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Target-mediated drug disposition model for drugs with N > 2 binding sites that bind to a target with one binding site

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Abstract

The paper extended the TMDD model to drugs with more than two ($N>2$) identical binding sites (N-to-one TMDD). The quasi-steady-state (N-to-one QSS), quasi-equilibrium (N-to-one QE), irreversible binding (N-to-one IB), and Michaelis–Menten (N-to-one MM) approximations of the model were derived. To illustrate properties of new equations and approximations, $N=4$ case was investigated numerically. Using simulations, the N-to-one QSS approximation was compared with the full N-to-one TMDD model. As expected, and similarly to the standard TMDD for monoclonal antibodies (mAb), N-to-one QSS predictions were nearly identical to N-to-one TMDD predictions, except for times of fast changes following initiation of dosing, when equilibrium has not yet been reached. Predictions for mAbs with soluble targets (slow elimination of the complex) were simulated from the full 4-to-one TMDD model and were fitted to the 4-to-one TMDD model and to its QSS approximation. It was demonstrated that the 4-to-one QSS model provided nearly identical description of not only the observed (simulated) total drug and total target concentrations, but also unobserved concentrations of the free drug, free target, and drug-target complexes. For mAb with a membrane-bound target, the 4-to-one MM approximation adequately described the data. The 4-to-one QSS approximation converged 8 times faster than the full 4-to-one TMDD.

Keywords Target-mediated drug disposition · Quasi-equilibrium approximation · Quasi-steady-state approximation · Irreversible binding approximation · Michaelis–Menten approximation · Nonlinear pharmacokinetics · Drugs with many binding sites

Introduction

It has been shown that monospecific biologics that bind to a single target receptor with high affinity is not optimal for many therapeutic applications. For example, multivalent binding is needed for optimal IgG antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) to improve target cell killing [1, 2]. Furthermore, biologics that can bind to multiple receptors can neutralize pathogens, diseased cells, and soluble proteins much better than monospecific biologics [3, 4]. As a result, many avidity-based biologics that can bind multiple receptors have been developed to overcome the therapeutic limitation of monospecific biologics [4, 5].

A commonly used target-mediated drug disposition (TMDD) model [6] and its approximations [7, 8] assume that both, the drug and the target have only one binding site (one-to-one binding). In [9], the TMDD model and its approximations were derived for drugs that have two identical binding sites (two-to-one binding). This was an important extension as most therapeutic monoclonal IgG antibodies (mAbs) belong to this class [10, 11]. Here we extend the TMDD model and its approximations to drugs that have more than two ($N>2$) identical binding sites (N-to-one binding). This extension can be helpful for modeling IgA antibodies that have 2 or 4 binding sites [12], IgM antibodies that have 10 or 12 binding sites [13], or engineered antibodies or other biologics with more than 2 binding sites [2, 5, 14].

While the TMDD model with one-to-one binding assumption describes biologics sufficiently accurately in most cases, development of mathematical models that describe N-to-one binding more mechanistically may facilitate understanding of drug-target interactions and their influence on pharmacokinetic and pharmacodynamic properties of the system. To simplify notations, the models for N-to-one binding will be referred

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to as N-to-one TMDD, N-to-one quasi-steady state (QSS), N-to-one quasi-equilibrium (QE), N-to-one irreversible binding (IB), and N-to-one Michaelis–Menten (MM) models.

Theoretical

TMDD model for a drug with *N* binding sites

A schematic representation of binding interactions for the drug that has $N=4$ identical binding sites with the target that has one binding site is presented in Fig. 1. We assume that all sites are identical, i.e. binding constants k_{on} and k_{off} are the same for all sites and are independent of the number of occupied sites. The free drug (C) and the free target (R) are defined as the drug and the target that are not bound to each other. The binding processes form drug-target complexes, R_kC, $k=1, \ldots, N$, where k is the number of drug binding sites occupied by the target. When $k<N$, these complexes correspond to the partially bound drug. The complex R_NC corresponds to the fully bound drug, with all binding sites occupied by the target. For future derivations it is convenient to extend the notation to $k=0$ and $k=N+1$ and define $R_0C=C$ and $R_{N+1}C=0$.

Concentrations of the total drug (C_{tot}) and the total target (R_{tot}) can be then expressed as

$$C_{tot} = C + \sum_{k=1}^{N} R_k C, \tag{1}$$

$$R_{tot} = R + \sum_{k=1}^{N} k \cdot R_k C. \tag{2}$$

We assume that the drug is described by a two-compartment model with combined linear elimination and target-mediated drug disposition/elimination following intravenous (IV) and subcutaneous (SC) dosing and that elimination rate of all drug-target complexes (k_{int}) is the same and is independent of the type of the complex (number of binding sites occupied). We also assume that binding constants are the same for all binding sites and they do not depend on the occupancy of the other binding sites. Then, equations of the system can be written as 4 equations that describe total drug and total target concentrations:

$$\frac{dA_d}{dt} = -k_a \cdot A_d; A_d(0) = F_{SC} \cdot D_1;\ C_{tot}(0) = \frac{D_2}{V_c}; \tag{3}$$

$$\frac{dC_{tot}}{dt} = \frac{In(t) + k_a \cdot A_d + k_{tp} \cdot A_T}{V_c} - \left(k_{el} + k_{pt}\right) \cdot C - k_{int} \cdot (C_{tot} - C); \tag{4}$$

$$\frac{dA_T}{dt} = k_{pt} \cdot C \cdot V_c - k_{tp} \cdot A_T; A_T(0) = 0;\ R_{tot}(0) = \frac{k_{syn}}{k_{deg}}; \tag{5}$$

$$\frac{dR_{tot}}{dt} = k_{syn} - k_{deg} \cdot R - k_{int} \cdot (R_{tot} - R); \tag{6}$$

and N equations that describe drug-target complexes:

$$\frac{dR_kC}{dt} = (N-k+1) \cdot k_{on} \cdot R_{k-1}C \cdot R - (k \cdot k_{off} + k_{int} + (N-k) \cdot k_{on} \cdot R) \cdot R_kC + (k+1) \cdot k_{off} \cdot R_{k+1}C; k = 1, \ldots, N. \tag{7}$$

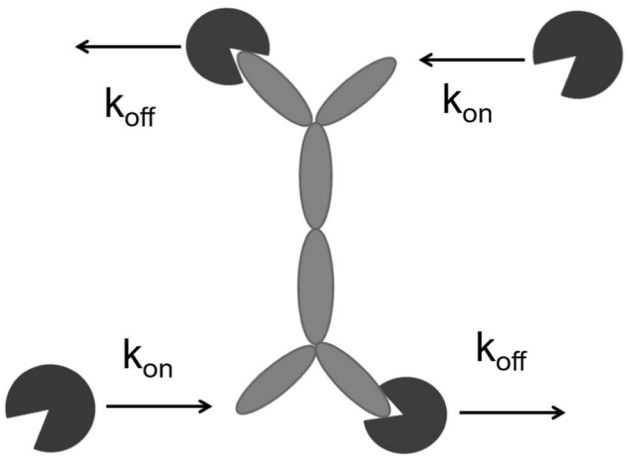

Fig. 1 Schematic representation of 4-to-one binding

Here k_{el} is the linear elimination rate constant, k_{pt} and k_{tp} are inter-compartment rate constants, k_{on}, k_{off}, and k_{int} are the binding, dissociation, and internalization (elimination of the complex) rate constants; k_{deg} and k_{syn} are the degradation (elimination of the target) and target production rate constants; V_c is the central compartment volume; $In(t)$ is the infusion rate; F_{SC} is the absolute bioavailability of the subcutaneous dose. Initial conditions correspond to the case where the free drug that is not present endogenously is administered as a subcutaneous dose D_1 and bolus dose D_2.

Quasi-steady-state approximation

Similarly to TMDD equations with one-to-one binding, the quasi-steady-state approximation of the TMDD N-to-one system can be derived by assuming that the free (unbound)

drug C, the free (unbound) target R, and the drug-target complexes R_kC are in quasi-steady-state [8], where binding rates are balanced by the sum of dissociation and internalization rates on the scale of the other processes:

$$\frac{dR_kC}{dt} = (N-k+1)\cdot k_{on}\cdot R_{k-1}C\cdot R - (k\cdot k_{off} + k_{int} + (N-k)\cdot k_{on}\cdot R)\cdot R_kC + (k+1)\cdot k_{off}\cdot R_{k+1}C = 0; k = 1,\ldots,N. \quad (8)$$

The QSS approximation is then described by Eqs. (3)-(6) supplemented by the algebraic Eqs. (1)-(2) and (8).

A recent work by Ng and Bauer [15] demonstrated that the QSS system of differential and algebraic equations can be successfully solved numerically. However, it is rather challenging and requires significant expertise in numerical methods. Here we demonstrate an example of analytical solution of algebraic part of the system, allowing for simple and fast numerical implementation.

