Chapter 2. The long residing negligence of target saturation

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The interaction between a drug and its biological target molecule is a key step in the causal chain between drug dosing and drug effect in the human body. The strength of this interaction may be represented by the drug-target dissociation constant (K_D), which describes the drug concentration that results in 50% target occupancy (i.e. the percentage of target molecules that is bound to a drug molecule) in equilibrium. However, the K_D does not inform on the rate at which target binding equilibrium is reached after a change in the drug concentration. The kinetics of target binding is described by two rate constants: the second order association rate constant k_{on} and the first order dissociation rate constant k_{off}. From the value of k_{off}, the average time that each drug molecule spends bound at the target after drug-target association (the drug-target residence time or RT) can be calculated as $1/k_{off}$.

The significance of drug-target residence time has received increasing attention in drug discovery following the publication of an Opinion article in 2006, which discussed the beneficial effect of a long dissociation half-life on (selective) prolongation of target occupancy (*Nat. Rev. Drug Discov.* **5,** 730–9 (2006))[1]. However, the role of target saturation (i.e. target occupancy close to 100%) on prolongation of target occupancy was not fully considered in this, as well as other subsequent publications.[2–5]

By using simulations, we demonstrate the impact of target saturation on prolongation of target occupancy and show that lack of consideration of this role may contribute to inaccurate conclusions about the influence of drug-target binding kinetics.[1,2,4,5] Moreover, we demonstrate that stating that a drug-target dissociation rate constant lower than the pharmacokinetic elimination rate constant prolongs the duration of target occupancy[3,6,7], does not incorporate the role of target saturation and therefore does not always hold, especially if target occupancy values are higher than 50%. However, it should be noted that not all simulations demonstrating the influence of k_{off} on the duration of target occupancy are misleading because of target saturation. Most notably, when differential equation models are used and the k_{off} is changed simultaneously with the k_{on} to keep the K_D constant, the target saturation is not obscuring the influence of k_{off} on the duration of target occupancy.[6,8]

The fact that a higher drug concentration leads to an increased duration of drug effects has been described in quantitative terms in the early days of PKPD modelling.[9,10] More recently, the relationship between target saturation and the duration of target occupancy has also been explained quantitatively with respect to drug-target binding kinetics.[11,12]The role of target saturation that we describe here should be taken into account for the decision whether or not to select drug candidates with low k_{off} values in drug discovery and for understanding the role of drug-target binding kinetics in pharmacotherapy.

In the initial opinion article of Copeland et al.[1], the influence of target saturation has been attributed to a low dissociation rate constant for the calculated target occupancy.[13] However, in fact, the high dissociation rate constants (0.009 – 1.0 s^{-1}) compared to the low elimination rate constant of the unbound drug (0.0002 s^{-1}) indicate that the observed long duration of target occupancy cannot be influenced by the dissociation rate constant.[6,11] Later publications from these authors[2,4] showed that the target occupancy was calculated according to the equilibrium equation[4], which challenges the conclusions about the role of binding kinetics, since this assumes binding equilibrium has been reached.[14]

To demonstrate that the duration of target occupancy in the simulations of Copeland et al.[1] is influenced by target saturation, we have performed similar simulations with a simple single-step drug-target binding model (Figure 1). Changing the value of the association rate constant (k_{on}) instead of the dissociation rate constant (k_{off}), resulted in similar target occupancy profiles as observed in the simulations of Copeland et al[1]. This means that the duration of target occupancy is mostly influenced by the affinity and not by the binding kinetics. To exclude the influence of the drug target affinity, we performed additional simulations with a constant affinity. In these simulations, k_{off} had to be lower than 2 h^{-1} to prolong the occupancy significantly (Figure 2).

