Chapter 3
Models of Open and Closed State Blockers

So far we have studied a one-dimensional model of calcium-induced calcium release. The analysis started with a stochastic differential equation modeling release from internal storage to the dyad. We found that this model could be analyzed using Monte Carlo simulations or a system of deterministic partial differential equations giving the probability density functions of the open and the closed states. Furthermore, we found analytical solutions of the stationary solutions of the probability density system.

The aim of the present chapter is to introduce mathematical models of a drug and then show how the parameters defining the drug can be computed so that it works as well as possible. For simplicity, we will focus on closed to open mutations (CO-mutations; see page 16), but it will become clear how to handle open to closed mutations (OC-mutations) in later chapters.

Let us start by recalling that the Markov model governing the states of the channel is given by

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O. \tag{3.1}$$

When a CO-mutation is present, we introduce the mutation severity index μ and replace the reaction rate k_{co} by μk_{co},

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O. \tag{3.2}$$

Obviously, $\mu = 1$ represents the wild type case and the size of $\mu > 1$ gives the strength of the mutation. By recalling what the Markov model means, we see that the mutation increases the probability of going from the closed to the open state and thus the open state probability will increase.

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In this chapter, we will study theoretical open and closed state blockers. We recall from Chap. 1 that open and closed state blockers can be presented in the forms

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \underset{k_{ob}}{\overset{k_{bo}}{\leftrightarrows}} B \tag{3.3}$$

and

$$B \underset{k_{bc}}{\overset{k_{cb}}{\leftrightarrows}} C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{3.4}$$

respectively. The reasoning behind this way of modeling the effect of a drug was discussed on page 18 above; see in particular Fig. 1.9. Basically, we assume that the drug introduces a new conformational state of the channel protein that can be attained via the open state (for open state blockers) or via the closed state (for closed state blockers). The blocked states are always assumed to be non-conducting.

The mathematical problem of finding a suitable theoretical drug is now to find the parameters k_{bc} and k_{cb} for the closed state blockers and k_{bo} and k_{ob} for the open state blockers such that the effect of the mutation is reduced as much as possible. We will see that this problem is much easier using the probability density approach than using Monte Carlo simulations.

To compute optimal drugs for the CO-mutation, we will first consider the equilibrium states of the reactions. For closed state blockers, we can use the equilibrium considerations to reduce the number of free parameters from two to one. In principle, this can also be done for open state blockers, but some averaging is needed in the process and optimality is not obtained. For the closed state blocker, we can use the steady state system derived above to completely characterize both parameters of the drug to obtain optimality and computations will show that the resulting drug is theoretically extremely good and asymptotically perfect in the sense that it completely reverses the effect of the mutation. We are also able to derive a good open state blocker, but the method is less satisfactory and the results are not as good as for the closed state blocker.

3.1 Markov Models of Closed State Blockers for CO-Mutations

We start the derivation of theoretical drugs by considering closed state blockers. The reaction scheme of a closed state blocker takes the form

$$B \underset{k_{bc}}{\overset{k_{cb}}{\leftrightarrows}} C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{3.5}$$

where the reaction rates of the drug given by k_{cb} and k_{bc} must be determined so that the mutated cell behaves as similarly to the wild type cell as possible. We regard these parameters as free and we seek to compute them to obtain optimal efficiency of the theoretical drug. Allow us also to briefly repeat that this is basically our definition of a theoretical drug as discussed on page 18.

3.1.1 Equilibrium Probabilities for Wild Type

Consider the Markov model given by

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O$$

and let o denote the probability of being in the open state and c the probability of being in the closed state. Suppose the channel just flickers between open and closed and nothing else happens. Then the equilibrium probabilities are characterized by

$$k_{co}c = k_{oc}o. \tag{3.6}$$

This means that the channel keeps on flickering in equilibrium and the probabilities of the open and closed states satisfy the relation (3.6). From this relation it follows that

$$c = \frac{k_{oc}}{k_{co}}o$$

and then, since $o + c = 1$, we obtain

$$o = \left(1 + \frac{k_{oc}}{k_{co}}\right)^{-1}.$$

3.1.2 Equilibrium Probabilities for the Mutant Case

In the CO-mutation case, we assume that the rate from C to O is increased and we define

$$k_{co,\mu} = \mu k_{co}, \tag{3.7}$$

where $\mu \geqslant 1$ and $\mu = 1$ denotes wild type. The equilibrium open probability of the mutant is given by

$$o_\mu = \left(1 + \frac{k_{oc}}{\mu k_{co}}\right)^{-1},$$

which clearly increases with increasing values of μ.

