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Supplementary Material

SBMLsqueezer: Kinetic Laws

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1 Assignment of specific rate laws to SBGN process diagrams

Models of biological systems consist of reacting species. The rate of change of each species' concentration is determined by the reactions it is involved in. This rate depends on the velocities of the reactions. Each reaction velocity can basically be influenced by three types of modifiers: catalysts, inhibitors, and activators. Catalysts lower the activation energy and stabilize the transition state of the reaction. Inhibitors lower the reaction velocity whereas activators speed up or enable the reaction. Any interaction of modulators with the reaction must be reflected in the rate law. An activated molecule can be an activator but this is not necessarily true. Here, activation means activation of a reaction, in contrast to the activation of a molecule, which is a reaction in which the non-activated form of the molecule turns into the active form.

The program called SBMLsqueezer was originally designed to interpret process diagrams created by CellDesigner and to apply rate laws for each reaction depending on the context (Dräger *et al.*, 2008, 2010). Since version 1.3 it as been extended to interpret annotations in form of Systems Biology Ontology (SBO) terms as well and can therefore also be used in a stand-alone mode. This document describes how SBMLsqueezer extracts the desired information from process diagrams and gives an overview of all currently supported rate laws. Each rate law is explained with an example process diagram, the general formula and the result yielded by SBMLsqueezer. At the end of each section we discuss the SBO terms which correspond to the introduced rate law and all of its parameters together with a discussion on the units of all elements. The appendix of this document contains an overview of all currently available SBO terms for mathematical expressions in cellular systems (Section B on page 42).

The graphical notation of process diagrams used by CellDesigner extends standard SBML[1] (Hucka *et al.*, 2003) with additional information that can be interpreted to automatically assign appropriate rate laws to each reaction. Since SBML Level 2 Version 3 this information can also be included in standard SBML using SBO designated terms. CellDesigner allows including reaction-specific information to only a certain level of detail, thus several reaction mechanisms cannot be distinguished. For instance, it is not possible to include exact formulas for inhibition and activation as can be done for selected mechanisms in relevant text books such as Segel (1993) and Cornish-Bowden (2004). Often the process diagrams do not show at which state the modifier affects the reaction, i.e., whether the inhibitor reacts with the first substrate or with the second one, with the ES_1, the ES_2 or with the EP_1P_2 complex, and so on.

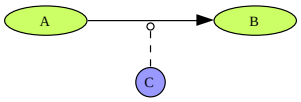
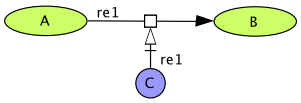
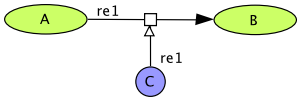
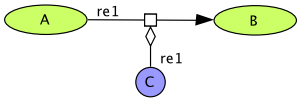
To overcome this difficulty, Liebermeister and Klipp (2006) define a generic inhibition and activation term. This function is a prefactor, that can be multiplied with a kinetic equation to introduce the effects of such a modification. SBMLsqueezer applies this prefactor also to rather more detailed rate laws, e.g., the random order, ping-pong, or the ordered ternary-complex mechanism.

CellDesigner 4.0α and higher versions support specialized arrows for two types of activating modification: one for transcriptional and another one for translational activation. A specific arrow for

[1] `http://sbml.org`

activation of enzyme reactions has been available since version 4.0β: the trigger symbol and—depending on the context—the symbol for physical stimulation. For SBMLsqueezer versions 1.0 through 1.2.* we reached the following accommodation: Besides the trigger and physical stimulation symbol, SBMLsqueezer also interprets an unknown catalysis arrow as an activation of the respective reaction (Table 1.1). This also allows modeling an activation with the α-version of CellDesigner 4.0. This feature has been removed with SBMLsqueezer 1.3 and now unknown catalysis is treated in the same way as regular catalysis because with the availability of specialized arrows for physical stimulation and trigger this feature becomes deprecated. For a complete list of all symbols used in CellDesigner reference can be made to Kitano *et al.* (2005) and the CellDesigner homepage[2]. The

