**Supplemental material**

**for**

**Target-mediated Drug Disposition Model for Bispecific Antibodies: Properties, Approximation and Optimal Dosing Strategy**

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2. **QE and constant total receptor approximation**
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11. **QE approximation in free drug concentration and free receptors**

The full BsAb model in total drug concentration and total receptor variables reads with Eqs. (1)-(8),(11)-(13):

|  |  |
| --- | --- |
|  | (A10) |
|  | (A11) |
|  | (A12) |
|  | (A13) |
|  | (A14) |
|  | (A15) |
|  | (A16) |
|  | (A18) |

We assume that all four binding occasions shown in Figure 1a are rapid and that the principle of microscopic reversibility (18) holds. This leads to the algebraic equations

|  |  |
| --- | --- |
|  | (A19) |
|  | (A20) |
|  | (A21) |
|  | (A22) |

Eqs. (A19)-(A20) directly lead to Eqs. (20)-(21). The remaining Eqs. (A21)-(A22) yield

|  |  |
| --- | --- |
|  | (A23) |

Using , , Eqs. (20)-(21), then both realizations of Eq. (A23) are equivalent and we obtain

|  |  |
| --- | --- |
|  | (A24) |

The derivatives of the binary complex Eqs. (20)-(21) and ternary complex Eq. (A24) are

|  |  |
| --- | --- |
|  | (A25) |
|  | (A26) |
|  | (A27) |

Inserting Eqs. (A25)-(A27) into the total variables Eqs. (11)-(13) provides

|  |  |
| --- | --- |
|  | (A28) |
|  | (A29) |
|  | (A30) |

Combining Eqs. (A28)-(A30) with Eqs. (A11)-(A13) results in

|  |  |
| --- | --- |
|  |  |

with

and Eq. (19). Inverting the matrix gives matrix from Eq. (18) and we obtain the QE approximation Eqs. (15)-(19).

1. **QE and constant total receptor approximation**

Let relationship Eq. (23) hold. Using Eqs. (A12)-(A13) we obtain

|  |  |
| --- | --- |
|  | (A31) |

for Substituting the complexes Eqs. (20)-(22) in Eq. (A31) provides

|  |  |
| --- | --- |
|  | (A32) |
|  | (A33) |

In the following we solve the coupled equation system Eqs. (A32)-(A33) with respect to and . Rearranging Eq. (A32) regarding and using Eq. (23)-(24) yields Eq. (26). Substituting Eq. (26) in Eq. (A33) provides the quadratic equation

|  |  |
| --- | --- |
|  | (A34) |

for with according to Eqs. (28)-(30). Eq. (A34) possesses a unique positive solution for and for . This shows see Eqs. (27)-(30).

Finally, the reduced system for the QE approximation with both constant total receptors reads

|  |  |
| --- | --- |
|  | (A35) |

with

|  |  |
| --- | --- |
|  | (A36) |

and

|  |  |
| --- | --- |
|  | (A37) |

and according to Eqs. (26)-(30).

1. **Equivalence of the EB model representations**

We show here the equivalence of the two representations Eqs. (16), (18)-(22) with (31) and Eqs. (20)-(22), (26)-(31) of the EB model. Rewriting the EB model representation Eqs. (16), (18)-(22) with (31) in total concentrations, i.e. for an IV bolus with at yields

|  |  |  |
| --- | --- | --- |
|  |  | (A38) |
|  |  | (A39) |
|  |  | (A40) |

and the equations (20)-(22) for the BCs and the TC. The solution of Eqs. (A38)-(A40) is , , Moreover, since Eq. (31) implies Eq. (23) we can apply the results of section 2, supplemental material and obtain the representations Eqs. (26)-(30) for the receptors and .

1. **Derivation of Eq. (35)**

Our proof works in two steps. We first show that the binary complexes and saturate at , respectively, and second, that these saturations enforce the ternary complex to vanish, if the concentration of the free BsAb tends to infinity.

