Supplementary Document for Pedersen et al. (2013)

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This document is the Supplementary Document for the manuscript entitled "A kinetic model explains why shorter and less affine enzyme-recruiting 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:

`Trel`	`TrelNO`	`Trelstoc`
`plot.doseresponse`	`EP`	`diffASO`
`EC50NO`	`EC50stoc`	`EC50`

Contents

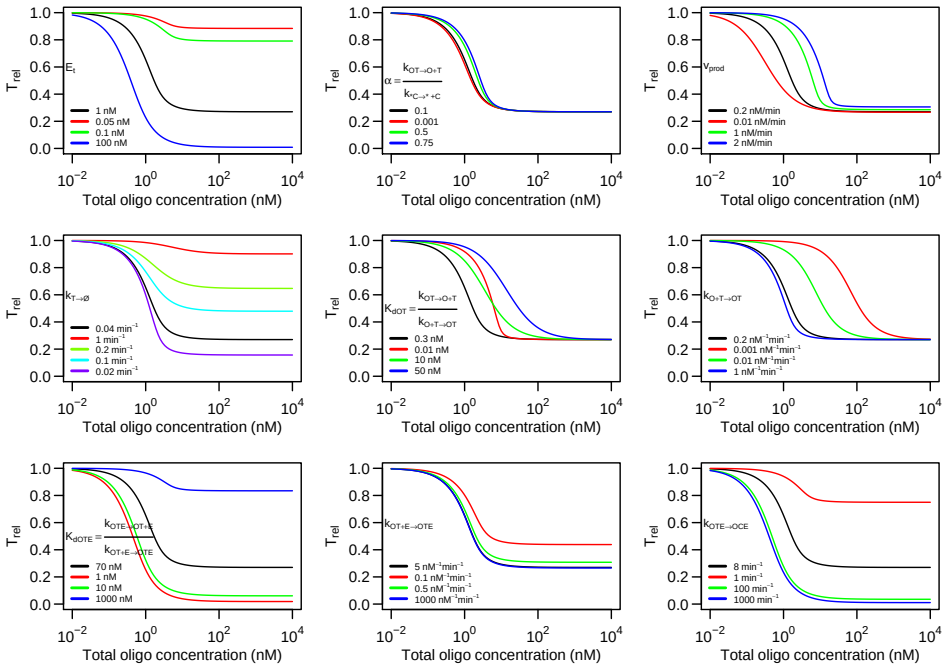
Supplementary Figure S1

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
```

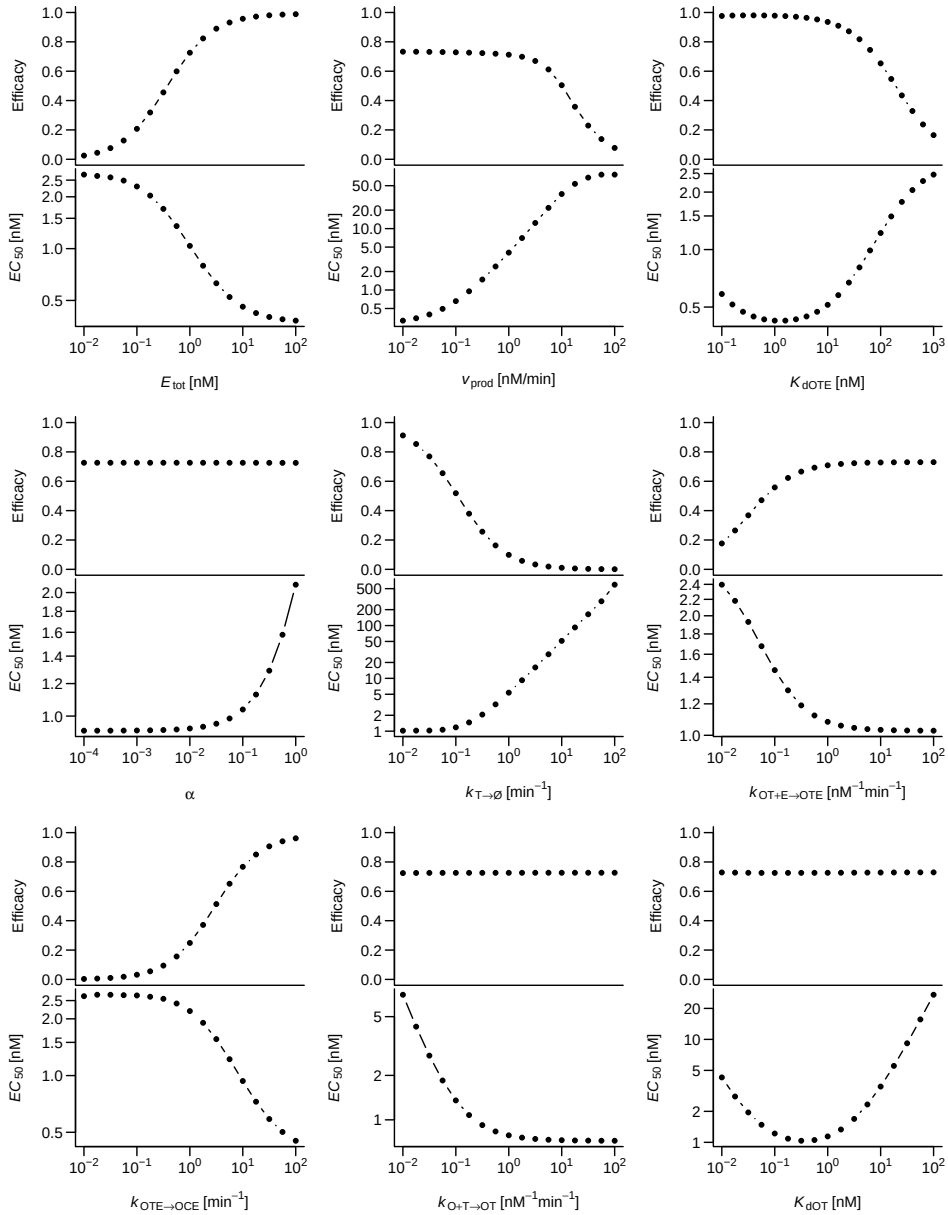
T_{rel} can be calculated for a range of different oligonucleotide concentrations (O_t) and from this a dose-response curve is obtained. Supplementary Figure S1 shows the change in the dose-reponse curves as the parameters vary. These plots are produced using `plot.doseresponse()`.



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 and the efficacy. It is observed that the potency and efficacy varies for varying parameter values as shown in Supp. Fig. S2.