Table 1.1: Redefinition of unknown catalysis to cover activation in process diagrams

Graphical Notation	CellDesigner Convention	SBMLsqueezer Interpretation
	Unknown Catalysis	Activation
	Trigger	Activation
	Physical Stimulation	Activation
	Modulation	Activation and Inhibition

term *modulation* was also introduced by CellDesigner 4.0β, although this term already existed in the specification of SBML beforehand. It does not specify which kind of interplay takes place between reaction and modulator. Hence its meaning is decidedly not clear. With the help of the Systems Biology Ontology (SBO) (Le Novère, 2006) or annotations in the format of the Minimal Information Requested in the Annotation of Models (MIRIAM) (Le Novère *et al.*, 2005; Laible and Le Novère, 2007) its meaning can be rendered precisely. If such an annotation is missing, SBMLsqueezer considers a modulator as both inhibitor and activator because SBMLsqueezer assumes that in a later parameter optimization process an appropriate optimizer determines which role prevails. Since version 1.3 SBMLsqueezer ignores modulators without a clear annotation. These modulators cannot be included anymore when creating kinetic equations due to the missing annotation. The new approach of SBMLsqueezer is not to provide default settings for cases in which information is missing. It is up to the user to specify the meaning of model components.

In some cases, certain rate laws are special cases of other ones that only differ in their parameter settings. In these cases SBMLsqueezer always assigns the most general equation to the reaction, driven by the assumption that in a later parameter optimization process an optimizer will find the correct solution. Alternatively, all rate laws created by SBMLsqueezer may also be modified manually using the designated CellDesigner dialog boxes.

In SBML each species can be initialized with an amount, i.e., a molecule count, or a concentration, i.e., a molecule count per volume of the surrounding compartment. This value does, however, not

[2] `http://www.celldesigner.org`

decide whether a species is to be interpreted in terms of concentration or molecule counts when occurring in rate equations. The attribute `hasOnlySubstanceUnits` of the species distinguishes both cases. If this field is, for instance, set to `true`, a species is to be interpreted in terms of molecule counts even if it is initialized with a concentration. Furthermore, all kinetic equations within an SBML model should always evaluate to units of `substance`, i.e., molecule counts, per `time` instead of concentration units per `time`. This becomes important if multi-compartment models are to be created because otherwise a simulator would have to recalculate the concentrations of all species again and again when crossing compartment borders. (Hucka *et al.*, 2008)

Earlier versions of SBMLsqueezer than 1.3 always generated kinetic equations under the assumption a species would be interpreted in terms of concentration and did not assign units to newly created parameters. Hence, in most cases, the units of generated kinetic equations could not be derived. Now SBMLsqueezer offers two ways to ensure unit consistency that can be changed in the preferences menu. First, if option *bring species to substance units* is selected, species occurring in kinetic equations are multiplied with the size of the surrounding compartment if their `hasOnlySubstanceUnits` attribute is set to `false`. In the other case, i.e., if option *bring species to concentration units* is selected, species are interpreted in terms of concentration and are therefore divided by their surrounding compartment size when these occur in kinetic equations and their `hasOnlySubstanceUnits` attribute is `false`. In any case, SBMLsqueezer derives the units of newly created parameters accordingly ensuring that all newly created kinetic equations evaluate to units of `substance` per `time`. For the sake of simplicity, all kinetic equations described in the following sections assume that the `hasOnlySubstanceUnits` attribute of all reacting species is `false` and that the second option has been selected in SBMLsqueezer, i.e., the size of surrounding compartments will not occur in anyone of the described rate equations.

The remainder of this document has been adapted from (Dräger *et al.*, 2008; Dräger, 2010).