By construction, the free receptor satisfies Eq. (A34). Using , one simply derives

|  |  |
| --- | --- |
|  | (A41) |

with

|  |  |
| --- | --- |
|  |  |
|  |  |
|  |  |

for the binary complex . Moreover, and hold. Hence, satisfies the limit equation

|  |  |
| --- | --- |
|  |  |

which proves

|  |  |
| --- | --- |
|  | (A42) |

Then using Eqs. (26), (A42) we obtain

|  |  |
| --- | --- |
|  |  |

and, finally,

|  |  |
| --- | --- |
|  |  |

follows.

1. **Derivation of Eq. (38)**

In the total constant receptor situation without a peripheral compartment and we obtain from Eq. (A11)

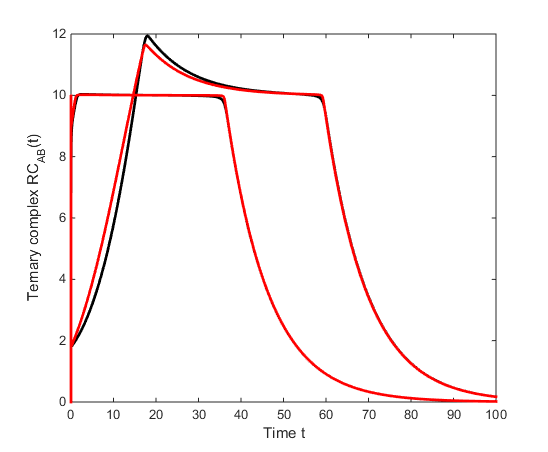
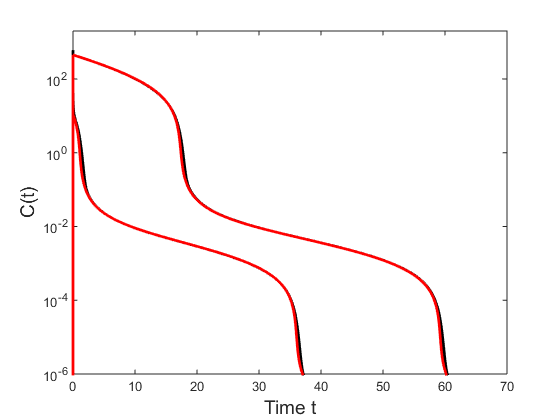
|  |  |
| --- | --- |
|  |  |

with and the solution representation Eq. (38) follows.

1. **Comparison of the free BsAb concentration from the full model with the QE approximation**

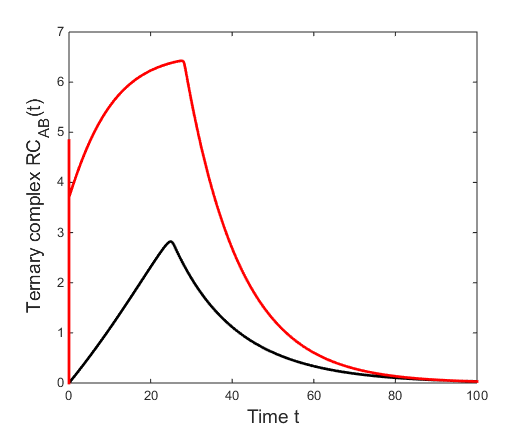
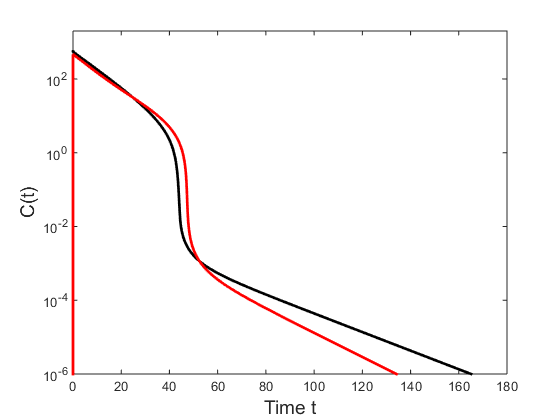
Comparison of the free BsAb concentration (panel A) and the ternary complex (panel B) from the full model with the QE approximation without a peripheral compartment and an IV bolus dose of 50 and 250 mg is shown in Figure S1 (black curve is the full BsAb model and red curve is the QE approximation). All model parameter values are taken from Table 2.

