Analysis of receptor binding kinetics models

Wijtkamp, C.

Leiden University

Mathematical Institute

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Author
C. Wijtkamp

Supervisor
Dr. V. Rottschäfer

March 31, 2020

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1 Introduction

Safe and effective drug use is a prerequisite in the pharmacological industry. A lot of research has to be performed before a potential new drug can be introduced into the market. There are various factors which influence a possible drug effect. These involve drug dosing, plasma exposure, target tissue exposure, drug-target binding kinetics, intra-tissue or target site distribution, transduction and body responses. Most of these factors are often examined in drug development except for drug-target binding kinetics.

Early understanding of the safety and effectiveness of a drug is important regarding three aspects: saving money, saving time and the safety of testing persons. The approximate cost of developing a new medicine is estimated to be $2870 million. This in an average cost over 106 randomly selected new drugs from 10 pharmaceutical firms [1]. The duration of development and clinical testing varies a lot between different drugs. This can vary between 1.8 up to 14.3 years [2]. Examining extra factors that have an influence on the effect of a drug in an early stage could help decreasing costs and safe time. There are many stages which a potential drug needs to complete successfully before it can be sold. The first safety and efficacy (the performance of an drug in a idealized or controlled setting) tests are performed on computerised models, cells and later on animals. The next stage is testing on a small group of healthy human beings. Then the drug is tested on patients with the disease the drug intents to treat. The last stage is testing on a larger group of patients [3]. Although drug testing is strictly monitored it is still desirable to test in vitro (outside the body of an organism) extra factors that influence drug response, since this could avoid human testing.

As stated earlier there are various factors which influence a possible drug effect. In this thesis we will focus on binding kinetics. Binding kinetics is the process in which a drug associates and disassociates with a particular rate to a molecule (receptor) on a cell. When bound, the medicine can trigger or block a response which is a measure for the efficacy. The article by Nederpelt et al [4] aims to investigate the role binding kinetics plays in drug response. In section 6 we will elaborate on the research described in this article. Only the experiments that are of interest for this thesis will be explained.

The aim of this thesis is to develop and analyze a model that represents an experiment where one or two drugs can bind to a single receptor. Unknown constants from a performed experiment from the article will be estimated with the use of these models. First in Chapter 2, some basic pharmacological concepts will be explained and models representing different situations will be given and analyzed. Then in Chapter 6 the methods and results from the article are discussed and linked to our models. Lastly in Chapter 7 and 8, research is performed in order to estimate unknown constants from the article and conclusions are drawn in Chapter 9.

2 Kinetic binding

A ligand is a substance which can bind to an other biomolecule such as a receptor. Receptors are localized on the cell wall or within a cell. Upon binding there will be an effect such as transduction of a signal. An example is a ligand named estrogen which can bind to the estrogen receptor (ER). The binding causes many protein activation cascades in the cell which eventually lead to development and regulation of the female reproductive system and secondary sex characteristics. A ligand can be divided into endogenous and exogenous ligands. Endogenous ligands are produced in the body (e.g. estrogen), while exogenous ligands are introduced to the body (e.g. drugs). Ligands can also be subdivided into agonists and antagonists. An agonist is a ligand that triggers a pharmacological response after binding

to a receptor. An antagonist on the contrary does not trigger a pharmacological response. Thus an antagonist can block a physiological response.

Ligands can bind, associate, to receptors and unbind, disassociate, from receptors. This is called binding kinetics as explained in the introduction. Free receptors (R) and ligands (L) bind with an association rate called K_{on} to form a receptor ligand complex (RL). Next the RL dissociates with a rate called K_{off}. This gives the follow schematic reaction:

$$R + L \leftrightharpoons RL. \tag{1}$$

This can be modelled by a system of differential equations and we call this model 1. The first ligand will be indicated with L_2 and the ligand-receptor complex previously denoted by RL will be indicated with B_2. Later in this thesis it will become clear why this notation is used. The factors K_{on2} and K_{off2} are the K_{on} and K_{off} values that are particular for the ligand L_2.

The system of differential equations for the target binding are given by:

$$\frac{dL_2}{dt} = -K_{on2}L_2R + K_{off2}B_2, \tag{2}$$

$$\frac{dB_2}{d2} = K_{on2}L_2R - K_{off2}B_2. \tag{3}$$

The first factor represents the binding of ligand and receptor into the complex while the second factor represents the decomposition of the complex into free ligand and receptor. Note that $\frac{dB_2}{dt} = -\frac{dL_2}{dt}$. This is evident since the first factor decreases the L_2 concentration and increases the B_2 concentrations and the second factor increases the L_2 concentration and decreases the B_2 concentrations. Furthermore the total amount of receptor is assumed constant and is the free amount of receptor combined with the bound receptor, thus $B_2 + R = R_{tot}$.

Therefore R can be replaced by $R_{tot} - B_2$, which gives the following equations:

$$\frac{dL_2}{dt} = -K_{on2}L_2(R_{tot} - B_2) + K_{off2}B_2, \tag{4}$$

$$\frac{dB_2}{dt} = K_{on2}L_2(R_{tot} - B_2) - K_{off2}B_2. \tag{5}$$

Now model 2 is explained. The first ligand that is added to the receptors will be called L_1 with ligand-receptor complex B_1 and K_{on1} and K_{off1} are the K_{on} and K_{off} values that are particular for the ligand L_1. Then the second ligand is added to the receptors and is called L_2. This ligand can also bind to the same receptor R. This leads to two reactions: $R + L_1 \leftrightharpoons B_1$ and $R + L_2 \leftrightharpoons B_2$ that take place at the same time. Figure 1 shows a schematic representation of this situation. It can be seen that there is competition to bind to R between L_1 and L_2. Now the total amount of receptor is the free amount of receptor combined with the two receptor complexes, thus: $B_1 + B_2 + R = R_{tot}$. The following equations are obtained similarly as previous:

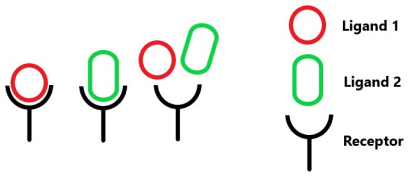

Figure 1: Schematic representation of two different ligands that can bind to the same receptor.

$$\frac{dL_1}{dt} = -K_{on1}L_1(R_{tot} - B_1 - B_2) + K_{off1}B_1, \tag{6}$$
$$\frac{dB_1}{dt} = K_{on1}L_1(R_{tot} - B_1 - B_2) - K_{off1}B_1, \tag{7}$$
$$\frac{dL_2}{dt} = -K_{on2}L_2(R_{tot} - B_1 - B_2) + K_{off2}B_2, \tag{8}$$
$$\frac{dB_2}{dt} = K_{on2}L_2(R_{tot} - B_1 - B_2) - K_{off2}B_2. \tag{9}$$

3 Steady state

In this section we will give the analysis of the steady state for both models. Steady state is the situation where the concentration free ligand and ligand-receptor complex are constant. This means that $\frac{dL_2}{dt} = 0$ and $\frac{dB_2}{dt} = 0$ in model 1 and that $\frac{dL_1}{dt} = 0$, $\frac{dB_1}{dt} = 0$, $\frac{dL_2}{dt} = 0$ and $\frac{dB_2}{dt} = 0$ in model 2. It is important to get an expression for the steady state, because in one experiment performed by Nederpelt et al results were obtained when steady state was reached [4]. We will address this experiment in more detail in section 6.

3.1 Model 1

First model 1 is considered. Equation (4) + (5) gives $\frac{dL_2}{dt} + \frac{dB_2}{dt} = 0$. Integration gives that $L_2 + B_2 = C$. This equals for $t = 0$ to $C = L_2(0) + 0 = L_2(0)$. Thus $L_2 = L_2(0) - B_2$ and $B_2 = L_2(0) - L_2$. Now L_2 is substituted in equation (5) and this equation is equaled to zero since this represents the steady state:

$$K_{on2}(L_2(0) - B_2)(R_{tot} - B_2) - K_{off2}B_2 = 0.$$

This leads to the following solutions for B_2:

$$B_2 = \frac{K_{on2}R_{tot} + K_{on2}L_2(0) + K_{off2} + \sqrt{(-K_{on2}R_{tot} - K_{on2}L_2(0) - K_{off2})^2 - 4K_{on2}^2L_2(0)R_{tot}}}{2K_{on2}} \tag{10}$$
$$B_2 = \frac{K_{on2}R_{tot} + K_{on2}L_2(0) + K_{off2} - \sqrt{(-K_{on2}R_{tot} - K_{on2}L_2(0) - K_{off2})^2 - 4K_{on2}^2L_2(0)R_{tot}}}{2K_{on2}}. \tag{11}$$

We obtain the solutions for L_2 by substituting (10) and (11) in $L_2 = L_2(0) - B_2$:

$$L_2 = \frac{-K_{on2}R_{tot} + K_{on2}L_2(0) - K_{off2} - \sqrt{(-K_{on2}R_{tot} - K_{on2}L_2(0) - K_{off2})^2 - 4K_{on2}^2 L_2(0)R_{tot}}}{2K_{on2}} \tag{12}$$

$$L_2 = \frac{-K_{on2}R_{tot} + K_{on2}L_2(0) - K_{off2} + \sqrt{(-K_{on2}R_{tot} - K_{on2}L_2(0) - K_{off2})^2 - 4K_{on2}^2 L_2(0)R_{tot}}}{2K_{on2}}. \tag{13}$$

We have the next two pairs for (B_2, L_2): $((10), (12))$ and $((11), (13))$. However not all these solutions are actual possible. Note that the expression under the square root in (12) is positive and bigger than the absolute value of $|-K_{on2}R_{tot} + K_{on2}L_2(0) - K_{off2}|$. Therefore solution (12) is not possible, because this results in a negative concentration for L_2. Furthermore solution (10) is not possible because this results in a B_2 concentration that is higher than the total amount of receptor R_{tot}. Thus the pair $((10), (12))$ is not possible. Note again that the the expression under the square root in (11) is positive. Therefore B_2 cannot become complex. Furthermore the expression under the square root in (11) is smaller than the absolute value of $|K_{on2}R_{tot} + K_{on2}L_2(0) + K_{off2}|$. Thus B_2 is non-negative. The solutions for B_2 and L_2 are given by the pair $((11), (13))$.

3.2 Model 2

Now the steady state for model 2 is considered. First note that (6) + (7) gives $\frac{dL_1}{dt} + \frac{dB_1}{dt} = 0$. In model 2 first ligand 1 is added to the receptors. When L_1 and B_1 reach steady state ligand 2 is added as well. The moment when ligand 2 is added corresponds to t=0. Therefore, we introduce $L_1^*(0)$ and $B_1^*(0)$ as the steady state values for L_1 and B_1 at t=0. Thus $L_1 + B_1 = C = L_1^*(0) + B_1^*(0)$. Furthermore $L_1^*(0) + B_1^*(0) = L_1(0)$ with $L_1(0)$ the original dose of ligand 1, thus $L_1 + B_1 = L_1(0)$. Now equation (8) + (9) is considered. Again $\frac{dL_2}{dt} + \frac{dB_2}{dt} = 0$ and thus $L_2 + B_2 = L_2(0) + B_2(0) = L_2(0)$ with $L_2(0)$ the original dose of ligand 2. The next expressions are obtained for L_1 and L_2: $L_1 = L_1(0) - B_1$ and $L_2 = L_2(0) - B_2$. These expressions are substituted in (7) and (9) and the next system for B_1 and B_2 is obtained:

$$\frac{dB_1}{dt} = K_{on1}(L_1(0) - B_1)(R_{tot} - B_1 - B_2) - K_{off1}B_1, \tag{14}$$

$$\frac{dB_2}{dt} = K_{on2}(L_2(0) - B_2)(R_{tot} - B_1 - B_2) - K_{off2}B_2. \tag{15}$$

Now we consider the steady state condition, thus $\frac{dB_1}{dt} = \frac{dB2}{dt} = 0$. After equalizing (14) and (15) to zero we multiply (14) by $K_{on2}(L_2(0) - B_2)$ and (15) by $K_{on1}(L_1(0) - B_1)$. Next we take the difference between these two equations. Then the following expression for B_1 is obtained:

$$B_1 = \frac{K_{off2}K_{on1}L_1(0)B_2}{K_{on2}K_{off1}L_2(0) - K_{on2}K_{off1}B_2 + K_{off2}K_{on1}B_2}. \tag{16}$$

Then the next expression for the steady state for B_2 is obtained where (16) is substituted in equation (15) and equalized to zero (since $\frac{dB2}{dt} = 0$):

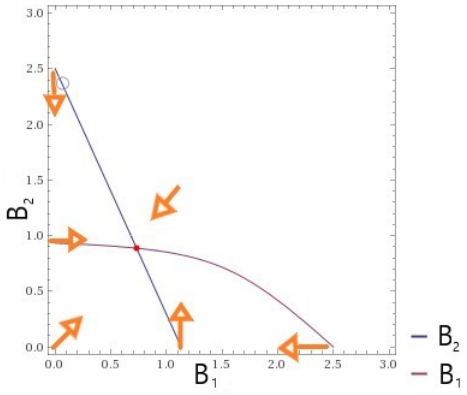

Figure 2: Plot of the nullclines described in (18) (purple) and (19) (blue) with some drawn vectors (arrows). The used values are $K_{on1} = 0.001$ nM^{-1}min^{-1}, $K_{off1} = 0.19$ min^{-1}, $L_1(0) = 158.49$ nM, $K_{on2} = 0.24$ nM^{-1}min^{-1}, $K_{off2} = 0.027$ min^{-1}, $L_2(0) = 1$ nM and $R_{tot} = 2.5$ nM.

$$K_{on2}(L_2(0) - B_2)(R_{tot} - \frac{K_{off2}K_{on1}L_1(0)B_2}{K_{on2}K_{off1}L_2(0) - K_{on2}K_{off1}B_2 + K_{off2}K_{on1}B_2} - B_2) - K_{off2}B_2 = 0. \tag{17}$$

The solution for B_2 can be obtained by solving this cubic equation. We only obtain an expression for B_2 since this value is of interest later on in this thesis.