2 Supported kinetic equations

This chapter gives a complete list of all currently supported kinetic formulas and shows examples in the graphical notation of CellDesigner[1] (Funahashi *et al.*, 2003; Kitano *et al.*, 2005). For an up-to-date list, refer to the project web page[2].

2.1 Kinetic equations for metabolic reactions

According to the process diagram, it often remains unclear at which state of the reaction the inhibitor or activator binds to enzyme, substrate or some intermediate complex. As stated in Section 1 on page 4, SBMLsqueezer applies a generic formula for inhibition and activation for many rate laws such as the generalized mass action kinetics or the rather detailed ternary-complex mechanisms. Eq. (2.1), which was defined by Liebermeister and Klipp (2006) in the context of convenience kinetics (Section 2.1.7 on page 21), gives the general formula for this prefactor of the desired rate equation:

$$F_j(\vec{\mathrm{S}},\vec{p}) = \prod_m h_{\mathrm{A}}([\mathrm{S}_m],k^{\mathrm{A}}_{jm})^{w^+_{jm}} h_{\mathrm{I}}([\mathrm{S}_m],k^{\mathrm{I}}_{jm})^{w^-_{jm}} \,. \tag{2.1}$$

where $\vec{S}$ and $\vec{p}$ are vectors of the concentrations of all reacting species in the system and parameter values. The matrices $\mathbf{W}^{\pm}$ are derived from the modulation matrix $\mathbf{W}$ and contain the absolute values of all positive or negative elements of this ternary matrix (-1 for inhibition, $+1$ for activation and 0 for no interaction). The modulation functions read (Liebermeister and Klipp, 2006):

$$h_{\mathrm{A}}([\mathrm{S}_m],k^{\mathrm{A}}_{jm}) = \frac{[\mathrm{S}_m]}{k^{\mathrm{A}}_{jm}+[\mathrm{S}_m]} \tag{2.2}$$

$$h_{\mathrm{I}}([\mathrm{S}_m],k^{\mathrm{I}}_{jm}) = \frac{k^{\mathrm{I}}_{jm}}{k^{\mathrm{I}}_{jm}+[\mathrm{S}_m]} \,. \tag{2.3}$$

SBO:0000363 defines the *activation constant* k^{A}_{jm}. The units of this parameter are the concentration units of the corresponding species, or, depending on the type of unit consistency, just the `substance` units of the species. In the following we will assume that species are always brought to concentration units and omit this case distinction. The *inhibitory constant* k^{I}_{jm}, defined by SBO:0000261, is given in concentration units of the corresponding species.

As an alternative to this simplified approach, one has to include all possible parameters assuming for a single inhibitor that it potentially acts at each state during the reaction. If more details about the mechanism are known, the rate law generated by SBMLsqueezer may serve as an initial equation that can be modified manually.

For reactions with two or more catalysts, one individual rate law will be generated for each catalyst. The total rate law for this particular reaction is given as the sum of the individual rates of all participating catalysts. If the reaction is one of those, whose modification is modeled according to Eq. (2.1),

[1] `http://www.celldesigner.org`

[2] `http://www.ra.cs.uni-tuebingen.de/software/SBMLsqueezer`

the whole rate law will be multiplied by the modification term F_j.

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \sum_{c=1}^{|\text{catalysts}|} v_{jc}(\vec{S},\vec{p}) \tag{2.4}$$

According to the Systems Biology Graphical Notation (SBGN) specification of process diagrams one separate reaction arc should be created for each catalyst (Le Novére *et al.*, 2008, p. 28).