We aim to find the algebraic solution of Eqs. (1)-(2) and (8), that is, to express all quantities C, R, R_kC, $k=1,\ldots,N$ as functions of C_{tot} and R_{tot} defined in (1)-(2).

First, we will derive equation for R. To do this, we compute the sum of Eqs. (8) multiplying each by the respective k (number of binding sites occupied by the target). The resulting equation is:

$$\sum_{k=1}^{N} k\cdot\frac{dR_kC}{dt} = 0. \quad (9)$$

Then, tedious but straightforward calculations presented in Appendix 1 result in the equation:

$$R_{tot} = R + \frac{N\cdot C_{tot}\cdot R}{K_D + K_{IB} + R}. \quad (10)$$

Here $K_D = k_{off}/k_{on}$ is the dissociation rate constant and $K_{IB} = k_{int}/k_{on}$ is the irreversible binding rate constant introduced in [9].

This equation is identical to the corresponding expression for one-to-one binding if we interpret $N\cdot C_{tot}$ as the total number of binding sites in the system. One can solve this equation for R to find:

$$R = \frac{1}{2}\left[-\left(N\cdot C_{tot} + K_{IB} + K_D - R_{tot}\right) + \sqrt{\left(N\cdot C_{tot} + K_{IB} + K_D - R_{tot}\right)^2 + 4\cdot(K_{IB}+K_D)\cdot R_{tot}}\right] \quad (11)$$

This is the key equation of the QSS approximation, but it is only half of the solution. We also need expressions for C and R_kC as functions of R, C_{tot}, and R_{tot}.

As shown in Appendix 2, R_NC can be expressed as

$$R_NC = \frac{N!\cdot C_{tot}\cdot R^N}{\prod_{k=1}^{N}(K_{IB} + k\cdot K_D + k\cdot R)} \quad (12)$$

while the remaining elements can be found recursively as follows:

$$R_{N-1}C = R_NC\cdot\frac{K_{IB} + N\cdot K_D}{R}, \quad (13)$$

$$R_kC = R_{k+1}C\cdot\frac{K_{IB} + (\mathrm{k}+1)\cdot K_D + (\mathrm{N}-\mathrm{k}-1)\cdot \mathrm{R}}{(\mathrm{N}-\mathrm{k})\cdot R} - R_{k+2}C\cdot\frac{(\mathrm{k}+2)\cdot K_D}{(\mathrm{N}-\mathrm{k})\cdot R}, k = (N-2),\ldots,0 \quad (14)$$

Note that free drug concentration C can be found from (14) when $\mathrm{k}=0$, or, equivalently, from the equation:

$$C = C_{tot} - \sum_{k=1}^{N} R_kC. \quad (15)$$

Thus, the N-to-one QSS approximation is described by differential Eqs. (3)-(6) supplemented by algebraic Eqs. (11)-(15). Equations (12)-(14) are explicitly written for $N=4$ in Appendix 3.

When internalization rate of the complexes k_{int} is equal to the degradation rate of the free target k_{deg}, the total target concentration R_{tot} is a constant parameter of the system, and the Eq. (6) is not needed.

Quasi-equilibrium approximation

QE equations can be obtained from the corresponding QSS equations by setting $K_{IB}=0$. They can be used to resolve binding equation for the in-vitro experiment with known drug and target concentrations, C_{tot} and R_{tot}, respectively. In this case, distribution of species is described by the system:

$$R = \frac{1}{2}\left[-\left(N{\cdot}C_{tot} + K_D - R_{tot}\right) + \sqrt{\left(N{\cdot}C_{tot} + K_D - R_{tot}\right)^2 + 4{\cdot}K_D{\cdot}R_{tot}}\right] \tag{16}$$

$$R_N C = \frac{C_{tot} \cdot R^N}{(K_D + R)^N} \tag{17}$$

$$R_{N-1} C = R_N C \cdot \frac{N \cdot K_D}{R}, \tag{18}$$

$$R_k C = R_{k+1} C \cdot \frac{(\mathrm{k}+1) \cdot K_D + (\mathrm{N}-\mathrm{k}-1) \cdot \mathrm{R}}{(\mathrm{N}-\mathrm{k}) \cdot R} - R_{k+2} C \cdot \frac{(\mathrm{k}+2) \cdot K_D}{(\mathrm{N}-\mathrm{k}) \cdot R}, k = N-2, \ldots, 0 \tag{19}$$

$$C = C_{tot} - \sum_{k=1}^{N} R_k C$$

In fact, in this case the system can be simplified (Appendix 4) and presented in the form:

$$R_k C = \frac{C(n,k) \cdot C_{tot} \cdot {K_D}^{N-k} \cdot R^k}{(K_D + R)^N}, C(n,k) = \frac{N!}{k! \cdot (N-k)!}, k = 0, \ldots, N. \tag{20}$$

This solution is intuitively obvious. When probability of the binding site being occupied in equal to $\mathrm{R}/(\mathrm{K_D}+\mathrm{R})$, and probability of the site being free is $\mathrm{K_D}/(\mathrm{K_D}+\mathrm{R})$, then Eqs. (20) show the probability of k sites being occupied and $(N\text{-}k)$ sites being free.

Irreversible binding approximation

The irreversible binding approximation can be obtained from the QSS approximation by assuming $k_{off} = 0$ (resulting in $K_D = 0$). Binding equations in this case have the form

$$R = \frac{1}{2}\left[-\left(N \cdot C_{tot} + K_{IB} - R_{tot}\right) + \sqrt{\left(N \cdot C_{tot} + K_{IB} - R_{tot}\right)^2 + 4 \cdot K_{IB}{\cdot}R_{tot}}\right] \tag{21}$$

$$R_N C = \frac{N!{\cdot}C_{tot} \cdot R^N}{\prod_{k=1}^{N} (K_{IB} + k \cdot R)} \tag{22}$$

$$R_{N-1} C = R_N C \cdot \frac{K_{IB}}{R}, \tag{23}$$

$$R_k C = R_{k+1} C \cdot \frac{K_{IB} + (\mathrm{N}-\mathrm{k}-1) \cdot \mathrm{R}}{(\mathrm{N}-\mathrm{k}) \cdot R}, k = N-2, \ldots, 0 \tag{24}$$

$$C = C_{tot} - \sum_{k=1}^{N} R_k C$$

In this case the system can be simplified and presented in the form (see Appendix 5):

$$R_k C = \frac{N!}{(N-k)!} \cdot C_{tot} \cdot \frac{K_{IB}{\cdot}R^k}{\prod_{i=N-k}^{N} (K_{IB} + i \cdot R)}, k = 0, \ldots, N. \tag{25}$$

Michaelis–Menten approximation

To further simplify the QSS equations, we will assume that the free drug concentration is approximately equal to the total drug concentration and that the free target concentration R is small relative to $(K_{IB} + K_D)$. In this case, we can derive (see Appendix 6)

$$R_k C \approx \frac{N!{\cdot}K_{IB} \cdot C_{tot} \cdot R^k}{(N-k)! \prod_{i=0}^{k} (K_{IB} + i \cdot K_D + i \cdot R)}, k = 0, .., N \tag{26}$$

Since R is small and $R_k C$ is of the order R^k, we can conclude that

$$C_{tot} - C \approx R_{tot} - R \approx R_1 C, \tag{27}$$

and that there is negligible concentration of complexes with $k > 1$.

Disregarding the quadratic term when solving Eq. (10) for R we get:

$$R \approx \frac{(K_{IB} + K_D){\cdot}R_{tot}}{N{\cdot}C_{tot} + K_{IB} + K_D} \tag{28}$$

and then

$$R_1 C \approx \frac{N{\cdot}C_{tot} \cdot R_{tot}}{N{\cdot}C_{tot} + K_{IB} + K_D} \approx \frac{N \cdot C \cdot R_{tot}}{N \cdot C + K_{IB} + K_D} \tag{29}$$

Substituting $C_{tot} - C$ and $R_{tot} - R$ by $R_1 C$ in (4) and (6), keeping only the terms up to the order of R, and assuming that C_{tot} is approximately equal to C, we can arrive at equations of the Michaelis–Menten approximation:

$$\frac{dC}{dt} = \frac{In(t) + k_a A_d + k_{tp} A_T}{V_c} - \left(k_{el} + k_{pt}\right) C - k_{int} \frac{R_{tot} \cdot C}{\frac{(K_D + K_{IB})}{N} + C}, \tag{30}$$

$$\frac{dR_{tot}}{dt} = k_{syn} - k_{deg} \cdot R - k_{int} \frac{R_{tot} \cdot C}{\frac{(K_D + K_{IB})}{N} + C}. \tag{31}$$

These equations are equivalent to the MM approximation of the standard TMDD system where $K_{SS} = (K_D + K_{IB})/N$.