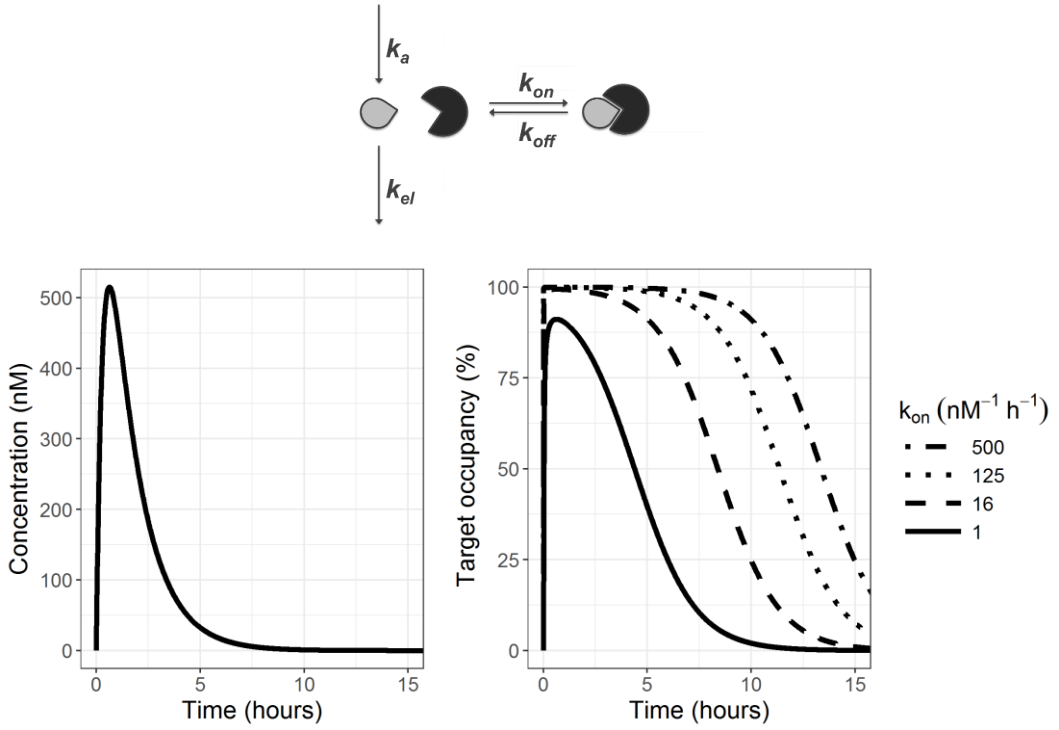

Figure 1. Simulations of plasma drug concentrations (left panel) and the resulting target occupancy profiles (right panel) for different values of k_{on}. All plasma concentration profiles overlap. The model structure is provided at the top. Here, k_a and k_{el} represent the first-order absorption constant (3.0 hr^{-1}) and elimination rate constant (0.69 h^{-1}), respectively. The value of k_{off} was fixed at 50 h^{-1} and the target concentration at 1 pM. For the associated differential equations, see supplementary information S1, and for the R simulation script see supplementary information S2. Note: similar simulations can be performed online at: wilbertdewitte.shinyapps.io/absorption_binding_elimination.

As shown in figure 2, a k_{off} value of 2 h^{-1} results in almost the same duration of target occupancy as a k_{off} value of 36 h^{-1}. To find the k_{off} value that gives a significant prolongation of target occupancy, we identified for what values of target occupancy the elimination rate constant (k_{el}) of the drug from plasma would have less influence on the duration of target occupancy than the k_{off}. The horizontal lines in figure 2 demonstrate that slow drug-target dissociation is the main determinant of the duration of target occupancy if both the dissociation rate constant and the target occupancy have values such that

$$BF < 1 - k_{off} / k_{el}$$

in which *BF* is the target fraction bound.[11,15] It should be noted that this equation is an approximation of the simple drug-target binding model and only holds for this model if the target concentration is lower than the ratio k_{el}/k_{on}, as described previously (which provides this equation in a slightly modified form as Equation 2).[11] However, the target occupancy versus time curves in figure 1 and figure 2 are independent from this approximation, as they are simulated with the full differential equation model. From this equation, it follows that when the clinical situation requires a target occupancy that, for example, should continuously be above 90%, the k_{off} needs to be more than tenfold smaller than the k_{el} for it to become the main determinant of the duration of target occupancy.[15] This equation also indicates that if $k_{off} > k_{el}$, the required target occupancy would be negative, which means that the k_{off} cannot be the main determinant of the duration of target occupancy for this condition.

