Supplementary Document for Pedersen et al. (2013)

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This document is the Supplementary Document for the manuscript entitled "A kinetic model of enzyme recruiting oligonucleotides predicts an optimal affinity and explains why shorter and less affine oligonucleotides can be more potent" and it is a vignette for the R-package ASOmodels.

With the aim of maximising reproducibility, the functions and data used to produce the figures in the main manuscript and this supplementary document are available after installing the ASOmodels package in R.

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

The ASOmodels package defines and documents the following functions that are used in this document:

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

Contents

Supplementary Results

The kinetic model governs seven ODEs of 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).

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

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

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

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

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

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

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

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 $k_{\mathrm{T}\to\emptyset}$.

We assume steady-state where the Eqs. (1)-(7) are equal to zero. Using Maple16 the steady-state concentrations are algebraicly 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}-value 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

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

Supplementary Table

Supplementary Table : Default values for the parameter-space of the 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 concentration	$\mathcal{O}(\mu M)$	
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	

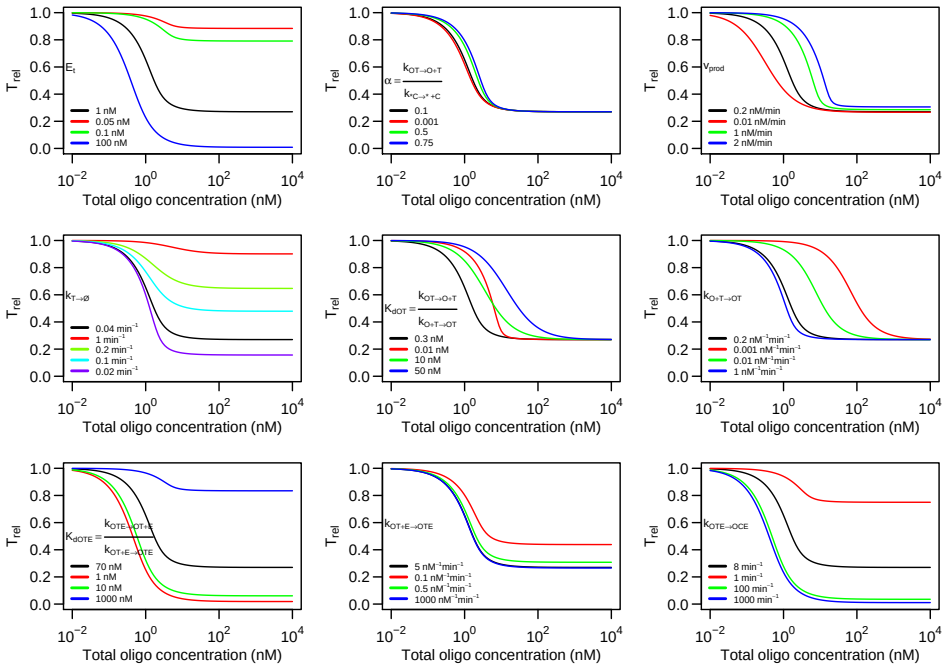
Supplementary Figure S1

The R-function `Trel()` calculates T_{rel} as a function of O_t and the set of parameters as in the example below:

```