Since SBML Level 2 every species has an identifier (ID) and may also have a name. The ID is an obligatory tag whereas the name may be empty (Hucka *et al.*, 2003). The name is intended to be a biologically meaningful identifier, which can in some cases be very long. Since the ID is supposed to be a short systematic identifier, SBMLsqueezer uses the ID for its LaTeX export. It should be noted that still the LaTeX export of SBMLsqueezer only provides limited functionality when compared to SBML2LaTeX (Dräger *et al.*, 2009b). If possible, users are encouraged to upload and convert their models with the help of the on-line version of SBML2LaTeX that can be accessed at `http://webservices.cs.uni-tuebingen.de/?tool=sbml2latex`. In SBML Level 1 the name attribute of the element had the same function as the ID in later Levels. Hence, if the user works with SBML Level 1, SBMLsqueezer and SBML2LaTeX use the name attribute of the element as the identifier.

2.1.1 Generalized mass-action kinetics

In SBMLsqueezer generalized mass-action kinetics utilizes Eq. (2.1) on the preceding page to include modification effects. This approach has already been successfully applied (Dräger *et al.*, 2007b,a, 2009a). Fig. 2.1 depicts an example of a reaction which is catalyzed by ion I_1. In contrast to enzymes such an-organic catalysts do not contain a catalytic center and are therefore unable to pass the substrates or intermediates from one catalytic center to another one. Hence, such a process can often not be modeled using enzyme kinetic approaches. This reaction may have an arbitrary mechanism in which the product P_1 acts as an inhibitor. Eq. (2.5) shows the general formula for the reversible, and Eq. (2.6) for the irreversible case. The reaction velocity v_j of reaction R_j depends on a vector of all reacting species $\vec{S}$ and a parameter vector $\vec{p}$. Eq. (2.7) gives the kinetic equation generated for the process in Fig. 2.1:

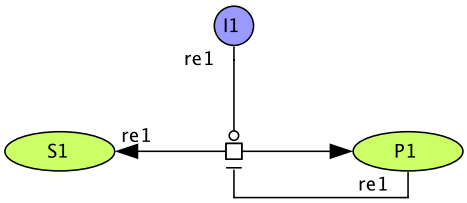


Figure 2.1: Example of a reaction to be modeled using the generalized mass-action rate law

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \left(k_{+j} \prod_i [S_i]^{n_{ij}^-} - k_{-j} \prod_i [S_i]^{n_{ij}^+} \right) \tag{2.5}$$

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot k_{+j} \prod_i [S_i]^{n_{ij}^-} \tag{2.6}$$

$$v_{\texttt{re1}} = \frac{\texttt{kic\_re1\_P1}}{\texttt{kic\_re1\_P1} + [\texttt{P1}]} \cdot (\texttt{kass\_re1\_I1} \cdot [\texttt{S1}] - \texttt{kdiss\_re1\_I1} \cdot [\texttt{P1}]) \cdot [\texttt{I1}]\,. \tag{2.7}$$

The matrices $\mathbf{N}^{\pm}$ contain the absolute values of all positive or negative elements of the stoichiometric matrix $\mathbf{N}$ or zero otherwise.

The association constant k_{+j} has the SBO identifier SBO:0000153[3] and SBO:0000162[4] in case of zeroth order reactions and the dissociation constant k_{-j} can be found in the SBO with the identifiers SBO:0000156[5] and SBO:0000352[6] in case of zeroth order reactions. To achieve that the rate equation evaluates to units of `substance` per `time`, the units of both parameters must be computed as follows:

$$\text{unit}\,(k_{\pm j}) = \frac{\texttt{substance}}{\texttt{time} \cdot \prod_i \text{unit}(\mathrm{S}_i)^{n_{ij}^{\mp}}} \tag{2.8}$$

with the model-wide valid units `substance` and `time`. In case of a species, the function unit returns either a concentration or a molecule count. Since the release of SBML Level 3 SBML models do no longer contain predefined units (Hucka *et al.*, 2010). Therefore, in this case the default values from previous SBML definitions are applied.