A phase plane for B_1 on the x-as and B_2 on the y-as is made in order to show that there is a critical point, and thus a steady state in model 2. Equations (14) and (15) are equaled to zero. Next the equations are being expressed in B_2 respectively B_1. This gives the following expressions for the nullclines:

$$B_1 = \frac{-K_{off2}B_2}{K_{on2}(L_2(0) - B_2)} - B_2 + R_{tot}, \tag{18}$$

$$B_2 = \frac{-K_{off1}B_1}{K_{on1}(L_1(0) - B_1)} - B_1 + R_{tot}. \tag{19}$$

In figure 2, we plot the nullclines for $K_{on1} = 0.001$ nM^{-1}min^{-1}, $K_{off1} = 0.19$ min^{-1}, $L_1(0) = 158.49$ nM, $K_{on2} = 0.24$ nM^{-1}min^{-1}, $K_{off2} = 0.027$ min^{-1}, $L_2(0) = 1$ nM and $R_{tot} = 2.5$ nM with B_1 on the horizontal axis and B_2 on the vertical axis. Furthermore the blue line represents the nullcline for B_2 and the purple line represents the nullcline for B_1. The arrows represent the vector field. It can be seen that in every situation the solution wants to go in the direction of the critical point (the red dot when the two nullclines intersect) and thus the steady state.

4 Analytic expression for B_2 from model 1

In this section we will give an analytic expression for B_2 as function over time for model 1. This is important since it will help to estimate unknown constants from the article which will be addressed in section 6.

First we replace L_2 in equation (5) with $L_2(0) - B_2$. Then we rewrite the equation and obtain:

$$\frac{dB_2}{dt} = {B_2}^2 K_{on2} + B_1(-K_{on2}L_2(0) - R_{tot}K_{on2} - K_{off2}) + K_{on2}L_2(0)R_{tot}.$$

Then using separation of variables we find that:

$$\int \frac{1}{{B_2}^2 K_{on2} + B_2(-K_{on2}L_2(0) - R_{tot}K_{on2} - K_{off2}) + K_{on2}L_2(0)R_{tot}} dB_2 = \int 1dt. \tag{20}$$

The left-hand side of this relation can be written as

$$\int \frac{dB_2}{aB_2^2 + bB_2 + c}$$

where $a = K_{on2}$, $b = -K_{on2}L_2(0) - R_{tot}K_{on2} - K_{off2}$, $c = K_{on2}L_2(0)R_{tot}$,
$A_+ = \frac{-b+\sqrt{b^2-4ac}}{2a}$, $A_- = \frac{-b-\sqrt{b^2-4ac}}{2a}$, $A_+ - A_- = \frac{\sqrt{b^2-4ac}}{a}$.
Then by rewriting the denominator we find:

$$\begin{aligned}\int \frac{1}{aB_2^2 + bB_2 + c} dB_2 &= \int \frac{1}{(B_2 - A_+)(B_2 - A_1)} dB_2 \\ &= \frac{log(B_2 - A_+) - log(B_2 - A_-)}{A_+ - A_-} + c.\end{aligned}$$

So (20) becomes:

$$log(\frac{B_2 - A_+}{B_2 - A_-}) = (t + \tilde{c})(A_+ - A_-) \tag{21}$$

and hence by taking the exponential and performing equation manipulation we obtain:

$$\frac{A_- - A_+}{B_2 - A_-} = e^{(t+\tilde{c})(A_+ - A_-)} - 1.$$

This yields:

$$B_2(t) = \frac{A_- - A_+}{e^{(t+\tilde{c})(A_+ - A_-)} - 1} + A_-.$$

The initial condition $B_2(0) = 0$ nM is used in order to determine the constant $\tilde{c}$. The following expression for $\tilde{c}$ is obtained after substituting this initial condition in (20):

$$\tilde{c} = \frac{log(\frac{A_+}{A_-})}{A_+ - A_-}.$$

Thus we obtain the final expression for $B_2(t)$:

$$B_2(t) = \frac{A_- - A_+}{e^{(t+\frac{log(\frac{A_+}{A_-})}{A_+ - A_-})(A_+ - A_-)} - 1} + A_- = \frac{A_- - A_+}{e^{(t(A_+ - A_-)}\frac{A_+}{A_-} - 1} + A_-. \tag{22}$$

Note that the expression for $L_2(t) = L_2(0) - B_2(t)$. Furthermore there is a relation between equation (22) and (11), namely:

$$\lim_{t\to\infty} B_2(t) = \frac{K_{on2}R_{tot} + K_{on2}L_2(0) + K_{off2} - \sqrt{(-K_{on2}R_{tot} - K_{on2}L_2(0) - K_{off2})^2 - 4K_{on2}^2 L_2(0)R_{tot}}}{2K_{on2}}.$$

This relation is evident since if $t \to \infty$, $B_2(t)$ will reach steady state and is thus equal to equation (11).

5 Models for binding kinetics

Recall that there are two situations, namely the first one when only one ligand can bind to a receptor and the second one when two different ligands can bind to a single receptor. This section will explain solutions obtained for model 1 and 2.

5.1 Model 1

First a simulation for solutions for model 1 is made in Matlab using equation (4) and (5). The code can be found in appendix 10.1. Figure 3 shows the result with the following chosen values: $K_{on2} = 1$ nM^{-1}min^{-1}, $K_{off2} = 0.01$ min^{-1}, $L_2(0) = 1$ nM and $R_{tot} = 1$ nM with the time (min) on the horizontal axis and the B_1 and L_1 concentration (nM) on the vertical axis. Furthermore the analytically determined steady state values for L_2 and B_2 (section 3) and the B_2 over time (section 4) are also displayed. The blue line represents the course from L_2 over time and the red line the course from B_2 over time using numerical simulation. At t=0 the value for B_2 is zero since no ligand has bound to the receptor yet and the value for L_2 is the initial dose and is chosen 1. Then B_2 concentration rapidly increases and the L_2 concentration decreases until both variables become constant. The progress of L_2 and B_2 concentrations over time is due to the chosen values $K_{on2} = 1$ nM^{-1}min^{-1} and $K_{off2} = 0.01$ min^{-1}. The ligand is more prone to bind than to unbind since the K_{on2} value is a lot higher than the K_{off2} value. Therefore at the beginning the free ligand will rapidly bind to free receptor. Then less free receptor and ligand is available and the binding and unbinding of ligand is slowly getting into balance until steady state is reached (when the lines are flat). It can be seen that the purple and green line that represents the analytically determined steady state values for B_2 and L_2 are similar to the steady state of the simulation. Furthermore the analytically determined expression for B_2 over time (yellow line) is also similar to the simulation for B_2 over time.

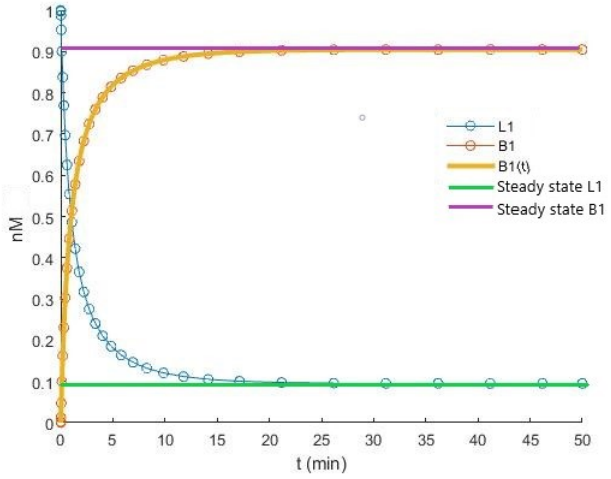

Figure 3: Progression of L_2 and B_2 with chosen values:$K_{on2} = 1$ nM^{-1}min^{-1}, $K_{off2} = 0.01$ min^{-1}, $L_2(0) = 1$ nM and $R_{tot} = 1$ nM. The blue and red line represent the simulation using Matlab for L_2 and B_2. The green and purple line are the analytically determined steady state conditions for L_2 and B_2. The yellow line is the analytically determined progression of B_2.

5.2 Model 2

Now a simulation for solutions for model 2 is made in Matlab using equation (6) up to (9). The code can be found in appendix 10.2. Model 2 represents a situation when first L_1 is added to free receptor. When steady state is reached L_2 is added as well and this moment represents t=0. Figure 4 shows the result of the simulation in Matlab and the analytically determined steady state for B_2. The following values are chosen: $K_{on1} = 0.1$ nM^{-1}min^{-1}, $K_{off1} = 0.01$ min^{-1}, $L_1(0) = 1.5$ nM, $K_{on2} = 1$ nM^{-1}min^{-1}, $K_{off2} = 0.001$ min^{-1}, $L_2(0) = 0.7$ nM and $R_{tot} = 1$ nM. At t=0 the values for L_1 (blue line) and B_1 (red line) are the steady conditions for a model 1 situation. The value for L_2 (yellow line) is the dose and the value for B_2 (purple line) is zero since no ligand 2 has bound yet. Then similar to model 1 B_2 concentrations increases and L_2 concentrations decreases until steady state is reached. Furthermore L_1 and B_1 change as well due to the competition for binding to the receptor between L_1 and L_2. The chosen K_{on2} value is higher than the K_{on1} value and the K_{off2} value is lower than the K_{off1} value. This means that L_2 is better in binding to the receptor and B_2 is harder to unbind compared to L_1 and B_1. Thus after adding L_2 to the receptors L_1 concentrations increases and B_1 concentrations decreases until a new steady state is reached. It can be seen that the green line that represents the analytically determined steady state for B_2 is similar to the steady state of the simulation.

6 Article

In this chapter we explain the performed experiments in the article [4] and give a summary of the results that are important for this thesis. Furthermore model 1 and 2 will be used to simulate an experiment performed in the article.

The aim of the study in [4] is to get an understanding of the role drug-target binding kinetics plays in

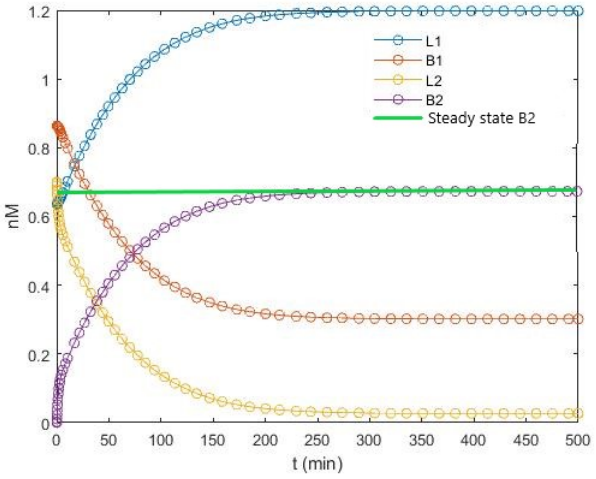

Figure 4: Progression of L_1, B_1, L_2 and B_2 with chosen values: $K_{on1} = 0.1$ nM^{-1}min^{-1}, $K_{off1} = 0.01$ min^{-1}, $L_1(0) = 1.5$ nM, $K_{on2} = 1$ nM^{-1}min^{-1}, $K_{off2} = 0.001$ min^{-1}, $L_2(0) = 0.7$ nM and $R_{tot} = 1$ nM. The blue, red, yellow and purple line represent the simulation using Matlab for L_1, B_1, L_2 and B_2. The green line is the analytically determined steady state condition for B_2.

the activation and blocking of a specific receptor and thereby be able to improve factors that can predict how well a drug works, so called drug efficacy. One performed experiment in this article represents a situation which can be described by the model given in section 4. The aim is to use this model in order to estimate unknown constants from the article. Only the performed experiments that are of importance for this thesis will be described.

In [4], the NK1 receptor was used as a model system. This receptor naturally occurs in the human body. Endogenous ligands (produced in the body) that normally bind to the receptor are NKA and SP. Recall that agonists are ligands that trigger a pharmacological response when they bind to a receptor and that antagonists are ligands that do not trigger a response when they bind and are thus blockers of the receptor. SP and NKA trigger a response after binding and are thus agonists. The used antagonists in the article are DFA and aprepitant and thus these block the NK1 receptor.

One of the aims of this thesis is to determine an estimate for the K_{on} and K_{off} values of the antagonists DFA and aprepitant. The K_{on} and K_{off} values for the agonists are already known and are shown in table 1.

Ligand	K_{on} (nM^{-1}min^{-1})	K_{off} (min^{-1})
SP	0.24 ± 0.046	0.027 ± 0.0025
NKA	0.001 ± 0.00018	0.19 ± 0.036
DFA	unknown	unknown
Aprepitant	unknown	unknown

Table 1: Known and unknown values for K_{on} and K_{off} for the agonists and antagonists [4].

When an agonist triggers a response many protein activation cascades are activated. An important molecule within this activation process is cAMP (cyclic adenosine monophosphate). CAMP can therefore

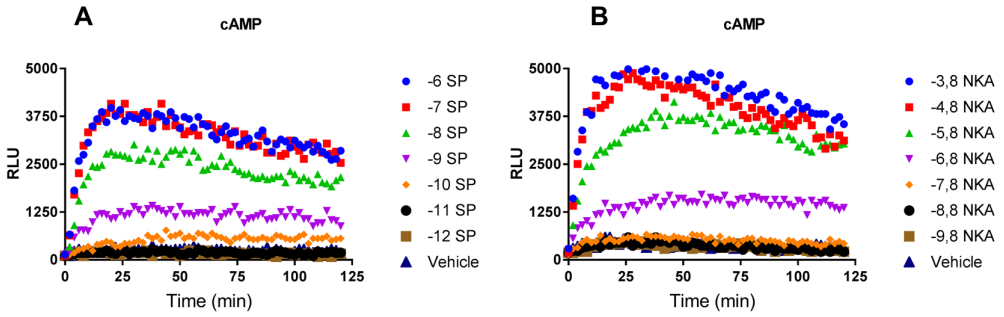

Figure 5: Results for the time dependent cAMP assay for different doses of SP (A) and NKA (B) in ^{10}log molar concentration from the original article [4].

be used as measurement for receptor activation and is measured in relative light units (RLU). The ratio RLU to cAMP is not known but the higher the RLU the higher the cAMP.

First the response over time of SP and NKA was measured using cAMP, a so called time dependent cAMP assay. In the different experiments different concentrations of both ligands were added. Figure 5 shows the result of this experiment. The x-as represents the time in minutes and the y-as represents the RLU values. The concentrations of both agonists are given in ^{10}log molar concentration (M). Molar concentration is the number moles per liter. It can be seen that an increase in concentration of SP or NKA leads to an increase in cAMP. This is in line with one would expect, since a higher concentration of agonist (SP and NKA) means more binding with the receptors which results in more activation and thus more cAMP.