As before, if k_{int} is equal to k_{deg}, the total target concentration is a constant parameter of the system, and the last equation can be removed. Then, the system is described by the two-compartment model with parallel linear and Michaelis–Menten elimination.

Investigation of the N-to-one model

To investigate the N-to-one model and its approximations, several simulation studies were performed for $N = 4$ binding sites. NONMEM 7.5.1® software [16] was used for simulations, estimation of parameters, and computation of predictions. FOCEI estimation method was used for model fitting.

First, the case of "slow elimination of complexes", with parameters typical for mAbs and soluble targets was evaluated with two examples.

In example 1, typical predictions of the free drug, the free target, the total drug, the total target, and each of the 4 drug target complexes were computed for the full 4-to-one TMDD model and the 4-to-one QSS approximation to compare the curves at different doses and times. Four consecutive intravenous (IV) doses of 10, 50, 100, and 500 nmol were

Table 1 "Slow elimination of complexes" case: true values and estimated population parameters of 4-to-one binding models. Log normal (that is, normal in the log-transformed parameter space) inter-subject variability was assumed. Log normal residual variability was implemented as additive residual errors in the log-transformed dependent variables space. The true values were used as initial values for estimation

Parameter	Description	True	4-to-one Full TMDD		4-to-one QSS
			a k_{on} estimated	*b* k_{on} fixed	
CL (L day^{-1})	Clearance	0.3	0.295	0.295	0.295
V_C (L)	Central volume	3.0	2.92	2.92	2.92
Q (L day^{-1})	Inter-compartment clearance	0.2	0.192	0.192	0.192
V_P (L)	Peripheral volume	3.0	2.88	2.88	2.86
F_{SC}	Bioavailability	0.7	0.686	0.686	0.687
k_a (day^{-1})	Absorption rate	0.5	0.498	0.498	0.498
k_{on} (nM L^{-1} day^{-1})	Binding rate	20	15.8	20 fixed	-
k_{off} (day^{-1})	Dissociation rate	2	1.81	2.32	-
k_{int} (day^{-1})	Internalization rate	0.2	0.193	0.193	0.193
k_{syn} (nM day^{-1})	Synthesis rate	1	1.00	1.00	1.00
k_{deg} (day^{-1})	Degradation rate	10	9.12	9.19	9.67
ω^2_{CL}	Variance of CL	0.04	0.0437	0.0437	0.0437
ω^2_{V1}	Variance of V_C	0.04	0.0363	0.0363	0.0364
ω^2_{Q}	Variance of Q	0.04	0.0403	0.0402	0.0402
ω^2_{V2}	Variance of V_P	0.04	0.0446	0.0446	0.0444
ω^2_{ka}	Variance k_a	0.04	0.0423	0.0423	0.0423
ω^2_{kint}	Variance of k_{int}	0.04	0.0492	0.0493	0.0501
ω^2_{ksyn}	Variance of k_{syn}	0.04	0.0376	0.0376	0.0372
ω^2_{kdeg}	Variance of k_{deg}	0.04	0.0198	0.0202	0.0242
σ^2_{drug}	Variance of residual error	0.0225	0.0221	0.0221	0.0221
σ^2_{target}	Variance of residual error	0.04	0.0395	0.0395	0.0395
K_D	k_{off}/k_{on}	0.1	0.115	0.116	0.109
K_{IB}	k_{int}/k_{on}	0.01	0.0122	0.00965	0.00965
K_{SS}	$(K_D + K_{IB})/4$	0.0275	0.0318	0.0314	0.0297
Minimum objective function value		15,701.7	15,678.7	15,678.8	15,683.1
Run time (sec)		-	7240	2627	349
Number of function evaluations		-	1578	637	743
Run time for one function evaluation (sec)		-	4.59	4.12	0.47

Table 2 Dosing and sampling scheme in the simulated study

Study	N	Dosing	Sampling Times	Number of Samples
1	6	IV, 100 nmol	1, 6, 12, 24 h; then 3, 7, 14, 21, 28, 35, 42, 49, and 56 days	3311 total drug concentrations and 3270 total target concentrations were above BQL levels of 0.1 nmol
	6	IV, 300 nmol		
	6	IV, 600 nmol		
	6	SC, 1000 nmol		
2	100	IV, 600 nmol	1, 24 h; then 1, 7, 14, 21, 28, 56, 63, 70, 77, 84, 91, 98, and 105 days	
	100	SC, 1000 nmol		

Table 3 "Fast elimination of complexes" case: true model parameters of 4-to-one binding models. Parameters not listed in this table coincide with those in Table 1

k_{on} (nM L^{-1} day^{-1})	Binding rate	10
k_{off} (day^{-1})	Dissociation rate	0.10
k_{int} (day^{-1})	Internalization rate	10
$K_D = k_{off}/k_{on}$		0.01
$K_{IB} = k_{int}/k_{on}$		1
$K_{SS} = (K_D + K_{IB})/4$		0.2525

administered 21 days apart. The parameters from Table 1 ("True" values of fixed-effect parameters) were used for simulation; all variance parameters were set to zero.

In example 2, population PK simulation/estimation was performed. The population PK data set that imitated data from combined typical phase 1 and phase 2 studies (Table 2) was used to simulate concentrations of various quantities from the full 4-to-one TMDD model. The parameters from Table 1 ("True" values) were used for simulations. The simulated concentrations of the free drug and of the total target were then used to fit the following models:

a. Full 4-to-one TMDD model with k_{on} parameter estimated.
b. Full 4-to-one TMDD model with k_{on} parameter fixed at the true value.
c. QSS 4-to-one approximation.

For these models, the estimated parameters were compared with the true values. Predictions of all quantities (including unobserved ones) were also computed and compared with the true (i.e. simulated from the full 4-to-one TMDD model) values. Conversion times were recorded. For all estimations, the true values were used as the initial values, thus the ability of models to converge was not tested and conversion times may have been optimistic (especially for the full model).

Then, the case of "fast elimination of complexes", with parameters typical for mAbs and membrane-bound targets where Michaelis–Menten equations should be valid was evaluated. The same simulations as in example 1 of the "slow elimination of complexes" case were performed for the 4-to-one full TMDD, QSS, and MM models. The parameters from Table 3 were used in the simulations.

Results

For the "slow elimination of complexes" case, typical predictions of the full 4-to-one TMDD model and the 4-to-one QSS approximation (Fig. 2) were almost identical for all quantities.

In the "fast elimination of complexes" case, k_{int} is equal to k_{deg}, so R_{tot} is constant and only C, R, and R_1C are changing with time. As shown in Fig. 3, both 4-to-one QSS and the 4-to-one Michaelis–Menten models were able to reproduce the predictions of the full 4-to-one TMDD model for all these quantities.

In the population study for the "slow elimination of complexes" case, the full 4-to-one TMDD model (Model a) converged and provided accurate estimates for all model parameters except k_{on} (Table 1). Model b. where k_{on} was fixed to the true value converged and provided accurate estimates for all the other model parameters. The 4-to-one QSS model provided accurate estimates for all the parameters of the QSS model.

Model fit (objective function value [OFV]) and run times of all the models are shown in Table 1. The run time of the full 4-to-one TMDD model with the fixed k_{on} value was approximately 8 times longer than that of the 4-to-one QSS model. The run time of the full 4-to-one TMDD model with the estimated k_{on} value was approximately 21 times longer than that of the 4-to-one QSS model. Each function evaluation of the full model was taking approximately 9–10 times longer of the QSS model due to longer model integration time.