$F_j(\vec{\mathrm{S}}, \vec{p})$ is allowed to be any positive function (Heinrich and Schuster, 1996). Thus, all kinetic equations presented in the remainder of this section are special forms of this general formula as they can be derived from Eq. (2.5) on the facing page by setting $F_j(\vec{\mathrm{S}}, \vec{p})$ appropriately. Because of the availability of more specific equations, SBMLsqueezer restricts F_j to the function defined in Eq. (2.1) on page 7 rather than providing arbitrarily defined positive functions. The generalized mass action kinetics allows modeling of reactions with any number of reactant and product molecules. However, since reactions with more than two reactants are unlikely to take place (Cornish-Bowden, 2004, p. 6), warnings can be displayed.

SBMLsqueezer applies Eqs. (2.5) on the facing page and (2.6) on the preceding page to all reactions which are not catalyzed by an enzyme or catalyzed by non-enzymes. Gene regulation (transcription) processes that are neither activated nor inhibited by other factors proceed at a constant rate (basal gene expression) and hence follow a zeroth order mass-action rate law. Another example of where SBMLsqueezer applies mass-action kinetics is in degradation processes.

The Systems Biology Ontology (SBO) defines several special cases of generalized mass action kinetics (Figs. B.3 to B.4 on pages 45–46). None of the formulas defined therein includes the presence of any modulators. *Monoexpotential decay*

$$v_j(S_i, k_j) = \frac{[S_i]}{k_j} \tag{2.9}$$

(SBO:0000333) as a special case of *first order irreversible mass action kinetics* (SBO:0000049) is indirectly supported by SBMLsqueezer because the rate constant k_{+j} can be set to a value less than one. SBMLsqueezer was designed to create rate laws for continuous simulators and does not support any derivatives of *irreversible mass action kinetics, discrete scheme* (SBO:0000166). Note that the discrete formulas SBO:0000140[7], SBO:0000141[8], SBO:0000143[9] and SBO:0000146[10] are formally identical to their corresponding continuous forms. All other special cases of the mass-action kinetics in SBO can be created by SBMLsqueezer. Whenever a mass-action rate law is applicable,

[3] Forward rate constant

[4] Forward zeroth order rate constant

[5] Forward rate constant

[6] Reverse zeroth order rate constant

[7] The zeroth order irreversible mass action kinetics, discrete scheme corresponds to the continuous form SBO:0000047.

[8] The first order irreversible mass action kinetics, discrete scheme corresponds to the continuous form SBO:0000049.

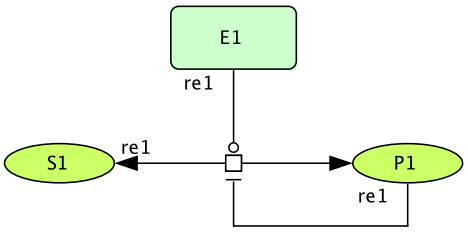
[9] The second order irreversible mass action kinetics, two reactants, discrete scheme corresponds to the continuous form SBO:0000054.

[10] The third order irreversible mass action kinetics, three reactants, discrete form corresponds to SBO:0000061 as its continuous form.

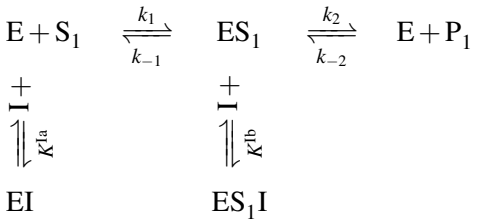
SBMLsqueezer also offers selection of a zeroth order rate law (either for the forward or the reverse reaction).