> #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.

Supplementary Figure S2

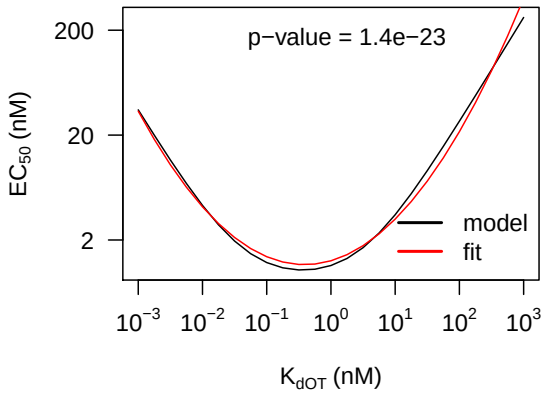
Using the R-function `drm()` from the drc package (v2.3-0) a dose-response curve is fitted to T_{rel} as a function of O_t to obtain an EC_{50}-value. We are interested in EC_{50} as a function of K_{dOT}. This is calculated through the ASOmodel-function `EC50()` that takes K_{dOT} and the set of parameters 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 parabola using the R-function `lm()`, see Supplementary Figure S2.

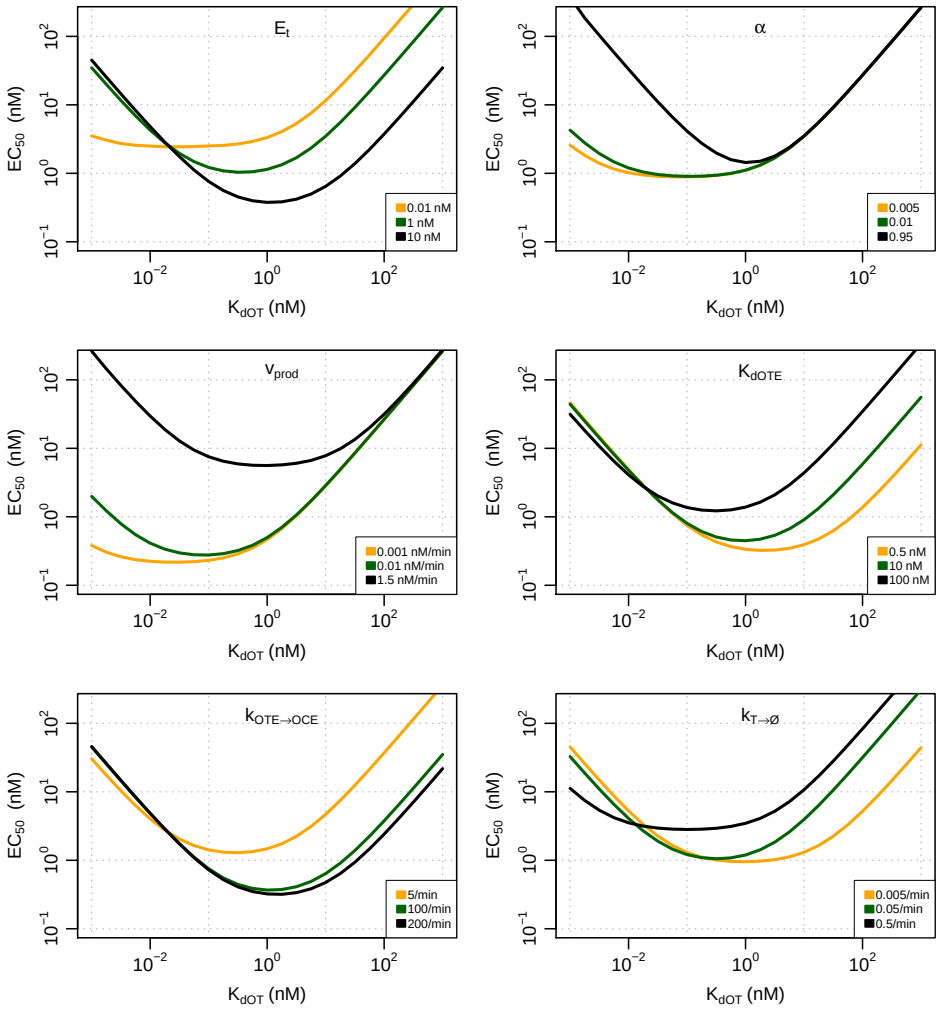
```
> D1_seq <- 10^seq(-3,3.2,by=0.25)
> ECseq <- sapply(D1_seq,EC50)
> FitPar <- lm(log10(ECseq) ~ log10(D1_seq) + I(log10(D1_seq)^2))
```



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

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 optimal affinity, quantified by K_{dOT}, changes as parameters are changed. A lower value of K_{dOT} correponds to a better affinity for the oligonucleotide.



Supplementary Figure S3: The optimal 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 target production (v_{prod}), the dissociation constant for the OTE complex (K_{dOTE}), the rate of target cleavage ($k_{\mathrm{OTE}\rightarrow\mathrm{OCE}}$), and the target degradation ($k_{\mathrm{T}\rightarrow\emptyset}$).

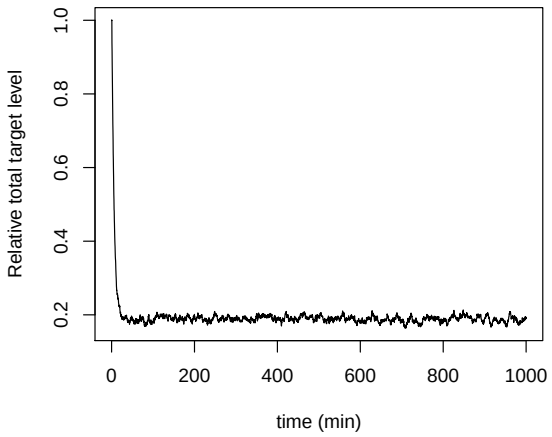
Supplementary Figure S4

The stochastic simulation of the model 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.

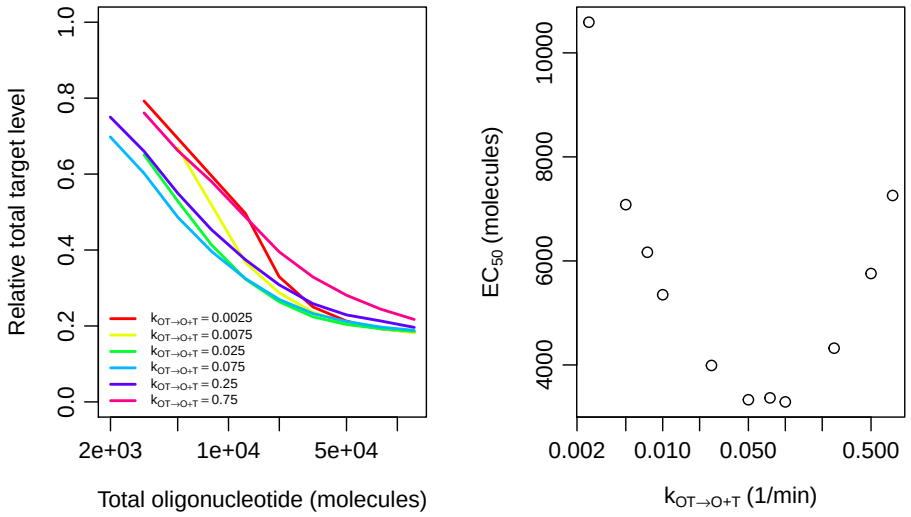


Supplementary Figure S4: The time-trace for the relative total target level when the model is simulated stochastically.

Supplementary Figure S5

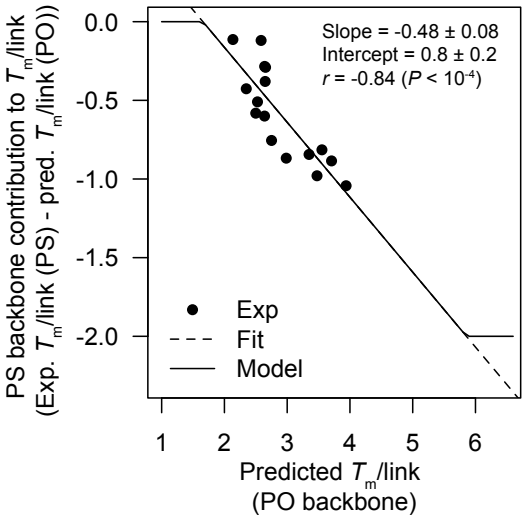
After a while the stochastic simulation reaches a plateau. In Supplementary Figure S4 the plateu starts around 50min. The mean of T_{rel} within the plateu is calculated through the R-function `Trelstoc()`. Using this function we can generate dose-response curves (Supplementary Figure S5,left). From 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). Note that as in the deterministic case (see main manuscript) an optimal affinity is observed.

