function of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$. A high value of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$ corresponds 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°C for 3min and slowly cooled to 20°C prior to measurements. Melting curves were recorded at 260nm using a heating rate of 1°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

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