Now the most important experiment will be described which is used to estimate the unknown constants. The authors have performed a test to investigate the amount of cAMP, a so called cAMP assay, with different concentrations of antagonist (DFA, aprepitant) and agonist (SP, NKA). Figure 6 shows a schematic overview of this experiment. First antagonist was added to cells. After reaching steady state agonist was added to the cells. Thereafter, the experiment again runs until it reaches steady state. In the figures the cAMP output after reaching steady state is given. There was also a control were only agonist was added to the cells. Again measurements were taken after reaching steady state. This is done in order to see that the antagonist has an effect on the binding between the agonist and the receptor. Figures 7 and 8 shows the result of this experiment. On the x-as is the ^{10}log concentration of the agonist in molar concentration (M). On the y-as is the RLU in percentage. The 100% corresponds to the RLU measurement at t=125 for the highest agonist concentration from the previous explained time dependent cAMP assay (figure 5). We call this value M_{SP} for agonist SP and is circa 3000 RLU (the RLU value for the blue dot at t=125 in figure 5A). For agonist NKA we call this value M_{NKA} and is circa 3750 RLU (the RLU value for the blue dot at t=125 in figure 5B). The dots, triangles and squares represent the measurements when steady state was reached and are divided by M_{SP} or M_{NKA} and multiplied by 100. To guide the eye a curve is plotted through these measure points. The results show that the highest activation rate (most cAMP) is achieved when only agonist (control) is added. This is the blue line in figures 7 and 8. Furthermore it shows that an increase in antagonist concentration lowers the cAMP production. This is due to the competition between the agonist (SP and NKA) and the antagonist (DFA and aprepitatant) for binding to the receptor. Less agonist is able to bind when more antagonist is added. This is why in most of the cases the red line lies lower than the blue line, the green line lies lower than the red line and the purple line lies lower than the green line.

In section 2 we have formulated two models and obtained figures (section 5) for a general situation when

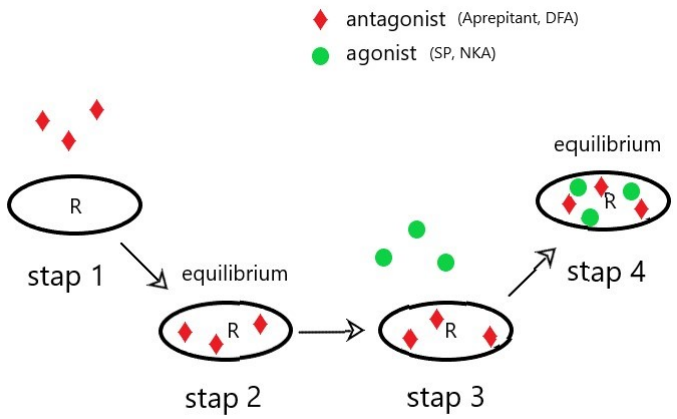

Figure 6: Schematic overview of the method for the cAMP assay. First antagonist is added followed by agonist after reaching steady state (equilibrium).

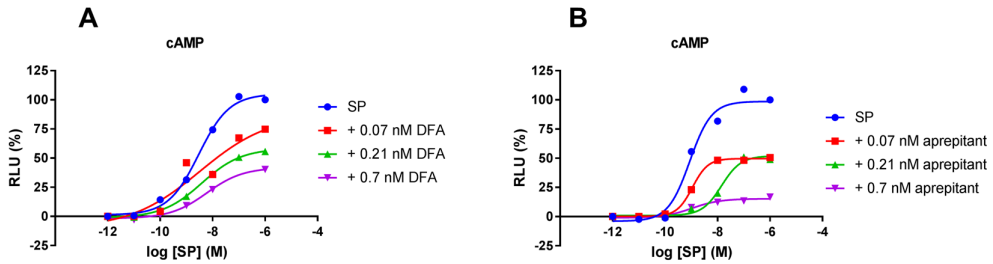

Figure 7: Results for the concentration dependent cAMP assay with agonist SP with DFA (A) and aprepitant (B) from the original article. On the x-as is the ^{10}log dose of SP in molar concentration (M). On the y-as is the RLU in percentage [4].

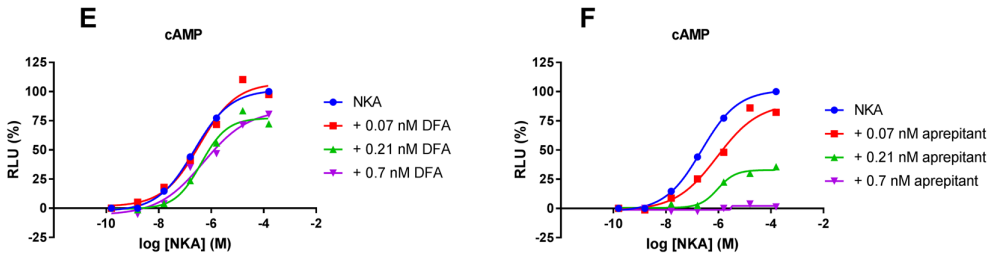

Figure 8: Results for the concentration dependent cAMP assay with agonist NKA with DFA (E) and aprepitant (F) from the original article. On the x-as is the ^{10}log dose of NKA in molar concentration (M). On the y-as is the RLU in percentage [4].

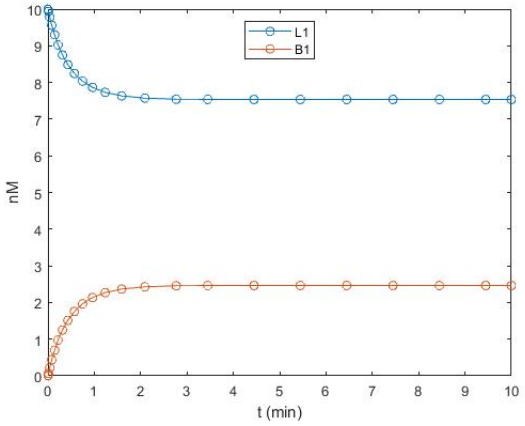

Figure 9: The progression of SP (blue) and SP-receptor complex (red) for model 1. The concentration of SP at t=0 is 10 nM and R_{tot} is estimated at 2.5 nM.

one or two ligands can bind to a single receptor. Note that the performed experiment when first different concentrations of antagonist (DFA, aprepitant) and then agonist (SP, NKA) is added to cells is the model 2 situation. The control, when only agonist is added is the model 1 situation. The chosen notation for model 1 e.g. L_2, B_2, K_{on2} and K_{off2} will now become clear. This notation is used because K_{on1} and K_{off1} correspond to the K_{on} and K_{off} values particular for the antagonists and K_{on2} and K_{off2} correspond to the K_{on} and K_{off} values particular for the agonists in both model 1 and 2. Furthermore, an important assumption that we make is that R_{tot} is the same in all performed experiments. This holds for the rest of this thesis. Now some figures will be given that represent this experiment.

Figures were made using the agonist SP. Thus in the differential equations L_2 is equal to SP concentrations and B_2 to SP-receptor complex concentrations and $K_{on2} = 0.24$ nM^{-1}min^{-1} and $K_{off2} = 0.027$ min^{-1} are constants that are particular for SP (table 1). The only constant unknown is the R_{tot} and therefore needs to be chosen. We have chosen $R_{tot} = 2.5$ nM for reasons that will become clear later in this thesis. For now this value is chosen since it provides clear figures. Figure 9 shows the result for the model 1 equations. The ligand SP was chosen with an initial concentration of 10 nM. This dose is also used in the experiment and equals to -8 SP in the figures. Recall that the the concentration in the article is given by ^{10}log [SP](M). Therefore we obtain $10^{-8} * 10^9 = 10$ nM. SP concentrations (blue) declines while complex concentrations (red) increases until steady state is reached. This behavior is similar to figure 3 seen in section 5.1. The measurements in the experiment (the blue line in figure 7 and 8) were taken when steady state is reached, thus in our model this is when the line is horizontally flat.

Now model 2 is considered. Figure 10 shows the result for a simulation of the experiment. Note that the K_{on} and K_{off} values for the antagonists are unknown and are thus chosen. We show two graphs with different chosen values in order to gain intuition about the effect of different values. To illustrate this, a graph with little influence of the ligands on each other (left) and a graph to show that the agonist and antagonist have an influence on each other (right) have been made. First the left graph is described. The K_{off1} and K_{on1} are chosen 0.001 min^{-1} respectively 0.01 nM^{-1}min^{-1} and the initial concentration of the antagonist is 0.7 nM. The R_{tot} value is still chosen at 2.5 nM. Furthermore, the initial concentration of SP is chosen at 1 nM. Recall that antagonist L_1 was first added to the cells (and thus receptors). When L_1 and B_1 are in steady state agonist L_2 is added at well and this corresponds to t=0. Thus the concentrations at t=0 for free antagonist L_1 (blue) and antagonist-receptor complex B_1 (red) are

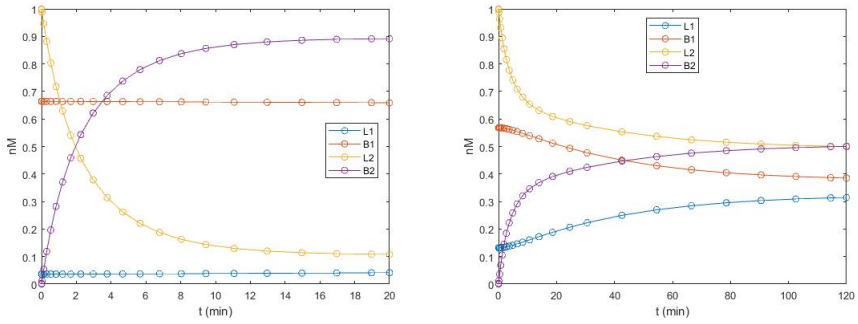

Figure 10: The progression of SP (yellow), SP-receptor complex (purple), antagonist (blue) and antagonist-receptor complex (red) for model 2. Left figure: the concentration of SP at t=0 is 1 nM, R_{tot} is chosen at 2.5 nM. At t=0 the values for antagonist and antagonist-receptor complex concentrations are the steady state values for a model 1 situation with concentration of 0.7 nM for the antagonist. The K_{on1} is chosen at 0.01 $\text{nM}^{-1}\text{min}^{-1}$ and the K_{off1} is chosen at 0.001 min^{-1}. Right figure: the concentration of SP at t=0 is 1 nM and R_{tot} is chosen at 1 nM. The concentration for the antagonist is 0.7 nM. The K_{on1} is chosen at 0.1 $\text{nM}^{-1}\text{min}^{-1}$ and the K_{off1} is chosen at 0.01 min^{-1}.

the output of the steady state for a model 1 situation. The concentration for agonist L_2 (yellow) at t=0 is the dose and agonist-receptor complex B_2 (purple) is zero since no agonist has bound to the receptor yet. Free receptor binds to the agonist L_2 when L_2 is added and agonist-receptor complex B_2 concentration increases. There is little difference in the antagonist L_1 and antagonist-receptor complex B_1 concentrations since there is still enough receptor to bind for the agonist L_2 and the antagonist L_1 binds faster and dissociates slower than the agonist. Thus there is little competition between L_1 and L_2 to bind to the receptor. The right graph shows the result with $R_{tot} = 1$ nM, $K_{on1} = 0.1$ $\text{nM}^{-1}\text{min}^{-1}$ and $K_{off1} = 0.01$ min^{-1} and again with agonist SP with initial condition of 1 nM. This graph does show a change in antagonist-receptor complex B_1 concentrations after SP is added (as expected when equations (6)-(9) are considered). This is due to the lower value for R_{tot}, since this means that the antagonist L_1 and agonist L_2 have to 'battle' for the free receptors.

6.1 Comparison of the experiment with the model for B_2

The expression (22) for B_2 from section 4 is used to compare the results obtained in the article and our model in Matlab (appendix 10.3). This is important because it can give more information about an other unknown constant, namely R_{tot}. A better estimate of R_{tot} will improve the estimate of the unknown constants for K_{on2} and K_{off2} for the antagonists since R_{tot} has a big influence on the amount of ligand binding.

Figure 5 shows the amount of cAMP over time when only agonist is added. This is thus a model 1 situation. Therefore the expression for B_2 we obtained in equation (22) can be used to see if there is some similarity between the model and the actual experiment. All the constants except one, R_{tot}, are known a priori. Three values for R_{tot} have been chosen based on the results in figures 7 and 8. The value for R_{tot} cannot be too low since this would cause very low cAMP measurement when antagonist is added. In other words especially the purple and green line would be too low. The value can also not be too high since this would cause very little difference between the different dose of antagonist. This means that there is no competition for binding to the receptor between the agonist and antagonist due

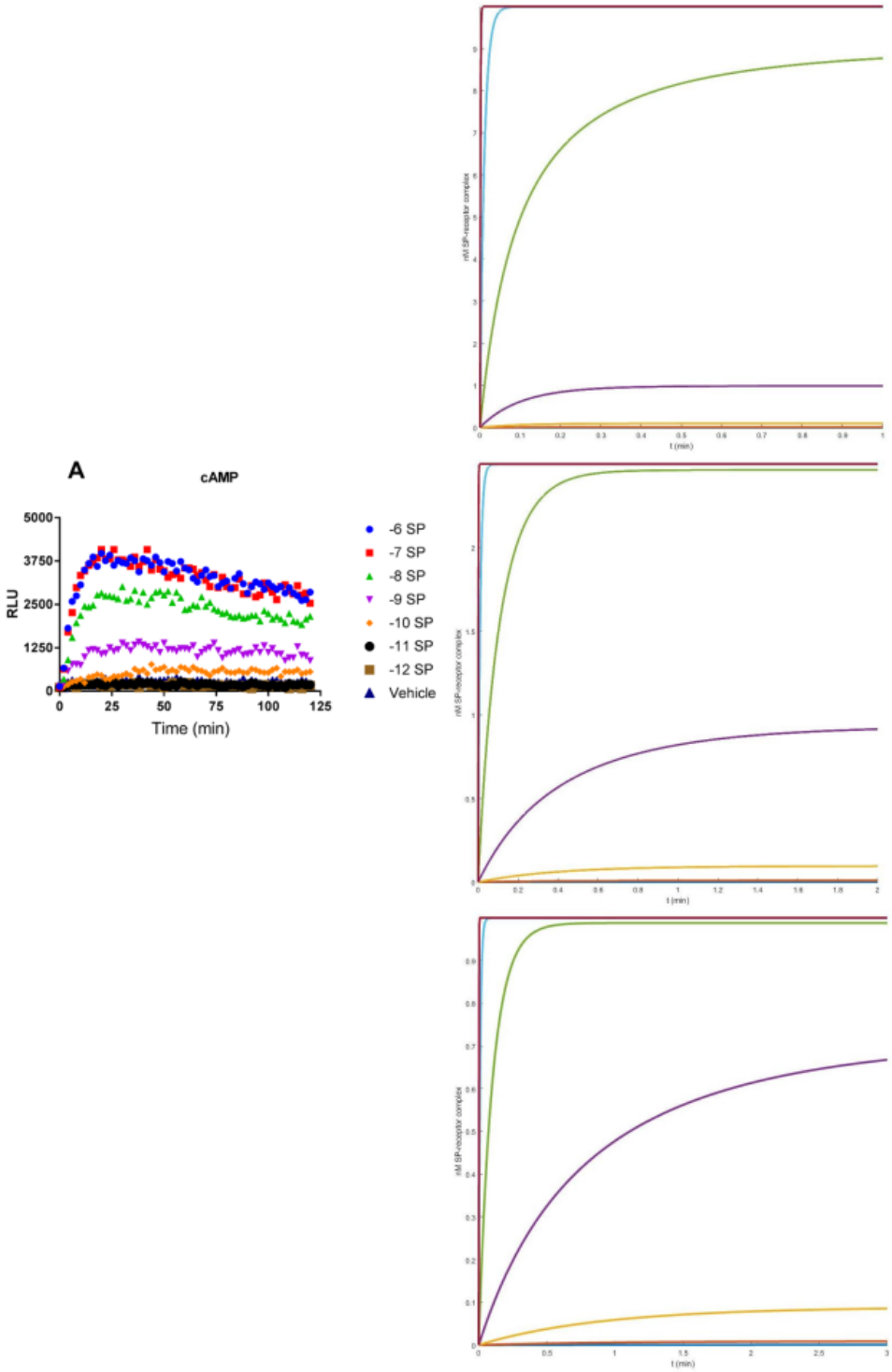

Figure 11: Left the results from the original article with concentrations given in ^{10}log[SP](M). Right the results of the B_2 concentration over time model with on the x-as the time in minutes and on the y-as the SP-receptor complex concentration in nM. Furthermore we have the next R_{tot} values from top to bottom of 10, 2.5 and 1 nM and SP concentrations in nM from bottom to top :0.001 (blue), 0.01 (brown), 0.1 (yellow), 1 (purple), 10 (green), 100 (light blue) and 1000 (red) nM.