**A B**



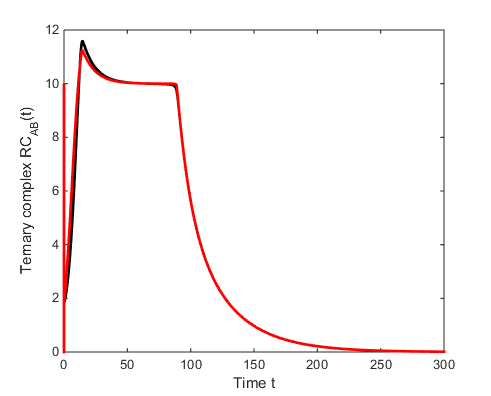
*Figure S1*

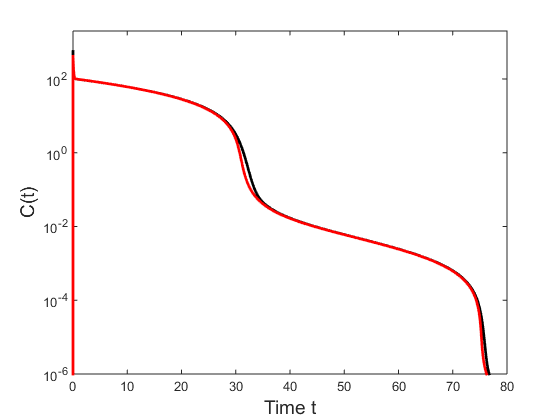
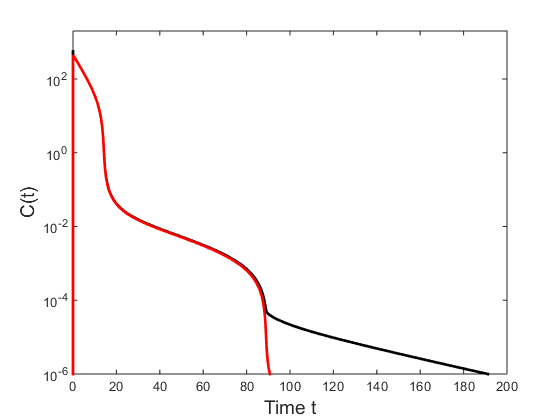
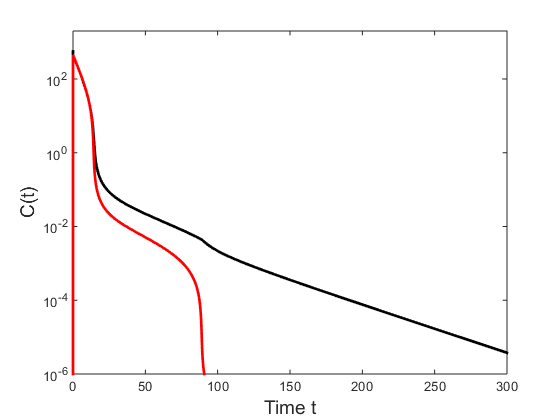
In Figure S2 the comparison is shown, if rapid binding is violated by = 0.0001 1/(nM day). Hence, we see that rapid binding is essential to guarantee a good approximation in free BsAb concentration as well as in the other model components as the ternary complex