Discussion

Equations of the TMDD model and its QSS, QE, IB, and MM approximations were derived for drugs with $N > 2$ binding sites. The N-to-one QSS model was the most general approximation. The N-to-one QE and N-to-one IB approximations can be obtained from N-to-one QSS by setting $K_{IB} = 0$ or $K_D = 0$, respectively. The QSS 4-to-one

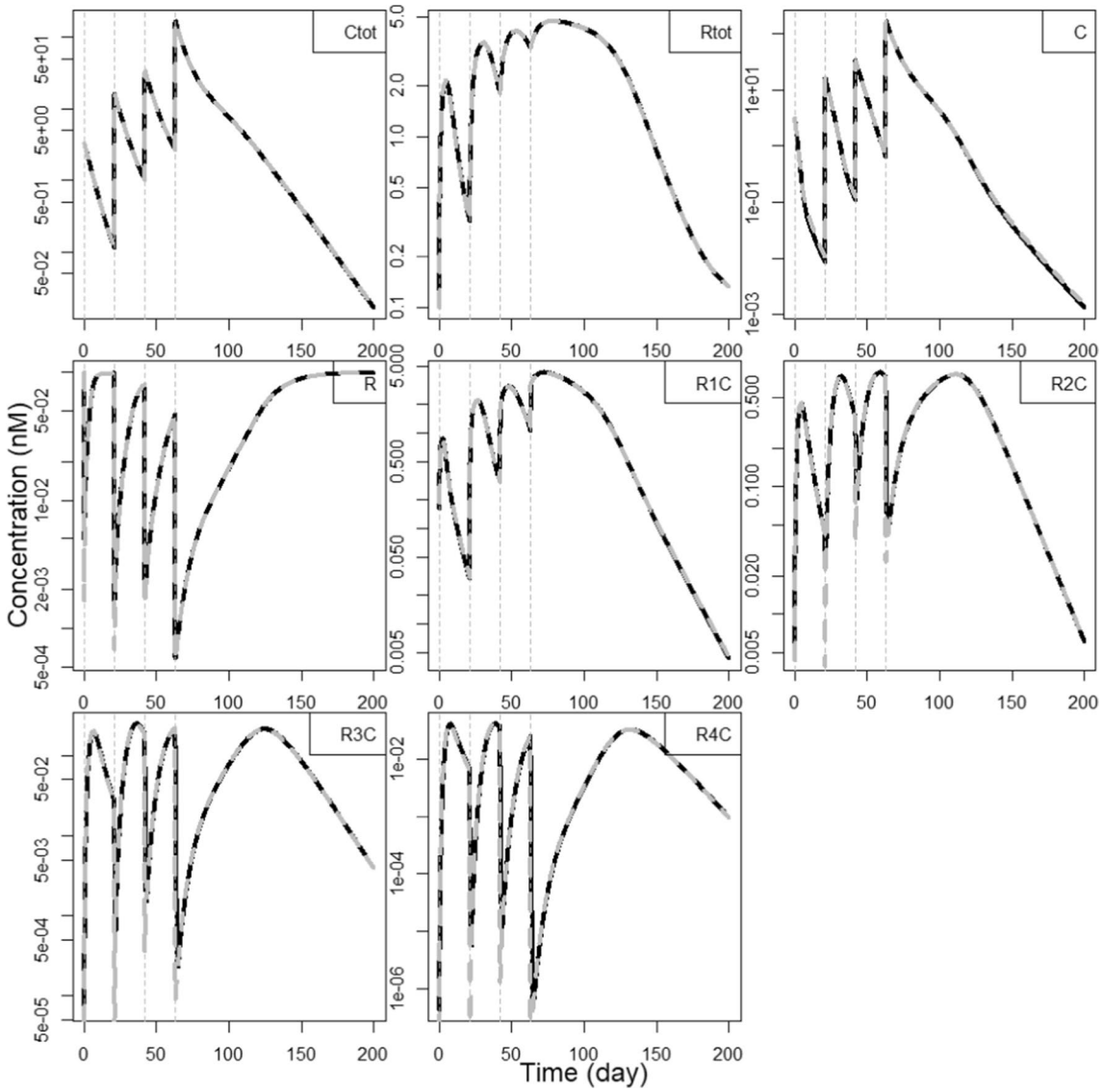

Fig. 2 'Slow Elimination of Complexes' Case: Comparison of Full TMDD 4-to-one and QSS 4-to-one Model Predictions. Black: full TMDD model, gray: QSS approximation. Vertical dash lines: 10, 50, 100, 500 nmol IV doses

model correctly estimated model parameters and predicted drug and target concentrations over time when it was fitted to the data simulated from the full 4-to-one TMDD model.

While advances in computer power and the software made numerical solution of the full N-to-one TMDD model possible, they did not (and could not) resolve the identifiability issue: binding parameters of the system cannot be determined from the routinely available data. Therefore, when the parameter k_{on} was fixed at the true value, the full model converged much faster and was able to estimate all parameters correctly, while the model with the estimated value of k_{on} took 2.5 times longer time to converge and was not able to estimate this parameter precisely even though dense sampling in the range of interest (immediately after the end of infusion) was available.

The system of Eqs. (1)-(4) was presented using differential equations for the total drug and total target concentrations rather than in the (equivalent and more traditional) form that uses differential equations for free drug and free target concentrations. The advantage of this form is that the large terms (terms that contain the parameter k_{on}) are localized in equations for the R_kC complexes rather than distributed throughout the entire system. We demonstrated that this is convenient for theoretical analysis of the system. Authors' experience with numerical investigations using the full TMDD model indicated that this version is also more stable numerically. We speculate that this is because the processes with high derivatives (that involve fast binding) are localized in Eq. (7) while derivatives in Eqs. (1)-(4) are relatively

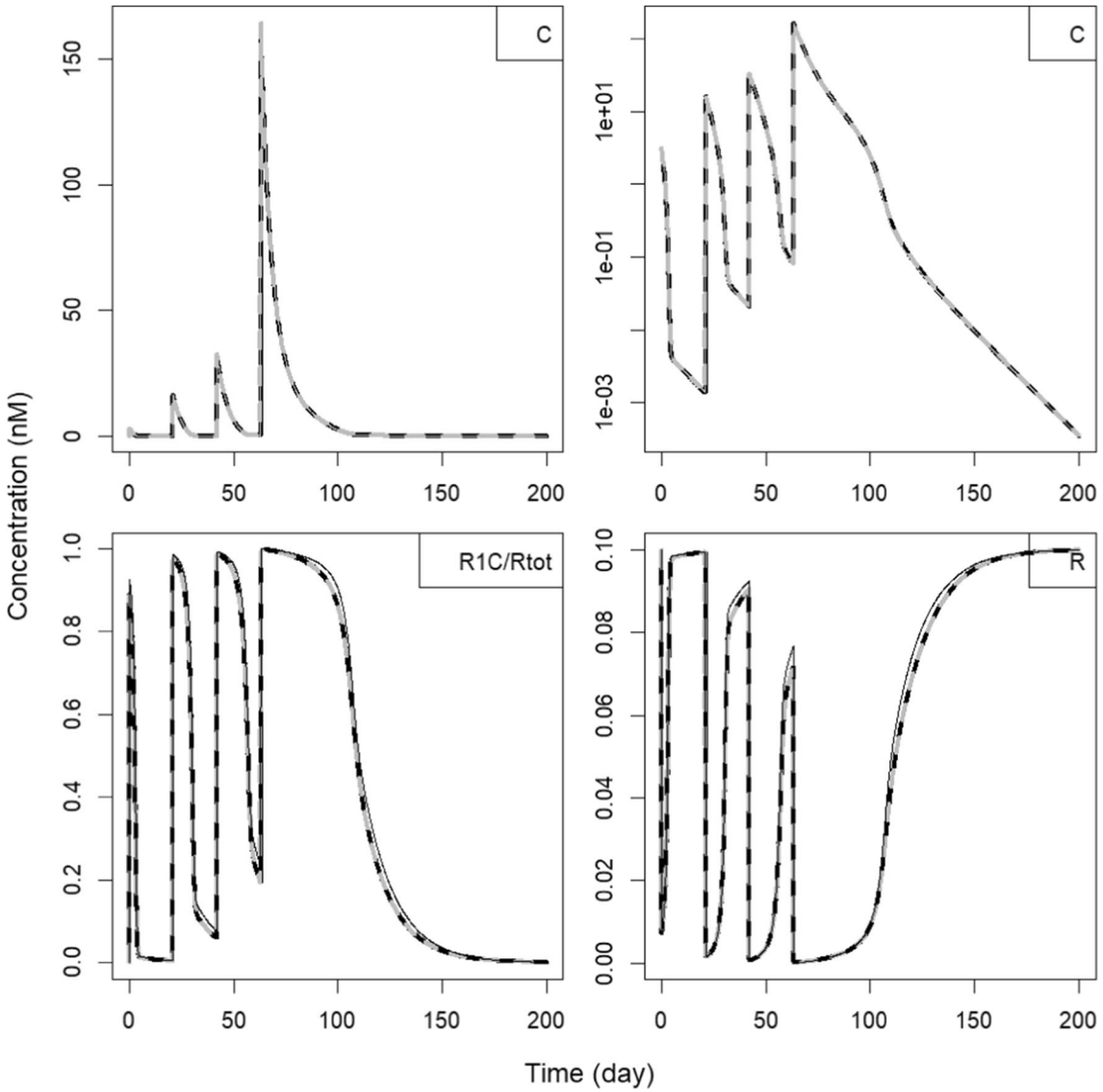

Fig. 3 'Fast Elimination of Complexes' Case: Comparison of Model Predictions for 4-to-one Models for Full TMDD, QSS, and MM. Black: full TMDD model, gray: QSS approximation, thin black: Michaelis–Menten approximation. Vertical dash lines: 10, 50, 100, 500 nmol IV doses

small. This may allow more stable integration of the part of the system that describes slow-changing variables.

