

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

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This document is the supplementary file for the manuscript entitled “A kinetic model of enzyme recruiting oligonucleotides predicts an optimal affinity and thus explains why shorter and less affine oligonucleotides may be more potent” (2013) and it is a vignette for the R-package ASOmodel.

The functions and data used to produce the figures in the main manuscript and this supplementary file are available after installing and requiring the ASOmodel 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. IC50
6. IC50NO
7. IC50stoc
8. diffASO
9. pretty10expLP

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## 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 ( $OC$ ). The ASO model is described by the seven equations

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

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

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

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

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

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

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

Complex formation and breaking are denoted by rate constants  $k$  with subscripts. Target production and degradation rates are denoted by  $v_{\text{prod}}$  and  $v_{\text{degrad}}$ , respectively. The default parameter values are listed in Supplementary Table S1.

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

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

$$[OTE] + [OCE] + [E] = E_t \quad ,$$

is chosen.

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

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

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

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

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

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

## 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	Ref. [1]
$O_t$	Total oligonucleotide conc	$\mathcal{O}(\mu M)$	
$v_{prod}$	Production of target	0.2 nM/min	Ref. [5]
$v_{degrad}$	Degradation of target	$0.04 \text{ min}^{-1}$	Ref. [7]
$D_{OT}$	Dissociation constant of $OT$	0.3 nM	Ref. [2]
$D_{OTE}$	Dissociation constant of $OTE$	70 nM	Ref. [1]
$k_{O+T \rightarrow OT}$	Rate of $O + T \rightarrow OT$	$0.2 (\text{nM min})^{-1}$	Ref. [2]
$k_{OT+E \rightarrow OTE}$	Rate of $OT + E \rightarrow OTE$	$5 (\text{nM min})^{-1}$	Ref. [1]
$k_{OTE \rightarrow OCE}$	Rate of cleavage	$8 \text{ min}^{-1}$	Ref. [1]
$\alpha$	Ratio of $\frac{k_{OT \rightarrow O+T}}{k_{*C \rightarrow **+C}} \leq 1$	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:

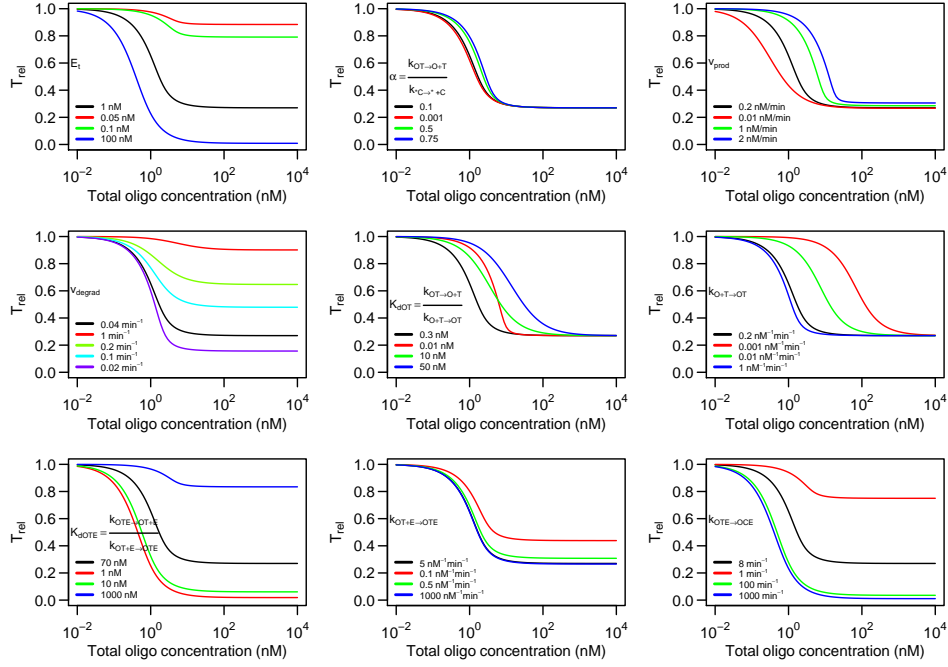
```
> #The parameters are in vector-format
> parms <- c(Et = 1, KdOT = 0.3, kOpT = 0.2, KdOTE = 70, kOTpE = 5,
+           vprod = 0.2, vdegrad = 0.04, alpha=0.1, kcleav = 8)
> Trel(Ot=1, param=parms)
```

```
[1] 0.6538694
```

For a sequence of different oligonucleotide concentrations ( $O_t$ ),  $T_{\text{rel}}$  can be calculated for varying parameter values. From this a dose-response curve is obtained. Supplementary Figure S1 shows the change in the dose-reponse curve as the parameters vary. These plots are produced using `plot.doseresponse()`.