2.1.2 Uni-uni Michaelis-Menten kinetics

Fig. 2.2b shows the uni-uni enzyme-catalyzed reaction scheme including inhibition. Fig. 2.2a depicts an example of one possible corresponding CellDesigner process diagram, where the product interferes with the reaction in a feedback inhibition loop. We also grant that the enzyme may be



(a) Example of a process diagram



(b) Reaction scheme

Figure 2.2: Uni-uni enzyme-catalyzed reaction

omitted from the process diagram for the sake of simplicity and clear arrangement of the reacting species. The context menu of SBMLsqueezer for single reactions considers RNA and asRNA, simple and unknown molecules, complexes, truncated as well as generic proteins, and receptors all as enzymes to allow the user to apply any possible kinetic formula to a certain reaction whereas the plug-in window provides user settings to restrict this list to a more detailed selection of possible enzymes. SBMLsqueezer therefore offers a checkbox asking whether all reactions should be considered as being enzyme-catalyzed. In this case, all factors $[\mathrm{E}]_0 \cdot k^{\mathrm{cat}}_{\pm j}$ are replaced by the parameters $V^{\mathrm{m}}_{\pm j}$ that hide the enzyme concentration and allow estimation of the whole factor by appropriate optimizers. In the case of the enzyme-catalyzed uni-reactant reaction, SBMLsqueezer can use the Michaelis-Menten equation. It includes both constants K^{Ia} and K^{Ib}. This allows for optimization of the model to fit the parameters and to decide which kind of inhibition is the most appropriate one if it is not known, including the following three special cases, that are often of particular interest:

1. Competitive inhibition for $K^{\mathrm{Ia}} \in (0,\infty)$ and $K^{\mathrm{Ib}} \to \infty$

2. Noncompetitive inhibition for $K^{\mathrm{Ia}} = K^{\mathrm{Ib}} \wedge K^{\mathrm{Ia}} \in (0,\infty)$

3. Uncompetitive inhibition for $K^{\mathrm{Ia}} \to \infty$ and $K^{\mathrm{Ib}} \in (0,\infty)$

A detailed explanation of all kinds of modification can be found in (Heinrich and Schuster, 1996, p. 21 et sqq.). In addition to the well-described inhibition in the detailed Michaelis-Menten kinetics, we employ the activation prefactor of Eq. (2.1) on page 7 for approximative rate laws.

The general Michaelis-Menten equation is given in Eq. (2.10) on the next page with its corresponding irreversible form in Eq. (2.11) on the facing page and the example generated for the process diagram in Fig. 2.2a is written in Eq. (2.12) on the facing page and the corresponding generated

irreversible formula can be found in Eq. (2.13).

$$v_j(\vec{S},\vec{p}) = [\mathrm{E}]_0 \cdot \prod_m h_\mathrm{A}([\mathrm{S}_m], k_{jm}^\mathrm{A})^{w_{jm}^+} \cdot \frac{\frac{k_{+j}^\mathrm{cat}}{K_{j,\mathrm{S}_1}^\mathrm{M}}[\mathrm{S}_1] - \frac{k_{-j}^\mathrm{cat}}{K_{j,\mathrm{P}_1}^\mathrm{M}}[\mathrm{P}_1]}{1 + \frac{[\mathrm{I}]}{K_j^\mathrm{Ia}} + \left(\frac{[\mathrm{S}_1]}{K_{j,\mathrm{S}_1}^\mathrm{M}} + \frac{[\mathrm{P}_1]}{K_{j,\mathrm{P}_1}^\mathrm{M}}\right)\left(1 + \frac{[\mathrm{I}]}{K_j^\mathrm{Ib}}\right)} \tag{2.10}$$

$$v_j(\vec{S},\vec{p}) = [\mathrm{E}]_0 \cdot \prod_m h_\mathrm{A}([\mathrm{S}_m], k_{jm}^\mathrm{A})^{w_{jm}^+} \cdot \frac{k_{+j}^\mathrm{cat}[\mathrm{S}_1]}{K_{j,\mathrm{S}_1}^\mathrm{M} \cdot \left(1 + \frac{[\mathrm{I}]}{K_j^\mathrm{Ia}}\right) + [\mathrm{S}_1] \cdot \left(1 + \frac{[\mathrm{I}]}{K_j^\mathrm{Ib}}\right)} \tag{2.11}$$