3.1.3 Equilibrium Probabilities for Mutants with a Closed State Drug

The equilibrium probabilities of reaction (3.5) are characterized by

$$\begin{aligned} \mu k_{co} c &= k_{oc} o, \\ k_{bc} b &= k_{cb} c; \end{aligned}$$

so

$$\begin{aligned} c &= \frac{k_{oc}}{\mu k_{co}} o, \\ b &= \frac{k_{cb}}{k_{bc}} c = \frac{k_{cb}}{k_{bc}} \frac{k_{oc}}{\mu k_{co}} o, \end{aligned}$$

and, since $o + c + b = 1$, we obtain

$$\left(1 + \frac{k_{oc}}{\mu k_{co}} + \frac{k_{cb}}{k_{bc}} \frac{k_{oc}}{\mu k_{co}}\right) o = 1.$$

So

$$o = \left(1 + \frac{k_{oc}}{\mu k_{co}} \left(1 + \frac{k_{cb}}{k_{bc}}\right)\right)^{-1}.$$

Define

$$\delta_c = \frac{k_{cb}}{k_{bc}} \tag{3.8}$$

and note that, in equilibrium, the wild type open probability is given by

$$o = \left(1 + \frac{k_{oc}}{k_{co}}\right)^{-1}$$

and the drugged mutant open probability is given by

$$o_{\mu,\delta_c} = \left(1 + \frac{k_{oc}}{k_{co}} \frac{1+\delta_c}{\mu}\right)^{-1}.$$

Now, we want to choose the drug characterization δ_c such that $o_{\mu,\delta_c} \approx o$ and this can clearly be achieved by requiring that

$$\frac{1+\delta_c}{\mu} \approx 1$$

or

$$\delta_c \approx \mu - 1.$$

So we obtain the characterization

$$k_{cb} = (\mu - 1)k_{bc}. \tag{3.9}$$

This means that, for the closed state blocker, we reduced the number of parameters characterizing the blocker from two to one. We will use the probability density approach to determine the remaining degree of freedom.

3.2 Probability Density Functions in the Presence of a Closed State Blocker

The probability density approach to the stochastic model in the presence of a closed state drug is

$$\begin{aligned}
\frac{\partial \rho_o}{\partial t} + \frac{\partial}{\partial x}(a_o \rho_o) &= \mu k_{co} \rho_c - k_{oc} \rho_o, \\
\frac{\partial \rho_c}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_c) &= k_{oc} \rho_o - (\mu k_{co} + k_{cb})\, \rho_c + k_{bc} \rho_b, \\
\frac{\partial \rho_b}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_b) &= k_{cb} \rho_c - k_{bc} \rho_b,
\end{aligned}$$

where

$$\begin{aligned}
a_o &= v_r(c_1 - x) + v_d(c_0 - x), \\
a_c &= v_d(c_0 - x).
\end{aligned}$$

From (3.9), the parameters of the drug are related by

$$k_{cb} = (\mu - 1)\, k_{bc}; \tag{3.10}$$

so the system is

$$\begin{aligned}
\frac{\partial \rho_o}{\partial t} + \frac{\partial}{\partial x}(a_o \rho_o) &= \mu k_{co} \rho_c - k_{oc} \rho_o, \\
\frac{\partial \rho_c}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_c) &= k_{oc} \rho_o - (\mu k_{co} + (\mu - 1)\, k_{bc})\, \rho_c + k_{bc} \rho_b, \\
\frac{\partial \rho_b}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_b) &= (\mu - 1)\, k_{bc} \rho_c - k_{bc} \rho_b.
\end{aligned}$$

In the stationary case, we obtain the system

$$\frac{\partial}{\partial x}(a_o \rho_o) = \mu k_{co} \rho_c - k_{oc} \rho_o, \tag{3.11}$$

$$\frac{\partial}{\partial x}(a_c \rho_c) = k_{oc} \rho_o - (\mu k_{co} + (\mu - 1)\, k_{bc})\, \rho_c + k_{bc} \rho_b, \tag{3.12}$$

$$\frac{\partial}{\partial x}(a_c \rho_b) = (\mu - 1)\, k_{bc} \rho_c - k_{bc} \rho_b. \tag{3.13}$$

In this system, the mutation severity is given by μ and the drug is characterized by a single parameter given by k_{bc}. For a given value of μ our aim is now to compute the value of k_{bc} such that the probability density function of the open state given by this system is as similar as possible to the probability density function of the open state in the case of $\mu = 1$, that is, the wild type solution when no drug is applied.