to an overload of receptors. It is difficult to determine which R_{tot} value is too low and which value is too high. Therefore we have chosen the next values for R_{tot}: 1, 2.5 and 10 nM. Figures 11 and 13 show the results from the experiment (figure 5) and from the model using equation (22) for both agonists SP and NKA with the three different chosen values for R_{tot}. First, we focus on the results for SP (figure 11). The left figure shows the results from the original article (figure 5A). It can be seen that the most RLU (and therefore the most cAMP) is measured at t≈25 min, especially with SP doses of 1000, 100 and 10 nM. Furthermore for different doses at t=0 it can be seen that some have the same RLU measurement at the end of the experiment, thus at t≈125 min. These are doses 1000 and 100 and 0.01 and 0.001 nM SP. There are also differences between different doses. It can be seen that there is a small difference in RLU between 0.1 and 0.01. There is a larger difference in RLU between doses 100 and 10 and between doses 1 and 0.1 nM. Furthermore there is a largest difference between 10 and 1 nM SP. This behavior is wanted in the graphs for B_2 from model 1. The right graphs show the results with from top to bottom R_{tot} values of 10, 2.5 and 1 nM. The colored lines correspond to the following initial SP concentrations: 0.001 (blue), 0.01 (brown), 0.1 (yellow), 1 (purple), 10 (green), 100 (light blue) and 1000 (red) nM. All the three graphs show that the two doses 1000 and 100 and the two doses 0.01 and 0.001 nM SP have (almost) the same B_2 concentration over time. However there are also some differences in B_2 concentration for different doses between the three graphs. The graph with $R_{tot} = 10$ nM shows a very small difference between 0.1 and 0.01 a small difference between doses 100 and 10 and between doses 1 and 0.1 and a large difference between doses 10 and 1 nM SP. The graph with $R_{tot} = 2.5$ nM shows a little difference between doses 100 and 10 and between doses 0.1 and 0.01 and a larger difference between doses 1 and 0.1 and the largest difference between doses 10 and 1 nM SP. The graph with $R_{tot} = 1$ nM shows a little difference between doses 100 and 10 a little larger difference between doses 0.1 and 0.01 and a large difference between doses 10 and 1 and the largest difference between doses 1 and 0.01 nM SP. It is concluded that the graphs with R_{tot} value of 10 and 2.5 nM have the best similarities with the original graph based on the size of the differences between different doses of SP. Thus it is believed, based on these results, that the R_{tot} value lies between circa 2.5 and 10 nM. We assume that R_{tot} will not be greater than 10 nM based on the results from figures 7 and 8 as explained above.

Note that equation (11) can be used to obtain a figure with R_{tot} on the horizontal axis and B_2 (when in steady state) on the vertical axis. These B_2 values correspond to the B_2 values in figure 11 when steady state is reached (flat line) for particular values for R_{tot}. Figure 12 shows the result for R_{tot} between zero up to 10 nM with SP as agonist. Again the colored lines correspond to the following initial SP concentrations: 0.001 (blue), 0.01 (brown), 0.1 (yellow), 1 (purple), 10 (green), 100 (light blue) and 1000 (red) nM. First note that not all initial SP concentrations are visible, namely: 0.001 nM (blue line) and 100 nM (light blue line). This is due to similarity between 0.001 nM and 0.01 nM (brown line) and between 100 nM and 1000 nM (red line). This behavior is also seen in the right three graphs in figure 11. Furthermore it can be seen that when R_{tot} increases differences between 1 nM (purple line) and 10 nM (green line) increases as well. There is also an increase in difference between 10 nM and 100/1000 nM (red line) from circa R_{tot} between 5 up to 10 nM. Furthermore differences between 0.01 nM (brown line) and 0.1 nM (yellow line) stay the same despite increasing R_{tot} values. This also applies to 0.1 nM (yellow line) and 1 nM (purple line) from circa R_{tot} between 1.5 up to 10 nM. This behavior corresponds to the differences in B_2 concentration for different doses between the right three graphs in figure 11. We conclude that the results from figure 11 are as expected.

Now, we focus on the results for NKA (figure 13). Again the left figure shows the results from the original article (figure 5B). It can be seen that the most RLU (and therefore the most cAMP) is measured at t≈25 min, especially with NKA doses of $10^{5.2}$, $10^{4.2}$ and $10^{3.2}$ nM. Furthermore, for different doses at t=0 it can be seen that some different doses of NKA have the same RLU measurement at the end of the experiment, thus at t≈125 min. These are the three doses $10^{-0.8}$, $10^{0.2}$ and $10^{1.2}$ nM NKA. The three doses $10^{5.2}$, $10^{4.2}$ and $10^{3.2}$ are really close to one another and the doses $10^{2.2}$ and $10^{1.2}$ have a large

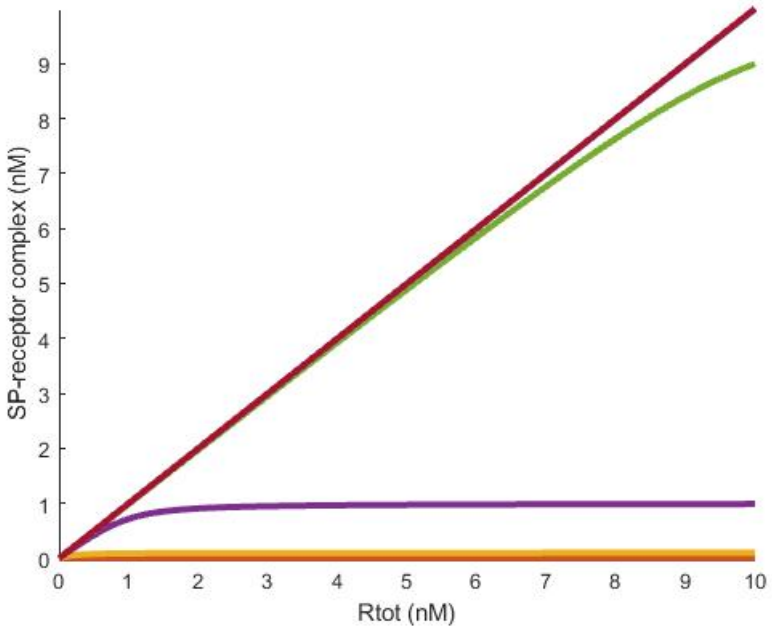

Figure 12: Comparison of the steady state SP-receptor complex concentration as function of R_{tot} for different concentrations of SP with R_{tot} (nM) on the horizontal axis and SP-receptor complex concentration (nM) (when in steady state) on the vertical axis. The colored lines correspond to the following initial SP concentrations: 0.001 (blue), 0.01 (brown), 0.1 (yellow), 1 (purple), 10 (green), 100 (light blue) and 1000 (red) nM.

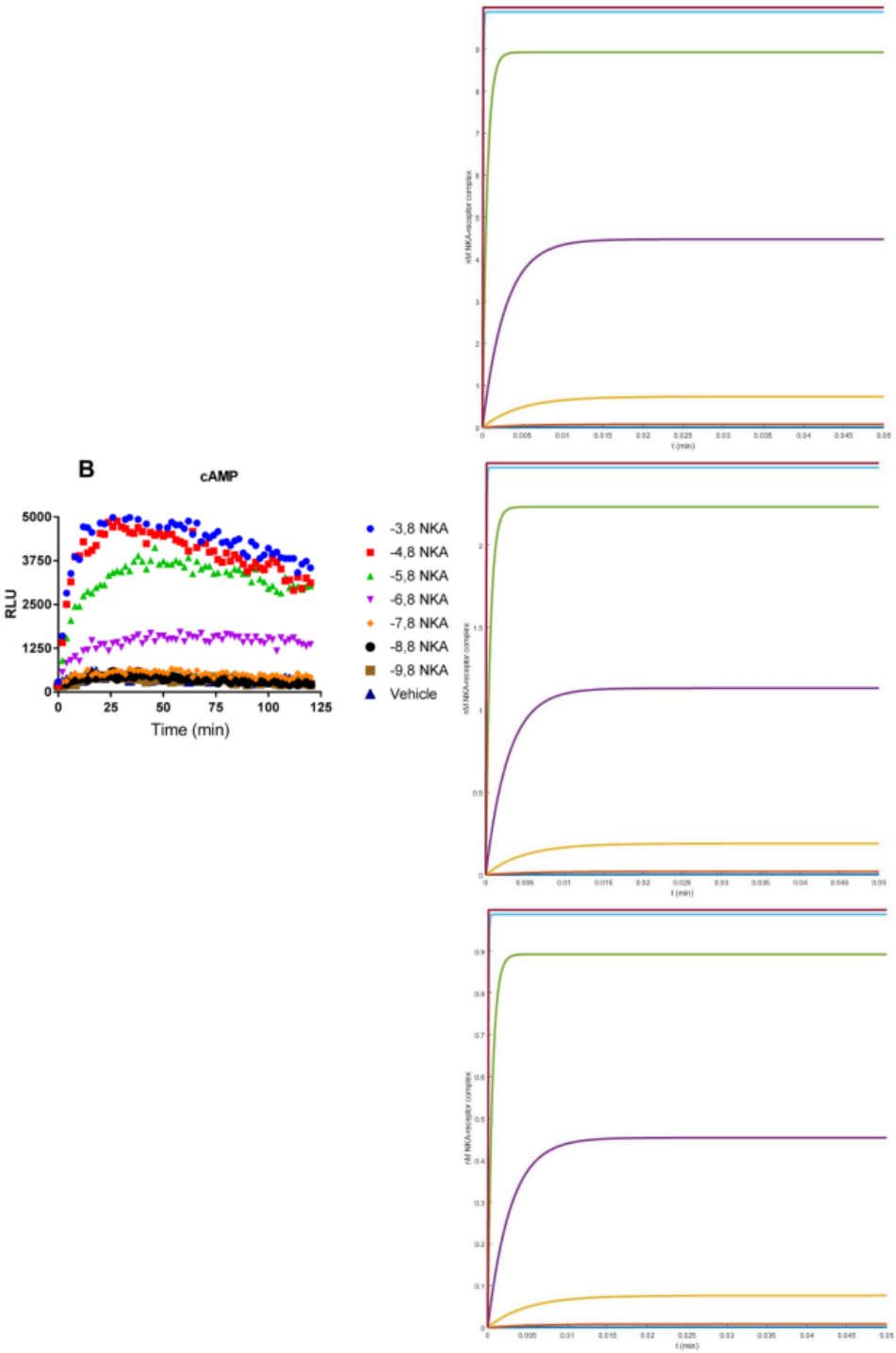

Figure 13: Left the results from the original article with concentrations given in ^{10}log[NKA](M). Right the results of the B_2 concentration over time model with on the x-as the time in minutes and on the y-as the NKA-receptor complex concentration in nM. Furthermore we have the next R_{tot} values from top to bottom of 10, 2.5 and 1 nM and NKA concentrations from bottom to top: $10^{-0.8}$ (blue), $10^{0.2}$ (brown), $10^{1.2}$ (yellow), $10^{2.2}$ (purple), $10^{3.2}$ (green), $10^{4.2}$ (light blue) and $10^{5.2}$ (red) nM.

difference and the doses $10^{3.2}$ and $10^{2.2}$ have the largest difference. This behavior is wanted in the graphs for B_2 from model 1. The right graphs show the results with from top to bottom R_{tot} values of 10, 2.5 and 1 nM. The colored lines correspond to the following initial NKA concentrations: $10^{-0.8}$ (blue), $10^{0.2}$ (brown), $10^{1.2}$ (yellow), $10^{2.2}$ (purple), $10^{3.2}$ (green), $10^{4.2}$ (light blue) and $10^{5.2}$ (red) nM. It can be seen that there is little difference between the three graphs. All three graphs show that doses $10^{5.2}$ and $10^{4.2}$ and doses $10^{-0.8}$ and $10^{0.2}$ are very close to one another. The doses $10^{5.2}$ and $10^{4.2}$ are very close to one another because the amount of ligand is very high in comparison to the amount of receptor. The doses $10^{-0.8}$ and $10^{0.2}$ are very close to one another because the amount of ligand is very low in comparison to the amount of receptor. Small differences are visible between doses $10^{4.2}$ and $10^{3.2}$ and $10^{0.2}$ and $10^{1.2}$. A large difference is visible between doses $10^{1.2}$ and $10^{2.2}$ and the largest difference is visible between doses $10^{2.2}$ and $10^{3.2}$. This behavior is very similar to the difference between different doses of NKA in the real experiment. Thus no conclusions can be made based on these results with NKA as agonist. Conclusions about possible values for R_{tot} are drawn from the graphs with SP as agonist since all three graphs with NKA as agonist show similar results.