$$v_\mathtt{re1} = [\mathrm{E1}] \cdot \frac{\frac{\mathtt{kcrf\_re1\_s3}}{\mathtt{kmc\_re1\_s1\_s3}} \cdot [\mathrm{S1}] - \frac{\mathtt{kcrr\_re1\_s3}}{\mathtt{kmc\_re1\_s2\_s3}} \cdot [\mathrm{P1}]}{1 + \frac{[\mathrm{P1}]}{\mathtt{kic\_1\_re1\_s2}} + \left(\frac{[\mathrm{S1}]}{\mathtt{kmc\_re1\_s1\_s3}} + \frac{[\mathrm{P1}]}{\mathtt{kmc\_re1\_s2\_s3}}\right) \cdot \left(1 + \frac{[\mathrm{P1}]}{\mathtt{kic\_2\_re1\_s2}}\right)} \tag{2.12}$$

$$v_\mathtt{re1} = [\mathrm{E1}] \cdot \frac{\mathtt{kcat\_re1\_s3} \cdot [\mathrm{S1}]}{\mathtt{kmc\_re1\_s1\_s3} \cdot \left(1 + \frac{[\mathrm{P1}]}{\mathtt{kic\_1\_re1\_s2}}\right) + [\mathrm{S1}] \cdot \left(1 + \frac{[\mathrm{P1}]}{\mathtt{kic\_2\_re1\_s2}}\right)} \tag{2.13}$$

The catalytic rate constants $k_{\pm j}^\mathrm{cat}$ have the SBO term identifiers SBO:0000320[11] and SBO:0000321[12]. Their units depend on the unit of the catalyzing enzyme. In case that the enzyme has a `substance` unit, the unit of both $k_{\pm j}^\mathrm{cat}$ is $\mathtt{time}^{-1}$. Otherwise, it must be multiplied with the `size` unit of the compartment, in which the enzyme is located. In case of absence of an explicit enzymatic catalyst, the catalytic rate constants are replaced with the *limiting rate* $V_{\pm j}^\mathrm{m}$ (in `substance` per `time`). In case of an irreversible reaction, this parameter has the SBO term SBO:0000186[13], otherwise SBO:0000324[14] for the forward reaction and SBO:0000325[15] for the reverse reaction. Unless otherwise stated, whenever a catalytic rate constant, a Michaelis or an inhibitory constant, or a limiting rate occurs in one of the following equations, these parameters will have the same unit and the same SBO term identifiers.

The Michaelis constants $K_{j,\mathrm{S}_1}^\mathrm{M}$ and $K_{j,\mathrm{P}_1}^\mathrm{M}$ for substrate and product have the SBO identifiers SBO:0000322[16] and SBO:0000323[17]. Their units are the same as the unit of the corresponding species. The same holds for the units of the inhibitory constants $K_j^{\mathrm{Ia|b}}$, which can be found in the SBO with the identifier SBO:0000261[18].

The SBO defines several special cases of Eq. (2.11) and provides one special case of Eq. (2.10) without inhibition (SBO:0000326[19]). Currently, SBMLsqueezer does not support any of the rate laws in the SBO subgraph SBO:0000443[20]. In the case of no modulation, Eq. (2.11) covers SBO:0000028[21] to SBO:0000031[22] and SBO:0000199[23]. If exactly one inhibitor interferes with the reaction, Eq. (2.11)

[11] Product catalytic rate constant
[12] substrate catalytic rate constant
[13] Maximal velocity
[14] Forward maximal velocity
[15] Reverse maximal velocity
[16] Michaelis constant for substrate
[17] Michaelis constant for product
[18] Inhibitory constant
[19] Enzymatic rate law for non-modulated unireactant enzymes
[20] Enzymatic rate law for reversible essential activation
[21] Enzymatic rate law for irreversible non-modulated non-interacting unireactant enzymes
[22] Briggs-Haldane rate law
[23] Normalized enzymatic rate law for unireactant enzymes

on the previous page equals SBO:0000265[24]. If $K_j^{\mathrm{Ia}} \equiv K_j^{\mathrm{Ib}}$ Eq. (2.11) on the preceding page and SBO:-0000266[25] are identical. This equation also covers competitive inhibition with appropriate parameter settings: SBO:0000262[26] for $K_j^{\mathrm{Ia}} \to \infty$ and SBO:0000260[27] for $K_j^{\mathrm{Ib}} \to \infty$.