3.2.1 *Numerical Simulations with the Theoretical Closed State Blocker*

We consider a mutation characterized by $\mu = 3$ and we apply closed state blockers (see reaction scheme (3.5)) with parameters satisfying the relation (3.10). In Fig. 3.1, we show the results of these simulations using the Monte Carlo approach: The lower panel of the figure is the same as the upper panel, except that we focus on concentrations ranging from 80 to 91 μM . We observe significant differences between the wild type solution and the solution representing the mutation. Furthermore, we observe that the drug works quite well. Similar results are given in Fig. 3.2, where the computations are based on the probability density approach: Here the lower panel focuses on very high concentrations ranging from 89 to 91 μM. We also see

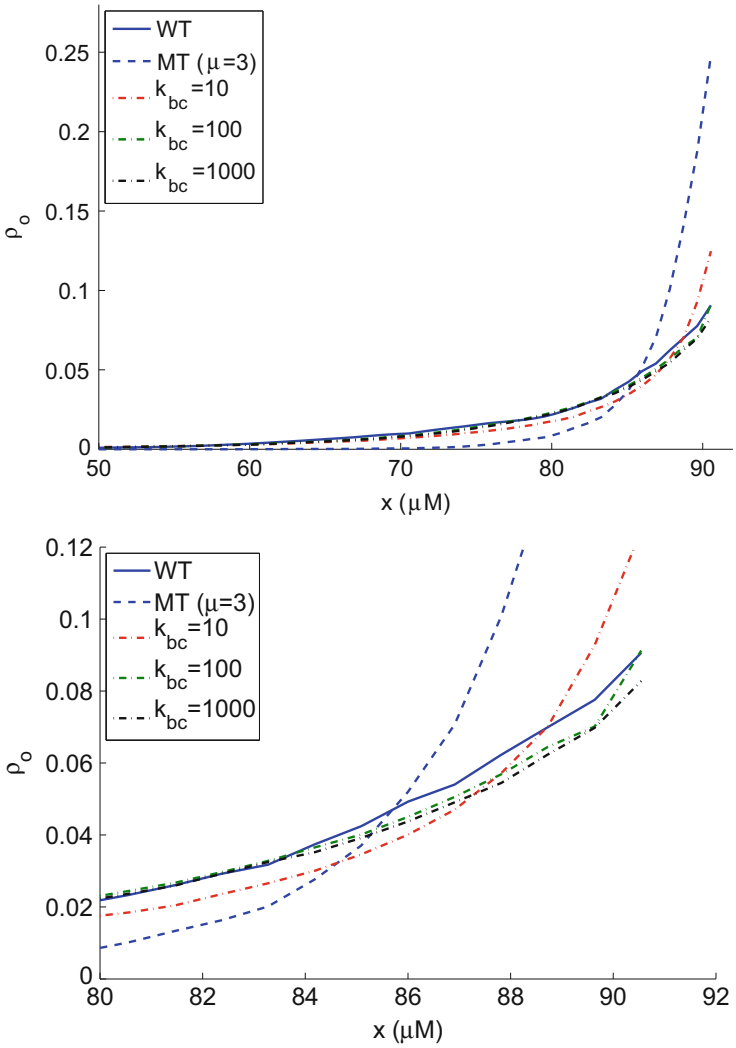

Fig. 3.1 Monte Carlo simulations using the theoretical closed state blocker given by the reaction scheme (3.5), where the reaction rates are related by (3.10) and the mutation severity index is given by $\mu = 3$. The lower panel focuses on higher levels of concentrations

that the closed state drug improves as the value of k_{bc} increases. In fact, the result seems to indicate that the drug is asymptotically perfect in the sense that the solution converges toward the wild type solution when $k_{bc} \to \infty$. Model parameters for these simulations are given in Table 3.1.