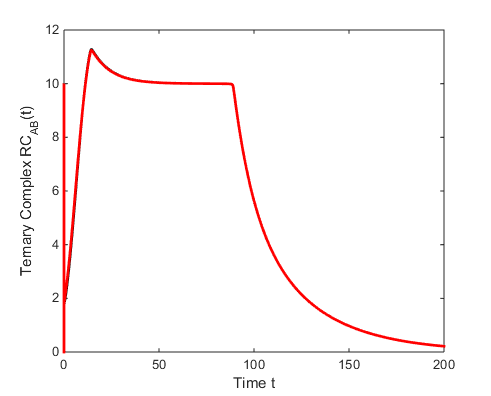


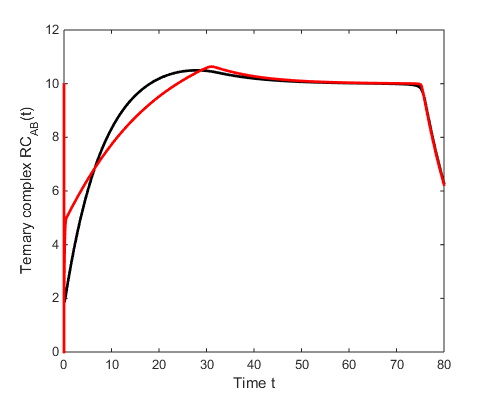
*Figure S2*

In the last comparison, a peripheral compartment is used. Figure S3, panel A shows the situation with the approximation for lower free BsAb concentrations. In fact, the full model (black) behaves quite differently than the approximation, suggesting that the rapid binding is partially violated. In panel B, the kon’s and koff’s are multiplied by the factor 100; please note that the dissociation constants stay the same. The full model is now closer to the approximation. In panel C, the rates between the central and peripheral compartment are multiplied by the factor 100, and full model and approximation behave equally. Panels D-F show the ternary complex for each situation.

**A D**



**B E**

** C** **F**

*Figure S3*

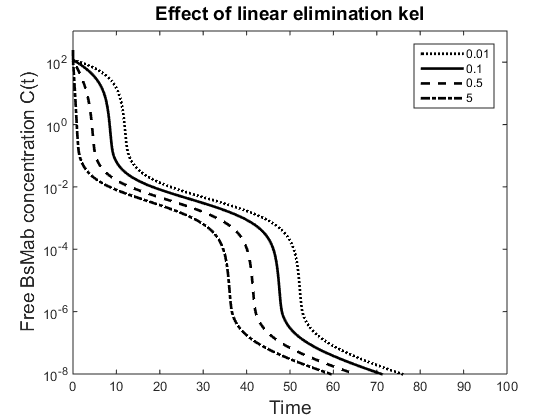
1. **Effects of varying binding parameters and total receptor concentration on the ternary complex**

To visualize the effect of varying binding parameters and total receptor concentration on the bell-shaped curve describing the relationship between and , we provide the Matlab implementation of the model Eqs. (20)-(22),(26)-(30):

🡺 Please see file Supp\_Code\_0.docx

1. **Effect of varying linear elimination and internalization rates on the free BsAb concentration profile**

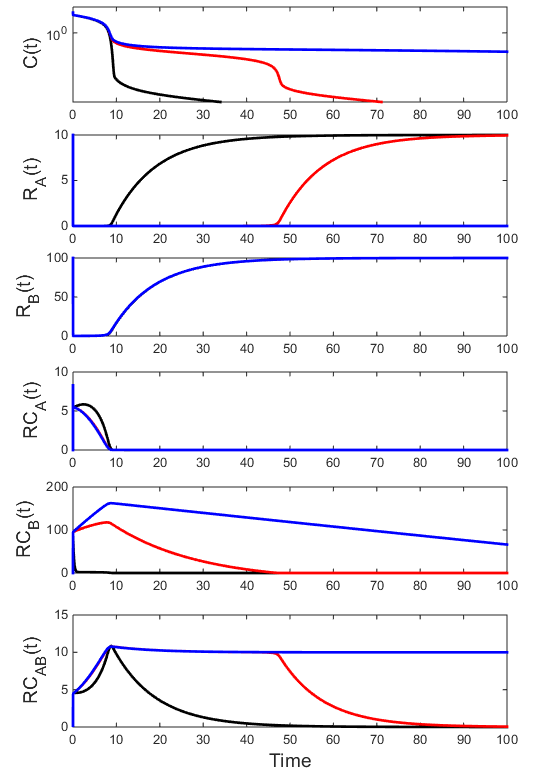
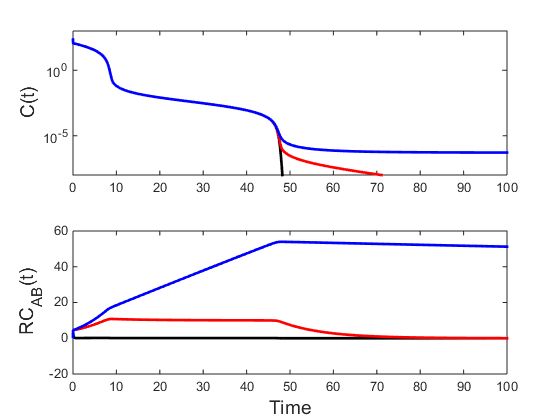
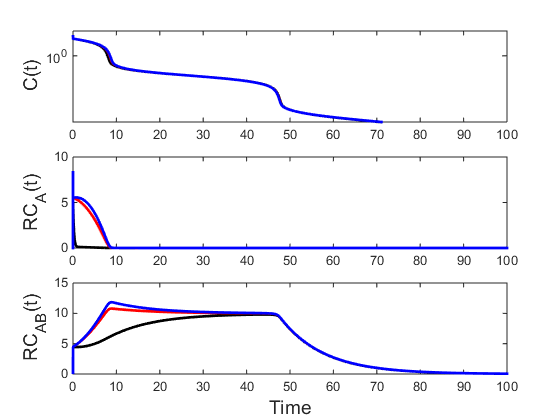
The effect of varying linear elimination rate is shown in Figure S4. For an increasing rate the free BsAb concentration profile shifts to the left.