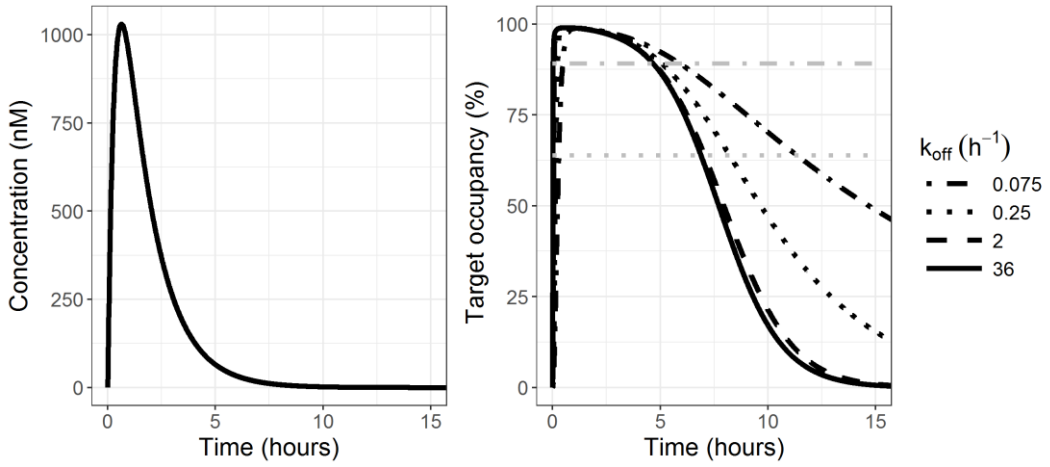

Figure 2. Simulations of plasma drug concentrations (left panel) and the resulting target occupancy profiles (right panel) for different values of k_{off}. All plasma concentration profiles overlap. The K_D was set at 10 nM for all simulations, resulting in k_{on} values of 3.6 (solid), 0.2 (dashed), 0.025 (dotted) and 0.0075 nM^{-1} h^{-1} (dash-dotted). The absorption and elimination rate constants k_a and k_{el} were 3.0 and 0.69 h^{-1}, respectively, and the concentration of the target was set at 1 pM. The grey lines denote the situation where the target fraction bound equals 1 - k_{off} / k_{el} for the corresponding line type (see text). Below that line, the condition is met for which k_{off} is the main determinant of the decline rate of target occupancy.

Our findings demonstrate the importance of target saturation on the duration of drug effects *in vivo*. These findings can directly be applied to the selection of drug candidates. A clear example where our insights should have been applied is the study of Lindström et al.[5] In this study, the *in vivo* drug effects of three NK1 antagonists are compared with their pharmacokinetics. Aprepitant demonstrated a much longer duration of drug effect, which can clearly be attributed to target saturation, considering the equation described above and the shape of the drug effect *versus* time curve (i.e. first a flat section close to the maximal effect and a subsequent rapid decline of the drug effect). In contrast, the authors conclude that the duration of the effect of Aprepitant cannot be explained by its pharmacokinetics. The other two compounds in this study did not show this target saturation and the authors conclude that this is likely explained by their faster binding kinetics. Aprepitant was therefore concluded to be the preferable drug of the three drugs due to its duration of effect. However, our findings above indicate that the other drugs may also exhibit this duration of effect at higher drug concentrations.

Our insights can also be applied to the decision as to whether to include target binding kinetics in hit or lead selection. For CCR2 antagonists, an occupancy of above 90% is considered to be required for a sufficient drug effect. This means that the dissociation half-life needs to be 10 times larger than the plasma elimination half-life. Together with an average plasma half-life of 5 hours[6], this means that the dissociation half-life needs to be 50 hours or longer before it becomes the main determinant of the duration of drug effect. In combination with the knowledge that such long dissociation half-lives are rarely observed[11], this suggests that inclusion of drug target binding kinetics for CCR2 antagonist screening should not be prioritized. In conclusion, target saturation is an important factor that should be included in the analysis of the influence of drug-target binding kinetics on target occupancy. By doing so, drug discovery scientists would be better equipped to decide on the relevance of drug-target binding kinetics for each specific project, depending on the required level of target occupancy and the (predicted) pharmacokinetics.

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Supplementary information S1. Differential equations for Figure 1 and Figure 2.

The concentrations in the depot and the plasma compartment were modeled according to equation S.1 - S.3. In these equations, [*DEP*] is the drug concentration in the depot compartment, [*C*] is the unbound drug concentration in the plasma compartment, [*R*] is the unbound target concentration in the plasma compartment, [R_{tot}] is the total target concentration and [*LR*] is the bound drug concentration. k_a is the first order absorption rate constant, k_{el} is the first order elimination rate constant, k_{on} is the second order drug-target association rate constant and k_{off} is the first order drug-target dissociation rate constant. For this model, the total target concentration is assumed to be constant, which allows the calculation of the free target concentration according to equation S.4. All initial concentrations were equal to zero and the dose was administered in the depot compartment.