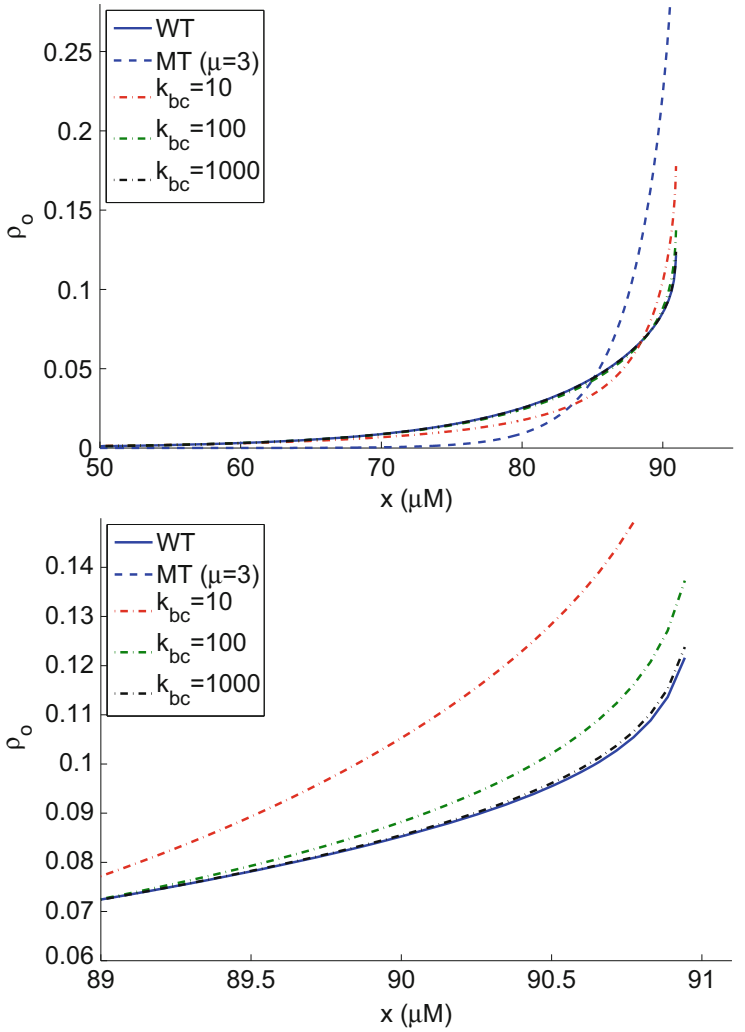

Fig. 3.2 Numerical solutions of the steady state probability density functions defined by the system (3.11)–(3.13), where the reaction rates are related by (3.10) and the mutation severity index is given by $\mu = 3$. The *lower panel* focuses on higher levels of concentrations. Note that the concentration axis of this figure is different from that of the *lower panel* of Fig. 3.1

Table 3.1 Parameter values for the undrugged case

v_d	1 ms^{-1}
v_r	0.1 ms^{-1}
c_0	0.1 μM
c_1	1,000 μM
$k_{co}(x)$	$0.1x$ ms^{-1} μM^{-1}
k_{oc}	1 ms^{-1}

3.3 Asymptotic Optimality for Closed State Blockers in the Stationary Case

In the simulations above, we observed that the closed state blocker worked well and that the drug became more effective as the value of k_{bc} increased. Our aim is now to indicate that, when $k_{bc} \to \infty$, the drug will completely repair the mutation. It is worth mentioning that the possibility of making a drug with $k_{bc} = \infty$ is quite unlikely, but the asymptotic result is still of theoretical interest.