Again a figure is made with NKA-receptor complex concentration in steady state as function of R_{tot}. Figure 14 shows the result with NKA as agonist and values for R_{tot} between zero up to 10 nM on the horizontal axis and NKA-receptor complex concentration (nM) (when in steady state) on the vertical axis. Again the colored lines correspond to the following initial NKA concentrations: $10^{-0.8}$ (blue), $10^{0.2}$ (brown), $10^{1.2}$ (yellow), $10^{2.2}$ (purple), $10^{3.2}$ (green), $10^{4.2}$ (light blue) and $10^{5.2}$ (red) nM. Note that the results for all concentrations are linear. This means that the ratio between different doses of NKA for different values of R_{tot} stays the same. This is also clearly seen in figure 13. We conclude that the results from figure 13 are as expected.

A big difference between the graphs from the article and the graphs from the model is the time until steady state is reached. The results from the experiment show a peak followed by a slowly decreasing measurement of cAMP. However the model shows that the free ligand binds very fast to the available receptors and steady state is reached within seconds up to a few minutes without showing any peak. This can be partly explained by the different values that are measured (RLU versus agonist-receptor complex concentration) between the experiment and the model. For example there is a delay in measurement and production of cAMP. The samples have to be put in the machine (that measures the cAMP) when the agonist is added. Furthermore this is a simplified model and there could be factors that have an influence on the binding, such as diffusion, the influence of other molecules and the production of cAMP. The model does not take into account that the ligand has to diffuse throughout the whole sample with cells containing the receptors. In other words in the model ligands are able to bind to all free receptor at t=0. However, in a real situation ligand is added at t=0 but has not immediately diffused over the whole sample. Furthermore, the model contains only ligand, receptor and complex, which is not the case in the real experiment. For instance, the sample contains cells and other molecules which can be an obstacle for the binding of ligands to receptors. As described previously, the amount of cAMP is measured in RLU. One should realize that for example when a complex is formed it is not that 1 or 2 cAMP molecules correspond to that binding. Thus it is unknown what the precise relation is between the amount of cAMP and the amount of binding. Lastly, it is assumed that the concentration R_{tot} is the same in both experiments. However, it could be possible that this is not the case and therefore has an influence on the results.

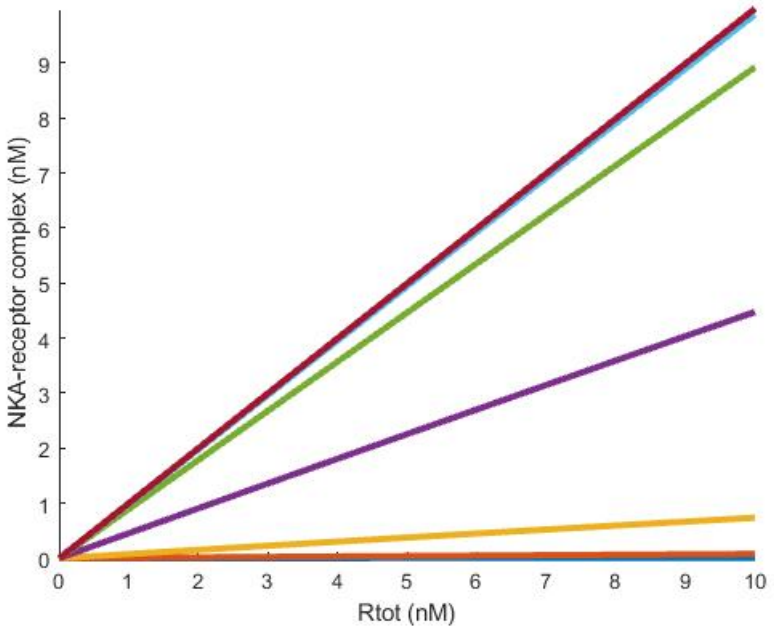

Figure 14: Comparison of the steady state NKA-receptor complex concentration as function of R_{tot} for different concentrations of NKA with R_{tot} (nM) on the horizontal axis and NKA-receptor complex concentration (nM) (when in steady state) on the vertical axis. The colored lines correspond to the following initial NKA concentrations: $10^{-0.8}$ (blue), $10^{0.2}$ (brown), $10^{1.2}$ (yellow), $10^{2.2}$ (purple), $10^{3.2}$ (green), $10^{4.2}$ (light blue) and $10^{5.2}$ (red) nM.

7 First analysis of the unknown constants

One of the main aims of this thesis is to give an estimate of the values for K_{on1} and K_{off1} for the two antagonists DFA and aprepitant. In this section we will give an initial estimate for these two constants. First we will give the method for estimating these values. Next the results will be discussed and we will end with a comparison between our obtained results and the results from the original article.

7.1 Method for determining the unknown constants

The K_{on} and K_{off} values of two different ligands have an influence on each other ability to bind to a single receptor. If for example the K_{on1} value from L_1 is higher than the K_{on2} value from L_2 then L_1 has more chance to bind to the receptor than L_2. Therefore we will consider the three different possible intervals in which the value for K_{on1} and K_{off1} lies compared to the already known values for the agonists. We already know that the K_{on} and K_{off} values for SP are 0.24 and 0.0027 and the K_{on} and K_{off} value for NKA are 0.001 and 0.19. Then the real values for K_{on1} for both antagonists can lie in one of the following intervals: $K_{on1} < 0.001$, $0.001 < K_{on1} < 0.24$ and $0.24 < K_{on1}$. The same holds for K_{off1}, thus: $K_{off1} < 0.027$, $0.027 < K_{off1} < 0.19$ and $0.19 < K_{off1}$. So there are nine possible combinations of intervals for K_{on1} and K_{off1}. We made plots for values for K_{on1} and K_{off1} chosen in each interval.

As stressed previously another important unknown constant is R_{tot}. This constant has an influence on the effect of the antagonists in the model. An estimate of R_{tot} has been determined in section 6.1 and therefore we consider $R_{tot} = 10$ and $R_{tot} = 2.5$ nM. The results are obtained by simulating model 1 and model 2 using Matlab. The values for B_2 are taken at circa t=45 min when steady state is already reached. Recall that the B_2 value is the agonist-receptor complex concentration. Then these values are divided by the agonist-receptor complex concentration from model 1 when the highest dose agonist is added to the cells. This is thus the case when no antagonist is added. Next all the values are multiplied by 100. This results in values that describe the agonist-receptor complex concentration in percentage with 100% representing the value when the highest dose of agonist is added to the cells. Now, we will give an example to illustrate this. Table 2 shows the results from simulating model 1 and 2 in Matlab with SP as agonist, $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} and $R_{tot} = 10$ nM. Note that the blue value corresponds to the highest SP dose and that no antagonist is added. Next all the values in table 2 are divided by the blue value and then multiplied by 100. This gives the results shown in table 3. The scaled values describe to a certain extent the measure points in figure 7 and 8 from the original article. The big difference is the value on the y-as. In the article this value is RLU percentage, whereas the results from the simulation are given in the agonist-receptor complex concentration (%).

	0.07	0.21	0.7	SP
-12 log [SP] (M)	0.000989	0.000989	0.000988	0.00099
-11 log [SP] (M)	0.00989	0.00989	0.00988	0.00989
-10 log [SP] (M)	0.0989	0.0989	0.0988	0.0989
-9 log [SP] (M)	0.988	0.987	0.987	0.988
-8 log [SP] (M)	8.96	8.89	8.606	8.994
-7 log [SP] (M)	9.91	9.775	9.303	9.977
-6 log [SP] (M)	9.942	9.788	9.32	9.989

Table 2: Table of the obtained results in Matlab for $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1}and $R_{tot} = 10$ nM. SP is used as agonist.

A figure from the article containing results from the simulation is made in order to illustrate which results from the article correspond to a certain extent to the obtained results by us. As explained above, table 3 shows the result of the just explained method of randomly chosen values $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} and $R_{tot} = 10$ nM for the agonist SP. Figure 15 shows which measure points from the experiment correspond to which results from the simulation. Only the values with SP concentrations of -8 up to -6 log [SP] (M) are displayed since the values would otherwise be too close to each other. The only means of this table and figure is to get an understanding for the meaning of the results from the simulation with respect to the original results from the experiment.

	0.07	0.21	0.7	SP
-12 log [SP] (M)	0,01	0,01	0,01	0,01
-11 log [SP] (M)	0,099	0,099	0,099	0,099
-10 log [SP] (M)	0,99	0,99	0,989	0,99
-9 log [SP] (M)	9,891	9,881	9,881	9,891
-8 log [SP] (M)	89,697	88,998	86,155	90,039
-7 log [SP] (M)	99,209	97,858	93,132	99,88
-6 log [SP] (M)	99,529	97,988	93,303	100

Table 3: Table of the scaled obtained results for $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} and $R_{tot} = 10$ nM. SP is used as agonist.

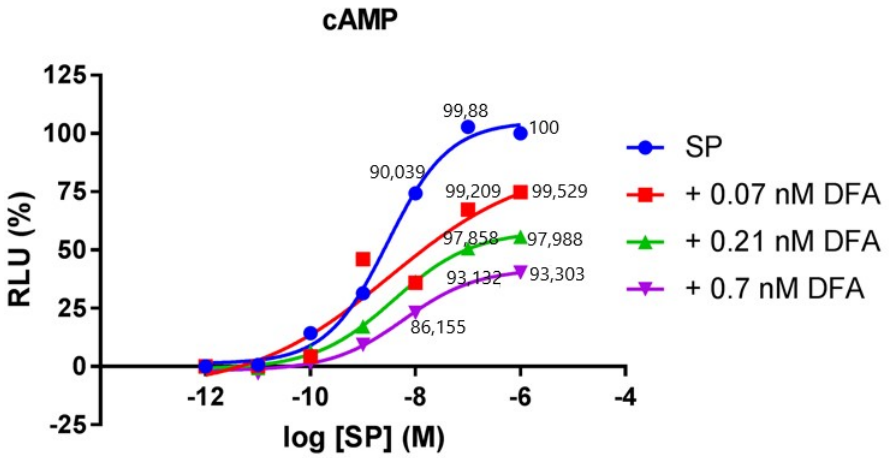

Figure 15: The results from the original article with values from the simulation given in table 3 to show which value corresponds to which point. Only the values for the last three SP concentrations are added to the figure.

7.2 Results

First, we analyze the results with $R_{tot} = 10$ nM. Figure 16 shows the nine graphs with the agonist SP. There are four situations: when only SP is added (yellow), when 0.07 nM antagonist is added followed bij SP (blue), when 0.21 nM antagonist is added followed bij SP (orange) and when 0.7 nM antagonist is added followed bij SP (grey). Each of the nine graphs is obtained with a chosen K_{on1} and K_{off1} value from a different interval: A: $K_{on1} = 0.0001$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1}. B:

K_{on1} = 0.01 nM^{-1}min^{-1}, K_{off1} = 0.001 min^{-1}.C: K_{on1} = 1 nM^{-1}min^{-1}, K_{off1} = 0.001 min^{-1}. D: K_{on1} = 0.0001 nM^{-1}min^{-1}, K_{off1} = 0.1 min^{-1}. E: K_{on1} = 0.0001 nM^{-1}min^{-1}, K_{off1} = 1 min^{-1}. F: K_{on1} = 0.01 nM^{-1}min^{-1}, K_{off1} = 0.1 min^{-1}. G: K_{on1} = 0.01 nM^{-1}min^{-1}, K_{off1} = 1 min^{-1}. H: K_{on1} = 1 nM^{-1}min^{-1}, K_{off1} = 0.1 min^{-1}.I: K_{on1} = 1 nM^{-1}min^{-1}, K_{off1} = 1 min^{-1}. It can be seen that the first five doses of SP give the same agonist-receptor complex concentration for different amounts of antagonist. Only graphs B and C show some minor differences between different antagonist concentrations. Figure 17 shows the graphs with NKA as agonist. Again we have four situations: when only NKA is added (yellow), when 0.07 nM antagonist is added followed bij NKA (blue), when 0.21 nM antagonist is added followed bij NKA (orange) and when 0.7 nM antagonist is added followed bij NKA (grey). The first four agonist concentrations does not show any difference between different amounts of antagonist. Graphs B and C show some minor differences between the last three agonist doses.

Next, we analyze the graphs with R_{tot} = 2.5 nM. Figures 18 and 19 show the graphs with the same values chosen for K_{on1} and K_{off1} for A up to I as described above. The first four doses of SP give similar results for different amounts of antagonist. Graph A shows minor difference when 0.7 nM antagonist is added and graphs B and C show larger differences when different concentrations of antagonist is added. The other graphs still do not show any difference. Figure 19 with NKA as agonist shows that the first three doses of NKA give similar results despite different concentrations of antagonist. This is different compared to the graphs with R_{tot} = 10 nM. Graph A shows some minor difference in the last three points and graphs B and C show larger differences compared to figure 16. Graph H shows some difference between the third and last point. The other graphs show similarity between different amount of antagonist concentrations.

7.3 Comparison

In this section we will compare the results from the simulation (figures 16, 17, 18 and 19) with the results from the experiment (figures 7 and 8). First, close attention is given to the results from the experiment. We begin with the figures with SP as agonist (figure 7). The lowest three concentrations of SP with and without antagonist give nearly the same RLU %, namely close to zero. For both antagonists DFA and aprepitant the steepest slope lies between the third and the fifth agonist concentration, thus for SP this is between -10 and -8 ^{10}log [SP] (M). The most observable difference between the antagonists DFA and aprepitant is that the values with the same concentration SP and different DFA concentrations are closer to one another compared to different concentrations of aprepitant. Next, the figures with agonist NKA are considered (figure 8). The first two concentrations of NKA give the same RLU response despite different antagonist concentrations. Again the steepest slope is between the third and fifth NKA concentration, thus between -7.8 and -5.8 ^{10}log [NKA] (M). Lastly, it is seen that values with different amounts of DFA with the same concentration NKA are much closer compared to different concentrations of aprepitant. Hence, we want to observe this behavior in the plots in figure 16-19.

The graphs with R_{tot} = 10 nM (figures 16 and 17) show less difference between different concentrations of antagonist than the graphs with R_{tot} = 2.5 nM (figures 18 and 19. Whereas we concluded above that we want more difference between different concentrations of antagonist. Therefore it is concluded that R_{tot} = 2.5 nM is a better estimate than R_{tot} = 10 nM. Furthermore, as seen in section 6.1 R_{tot} = 2.5 nM fits the results from the time dependent cAMP assay as well (figure 5).