$$\frac{d[DEP]}{dt} = -k_a \cdot [DEP] \tag{S.1}$$

$$\frac{d[C]}{dt} = k_a \cdot [DEP] - k_{el} \cdot [C] - k_{on} \cdot [C] \cdot [R] + k_{off} \cdot [LR] \tag{S.2}$$

$$\frac{d[LR]}{dt} = k_{on} \cdot [C] \cdot [R] - k_{off} \cdot [LR] \tag{S.3}$$

$$[R] = [R_{tot}] - [LR] \tag{S.4}$$

Supplementary information S2. R script for the simulations for Figure 1 and Figure 2.

```
# The following packages are required for the script below.
# Please install those by removing the hashtag and running the install commands below
# install.packages("deSolve")
# install.packages("ggplot2",dependencies = T)
# install.packages("gridExtra")
# install.packages("grid")
######################
library(deSolve)
library(ggplot2)
library(gridExtra)
library(grid)
rm(list = ls())  #clear environment
#-------------------------------------------------------------
# parameters
#-------------------------------------------------------------
parameters = c(
 ka = 3,
 kel = 0.693,       #h-1
 kon = 1,                                              #nM-1 h-1
 koff = 50,       #h-1
 Kd = expression(koff/kon),
 Rtot = 0.001, #nM
              dose =  800,
 dosetime = 0  )
#-------------------------------------------------------------
# ODE solving function
#-------------------------------------------------------------
solveivro<-function(allparams2){
   allparams2<-lapply(allparams2,FUN=eval,envir=allparams2)
   #-------------------------------------------------------------
 # initial states
 #-------------------------------------------------------------
  state<-  c(
   D = 0,
   C = 0,
   RL = 0    )
 #-------------------------------------------------------------
 # ODE system plus dosing function
 #-------------------------------------------------------------
   ivro <<- function(t, state, allparams2) {
   with(as.list(c(state, allparams2)),{
     dD = -ka*D
     dC =  - kon*C*(Rtot-RL)  + koff*RL - kel*C  +ka*D
     dRL=     kon*C*(Rtot-RL)  - koff*RL

         list(c(dD,dC,dRL))    })  }
 eventdat<-data.frame(var = "D",
                  time = with(allparams2,{dosetime}),
                  value = with(allparams2,{dose}),
                  method = "add"
                  )#dosing regiments
  time<-  seq(0,  24, by = 0.01)
 out <-  lsoda(y = state, times = time, func = ivro, parms = allparams2,
            events = list(data = eventdat))
  #-------------------------------------------------------------
 # derived output
 #-------------------------------------------------------------
 dout <- as.data.frame(out)
```

```
 outpar<-c(as.list(dout),as.list(allparams2))
 outderpar <- within(outpar, {
    TO = RL/Rtot     })
  outder<-outderpar[names(outderpar)[!(names(outderpar)%in%names(allparams2))]]
 doutder<-as.data.frame(outder)
 return(doutder)
} #end function solveivro
############################################################
# changing parameters for additional simulations
############################################################
params<-as.list(parameters)
changedpars1<-within(params, {  kon=16 })
changedpars2<-within(params, {  kon=125 })
changedpars3<-within(params, {  kon=500 })
############################################################
# executing the additional simulations
############################################################
doutder1<-NULL
doutder1<-solveivro(as.list(params))
doutder2<-NULL
doutder2<-solveivro(changedpars1)
doutder3<-NULL
doutder3<-solveivro(changedpars2)
doutder4<-NULL
doutder4<-solveivro(changedpars3)
#-----------------------------------------------------------
# plots over time
#-----------------------------------------------------------
# plot pharmacokinetics over time ------------------------------------------------------------------------
plotpk<-ggplot()+
 geom_line(data=(data=doutder1),aes(y=C,x=time),lty=1,size=1.5)+
 geom_line(data=(data=doutder2),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
 geom_line(data=(data=doutder3),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
 geom_line(data=(data=doutder4),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