*Figure S4*

For the effect of the three different internalization rates is shown. Only the relevant states with changes in their profile are visualized. For varying [0.0005, 0.05, 5] (blue, red, black) 1/day and fixed = 0.05 and = 0.1, nearly no effect on the free BsAb concentration profile could be seen, whereas the binary and ternary complex showed changes in their profile, see Figure S5 panel A. For varying [0.0005, 0.05, 5] (blue, red, black) and fixed = 0.05 and = 0.1, the free BsAb concentration profile in the first non-linear phase results in changing behaviour of the free receptors and additionally on all three complexes, see Figure S5 panel B. Finally, for [0.001, 0.1, 10] (blue, red, black) and fixed = 0.05 and = 0.01, an effect on the free BsAb concentration in the second non-linear phase is observed. Also the ternary complex showed strong dependency, see Figure S5 panel C.

**A B**



**C**

*Figure S5:* Effects of varying internalization rates are shown (panel A , panel B , and panel C ). Blue curves represent a very small value and black curves a very high value.

1. **General source codes for the implementation of the QE approximation and the full model and remarks regarding IV infusion and dataset**

As described in the Method Section, internal dosing mechanisms from Monolix and Nonmem cannot be used to handle IV infusion for the QE approximation. Therefore, we present a solution for Monolix and Nonmem to implement an IV infusion for the QE approximation. In both cases let tdur be the duration of infusion.

**Monolix**

As described in the Method Section, an additional dummy compartment Eq. (42)

can be used, which is simply added to the other model equations. The dummy compartment is then controlled via the tlag and “bioavailability” functionality. More precisely, with the depot command the dummy state is increased to a certain value for where is the dosing time point. The first depot command

depot(adm = 1,target = In,p = 1/tdur)

sets

for by using the “bioavailability” option p = 1/tdur and therefore starts the infusion. The second depot command

depot(adm = 1,target = In,p = -1/tdur, Tlag = tdur)

sets

for. Hence, after time tdur, the tlag option together with the “bioavailability” option is used to stop the infusion.

The drug amount is taken from the data file

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| #ID | TIME | DV | AMT | EVID | MDV | ADM |
| 1 | 0 | . | 670 | 1 | 1 | 1 |

With a tdur small, e.g. 1e-4, an IV bolus can be mimicked by an IV short infusion.

Example code of the QE approximation with non-constant total receptors

🡺 Please see file Supp\_Code\_1.docx

Example code of the full model:

🡺 Please see file Supp\_Code\_2.docx

**Nonmem**

In Nonmem it is not clear how a negative “bioavailability” can be used. Consequently application of an additional dummy compartment to describe the input function seems not to be applicable. Therefore, we “hard-code” the infusion mechanism as presented in the Method Section. To be as general as possible, both routes of administration, SC and IV, are included in the Nonmem code. In case of an SC administration everything is as usual and the dose is provided by the AMT column. For the IV administration, we have to set up a very low dummy dose, such as 1e-16, in the AMT column. The real dose is provided in the Nonmem code.