The run time of the 4-to-one QSS model was about 8 times faster than that of the full 4-to-one TMDD model with fixed k_{on}, and it was about 21 times faster than that of the full 4-to-one TMDD model with the estimated k_{on} value. All estimations used the true values as initial values. Further study is needed to determine and compare the ability of these models to obtain precise and robust parameter estimates from the real study data. The NONMEM code of the full 4-to-1 TMDD model, the QSS approximation of this model, and the corresponding Michelis-Menten approximation are provided in Appendix 7, 8, and 9, respectively.

We should repeat the limitation of the suggested analytical approach. We assumed that binding constants k_{on} and k_{off} are the same for all sites and they are independent of the number of occupied sites. Indeed, the parameters k_{on} and k_{off} are expected to be similar due to structural symmetry (as in most cases they are replicas of the same structure). However, it is unknown how much these parameters (and other parameters of the drug-target complexes, including k_{int}) change when some or many of the binding sites are occupied, thus changing the structure (protein folding) of the drug-target complexes and their molecule weight. If this assumption does not hold, the analytical approach is intractable. For example, this approach does not describe systems with cooperative or allosteric binding.

Appendix 1: Derivation of Eq. (10)

From (8) we have:

$$\sum_{k=1}^{N} k \cdot \left\{(N-k+1)\cdot k_{on}\cdot R_{k-1}C\cdot R - k\cdot k_{off}\cdot R_kC - (N-k)\cdot k_{on}\cdot R_kC\cdot R + (k+1)\cdot k_{off}\cdot R_{k+1}C - k_{int}\cdot R_kC\right\} = 0 \tag{32}$$

Introducing the dissociation rate constant $K_D = k_{off}/k_{on}$ and the irreversible binding rate constant $K_{IB} = k_{int}/k_{on}$ one can arrive at

$$\sum_{k=1}^{N} k\cdot(N-k+1)\cdot R_{k-1}C\cdot R - \sum_{k=1}^{N} k\cdot\left(k\cdot K_D + (N-k)\cdot R\right)\cdot R_kC + \sum_{k=1}^{N} k\cdot(k+1)\cdot k_D\cdot R_{k+1}C = K_{IB}\cdot(R_{tot}-R) \tag{33}$$

For any function F(k), where we use the notation

$$R_oC = C; R_{N+1}C = 0,$$

we can derive:

$$\begin{aligned}\sum_{k=1}^{N} F(k)\cdot R_{k-1}C &= \sum_{k=0}^{N-1} F(k+1)\cdot R_kC \\ &= F(1)\cdot C - F(N+1)\cdot R_NC \\ &+ \sum_{k=1}^{N} F(k+1)\cdot R_kC\end{aligned} \tag{34}$$

and

$$\sum_{k=1}^{N} F(k)\cdot R_{k+1}C = \sum_{k=2}^{N+1} F(k-1)\cdot R_kC = -F(0)\cdot C + \sum_{k=1}^{N} F(k-1)\cdot R_kC. \tag{35}$$

Therefore,

$$\begin{aligned}\sum_{k=1}^{N} k\cdot(N-k+1)\cdot R_{k-1}C\cdot R &= N\cdot C\cdot R \\ &+ \sum_{k=1}^{N}(k+1)\cdot(N-k)\cdot R_kC\cdot R\end{aligned} \tag{36}$$

and

$$\sum_{k=1}^{N} k\cdot(k+1)\cdot R_{k+1}C = \sum_{k=1}^{N} k\cdot(k-1)\cdot R_kC. \tag{37}$$

Using Eqs. (36) and (37) in Eq. (33) we get:

$$\sum_{k=1}^{N}((k+1)\cdot(N-k)\cdot R_kC\cdot R - k\cdot k\cdot K_D\cdot R_kC - k\cdot(N-k)\cdot R_kC\cdot R + k\cdot(k-1)\cdot k_D\cdot R_kC) + N\cdot C\cdot R = K_{IB}\cdot(R_{tot}-R). \tag{38}$$

Collecting the terms for R_kC and $R_kC\cdot R$ we have

$$N\cdot R\cdot\sum_{k=1}^{N} R_kC - \left(K_D+R\right)\cdot\sum_{k=1}^{N} k\cdot R_kC + N\cdot C\cdot R = K_{IB}\cdot(R_{tot}-R). \tag{39}$$

Using the definitions of C_{tot} and R_{tot} from Eqs. (1) and (2), we then have

$$N\cdot C_{tot}\cdot R = (K_{IB}+R+K_D)\cdot(R_{tot}-R) \tag{40}$$

Equation (10) immediately follows.

Appendix 2: Derivation of Eq. (12)

Derivation of this section is based on the matrix linear algebra that can be found in many textbooks. We can recommend [17].

The system of Eqs. (8) for k = 2, …, N together with Eq. (2) can be presented in the matrix form $A * x = b$.

We will present the case of $N=4$, but it will be clear from the derivation that the same procedure is valid for any N.

For $N=4$, A is the (N + 1)-by-(N + 1) matrix of the form:

$$A = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ 4 \cdot R & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 & 0 \\ 0 & 3 \cdot R & -(2 \cdot K_D + K_{IB} + 2 \cdot R & 3 \cdot K_D & 0 \\ 0 & 0 & 2 \cdot R & -(3 \cdot K_D + K_{IB} + R) & 4 \cdot K_D \\ 0 & 0 & 0 & R & -(4 \cdot K_D + K_{IB}) \end{bmatrix}$$

and x and b are the vectors:

$$\mathrm{x} = \begin{bmatrix} R_0C \\ R_1C \\ R_2C \\ R_3C \\ R_4C \end{bmatrix}, \ \mathrm{b} = \begin{bmatrix} C_{tot} \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

This equation has a solution $x = A^{-1} * b$, where A^{-1} is the inverse matrix of A.

We are interested in the expression for R_4C, the last element of x. Since all elements of vector b except for the first are zero, $R_4C = (A^{-1})_{N+1,1} \cdot C_{tot}$, where $(A^{-1})_{N+1,1}$ is the (N + 1,1) element of the matrix A^{-1}.

This element of the inverse matrix is equal to.

$\left(A^{-1}\right)_{N+1,1} = C_{1,N+1}/\det(\mathrm{A})$, where $C_{1,N+1}$ is the co-factor of the (1,N + 1) element of the matrix A.

$C_{1,N+1}$ is equal to $(-1)^{N+2}\det(X)$, where X is the N-by-N matrix of the form:

$$X = \begin{bmatrix} 4 \cdot \boldsymbol{R} & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ 0 & 3 \cdot \boldsymbol{R} & -(2 \cdot K_D + K_{IB} + 2 \cdot R & 3 \cdot K_D \\ 0 & 0 & 2 \cdot \boldsymbol{R} & -(3 \cdot K_D + K_{IB} + R) \\ 0 & 0 & 0 & \boldsymbol{R} \end{bmatrix}$$

The determinant of a triangular matrix is equal to the product of its diagonal elements. Therefore, $\det(X) = N! \cdot R^N$. Obviously, $(-1)^{N+2} = (-1)^N$.

Thus,

$R_4C = (-1)^N \bullet N! \cdot R^N \cdot C_{tot} / \det(\mathrm{A})$.

To complete the derivation we need to show that

$$(-1)^N \cdot det(A) = \prod_{k=1}^{N} (K_{IB} + k \cdot K_D + k \cdot R).$$

To compute $det(A)$ we will reduce the matrix A to the triangular form using a sequence of matrix transformations that do not change the determinant, i.e., adding one column (or row) of the matrix with some coefficients to the other column (row) of the matrix. The sequence of these transformations and all intermediate matrices are presented below.