 ylab("Concentration (nM)")+ xlab("Time (hours)")+
 theme_bw()+theme(text=element_text(size=15))+
 coord_cartesian(xlim = c(0,15)    )
plotpk
# plot target occupancy over time ------------------------------------------------------------------------
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2", "#D55E00", "#CC79A7")
plotTO<-ggplot()+
   geom_line(data=data.frame(doutder1,col=factor(1,levels = c(1,2,3,4))),aes(y=TO*100,x=time, col=col),lty=1,size=1.5)+
   geom_line(data=data.frame(doutder2,col=factor(2,levels = c(1,2,3,4))),aes(y=TO*100,x=time, col=col),lty=1,size=1.5)+
  geom_line(data=data.frame(doutder3,col=factor(3,levels = c(1,2,3,4))),aes(y=TO*100,x=time, col=col),lty=1,size=1.5)+
 geom_line(data=data.frame(doutder4,col=factor(4,levels = c(1,2,3,4))),aes(y=TO*100,x=time, col=col),lty=1,size=1.5)+
 ylab(" Target occupancy (%)")+ xlab("Time (hours)")+
 theme_bw()+theme(text=element_text(size=15),plot.margin = unit(c(5,5,5,5),"mm"))+
 scale_color_manual(name= expression(k[on]~(nM^-1~h^-1)),values = cbbPalette, breaks=c(4,3,2,1), labels =
           c(500,125,16,1))+
 coord_cartesian(ylim = c(0,100),xlim = c(0,15)
 )
plotTO
############################################################
# changing parameters for additional simulations
############################################################
changedpars8<-within(params, {  kon = 3.6
koff = 36.00
dose = 1600})
changedpars9<-within(params, {  kon = 0.2
koff = 2.00
dose = 1600})
```

```
changedpars10<-within(params, {  kon = 0.025
koff = 0.25
dose = 1600})
changedpars11<-within(params, {  kon = 0.0075
koff = 0.075
dose = 1600})

############################################################
# executing additional simulations
############################################################
doutder9<-NULL
doutder9<-solveivro(as.list(changedpars8))
doutder10<-NULL
doutder10<-solveivro(changedpars9)
doutder11<-NULL
doutder11<-solveivro(changedpars10)
doutder12<-NULL
doutder12<-solveivro(changedpars11)
# plot pharmacokinetics over time ------------------------------------------------------------------------
plotpk3<-ggplot()+
  geom_line(data=(data=doutder9),aes(y=C,x=time),lty=1,size=1.5)+
  geom_line(data=(data=doutder10),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
  geom_line(data=(data=doutder11),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
  geom_line(data=(data=doutder12),aes(y=C,x=time),lty=1,size=1.5, col = 1)+
  ylab("Concentration (nM)")+ xlab("Time (hours)")+
  theme_bw()+theme(text=element_text(size=15))+
  coord_cartesian(xlim = c(0,15)  )
plotpk3
# plot target occupancy over time ------------------------------------------------------------------------
plotTO3<-ggplot()+
  geom_line(data=(data=data.frame(doutder9,col=factor(1))),aes(y=TO*100,x=time,col=col),lty=1,size=1.5)+
  geom_line(data=(data=data.frame(doutder10,col=factor(2))),aes(y=TO*100,x=time,col=col),lty=1,size=1.5)+
  geom_line(data=(data=data.frame(doutder11,col=factor(3))),aes(y=TO*100,x=time,col=col),lty=1,size=1.5)+
  geom_line(data=(data=data.frame(doutder12,col=factor(4))),aes(y=TO*100,x=time,col=col),lty=1,size=1.5)+
  geom_line(aes(y=rep((1-0.075/0.693)*100,2),x=c(0,15)),lty=2, col = cbbPalette[4],size=1.5)+
  geom_line(aes(y=rep((1-0.25/0.693)*100,2),x=c(0,15)),lty=2, col = cbbPalette[3],size=1.5)+
  ylab(" Target occupancy (%)")+ xlab("Time (hours)")+
  theme_bw()+theme(text=element_text(size=15))+
  scale_color_manual(name= expression(k[off]~(h^-1)),values = cbbPalette, breaks=c(4,3,2,1),
                     labels = c(0.075,0.25,2,36))+
  coord_cartesian(ylim = c(0,100),xlim = c(0,15)
  )
plotTO3
fig1<-cbind(ggplotGrob(plotpk), ggplotGrob(plotTO),size="first")
grid.newpage()
grid.draw(fig1)
fig2<-cbind(ggplotGrob(plotpk3), ggplotGrob(plotTO3),size="first")
grid.newpage()
grid.draw(fig2)

tiff("fig1.tiff",width = 9, height = 4, units = "in", res = 300)
grid.newpage()
grid.draw(fig1)
dev.off()


tiff("fig2.tiff",width = 9, height = 4, units = "in", res = 300)
grid.newpage()
grid.draw(fig2)
dev.off()
```