```
> #### 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,])})
```



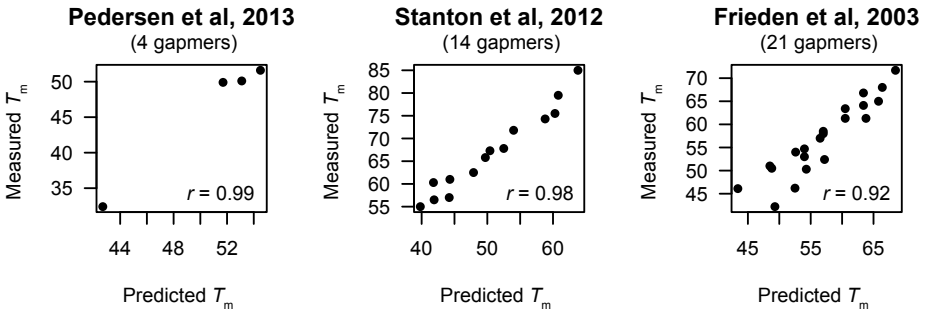
Supplementary Figure S5: Left: Dose-response curves for various values of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$ (compare to Supplementary Figure S1,middle). Right: EC_{50} as a function of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$. A high value of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$ corresponds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

Supplementary Figure S6



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

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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