Example code of the QE approximation with non-constant total receptors

🡺 Please see file Supp\_Code\_3.docx

Example code of the full model:

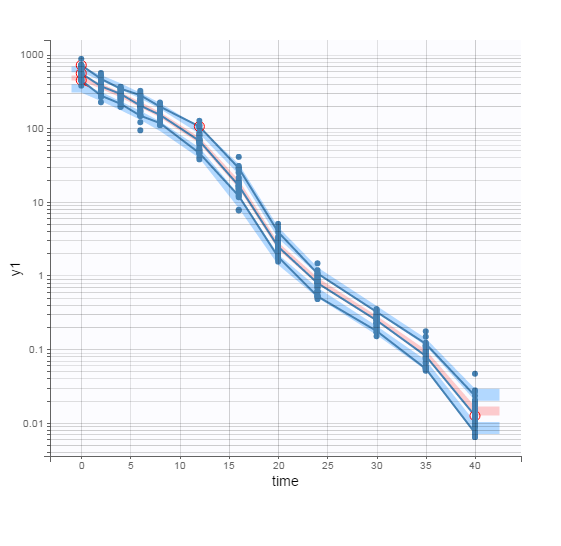
🡺 Please see file Supp\_Code\_4.docx

1. **Applied source codes and additional material for the simulation-estimation study**

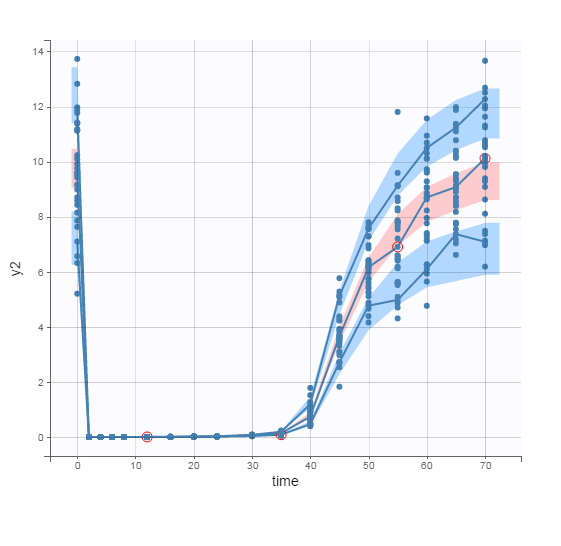
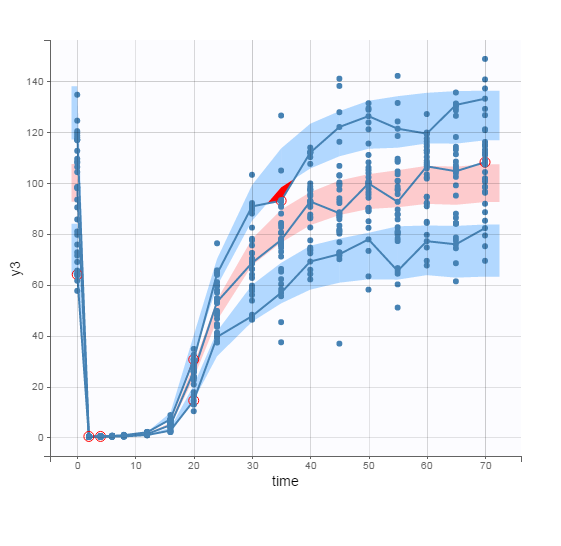
**Applied Monolix source code for Eqs. (15)-(23)**

🡺 Please see file Supp\_Code\_5.docx

Visual predictive checks:

BsAb concentration

Free receptor concentrations and :



**Alternative Monolix source code for Eqs. (15),(17),(25)-(30)**

In this formulation the setting odeType = stiff causes convergence problems and is therefore not recommended. Without a stiff solver, results were comparable to values obtained from the above source code and presented in Table 3.

🡺 Please see file Supp\_Code\_6.docx

**Applied Nonmem source code for Eqs. (15)-(23)**

🡺 Please see file Supp\_Code\_7.docx

**Alternative Nonmem source code for Eqs. (15),(17),(25)-(30)**

🡺 Please see file Supp\_Code\_8.docx