Consider the steady state system

$$\frac{\partial}{\partial x}\left(a_o \rho_o\right) = \mu k_{co} \rho_c - k_{oc} \rho_o, \tag{3.14}$$

$$\frac{\partial}{\partial x}\left(a_c \rho_c\right) = k_{oc} \rho_o - \left(\mu k_{co} + (\mu - 1)\, k_{bc}\right) \rho_c + k_{bc} \rho_b, \tag{3.15}$$

$$\frac{\partial}{\partial x}\left(a_c \rho_b\right) = (\mu - 1)\, k_{bc} \rho_c - k_{bc} \rho_b. \tag{3.16}$$

By adding all the equations, we obtain

$$\frac{\partial}{\partial x}\left(a_o \rho_o + a_c \left(\rho_c + \rho_b\right)\right) = 0. \tag{3.17}$$

From the boundary conditions, we obtain

$$a_o \rho_o + a_c \left(\rho_c + \rho_b\right) = 0 \tag{3.18}$$

and therefore

$$\rho_c = \frac{-1}{a_c}\left(a_o \rho_o + a_c \rho_b\right), \tag{3.19}$$

where we recall that $a_c < 0$ for $x \in (c_0, c_+)$. Now, the system (3.14)–(3.16) can be rewritten in the form

$$\frac{\partial}{\partial x}\left(a_o \rho_o\right) = -\mu k_{co} \rho_b - \left(\frac{\mu k_{co} a_o}{a_c} + k_{oc}\right) \rho_o, \tag{3.20}$$

$$\frac{1}{k_{bc}} \frac{\partial}{\partial x}\left(a_c \rho_b\right) = -\left(\mu - 1\right) \frac{a_o}{a_c} \rho_o - \mu \rho_b. \tag{3.21}$$

We are interested in solutions of this system as k_{bc} becomes very large and we therefore note that, in the limit as $k_{bc} \to \infty$, the second equation yields

$$\rho_b = -\frac{(\mu - 1)}{\mu} \frac{a_o}{a_c} \rho_o \tag{3.22}$$

and therefore the first equation becomes

$$\frac{\partial}{\partial x}(a_o\rho_o) = -\mu k_{co}\rho_b - \left(\frac{\mu k_{co}a_o}{a_c} + k_{oc}\right)\rho_o \tag{3.23}$$

$$= k_{co}(\mu - 1)\frac{a_o}{a_c}\rho_o - \left(\frac{\mu k_{co}a_o}{a_c} + k_{oc}\right)\rho_o \tag{3.24}$$

$$= -\left(k_{co}\frac{a_o}{a_c} + k_{oc}\right)\rho_o. \tag{3.25}$$

So

$$\frac{\partial}{\partial x}(a_o\rho_o) = -\left(k_{co}\frac{a_o}{a_c} + k_{oc}\right)\rho_o. \tag{3.26}$$

Recall that the wild type model is

$$\frac{\partial}{\partial x}(a_o\rho_o) = -\left(k_{co}\frac{a_o}{a_c} + k_{oc}\right)\rho_o \tag{3.27}$$

(see (2.36)). By comparing (3.26) and (3.27), we see that when the drug is chosen to be of the form

$$k_{cb} = (\mu - 1)k_{bc}$$

and when we let $k_{bc} \to \infty$, the drug *completely* repairs the probability density functions of the mutated cell.

3.4 Markov Models for Open State Blockers

Next, we want to consider models of open state blockers. The reaction scheme of an open state blocker for the mutant reads

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \underset{k_{ob}}{\overset{k_{bo}}{\leftrightarrows}} B.$$

The equilibrium probabilities are now characterized by

$$\mu k_{co}c = k_{oc}o,$$
$$k_{bo}b = k_{ob}o;$$

so

$$c = \frac{k_{oc}}{\mu k_{co}} o,$$
$$b = \frac{k_{ob}}{k_{bo}} o,$$

and since $o + c + b = 1$, we have

$$\left(1 + \frac{k_{oc}}{\mu k_{co}} + \frac{k_{ob}}{k_{bo}}\right) o = 1.$$

We now define the open state blocker characterization

$$\delta_o = \frac{k_{ob}}{k_{bo}}$$

and note that the open probability is given by

$$o_{\mu,\delta_o} = \left(1 + \frac{k_{oc}}{\mu k_{co}} + \delta_o\right)^{-1}.$$

Since the wild type open probability is given by

$$o = \left(1 + \frac{k_{oc}}{k_{co}}\right)^{-1},$$

we want to choose the drug such that $o_{\mu,\delta_o} \approx o$ and we therefore require

$$\frac{k_{oc}}{\mu k_{co}} + \delta_o \approx \frac{k_{oc}}{k_{co}}$$

or

$$\delta_{o,\mu} \approx \frac{k_{oc}}{k_{co}} \frac{\mu - 1}{\mu}, \tag{3.28}$$

where we recall that the mutation severity index $\mu \geqslant 1$. Since $\mu = 1$ is the wild type case, we note that in that case $\delta_o = 0$ is the optimal drug, which makes sense; there is no need to drug the wild type. However, for mutant cells, we have $\mu > 1$ and the characterization (3.28) of δ_o depends on the dyad calcium concentration, x. We will therefore use direct optimization to find suitable open state blockers.