We start with (N + 1)-by-(N + 1) matrix of the form:

$$A = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ 4 \cdot R & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 & 0 \\ 0 & 3 \cdot R & -(2 \cdot K_D + K_{IB} + 2 \cdot R & 3 \cdot K_D & 0 \\ 0 & 0 & 2 \cdot R & -(3 \cdot K_D + K_{IB} + R) & 4 \cdot K_D \\ 0 & 0 & 0 & R & -(4 \cdot K_D + K_{IB}) \end{bmatrix}$$

Subtract column (4) from column (5):

$$\begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ 4\cdot R & -(K_D+K_{IB}+3\cdot R) & 2\cdot K_D & 0 & 0 \\ 0 & 3\cdot R & -(2\cdot K_D+K_{IB}+2\cdot R & 3\cdot K_D & -3\cdot K_D \\ 0 & 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+R) & 7\cdot K_D+K_{IB}+R \\ 0 & 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Subtract column (3) from column (4):

$$\begin{bmatrix} 1 & 1 & 1 & 0 & 0 \\ 4\cdot R & -(K_D+K_{IB}+3\cdot R) & 2\cdot K_D & -2\cdot K_D & 0 \\ 0 & 3\cdot R & -(2\cdot K_D+K_{IB}+2\cdot R & 5\cdot K_D+K_{IB}+2\cdot R & -3\cdot K_D \\ 0 & 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+3\cdot R) & 7\cdot K_D+K_{IB}+R \\ 0 & 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Subtract column (2) from column (3):

$$\begin{bmatrix} 1 & 1 & 0 & 0 & 0 \\ 4\cdot R & -(K_D+K_{IB}+3\cdot R) & 3\cdot K_D+K_{IB}+3\cdot R & -2\cdot K_D & 0 \\ 0 & 3\cdot R & -(2\cdot K_D+K_{IB}+5\cdot R) & 5\cdot K_D+K_{IB}+2\cdot R & -3\cdot K_D \\ 0 & 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+3\cdot R) & 7\cdot K_D+K_{IB}+R \\ 0 & 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Subtract column (1) from column (2):

$$\begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 4\cdot R & -(K_D+K_{IB}+7\cdot R) & 3\cdot K_D+K_{IB}+3\cdot R & -2\cdot K_D & 0 \\ 0 & 3\cdot R & -(2\cdot K_D+K_{IB}+5\cdot R) & 5\cdot K_D+K_{IB}+2\cdot R & -3\cdot K_D \\ 0 & 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+3\cdot R) & 7\cdot K_D+K_{IB}+R \\ 0 & 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Now we can remove the first row and the first column of the matrix without changing the determinant:

$$\begin{bmatrix} -(K_D+K_{IB}+7\cdot R) & 3\cdot K_D+K_{IB}+3\cdot R & -2\cdot K_D & 0 \\ 3\cdot R & -(2\cdot K_D+K_{IB}+5\cdot R) & 5\cdot K_D+K_{IB}+2\cdot R & -3\cdot K_D \\ 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+3\cdot R) & 7\cdot K_D+K_{IB}+R \\ 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Add row (4) to row (3):

$$\begin{bmatrix} -(K_D+K_{IB}+7\cdot R) & 3\cdot K_D+K_{IB}+3\cdot R & -2\cdot K_D & 0 \\ 3\cdot R & -(2\cdot K_D+K_{IB}+5\cdot R) & 5\cdot K_D+K_{IB}+2\cdot R & -3\cdot K_D \\ 0 & 2\cdot R & -(3\cdot K_D+K_{IB}+2\cdot R) & 3\cdot K_D \\ 0 & 0 & R & -(4\cdot K_D+K_{IB}+R) \end{bmatrix}$$

Add row (3) to row (2):

$$\begin{bmatrix} -(K_D + K_{IB} + 7 \cdot R) & 3 \cdot K_D + K_{IB} + 3 \cdot R & -2 \cdot K_D & 0 \\ 3 \cdot R & -(2 \cdot K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ 0 & 2 \cdot R & -(3 \cdot K_D + K_{IB} + 2 \cdot R) & 3 \cdot K_D \\ 0 & 0 & R & -(4 \cdot K_D + K_{IB} + R) \end{bmatrix}$$

Add row (2) to row (1):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ 3 \cdot R & -(2 \cdot K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ 0 & 2 \cdot R & -(3 \cdot K_D + K_{IB} + 2 \cdot R) & 3 \cdot K_D \\ 0 & 0 & R & -(4 \cdot K_D + K_{IB} + R) \end{bmatrix}$$

The resulting matrix has a very simple form that can be easily generalized to any N. We then continue as follows:

Add rows (1), (2), (3) to row (4); add rows (1), (2) to row (3); add row (1) to row (2):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ -(K_D + K_{IB} + R) & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ -(K_D + K_{IB} + R) & -(K_D + K_{IB} + R) & -(K_D + K_{IB} + 2 \cdot R) & 3 \cdot K_D \\ -(K_D + K_{IB} + R) & -(K_D + K_{IB} + R) & -(K_D + K_{IB} + R) & -(K_D + K_{IB} + R) \end{bmatrix}$$

Subtract column 4 from columns (1), (2), (3):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ -(K_D + K_{IB} + R) & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ -(4 \cdot K_D + K_{IB} + R) & -(4 \cdot K_D + K_{IB} + R) & -(4 \cdot K_D + K_{IB} + 2 \cdot R) & 3 \cdot K_D \\ 0 & 0 & 0 & -(K_D + K_{IB} + R) \end{bmatrix}$$

Add rows (1) and (2) to row (3):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ -(K_D + K_{IB} + R) & -(K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ -3 \cdot (2 \cdot K_D + K_{IB} + 2R) & -2 \cdot (2 \cdot K_D + K_{IB} + 2 \cdot R) & -(2 \cdot K_D + K_{IB} + 2 \cdot R) & 3 \cdot K_D \\ 0 & 0 & 0 & -(\boldsymbol{K_D + K_{IB} + R}) \end{bmatrix}$$

Subtract column (3) multiplied by 3 from column (1) and then subtract column (3) multiplied by 2 from column (2):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ -(7 \cdot K_D + K_{IB} + R) & -(5 * K_D + K_{IB} + 3 \cdot R) & 2 \cdot K_D & 0 \\ 0 & 0 & -(2 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 2 \cdot \boldsymbol{R}) & 3 \cdot K_D \\ 0 & 0 & 0 & -(\boldsymbol{K_D + K_{IB} + R}) \end{bmatrix}$$

Add row (1) multiplied by 2 to row (2):

$$\begin{bmatrix} -(K_D + K_{IB} + 4 \cdot R) & K_D & 0 & 0 \\ -3 * (3 \cdot K_D + K_{IB} + 3 \cdot R) & -(3 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 3 \cdot \boldsymbol{R}) & 2 \cdot K_D & 0 \\ 0 & 0 & -(2 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 2 \cdot \boldsymbol{R}) & 3 \cdot K_D \\ 0 & 0 & 0 & -(\boldsymbol{K_D} + \boldsymbol{K_{IB}} + \boldsymbol{R}) \end{bmatrix}$$

Subtract column (2) multiplied by 3 from column (1):

$$\begin{bmatrix} -(4 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 4 \cdot \boldsymbol{R}) & K_D & 0 & 0 \\ 0 & -(3 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 3 \cdot \boldsymbol{R}) & 2 \cdot K_D & 0 \\ 0 & 0 & -(2 \cdot \boldsymbol{K_D} + \boldsymbol{K_{IB}} + 2 \cdot \boldsymbol{R}) & 3 \cdot K_D \\ 0 & 0 & 0 & -(\boldsymbol{K_D} + \boldsymbol{K_{IB}} + \boldsymbol{R}) \end{bmatrix}$$

The determinant of the triangular matrix is equal to the product of its diagonal elements. Thus, the proof for $N=4$ is complete. The derivation is general and can be repeated for any N.

Appendix 3: Specific examples

The expressions for 4-to-one binding:

$$R_4C = \frac{24 \cdot C_{tot} \cdot R^4}{(K_{IB} + K_D + R) \cdot (K_{IB} + 2 \cdot K_D + 2 \cdot R) \cdot (K_{IB} + 3 \cdot K_D + 3 \cdot R) \cdot (K_{IB} + 4 \cdot K_D + 4 \cdot R)};$$

$$R_3C = R_4C \cdot \frac{K_{IB} + 4 \cdot K_D}{R},$$

$$R_2C = R_3C \cdot \frac{K_{IB} + 3 \cdot K_D + \mathrm{R}}{2 \cdot R} - R_4C \cdot \frac{2 \cdot K_D}{R},$$

$$RC = R_2C \cdot \frac{K_{IB} + 2 \cdot K_D + 2 \cdot \mathrm{R}}{3 \cdot R} - R_3C \cdot \frac{K_D}{3 \cdot R},$$

$$C = C_{tot} - RC - R_2C - R_3C - R_4C.$$

Appendix 4: Derivation of the QE equations

We will use recursion to prove Eqs. (26). First, the base of the recursion for k = N and k = N-1 can be computed as:

$$R_NC = \frac{C_{tot} \cdot R^N}{(K_D + R)^N} \text{ and} \tag{41}$$

$$R_{N-1}C = R_NC \cdot \frac{N \cdot K_D}{R} = \frac{C_{tot} \cdot N \cdot K_D \cdot R^{N-1}}{(K_D + R)^N}. \tag{42}$$

Let us now assume that we proved Eq. (26) for all values of k greater than K. Then

$$\begin{aligned} R_KC = &\frac{N!}{(K+1)! \cdot (N-K-1)!} \frac{C_{tot} \cdot {K_D}^{N-K-1} \cdot R^{K+1}}{(K_D + R)^N} \cdot \frac{(\mathrm{K}+1) \cdot K_D + (\mathrm{N}-\mathrm{K}-1) \cdot \mathrm{R}}{(\mathrm{N}-\mathrm{K}) \cdot R} \\ &- \frac{N!}{(K+2)! \cdot (N-K-2)!} \frac{C_{tot} \cdot {K_D}^{N-K-2} \cdot R^{K+2}}{(K_D + R)^N} \cdot \frac{(\mathrm{K}+2) \cdot K_D}{(\mathrm{N}-\mathrm{k}) \cdot R} = \frac{N! \cdot {K_D}^{N-K} \cdot R^K}{(N-K)! \cdot K! \cdot (K_D + R)^N} \cdot C_{tot} \end{aligned} \tag{43}$$

Thus, we proved it for $k=K$ and, by recursion, for any $k \geq 0$.