The only intervals that show some difference between different amounts of antagonist are graphs B and C in figures 18 and 19 for agonists SP and NKA and graph H in figure 19 for agonist NKA. Interval H cannot be the right interval for one of the antagonists. This is due to lack of difference with agonist SP and no visible difference at the highest concentration of NKA, while this is clearly visible in the original results for both antagonists. Thus the intervals B and or C contain the real values for K_{on1} and K_{off1}

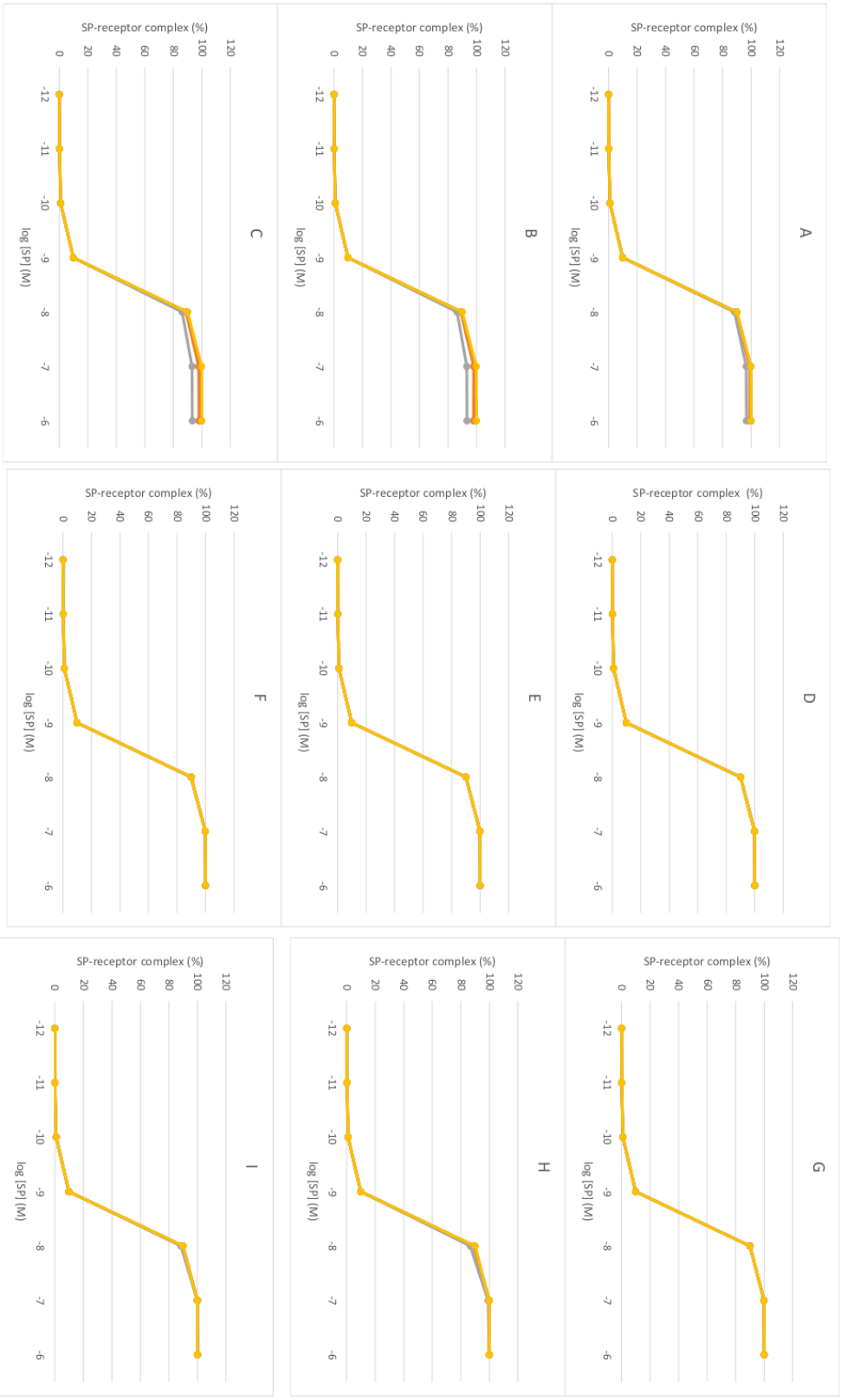

Figure 16: Graphs with different values for K_{on1} and K_{off1} with $R_{tot} = 10$ nM and agonist SP. There are four situations: when only SP is added (yellow), when 0.07 nM antagonist is added followed bij SP (blue), when 0.21 nM antagonist is added followed bij SP (orange) and when 0.7 nM antagonist is added followed bij SP (grey). A: $K_{on1} = 0.0001$, $K_{off1} = 0.001$. B: $K_{on1} = 0.01$, $K_{off1} = 0.001$.C: $K_{on1} = 1$, $K_{off1} = 0.001$. D: $K_{on1} = 0.0001$, $K_{off1} = 0.1$. E: $K_{on1} = 0.0001$, $K_{off1} = 1$. F: $K_{on1} = 0.01$, $K_{off1} = 0.1$. G: $K_{on1} = 0.01$, $K_{off1} = 1$. H: $K_{on1} = 1$, $K_{off1} = 0.1$. I: $K_{on1} = 1$, $K_{off1} = 1$.

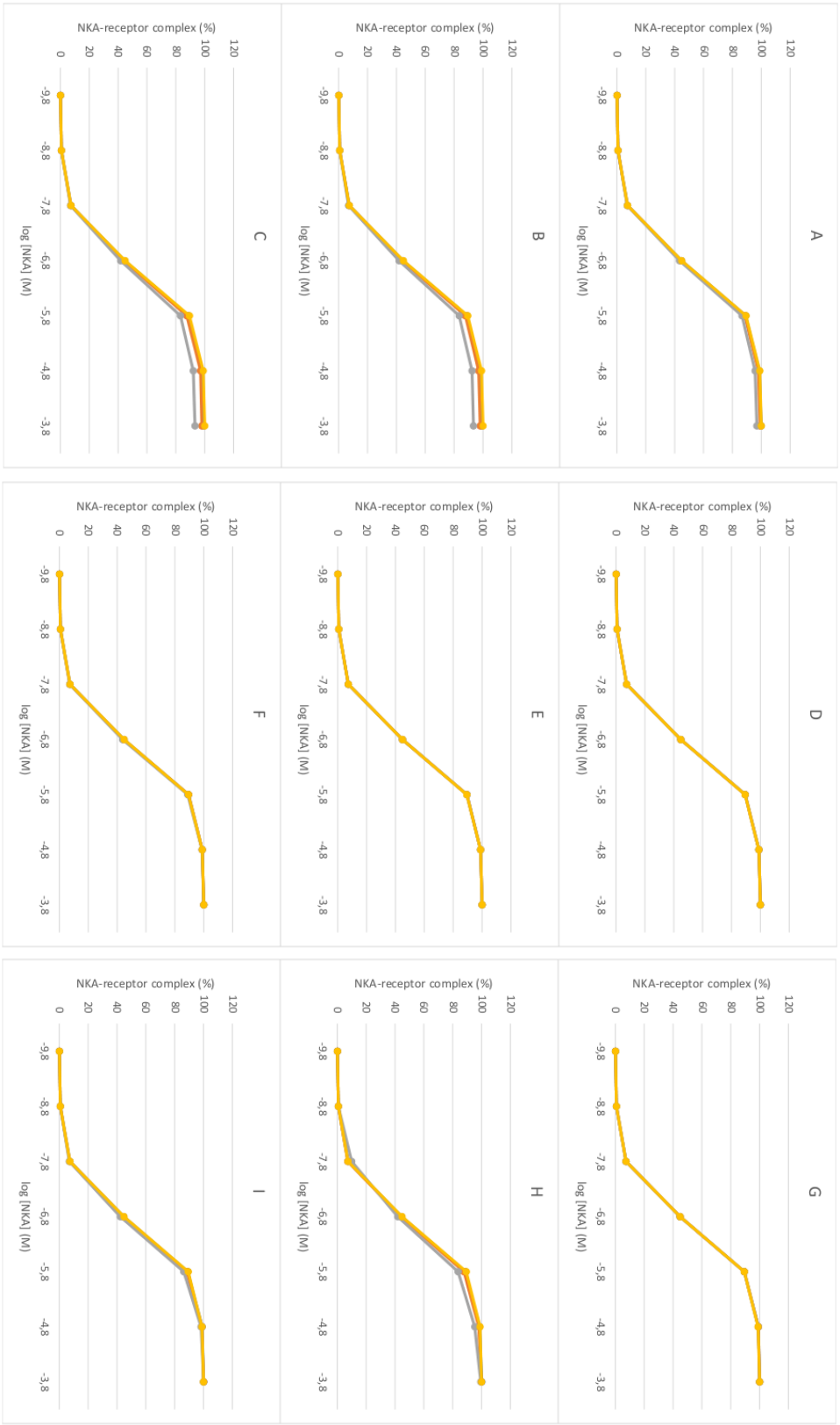

Figure 17: Graphs with different values for K_{on1} and K_{off1} with $R_{tot} = 10$ nM and agonist NKA. There are four situations: when only NKA is added (yellow), when 0.07 nM antagonist is added followed bij NKA (blue), when 0.21 nM antagonist is added followed bij NKA (orange) and when 0.7 nM antagonist is added followed bij NKA (grey). A: $K_{on1} = 0.0001$, $K_{off1} = 0.001$. B: $K_{on1} = 0.01$, $K_{off1} = 0.001$.C: $K_{on1} = 1$, $K_{off1} = 0.001$. D: $K_{on1} = 0.0001$, $K_{off1} = 0.1$. E: $K_{on1} = 0.0001$, $K_{off1} = 1$. F: $K_{on1} = 0.01$, $K_{off1} = 0.1$. G: $K_{on1} = 0.01$, $K_{off1} = 1$. H: $K_{on1} = 1$, $K_{off1} = 0.1$. I: $K_{on1} = 1$, $K_{off1} = 1$.

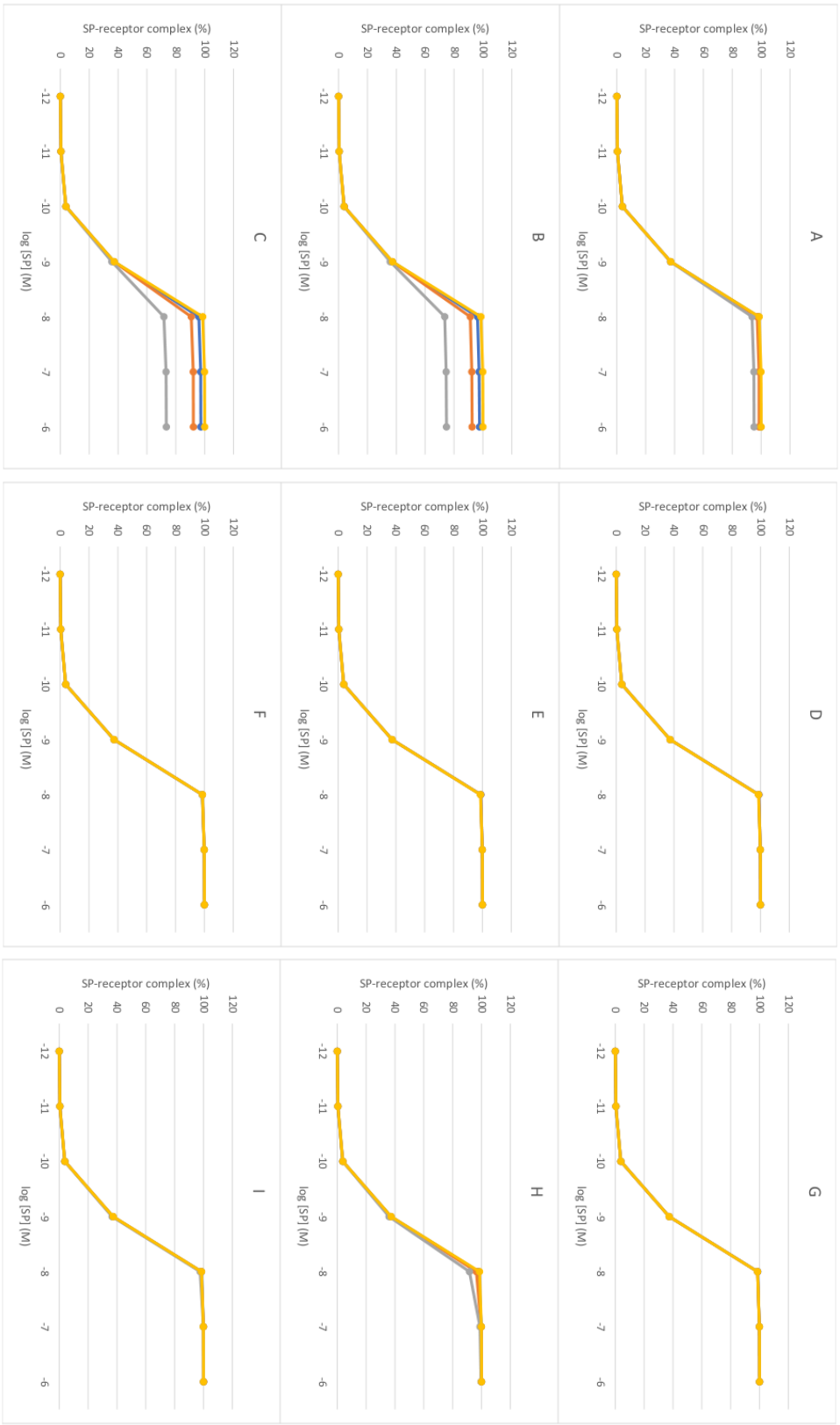

Figure 18: Graphs with different values for K_{on1} and K_{off1} with $R_{tot} = 2.5$ nM and agonist SP. There are four situations: when only SP is added (yellow), when 0.07 nM antagonist is added followed bij SP (blue), when 0.21 nM antagonist is added followed bij SP (orange) and when 0.7 nM antagonist is added followed bij SP (grey). A: $K_{on1} = 0.0001$, $K_{off1} = 0.001$. B: $K_{on1} = 0.01$, $K_{off1} = 0.001$.C: $K_{on1} = 1$, $K_{off1} = 0.001$. D: $K_{on1} = 0.0001$, $K_{off1} = 0.1$. E: $K_{on1} = 0.0001$, $K_{off1} = 1$. F: $K_{on1} = 0.01$, $K_{off1} = 0.1$. G: $K_{on1} = 0.01$, $K_{off1} = 1$. H: $K_{on1} = 1$, $K_{off1} = 0.1$. I: $K_{on1} = 1$, $K_{off1} = 1$.