3.4.1 Probability Density Functions in the Presence of an Open State Blocker

The probability density model in the presence of an open state drug is

$$\frac{\partial \rho_o}{\partial t} + \frac{\partial}{\partial x}(a_o \rho_o) = \mu k_{co} \rho_c - (k_{oc} + k_{ob})\rho_o + k_{bo}\rho_b, \tag{3.29}$$

$$\frac{\partial \rho_c}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_c) = k_{oc}\rho_o - \mu k_{co}\rho_c, \tag{3.30}$$

$$\frac{\partial \rho_b}{\partial t} + \frac{\partial}{\partial x}(a_c \rho_b) = k_{ob}\rho_o - k_{bo}\rho_b, \tag{3.31}$$

where we recall that

$$a_o = v_r(c_1 - x) + v_d(c_0 - x),$$
$$a_c = v_d(c_0 - x).$$

In the stationary case, we obtain the system

$$\frac{\partial}{\partial x}(a_o \rho_o) = \mu k_{co} \rho_c - (k_{oc} + k_{ob})\rho_o + k_{bo}\rho_b, \tag{3.32}$$

$$\frac{\partial}{\partial x}(a_c \rho_c) = k_{oc}\rho_o - \mu k_{co}\rho_c, \tag{3.33}$$

$$\frac{\partial}{\partial x}(a_c \rho_b) = k_{ob}\rho_o - k_{bo}\rho_b. \tag{3.34}$$

We let both k_{ob} and k_{bo} be free parameters and use the *Fminsearch* function in Matlab to optimize these parameters by minimizing the discrete l_2 difference[1] between the wild type and mutant ρ_o. The resulting parameters are $k_{ob} = 0.28\ \text{ms}^{-1}$, and $k_{bo} = 1.63\ \text{ms}^{-1}$ and the associated numerical results are given in Fig. 3.3, marked as *opt*.

3.5 Open Blocker Versus Closed Blocker

In Fig. 3.4, we compare the results of the best open state blocker (referred to as *opt* in Fig. 3.3) and closed state blocker, using $k_{bc} = 1{,}000\ \text{ms}^{-1}$ (see Fig. 3.2). We clearly see that the closed state blocker is better; in fact, at this resolution of

[1]The discrete l_2 difference between two vectors is given by $\|u - v\|_2 = (\sum_i (u_i - v_i)^2)^{1/2}$.

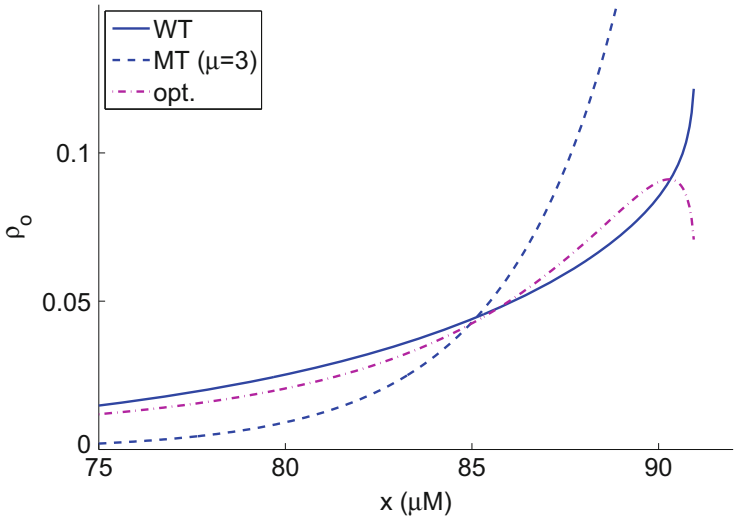

Fig. 3.3 Graphs of the numerical solutions using open state blockers. The open state blockers are based on optimization using the *Fminsearch* function in Matlab. In the simulation marked with *opt*, both parameters k_{ob} and k_{bo} are used in the minimization