Supplementary Figure S2: Efficacy and EC_{50} is plotted as functions of parameter values for E_{tot}, K_{dOTE}, v_{prod}, α, $k_{\text{O+T}\to\text{OT}}$, $k_{\text{OT+E}\to\text{OTE}}$, $k_{\text{T}\to\emptyset}$, $k_{\text{OTE}\to\text{OCE}}$.

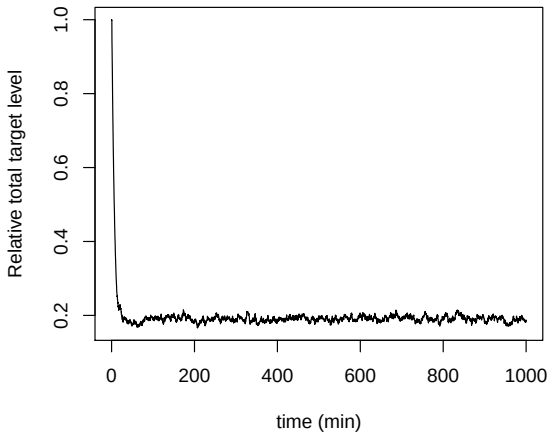
Supplementary Figure S3

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 S3 shows T_{rel} from the Gillespie simulation.

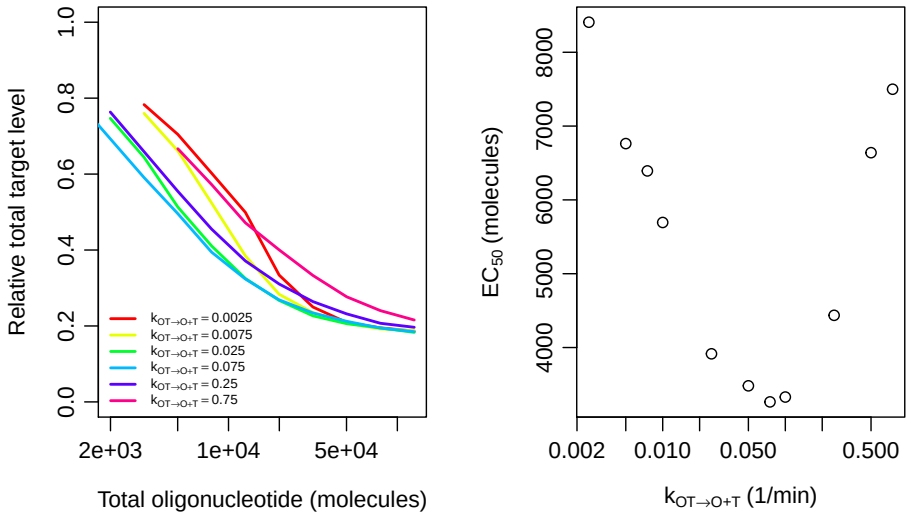


Supplementary Figure S3: The time-trace for the relative total target level when the model is simulated stochastically.

Supplementary Figure S4

After a while the stochastic simulation reaches a plateau. In Supplementary Figure S3 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 S4,left). From these EC_{50}-values can be calculated using `EC50stoc()` and they are subsequently plotted as a function of $k_{\mathrm{OT}\rightarrow\mathrm{O+T}}$ (Supplementary Figure S4,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 S4: Left: Dose-response curves for various values of $k_{\mathrm{OT}\to\mathrm{O+T}}$ (compare to Supplementary Figure S1,middle). Right: EC_{50} as a function of $k_{\mathrm{OT}\to\mathrm{O+T}}$. A high value of $k_{\mathrm{OT}\to\mathrm{O+T}}$ corresponds to a low affinity.

Supplementary Figure S5

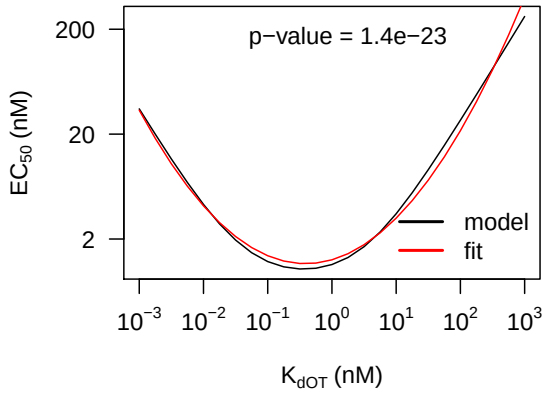
We are interested in EC_{50} as a function of K_{dOT}. This is calculated through the ASOmodel-function `EC50()` that takes K_{dOT} and the set of parameters as input:

```
> 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 S5: EC_{50} as a function of K_{dOT} is fitted on a log-log scale to a parabola.

References

[1] M. Frieden, S. M. Christensen, N. D. Mikkelsen, C. Rosenbohm, C. A. Thrue, M. Westergaard, H. F. Hansen, H. Ørum, and T. Koch. Expanding the design horizon of antisense oligonucleotides with alpha-L-LNA. *Nucleic Acids Res.*, 31(21):6365–6372, 2003.

[2] 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.

[3] 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.