Appendix 5: Derivation of the IB equations

We will use recursion to prove Eq. (31). First, the base of the recursion for k = N and k = N-1 can be computed as:

$$R_NC = \frac{N! \cdot C_{tot} \cdot R^N}{\prod_{k=1}^{N} (K_{IB} + k \cdot R)} \text{ and} \tag{44}$$

$$R_{N-1}C = R_NC \cdot \frac{K_{IB}}{R} = \frac{N! \cdot C_{tot} \cdot K_{IB} R^{N-1}}{\prod_{k=1}^{N} (K_{IB} + k \cdot R)}. \tag{45}$$

Let us now assume that we proved (31) for all values of k > K. Then

$$R_K C = \frac{N!}{(N-K-1)!} \cdot C_{tot} \cdot \frac{K_{IB} \cdot R^{K+1}}{\prod_{i=N-K-1}^{N} (K_{IB} + i \cdot R)} \cdot \frac{K_{IB} + (\mathrm{N} - \mathrm{K} - 1) \cdot \mathrm{R}}{(\mathrm{N} - \mathrm{K}) \cdot R} = \frac{N!}{(N-K)!} \cdot C_{tot} \cdot \frac{K_{IB} \cdot R^{K}}{\prod_{i=N-K}^{N} (K_{IB} + i \cdot R)} \quad (46)$$

Thus, we proved it for $k = K$ and, by recursion, for any $k \geq 0$.

Appendix 6: Derivation of the Michaelis–Menten equations

We will use recursion to prove Eq. (26). First, the base of the recursion for $k = N$ and $k = N\text{-}1$ can be computed starting from Eqs. (12) and (13) and assuming that R is small relative to $(K_{IB} + K_D)$ as:

$$R_N C = \frac{N! \cdot C_{tot} \cdot R^N}{\prod_{k=1}^{N} (K_{IB} + k \cdot K_D + k \cdot R)} \quad (47)$$

$$R_{N-1} C = R_N C \cdot \frac{K_{IB} + N \cdot K_D}{R} \approx \frac{N! \cdot C_{tot} \cdot R^{N-1}}{\prod_{k=1}^{N-1} (K_{IB} + k \cdot K_D + k \cdot R)}. \quad (48)$$

Assuming that Eq. (26) is valid for all $k > K$, from Eq. (14) we have:

$$\begin{aligned} R_K C \approx & \frac{N! \cdot K_{IB} \cdot C_{tot} \cdot R^K}{(N-K-1)! \prod_{i=0}^{K+1} (K_{IB} + i \cdot K_D + i \cdot R)} \\ & \cdot \frac{K_{IB} + (\mathrm{K}+1) \cdot K_D + (\mathrm{N} - \mathrm{K} - 1) \cdot \mathrm{R}}{(\mathrm{N} - \mathrm{K})} \\ & - \frac{N! \cdot K_{IB} \cdot C_{tot} \cdot R^{K+1}}{(N-K-2)! \prod_{i=0}^{K+2} (K_{IB} + i \cdot K_D + i \cdot R)} \\ & \cdot \frac{(\mathrm{K}+2) \cdot K_D}{(\mathrm{N} - \mathrm{K})} \end{aligned} \quad (49)$$

Removing the terms of the order of R^{K+1}, we have

$$\begin{aligned} R_K C \approx & \frac{N! \cdot K_{IB} \cdot C_{tot} \cdot R^K}{(N-K-1)! \prod_{i=0}^{K+1} (K_{IB} + i \cdot K_D + i \cdot R)} \\ & \cdot \frac{K_{IB} + (\mathrm{k}+1) \cdot K_D}{(\mathrm{N} - \mathrm{k})} \\ \approx & \frac{N! \cdot K_{IB} \cdot C_{tot} \cdot R^K}{(N-K)! \prod_{i=0}^{K} (K_{IB} + i \cdot K_D + i \cdot R)} \end{aligned} \quad (50)$$

Thus, we proved it for $k = K$ and, by recursion, for any $k \geq 0$.

Appendix 7: Nonmem code of the full TMDD model with 4-to-1 binding

```
$PROBLEM 101est, full TMDD model with 4 binding sites.
  $INPUT C=DROP,ID,TIME,AMT,DV,LDV=DROP,EVID,MDV,CMT,DOSE,TYPE.
  $DATA../../Data/DerivedData/SimulatedNonmemData101.csv IGNORE=C.
  $ABBREV DERIV2=NO.
  $SUBROUTINES ADVAN13 TOL=9.
  $MODEL.
  NCOMP=8.
  $PK.
  N=4.
  CL=THETA(1)*EXP(ETA(1)).
  V1= THETA(2)*EXP(ETA(2))
  Q=THETA(3)*EXP(ETA(3)).
  V2 = THETA(4)*EXP(ETA(4))
  K10= CL/V1
  K12= Q/V1
  K21= Q/V2
  F1= THETA(5)
  KA=THETA(6)*EXP(ETA(5)).
  KON=THETA(7).
  KOFF=THETA(8).
  KINT=THETA(9)*EXP(ETA(6)).
  KSYN=THETA(10)*EXP(ETA(7)).
  KDEG=THETA(11)*EXP(ETA(8)).
  BASE=KSYN/KDEG.
  KSS=(KOFF+KINT)/KON.
  KD=KOFF/KON.
  A_0(4)=BASE.
  $DES.
  RC=A(5).
  R2C=A(6)
  R3C=A(7)
  R4C =A(8)
  CTOT=A(2)/V1.
  RCTOT=R4C+R3C+R2C+RC.
  C=CTOT-RCTOT.
  RTOT=A(4).
  RPTOT=4*R4C+3*R3C+2*R2C+RC.
  R=RTOT-RPTOT.
  DADT(1)=-KA*A(1); Total Drug depot amount.
  ; Total Drug central amount.
```

```
DADT(2) = KA*A(1)-K10*C*V1-KINT*RCTOT*V1-K12*C*V1 + K21*A(3).
DADT(3) = K12*C*V1-K21*A(3); Free Drug second compartment amount.
DADT(4) = KSYN—KDEG*R—KINT*RPTOT; Total target central concentration.
; Drug-Target Complex RC, R2C, R3C, R4C concentrations:
DADT(5) = (N-0)*KON*C*R—(1*KOFF + KINT + (N-1)*KON*R)*RC + 2*KOFF*R2C.
DADT(6) = (N-1)*KON*RC*R- (2*KOFF + KINT + (N-2)*KON*R)*R2C + 3*KOFF*R3C.
DADT(7) = (N-2)*KON*R2C*R—(3*KOFF + KINT + (N-3)*KON*R)*R3C + 4*KOFF*R4C.
DADT(8) = (N-3)*KON*R3C*R—(4*KOFF + KINT + (N-4)*KON*R)*R4C.
$ERROR.
RRC = A(5).
RR2C = A(6).
RR3C = A(7).
RR4C = A(8).
CCTOT = A(2)/V1.
RRCTOT = RR4C + RR3C + RR2C + RRC.
CC = CCTOT-RRCTOT.
RRTOT = A(4).
RRPTOT = 4*RR4C + 3*RR3C + 2*RR2C + RRC.
RR = RRTOT-RRPTOT.
Y = CCTOT*EXP(EPS(1)).
IF(TYPE.EQ.2) Y = RRTOT*EXP(EPS(2)).
IPRED = Y.
$THETA.
(0,0.3); 1 CL
(0,3.00); 2 V1
(0,0.2); 3 Q
(0,3.0); 4 V2
(0,0.7); 5 F1
(0,0.5); 6 KA
(0,20,30); 7 KON
(0,2); 8 KOFF
(0,0.2); 9 KINT
(0,1);10 KSYN
(0,10);11 KDEG
$OMEGA.
0.04  ;1 CL
 0.04  ;2 V1
 0.04  ;3 Q
 0.04  ;4 V2
 0.04  ;5 KA
 0.04  ;6 KINT
 0.04  ;7 KSYN
 0.04  ;8 KDEG
$SIGMA.
0.0225
0.04
$EST MAXEVAL = 99999 METHOD = 1 INTER PRINT = 10 NOABORT.
NOTHETABOUNDTEST NOOMEGABOUNDTEST NOSIGMABOUNDTEST
$COV PRINT = E UNCONDITIONAL MATRIX = S.
$TABLE ID TIME IPRED DOSE CCTOT RRCTOT RR4C RR3C RR2C RRC.
CC RRTOT RRPTOT RR ONEHEADER NOPRINT FILE = ../101est.tab.
```