## 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  $IC_{50}$  value. This is



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

calculated through the R-function `IC50()` that takes  $K_{dOT}$  and the set of parameters as input:

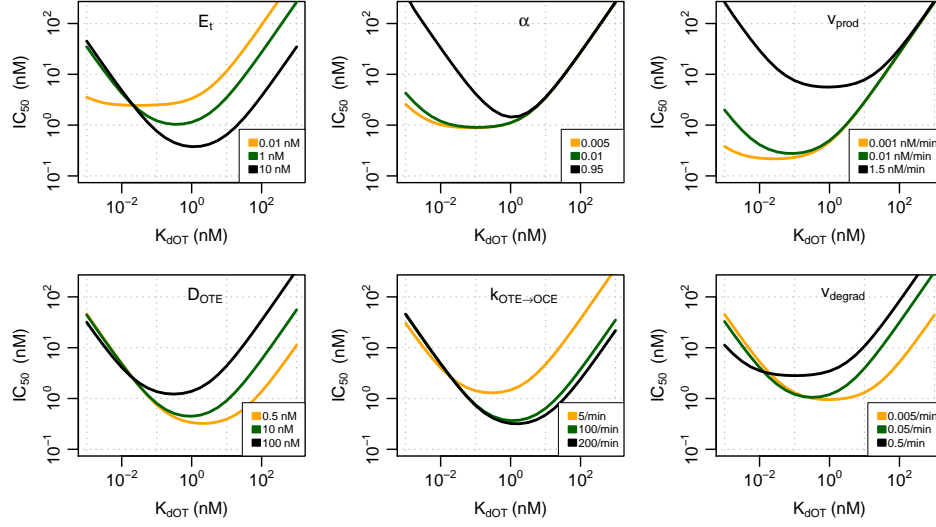
```
> IC50(KdOT=0.1,param=parms)
```

```
IC50
1.218908
```

For a sequence of  $K_{dOT}$  the corresponding  $IC_{50}$  values can be calculated. Supplementary Figure S2 shows  $IC_{50}$  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.

## S5 Supplementary Figure S3

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,



Supplementary Figure S2: The optimum affinity is dependent on the parameter settings. In the panels the  $IC_{50}$  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 ( $v_{degrad}$ ), and the dissociation constant for the OTE complex ( $D_{OTE}$ ).

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).

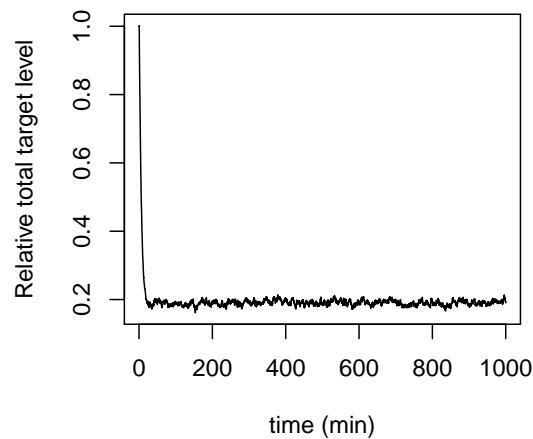
```
> library(GillespieSSA)
> #Model parameters
> parms1 <- c(kOpT = 2E-5, kOTpE = 50E-5, vprod = 150, vdegrad = 0.04,
+             kcleav = 2, kOT = 0.06, kOTE = 2, kC = 0.1)
> #Initital state vector
> x0 <- c(Tt=parms1["vprod"]/parms1["vdegrad"],
+         OT=0, OTE=0, E=1e3, O=1e5, OCE=0, OC=0)
> names(x0) <- c('Tt', 'OT', 'OTE', 'E', 'O', 'OCE', 'OC')
> #Propensity vector
> a <- c("vprod", "kOpT*O*Tt", "vdegrad*Tt", "kOT*OT", "kOTE*OTE", "vdegrad*OT",
+       "kOTpE*OT*E", "vdegrad*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','vdegrad*Tt')] <- -1
> #OT
> nu['OT',c('kOpT*O*Tt','kOTE*OTE')] <- 1
> nu['OT',c('kOT*OT','kOTpE*OT*E','vdegrad*OT')] <- -1
> #OTE
> nu['OTE',c('kOTpE*OT*E')] <- 1
> nu['OTE',c('kOTE*OTE','vdegrad*OTE','kcleav*OTE')] <- -1
> #E
> nu['E',c('kOTE*OTE','vdegrad*OTE','kOTE*OCE')] <- 1
> nu['E',c('kOTpE*OT*E')] <- -1
> #O
> nu['O',c('kOT*OT','vdegrad*OTE','vdegrad*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
> Gillespie <- ssa( x0=x0,a=a,nu=nu,
+      parms = parms1,tf=1E3,method = "ETL")

```

Supplementary Figure S3 shows  $T_{\text{rel}}$ , from the Gillespie simulation, as a function of time.