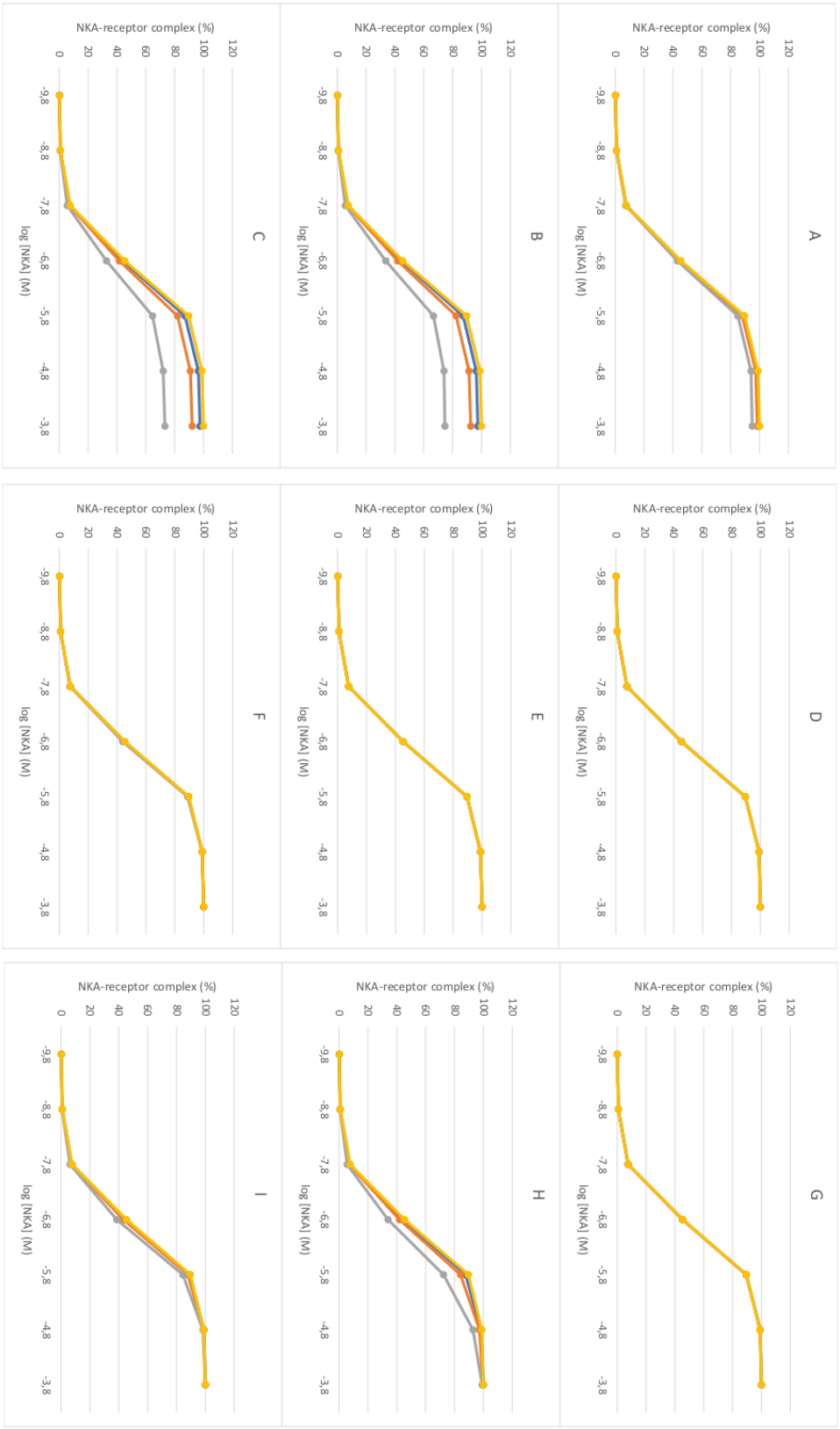

Figure 19: Graphs with different values for K_{on1} and K_{off1} with $R_{tot} = 2.5$ nM and agonist NKA. There are four situations: when only NKA is added (yellow), when 0.07 nM antagonist is added followed bij NKA (blue), when 0.21 nM antagonist is added followed bij NKA (orange) and when 0.7 nM antagonist is added followed bij NKA (grey). A: $K_{on1} = 0.0001$, $K_{off1} = 0.001$. B: $K_{on1} = 0.01$, $K_{off1} = 0.001$.C: $K_{on1} = 1$, $K_{off1} = 0.001$. D: $K_{on1} = 0.0001$, $K_{off1} = 0.1$. E: $K_{on1} = 0.0001$, $K_{off1} = 1$. F: $K_{on1} = 0.01$, $K_{off1} = 0.1$. G: $K_{on1} = 0.01$, $K_{off1} = 1$. H: $K_{on1} = 1$, $K_{off1} = 0.1$. I: $K_{on1} = 1$, $K_{off1} = 1$.

for the antagonists DFA and aprepitant. Note that the K_{on1} and K_{off1} value are particular to a given ligand thus DFA and aprepitant have different values for K_{on1} and K_{off1}. The graphs B and C are very similar. First the similarities with the results from the experiment are considered. Figure 7 from the original article with agonist SP shows for both antagonists no or little RLU measurement for the lowest three concentrations and with agonist NKA for the lowest two concentrations. This is also seen in graphs B and C in figure 18. Furthermore the steepest slope between the third and fifth concentration is also observable in graph B and C in figure 18. Lastly, the highest two doses of SP and NKA give the same value for a given concentration of antagonist. There are also a few features that do not match the original results in figure 7. The fourth dose of SP does not give any difference while this is observed in the original results. The original results in figures 7 and 8 show more differences between different amounts of antagonists, especially when antagonist aprepitant is used.

8 Second analysis of the unknown constants

The previous section showed that the best results were obtained in the intervals $0.001 < K_{on1} < 0.24$ and $0.24 < K_{on1}$ combined with the interval $K_{off1} < 0.027$. In this section we will examine these intervals further and try to improve the estimate for K_{on1} and K_{off1} for both antagonists.

B	0.07	0.21	0.7	SP
-12 log [SP] (M)	0,038	0,038	0,038	0,038
-11 log [SP] (M)	0,383	0,382	0,377	0,383
-10 log [SP] (M)	3,821	3,809	3,761	3,825
-9 log [SP] (M)	37,245	37,004	35,803	37,365
-8 log [SP] (M)	96,195	91,189	73,568	98,638
-7 log [SP] (M)	97,317	92,231	74,529	99,92
-6 log [SP] (M)	97,637	92,511	74,77	100
C	0.07	0.21	0.7	SP
-12 log [SP] (M)	0,038	0,038	0,038	0,038
-11 log [SP] (M)	0,383	0,382	0,377	0,383
-10 log [SP] (M)	3,821	3,809	3,757	3,825
-9 log [SP] (M)	37,245	37,004	35,643	37,365
-8 log [SP] (M)	95,875	90,669	71,766	98,638
-7 log [SP] (M)	97,237	92,07	73,248	99,92
-6 log [SP] (M)	97,317	92,151	73,408	100

Table 4: Obtained results for $K_{on1} = 0.01$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} (B) and $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} (C). These results were used to obtain graph B and C in figure 18. Here $R_{tot} = 2.5$ nM and SP is used as agonist.

Graphs B and C in figure 18 and 19 are very similar despite different values for K_{on1}. Table 4 and 5 show the obtained values for both intervals with agonists SP and NKA in order to determine if there are or are not (small) differences in agonist-receptor complex concentration. First we focus on table 4. In table 4B we give the agonist-receptor complex concentration % values for $K_{on1} = 0.01$ nM^{-1}min^{-1} and $K_{off1} = 0.001$ min^{-1} and in table 4C the agonist-receptor complex concentration % values for $K_{on1} = 1$ nM^{-1}min^{-1} and $K_{off1} = 0.001$ min^{-1}. These choices imply that the antagonist in the second situation binds better than the first one. Little differences between table 4B and C are visible, especially when the highest antagonist concentration of 0.7 nM is added and when the SP doses are high. These are the

B	0.07	0.21	0.7	NKA
-9,8 log [NKA] (M)	0,08	0,076	0,061	0,082
-8,8 log [NKA] (M)	0,798	0,754	0,601	0,814
-7,8 log [NKA] (M)	7,418	7,017	5,613	7,578
-6,8 log [NKA] (M)	44,226	41,82	33,561	45,389
-5,8 log [NKA] (M)	87,289	82,478	66,68	89,575
-4,8 log [NKA] (M)	96,391	91,339	73,978	98,957
-3,8 log [NKA] (M)	97,434	92,502	74,619	100
C	0.07	0.21	0.7	NKA
-9,8 log [NKA] (M)	0,0802	0,076	0,06	0,082
-8,8 log [NKA] (M)	0,798	0,75	0,593	0,814
-7,8 log [NKA] (M)	7,418	7,017	5,533	7,578
-6,8 log [NKA] (M)	44,146	41,62	32,759	45,389
-5,8 log [NKA] (M)	87,089	81,957	64,635	89,575
-4,8 log [NKA] (M)	96,431	90,858	72,053	98,957
-3,8 log [NKA] (M)	97,554	92,141	73,216	100

Table 5: Obtained results for $K_{on1} = 0.01$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} (B) and $K_{on1} = 1$ nM^{-1}min^{-1}, $K_{off1} = 0.001$ min^{-1} (C). These results were used to obtain graph B and C in figure 19. Here $R_{tot} = 2.5$ nM and NKA is used as agonist.

blue values in table 4. It can be seen that table B contains the same or slightly higher values than table C. This means that the antagonist with $K_{on1} = 1$ nM^{-1}min^{-1} has little bit better antagonistic effects (since there is slightly less binding between the agonist and the receptor). Table 5 shows similar results. Thus the interval $0.24 < K_{on1}$ combined with the interval $K_{off1} < 0.027$ give the best result.

8.1 Comparison within the intervals

The original results in figures 7 and 8 show, especially with antagonist aprepitant, more difference in RLU measurement between different concentrations of antagonist. This amount of difference has not been observed in the plots we made with the models. Thus further investigation is needed in order to improve the results. Again a simulation has been made with the same method as in the previous section with higher values for K_{on1} than $K_{on1} = 1$ nM^{-1}min^{-1} and lower values for K_{off1} than $K_{off1} = 0.01$ min^{-1} in order to investigate if this improves the results. The next values have been examined: $K_{on1} = 10$ nM^{-1}min^{-1}, $K_{off1} = 0.0001$ min^{-1} and $K_{on1} = 100$ nM^{-1}min^{-1}, $K_{off1} = 0.00001$ min^{-1}. Table 6 and 7 show the result of these values. It can be seen that the obtained values are very similar even though K_{on1} is increased a 10 or 100 fold and K_{off1} is decreased a 10 or 100 fold. This implies that at a certain point increasing the K_{on1} and decreasing the K_{off1} values does not effect the antagonistic effects of the antagonists. One explanation could be that the antagonist already has so much better affinity to the receptor compared to the agonist that improvement does not give any changes. In other words at a certain point the antagonist does not 'battle' anymore with the agonist over binding to the receptor. Thus changing the K_{on1} and K_{off1} value for better association and lesser disassociation does not improve the results. The similar results between these different values for K_{on1} and K_{off1} can also be explained with use of equation (17). When K_{off1} is chosen very low the first two terms in the denominator will be insignificantly low. Then K_{on1} can be removed from the numerator and the denominator. Thus K_{on1} has no influence on the B_2 concentration when in steady state for very low values for K_{off1}. The next subsection will investigate an other method in order to obtain more difference between different

concentrations of antagonist.

A	0.07	0.21	0.7	SP
-12 log [SP] (M)	0,383	0,038	0,0377	0,038
-11 log [SP] (M)	0,383	0,382	0,3765	0,383
-10 log [SP] (M)	3,821	3,809	3,757	3,825
-9 log [SP] (M)	37,245	37,004	35,643	37,365
-8 log [SP] (M)	95,795	90,308	71,085	98,638
-7 log [SP] (M)	97,117	91,549	72,006	99,92
-6 log [SP] (M)	97,237	91,63	72,127	100
B	0.07	0.21	0.7	SP
-12 log [SP] (M)	0,038	0,038	0,0377	0,038
-11 log [SP] (M)	0,383	0,382	0,377	0,383
-10 log [SP] (M)	3,821	3,809	3,757	3,825
-9 log [SP] (M)	37,245	37,004	35,643	37,365
-8 log [SP] (M)	95,915	90,388	71,125	98,638
-7 log [SP] (M)	97,117	91,51	71,926	99,92
-6 log [SP] (M)	97,197	91,63	72,006	100

Table 6: Table of the obtained results for $K_{on1} = 10$ nM^{-1}min^{-1}, $K_{off1} = 0.0001$ min^{-1} (A) and $K_{on1} = 100$ nM^{-1}min^{-1}, $K_{off1} = 0.00001$ min^{-1} (B). Here $R_{tot} = 2.5$ nM and SP is used as agonist.

A	0.07	0.21	0.7	NKA
-9,8 log [NKA] (M)	0,08	0,076	0,06	0,082
-8,8 log [NKA] (M)	0,794	0,75	0,593	0,814
-7,8 log [NKA] (M)	7,418	7,017	5,533	7,578
-6,8 log [NKA] (M)	44,146	41,62	32,719	45,389
-5,8 log [NKA] (M)	87,009	81,997	64,435	89,575
-4,8 log [NKA] (M)	96,191	90,658	71,411	98,957
-3,8 log [NKA] (M)	97,434	91,82	72,093	100
B	0.07	0.21	0.7	NKA
-9,8 log [NKA] (M)	0,08	0,076	0,06	0,082
-8,8 log [NKA] (M)	0,794	0,75	0,593	0,814
-7,8 log [NKA] (M)	7,418	7,017	5,493	7,578
-6,8 log [NKA] (M)	44,146	41,62	32,719	45,389
-5,8 log [NKA] (M)	87,009	81,997	64,435	89,575
-4,8 log [NKA] (M)	96,191	90,658	71,411	98,957
-3,8 log [NKA] (M)	97,434	91,62	72,173	100

Table 7: Table of the obtained results for $K_{on1} = 10$ nM^{-1}min^{-1}, $K_{off1} = 0.0001$ min^{-1} (A) and $K_{on1} = 100$ nM^{-1}min^{-1}, $K_{off1} = 0.00001$ min^{-1} (B). Here $R_{tot} = 2.5$ nM and NKA is used as agonist.