Appendix 8: Nonmem code of the QSS approximation of the TMDD model with 4-to-1 binding.

```
$PROBLEM 102est, QSS TMDD model with 4 binding sites.
$INPUT C = DROP,ID,TIME,AMT,DV,LDV = DROP,EVID,MDV,CMT,DOSE,TYPE.
$DATA../../Data/DerivedData/SimulatedNonmem-Data101.csv IGNORE = C.
$ABBREV DERIV2 = NO.
$SUBROUTINES ADVAN13 TOL = 9.
$MODEL.
NCOMP = 4.
$PK.
N = 4.
CL = THETA(1)*EXP(ETA(1)).
V1 = THETA(2)*EXP(ETA(2))
Q  = THETA(3)*EXP(ETA(3))
V2 = THETA(4)*EXP(ETA(4))
K10 = CL/V1
K12 = Q/V1
K21 = Q/V2
F1  = THETA(5)
KA = THETA(6)*EXP(ETA(5)).
KON = THETA(7).
KOFF = THETA(8).
KINT = THETA(9)*EXP(ETA(6)).
KSYN = THETA(10)*EXP(ETA(7)).
KDEG = THETA(11)*EXP(ETA(8)).
BASE = KSYN/KDEG.
KSS = (KOFF + KINT)/KON.
KD = KOFF/KON.
KIB = KINT/KON.
A_0(4) = BASE.
$DES.
CTOT = A(2)/V1.
RTOT = A(4).
R = 0.5*(-(N*CTOT + KIB + KD-RTOT) + sqrt((N*CTOT + KIB + KD-RTOT)**2 + 4*(KIB + KD)*RTOT)).
```

```
R4C = CTOT*(R/(KIB + KD + R))*(2*R/(KIB + 2*KD + 2*R))*(3*R/(KIB + 3*KD + 3*R))*(4*R/(KIB + 4*KD + 4*R))
R3C = R4C*(KIB+4*KD)/R
R2C = R3C*(KIB+3*KD+1*R)/(2*R)-R4C*4*KD/(2*R)
RC = R2C*(KIB + 2*KD + 2*R)/(3*R)-R3C*3*KD/(3*R).
RCTOT = R4C + R3C + R2C + RC.
C = CTOT-RCTOT.
RPTOT = 4*R4C + 3*R3C + 2*R2C + RC.
DADT(1) = -KA*A(1); Total Drug depot amount.
; Total Drug central amount.
DADT(2) = KA*A(1)-K10*C*V1-KINT*RCTOT*V1-K12*C*V1 + K21*A(3).
DADT(3) = K12*C*V1-K21*A(3); Free Drug second compartment amount.
DADT(4) = KSYN—KDEG*R—KINT*RPTOT; Total target central concentration.
$ERROR.
CCTOT = A(2)/V1.
RRTOT = A(4).
RR = 0.5*(-(N*CCTOT + KIB + KD-RRTOT) +
sqrt((N*CCTOT + KIB + KD-RRTOT)**2+4*(KIB+KD)*RRTOT)).
RR4C = CCTOT*(RR/(KIB + KD + RR))*(2*RR/(KIB + 2*KD + 2*RR))*
(3*RR/(KIB + 3*KD + 3*RR))*(4*RR/(KIB+4*KD+4*RR)).
RR3C=RR4C*(KIB+4*KD)/RR.
RR2C = RR3C*(KIB + 3*KD + 1*RR)/(2*RR)-RR4C*4*KD/(2*RR).
RRC = RR2C*(KIB + 2*KD + 2*RR)/(3*RR)-RR3C*3*KD/(3*RR).
RRCTOT=RR4C+RR3C+RR2C+RRC.
CC=CCTOT-RRCTOT.
RRPTOT=4*RR4C+3*RR3C+2*RR2C+RRC.
Y=CCTOT*EXP(EPS(1)).
IF(TYPE.EQ.2) Y=RRTOT*EXP(EPS(2)).
IPRED=Y.
$THETA.
(0,0.3); 1 CL
(0,3.00); 2 V1
(0,0.2); 3 Q
(0,3.0); 4 V2
(0,0.7); 5 F1
(0,0.5); 6 KA
20 FIX  ; 7 KON
(0,2); 8 KOFF.
(0,0.2); 9 KINT
(0,1);10 KSYN
(0,10);11 KDEG
$OMEGA.
0.04  ;1 CL
0.04  ;2 V1
0.04  ;3 Q
0.04  ;4 V2
0.04  ;5 KA
0.04  ;6 KINT
0.04  ;7 KSYN
0.04  ;8 KDEG
$SIGMA.
0.0225
0.04
$EST MAXEVAL=99,999 METHOD=1 INTER PRINT=10 NOABORT.
NOTHETABOUNDTEST NOOMEGABOUNDTEST NOSIGMABOUNDTEST
$COV PRINT=E UNCONDITIONAL MATRIX=S.
$TABLE ID TIME IPRED DOSE CCTOT RRCTOT RR4C RR3C RR2C RRC.
CC RRTOT RRPTOT RR ONEHEADER NOPRINT FILE=../102est.tab.
```

Appendix 9: Nonmem code of the Michelis-Menten approximation of the TMDD model with 4-to-1 binding.

```
$PROBLEM 103est, MM TMDD model with 4 binding sites.
$INPUT C=DROP,ID,TIME,AMT,DV,LDV=DROP,EVID,MDV,CMT,DOSE,TYPE.
$DATA../../Data/DerivedData/SimulatedNonmem-Data101.csv IGNORE=C.
$ABBREV DERIV2=NO.
$SUBROUTINES ADVAN13 TOL=9.
$MODEL.
NCOMP=4.
$PK
N=4
CL=THETA(1)*EXP(ETA(1))
V1=THETA(2)*EXP(ETA(2))
Q=THETA(3)*EXP(ETA(3))
V2=THETA(4)*EXP(ETA(4))
K10=CL/V1
K12=Q/V1
K21=Q/V2
F1=THETA(5)
KA=THETA(6)*EXP(ETA(5))
KON=THETA(7)
KOFF=THETA(8)
KINT=THETA(9)*EXP(ETA(6))
KSYN=THETA(10)*EXP(ETA(7))
KDEG=THETA(11)*EXP(ETA(8))
BASE=KSYN/KDEG
KSS=(KOFF+KINT)/KON
KD=KOFF/KON
```

```
KIB=KINT/KON
A_0(4)=BASE
$DES
 C = A(2)/V1
 RTOT = A(4)
 R=RTOT*(KIB+KD)/(KIB+KD+4*C)
 DADT(1) =-KA*A(1); Free Drug depot amount
; Free Drug central amount
 DADT(2) = KA*A(1)-K10*C*V1-KINT*A(4)*4*C*V1/
(KIB+KD+4*C)-K12*C*V1+K21*A(3)
 DADT(3) = K12*C*V1-K21*A(3) ; Free Drug second
compartment amount
 DADT(4) = KSYN-KDEG*R-KINT*A(4)*4*C/
(KIB+KD+4*C) ; Total target central concentration
$ERROR
 CC = A(2)/V1
 RRTOT = A(4)
 RRC = RRTOT*4*CC/(KIB+KD+4*CC)
 RR = RRTOT*(KIB+KD)/(KIB+KD+4*CC)
 Y = CC*EXP(EPS(1))
 IF(TYPE.EQ.2) Y = RRTOT*EXP(EPS(2))
 IPRED = Y
$THETA
(0,0.3); 1 CL
(0,3.00); 2 V1
(0,0.2)  ; 3 Q
(0,3.0)  ; 4 V2
(0,0.7)  ; 5 F1
(0,0.5)  ; 6 KA
 20 FIX  ; 7 KON
(0,2 )   ; 8 KOFF
(0,0.2)  ; 9 KINT
(0,1)    ;10 KSYN
(0,10)   ;11 KDEG
$OMEGA
 0.04 ;1 CL
 0.04 ;2 V1
 0.04 ;3 Q
 0.04 ;4 V2
 0.04 ;5 KA
 0.04 ;6 KINT
 0.04 ;7 KSYN
 0.04 ;8 KDEG
$SIGMA
0.0225
0.04
$EST MAXEVAL=99999 METHOD=1 INTER
PRINT=10 NOABORT
    NOTHETABOUNDTEST NOOMEGABOUNDTEST
NOSIGMABOUNDTEST
$COV PRINT=E UNCONDITIONAL MATRIX=S
$TABLE ID TIME IPRED DOSE RRC CC RRTOT RR
ONEHEADER NOPRINT FILE=../103est.tab
```

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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