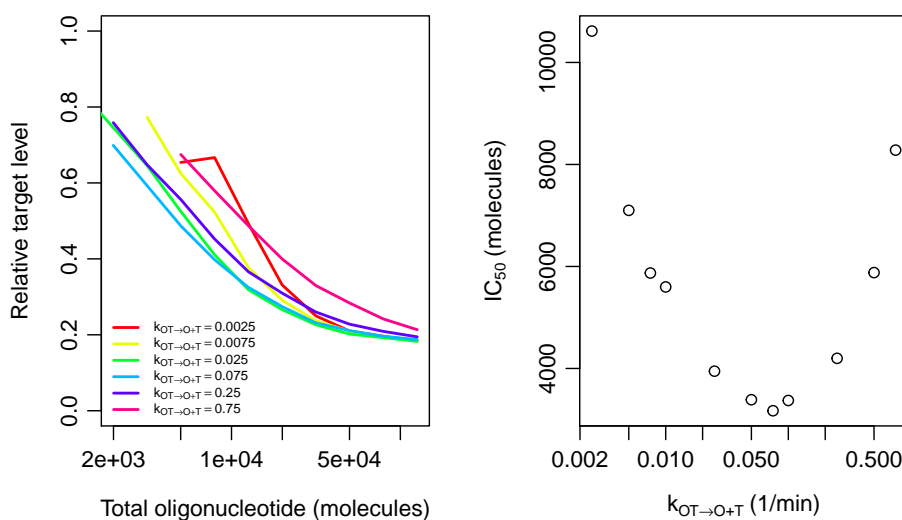


Supplementary Figure S3: The time-trace for the relative total target level when the ASO model is simulated stochastically.

## S6 Supplementary Figure S4

After a while the stochastic simulation reaches a plateau. In Supplementary Figure S3 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 S4, left). From these  $IC_{50}$  values can be calculated using `IC50stoc()` and they are subsequently plotted as a function of  $k_{OT \rightarrow O+T}$  (Supplementary Figure S4, right):

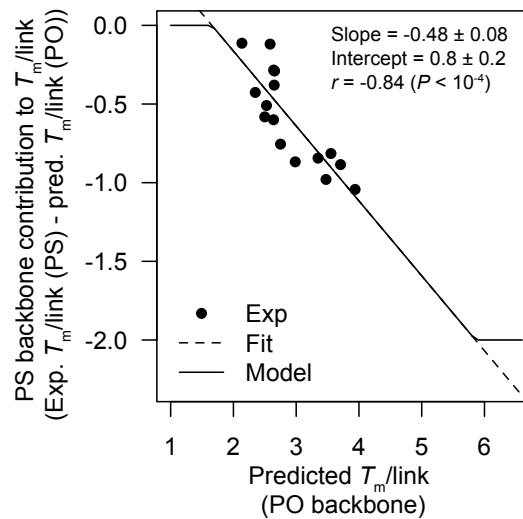
```
> ##### Sequence of k(OT -> O+T) values
> lseq <- c(1,2.5,5,7.5)
> lKOT <- c(1E-3*lseq[-1],1E-2*lseq,1E-1*lseq)
> ##### Generation of dose-response curves
> DRcurve <- lapply(lKOT,function(ki){
+       sapply(10^seq(2.5,6,by=0.2),
+       function(i) Trelstoc(i,kOT=ki)$Tstat)})
> DRc <- lapply(DRcurve,function(x) x[,!is.na(x[3,])])
> ##### Calculation of IC50
> IC50_lKOT <- sapply(1:length(DRc),
+       function(x){IC50stoc(DRc[[x]][2,],DRc[[x]][1,])})
```



Supplementary Figure S4: Left: Dose-response curves for various values of  $k_{OT \rightarrow O+T}$ . Right:  $IC_{50}$  as a function of  $k_{OT \rightarrow O+T}$ .

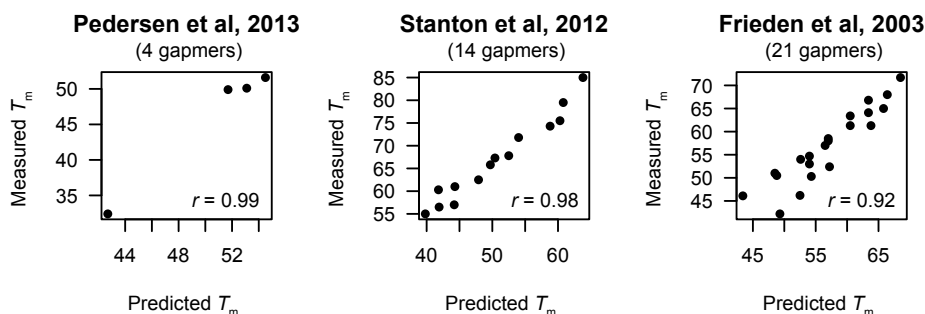


## S7 Supplementary Figure S5



Supplementary Figure S5: The effect on  $T_m$  of a phosphorothioate backbone was estimated using published data from Ref. [4].

## S8 Supplementary Figure S6



Supplementary Figure S6: Measured melting temperature vs 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].

## References

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