8.2 The influence of R_{tot}

As stressed multiple times R_{tot} is also an important unknown constant. This subsection illustrates the role R_{tot} plays in the agonist-receptor complex concentrations between different concentrations of antagonist with different doses of agonist. The same method is used as described in the previous section. Results

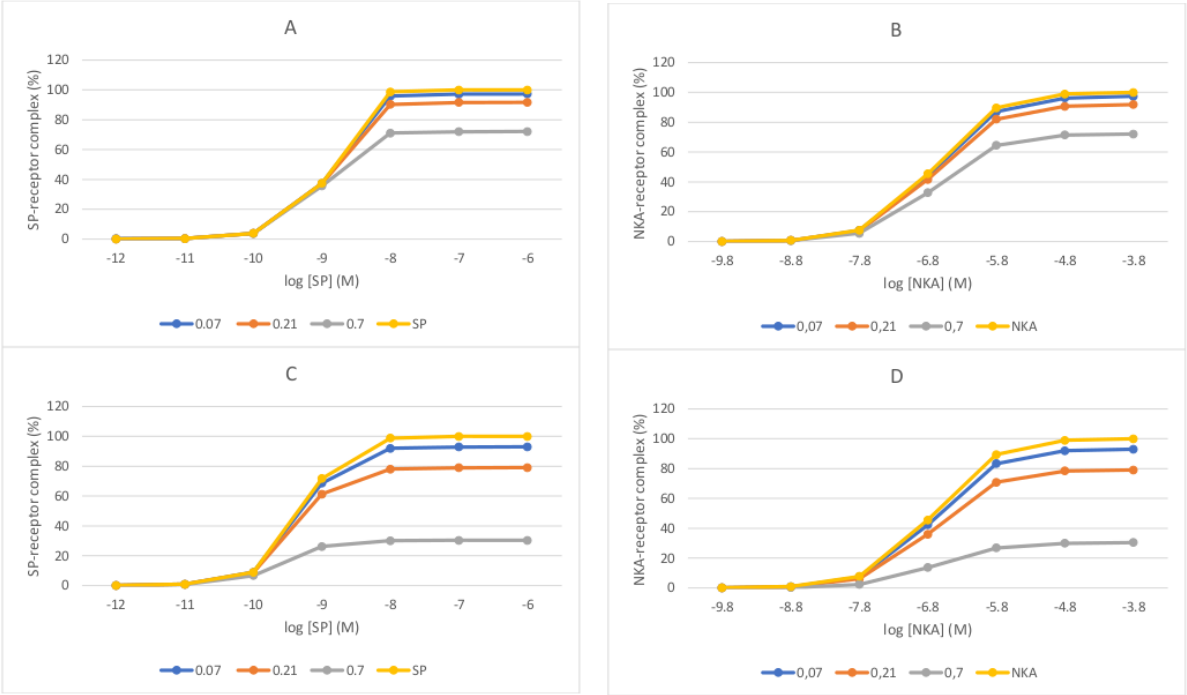

Figure 20: Results for different values for R_{tot} with $K_{on1} = 10$ nM^{-1}min^{-1} and $K_{off1} = 0.0001$ min^{-1}. A: $R_{tot} = 2.5$ and SP is used as agonist. B: $R_{tot} = 2.5$ and NKA is used as agonist. C: $R_{tot} = 1$ and SP is used as agonist. D: $R_{tot} = 1$ and NKA is used as agonist.

with $R_{tot} = 2.5$ and $R_{tot} = 1$ nM with $K_{on1} = 10$ nM^{-1}min^{-1}, $K_{off1} = 0.0001$ min^{-1} are compared. Figure 20 shows the results for the different values for R_{tot}. It can be seen that there is more difference between different concentrations of antagonist when $R_{tot} = 1$ nM compared to $R_{tot} = 2.5$ nM. This is true for both agonists. This can be explained as follows. The antagonist can block a bigger part of the total available receptors when less R_{tot} is available. Thus there is not enough receptor available for the agonists when more antagonist is added. Furthermore, the antagonist is more prone to bind compared to the agonist due to the chosen K_{on1} and K_{off1} value. This causes that free antagonist has more chance to bind than free agonist to free receptor and thus that agonist-receptor complex concentrations decreases. Thus the R_{tot} value has a big influence on the behavior of the plots. Therefore it is important to know the exact value of R_{tot}.

9 Conclusion

In this thesis, we formulated several models for binding of one or two ligands to a single receptor in terms of systems of differential equations. Analysis of these equations have been performed and an expression for the steady state is obtained. The models have been linked to experiments performed in the article [4]. Furthermore, several approaches have been taken in order to estimate the unknown K_{on1} and K_{off1} values of the antagonists DFA and aprepitant from the article. We will summarize these approaches and conclusions for these values will be drawn.

There are nine possible combinations of intervals for K_{on1} and K_{off1} which contain the values for antagonists DFA and aprepitant. A value in each interval was chosen and figures were made in order to compare with the original results (figures 7 and 8). There are a few difficulties regarding this comparison. First, an important constant, namely R_{tot}, is unknown. The model for the B_2 expression was used to compare results between the experiment with results shown in figure 5 and the results using this model with different amounts of R_{tot} concentrations. This showed that R_{tot} concentrations of 10 and 2.5 nM in comparison to 1 nM give the best features compared to the original results. A second difficulty is the difference in measurement on the y-as between the real experiment and the model. The model measures the agonist-receptor complex concentration (SP or NKA bound to a receptor), whereas the real experiment measures cAMP in RLU. The relationship between these two different measurement is as follows: cAMP is released when agonist is bound to a receptor. However, it is unknown how much cAMP is released per agonist-receptor complex and the ratio between cAMP and RLU is also unknown. Therefore, it is assumed that a higher amount of agonist-receptor complex concentration correspond to a higher amount of RLU in order to be able to compare the results. Lastly, the model does not take into account varying circumstances while this does happen in the real experiment. For example it is assumed that the R_{tot} value is the same in all experiments but it is likely that there is some variation between experiments. Furthermore, the experiments are performed by humans and this can cause (minor) differences between experiments. For example, doses of agonist or concentrations of antagonist can be slightly divergent. Simulations have been made with R_{tot} values of 10 and 2.5 nM and chosen values from all nine combination of intervals. Then figures were generated and these showed only two possible combinations of intervals for both antagonists DFA and aprepitant. Only the combination of intervals $0.001 < K_{on1} < 0.24$ with $K_{off1} < 0.027$ and $0.24 < K_{on1}$ with $K_{off1} < 0.027$ showed some difference between different amount of antagonist concentration. Furthermore, the results from the simulation with chosen R_{tot} of 2.5 nM showed more difference between different doses of antagonist and are therefore more similar to figure 7 and 8 than the results from the simulation with R_{tot} of 10 nM. The graphs B and C in figures 18 and 19 with the two combinations of intervals with R_{tot} of 2.5 nM are quite similar to figures 7 and 8, especially to the graphs with DFA as antagonist. However, further investigation is needed in order to obtain more differences between different amount of antagonist. This is seen in the figures 7 and 8 with especially aprepitant as antagonist. First it was seen that there were small differences between the intervals $0.001 < K_{on1} < 0.24$ combined with $K_{off1} < 0.027$ and $0.24 < K_{on1}$ combined with $K_{off1} < 0.027$. Therefore new results were obtained with higher K_{on1} and lower K_{off1} values in order to investigate if this betters the differences between different amounts of antagonist. It was seen that this does not significantly improve the results and the values between different K_{on1} and K_{off1} were very similar. This means that the antagonist already binds faster and unbinds slower to the receptor compared to the agonists SP and NKA that even improvement of the binding kinetics of the antagonist (higher K_{on1} and lower K_{off1}) does not make any difference in agonist-receptor complex concentrations. Again R_{tot} was considered in order to obtain more similar figures to the experiment. The results (figure 20) showed that reduction of R_{tot} led to more difference in agonist-receptor complex concentrations between different concentrations of antagonist. Thus R_{tot} has a big influence on the results.

It is only possible to give an estimate of a lower limit for K_{on1} and an upper limit for K_{off1} for the antagonists DFA and aprepitant due to lack of enough available results from the article. First the lower limit for K_{on1} is explained. The obtained results in Chapter 7.2, 7.3 and 8.1 showed clear difference between different amounts of antagonist for $K_{on1} \geq 0.01$ $\text{nM}^{-1}\text{min}^{-1}$. Therefore, the lower limit for both antagonists DFA and aprepitant is estimated at 0.01 $\text{nM}^{-1}\text{min}^{-1}$. Little improvements in difference between different amounts of antagonist could be seen when K_{on1} was chosen higher (table 4, 5, 6 and 7). However these differences when K_{on1} was chosen higher were at a maximum of circa 1 % decrease of agonist-receptor complex concentration. This could imply a slightly decrease in release of cAMP

(recall that when the agonist binds to the receptor cAMP is released). It is unknown, but unlikely assumed that this gives serious change in RLU measurement. Thus it is not assumed that a decrease less than 1 % of agonist-receptor complex concentration (simulation) would cause a notable difference in RLU measurement (experiment). The upper limit for K_{off1} is estimated at 0.001 min^{-1}. Higher values of K_{off1} gave no difference between different amount of antagonist. Again little improvements were seen when K_{off1} was chosen smaller, but not enough to conclude that that causes any changes in RLU measurement. It is not possible to distinguish lower and upper limits between the two different antagonists DFA and aprepitant due to lack of available results. However, based on figures 7 and 8 from the article it is hypothesized that the K_{on1} value for DFA is lower and the K_{off1} value is higher than aprepitant. Increasing concentrations of antagonist DFA shows less decrease in RLU compared to increasing concentrations of antagonist aprepitant. This implies that aprepitant has better antagonistic effects and therefore binds faster and unbinds slower to the receptor compared to DFA. This corresponds to a higher K_{on} and lower K_{off} value compared to DFA.

Further experiments are needed in order to improve the estimate for the values for K_{on1} and K_{off1} for the antagonists. The next experiments are recommended. First, it is desired to have a good estimate for R_{tot}. Literature has shown that there are multiple experiments that could give a good indication for the value for R_{tot}. The article by Pollard et al [5] discusses various methods that can give more information about the value for R_{tot}. These experiments will not be discussed here since a lot of knowledge on molecular biology of the cell is needed. The concentration dependent cAMP assay described in Chapter 6 (page 11) is recommended in order to determine an upper bound for K_{on1} and a lower bound for K_{off1}. Better comparisons can be made when this experiment is performed with other agonists. These agonists should have different values for K_{on} (higher than 0.01 $\text{nM}^{-1}\text{min}^{-1}$) and K_{off} (lower than 0.001 min^{-1}) and the same antagonists DFA and aprepitant should be used. The problem we face now with estimating the upper bound for K_{on1} is that increasing values for K_{on1} do not significantly improve the antagonistic effects (as seen in tables 4, 5, 6 and 7). The antagonist already has so much better affinity to the receptor compared to the agonist that improvement of the binding kinetics for the antagonist does not decrease the agonist-receptor complex concentrations. In other words at a certain point the antagonist does not 'battle' anymore with the agonist over binding to the receptor. Thus changing the K_{on1} and K_{off1} value for better association and lesser disassociation does not improve the results. However, if the same experiment is performed with agonists that have higher K_{on} values it is possible to see competition between the agonist and antagonist when higher K_{on1} values are chosen. The same holds for agonists with lower K_{off} values. Thus, we hypothesize that the simulation will show visible differences for higher K_{on1} and lower K_{off1} values compared to lower K_{on1} and higher K_{off1} values and this could then be compared to the results from the experiment.

References

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10 Appendix

This section contains the matlab files used in this thesis.

10.1 Code model 1

This code was used to generate model 1 figures.

```
function [res] = pareq(t,y)

Kon1=0.24;
Koff1=0.027;
Rtot=2.5;

ode1 = -Kon1*y(1)*(Rtot-y(2)) + Koff1*y(2);
ode2 = Kon1*y(1)*(Rtot-y(2)) - Koff1*y(2);

res = [ode1, ode2]';

clear

L1 = 10;
B1 = 0;
y0 = [L1; B1];
tspan = [0 10];
[t,y] = ode23(@pareq,tspan,y0);
plot(t,y,'-o');
xlabel('t (min)')
```

```
ylabel('nM')
legend('L1','B1','Location','North')
```

10.2 Code model 2

This code was used to generate model 2 figures.

```
function [res] = pareqtwee(t,y)

Kon1=0.01;
Koff1=0.001;
Kon2=0.24;
Koff2=0.027;
Rtot=2.5;

ode1 = -Kon1*y(1)*(Rtot-y(2)-y(4)) + Koff1*y(2);
ode2 = Kon1*y(1)*(Rtot-y(2)-y(4)) - Koff1*y(2);
ode3 = -Kon2*y(3)*(Rtot-y(2)-y(4))+ Koff2*y(4);
ode4 = Kon2*y(3)*(Rtot-y(2)-y(4))- Koff2*y(4);

res = [ode1,ode2,ode3,ode4]';

clear

a=0.01; %Kon1
b=0.001; %Koff1
c=2.5; %Rtot
d=0.7; %L1(0) normaal

L1 = ((-a*c)+(a*d)-b+sqrt(((-a*c)-(a*d)-b)^2-(4*a^2*d*c)))/(2*a);
B1 = ((a*c)+(a*d)+b-sqrt(((-a*c)-(a*d)-b)^2-(4*a^2*d*c)))/(2*a);
L2 = 0.1;
B2 = 0;
y0 = [L1; B1; L2; B2];
tspan = [0 55];
[t,y] = ode23(@pareqtwee,tspan,y0);
plot(t,y,'-o');
xlabel('t (min)')
ylabel('nM')
legend('L1','B1','L2','B2','Location','North')
```

10.3 Code expression B_2 over time

This code was used to generate figures that describe the progression of B_2 over time.

```
kon1 = 1;
L1 = NaN(1000,1);
```

```
L1(1) = 1;
Rtot = 1;
koff1 = 0.01;
tspan = 0:0.001:50;

%functions
Amin = @(a,b,c) (-b + sqrt(b^2-4*a*c))/(2*a);
Aplus = @(a,b,c) (-b-sqrt(b^2-4*a*c))/(2*a);

%constants
a = kon1;
b = -kon1*L1(1)-Rtot*kon1-koff1;
c = kon1*L1(1)*Rtot;


const = log(Aplus(a,b,c)/Amin(a,b,c))/(Aplus(a,b,c)-Amin(a,b,c));

B1 = @(t) ( (Amin(a,b,c) - Aplus(a,b,c)) / (exp((t+const)*(Aplus(a,b,c)-Amin(a,b,c)))

complex = NaN(size(tspan,2),1);
for i = 1:size(tspan,2)
    complex(i) = B1(tspan(i));
end

plot(tspan,complex,'LineWidth',3);
xlabel('t (min)')
ylabel('nM B1')
```