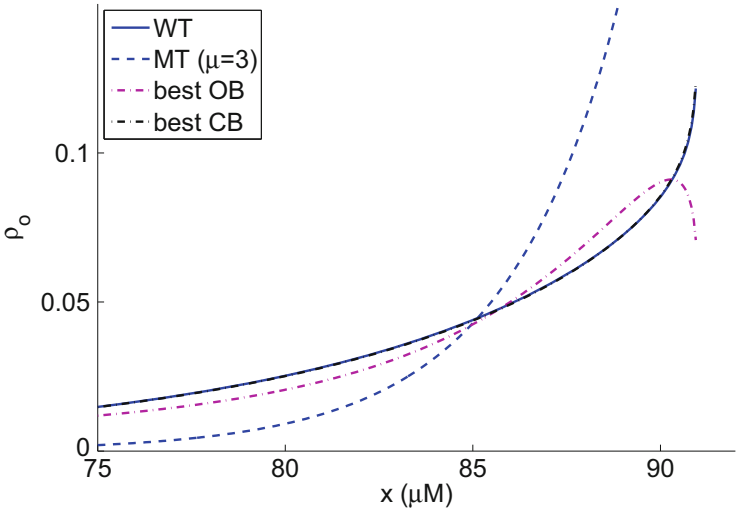

Fig. 3.4 Comparison of the best open state blocker and closed state blocker using $k_{bc} = 1{,}000$ ms^{-1} (see Table 3.2 for all the parameters of the two drugs). It is hard to distinguish between the wild type solution and the solution of the mutant case where the closed state blocker is applied

Table 3.2 Parameter values for the drugs used in Fig. 3.4

k_{ob}	0.28 ms^{-1}
k_{bo}	1.63 ms^{-1}
k_{cb}	2,000 ms^{-1}
k_{bc}	1,000 ms^{-1}

the graphs, it is hard to distinguish the wild type solution from the solution of the mutant case where the closed state blocker is applied.

3.6 CO-Mutations Does Not Change the Mean Open Time

To understand why the closed state blocker is much better than the open state blocker for CO-mutations, it is useful to recall that the mean open time of the Markov model

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \tag{3.35}$$

is given by

$$\tau_o = \frac{1}{k_{oc}}.$$

Thus the mean open time is independent of the mutation. If a closed state blocker is introduced as

$$B \underset{k_{bc}}{\overset{k_{cb}}{\leftrightarrows}} C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{3.36}$$

we clearly see that the mean open time is still given by

$$\tau_o = \frac{1}{k_{oc}}.$$

On the other hand, for an open state blocker of the form

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \underset{k_{ob}}{\overset{k_{bo}}{\leftrightarrows}} B, \tag{3.37}$$

the mean open time is changed and reads

$$\tau_o = \frac{1}{k_{oc} + k_{ob}}.$$

With a closed state blocker used to repair a CO-mutation, the mean open time is kept constant, as it should, but it is changed using an open state blocker. Consequently, it is hard to see how to derive an efficient open state blocker for a CO-mutation.

3.7 Notes

1. In this chapter we focused on CO-mutations (see page 16) and, for such mutations, closed state blockers are best suited from a theoretical perspective. We will see later that OC-mutations are more easily repaired using open state blockers.
2. The argument of asymptotic optimality given on page 63 is not a rigorous proof. To prove it mathematically, we have to take the boundary layer into consideration. Our derivation assumes smooth solutions but that assumption does not hold at the boundary.
3. In this section, we used probability density formulations for systems with more than two states. The general case of many states is presented in Appendix C of Huertas and Smith [35].
4. The mean open time will be introduced and analyzed in Chap. 13. In the present chapter we just used very basic properties.
5. We mentioned above that we used the function *Fminsearch* in Matlab to solve a minimization problem; see page 66. The *Fminsearch* function uses the Melder-Nead [58] algorithm studied by Lagarias et al. [46]. The method is very powerful and will be used routinely in these notes.
6. It is an underlying assumption for Markov models that the states of the model correspond to the conformational states of the channel protein. This should not be interpreted literally; rather, it has proved to be a useful modeling technique. A thorough discussion on the modeling of ion channels using Markov models and the models relation to the states of the protein is provided by Rudy and Silva [75].