If more than one inhibitor interacts with the enzyme during the irreversible uni-reactant reaction, SBMLsqueezer applies SBO:0000275[28]:

$$v_j(\vec{\mathrm{S}},\vec{p}) = \prod_m h_{\mathrm{A}}([\mathrm{S}_m], k_{jm}^{\mathrm{A}})^{w_{jm}^+} \cdot \frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{S}_1]}{K_{j,\mathrm{S}_1}^{\mathrm{M}} \cdot \left(1+\sum_{i=1}^n \frac{[\mathrm{I}_i]}{K_j^{\mathrm{Ib}i}}\right) + [\mathrm{S}_1] \cdot \left(1+\sum_{i=1}^n \frac{[\mathrm{I}_i]}{K_j^{\mathrm{Ia}i}}\right)} \,. \tag{2.14}$$

This equation includes SBO:0000276[29] and SBO:0000277[30] if exactly two inhibitors lower the reaction velocity v_j. The latter one applies if $\forall i : K^{\mathrm{Ia}i} \equiv K^{\mathrm{Ib}i}$, which depends on the parameter settings. Another special case of Eq. (2.14) emerges for $\forall i : K_j^{\mathrm{Ia}i} \to \infty$: this rate law then includes SBO:0000270[31] and all of its derivatives (Fig. B.1 on page 43).

SBO:0000273[32] and its derivative SBO:0000267[33] constitute a special case of Eq. (2.11) on the previous page only if exactly one inhibitor interferes with the reaction, $K_j^{\mathrm{Ib}} \to \infty$ and $\forall i : [\mathrm{R}_i] = 1$:

$$v_j(\vec{\mathrm{S}},\vec{p}) = \prod_m h_{\mathrm{A}}([\mathrm{S}_m], k_{jm}^{\mathrm{A}})^{w_{jm}^+} \cdot [\mathrm{E}]_0 \cdot \frac{k_j^{\mathrm{cat}}[\mathrm{S}_1]}{K_{j,\mathrm{S}_1}^{\mathrm{M}} \cdot \prod_{i=1}^n \left(1+\frac{[\mathrm{I}_i]}{K_i^{\mathrm{I}}}\right)^{m_i} + [\mathrm{S}_1]} \,. \tag{2.15}$$

Therefore, SBMLsqueezer offers this equation as an alternative for each irreversible reaction with one substrate molecule and more than one inhibitor. Activation is included using the prefactor from convenience kinetics.

Reversible uni-uni reactions with more than one inhibitor are modeled using the following equation which makes use of Eq. (2.1) on page 7 and is in this form not included in the SBO:

$$v_j(\vec{\mathrm{S}},\vec{p}) = [\mathrm{E}]_0 \cdot F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{\frac{k_{+j}^{\mathrm{cat}}}{K_{j,\mathrm{S}_1}^{\mathrm{M}}}[\mathrm{S}_1] - \frac{k_{-j}^{\mathrm{cat}}}{K_{j,\mathrm{P}_1}^{\mathrm{M}}}[\mathrm{P}_1]}{1+\frac{[\mathrm{S}_1]}{K_{j,\mathrm{S}_1}^{\mathrm{M}}}+\frac{[\mathrm{P}_1]}{K_{j,\mathrm{P}_1}^{\mathrm{M}}}} \,. \tag{2.16}$$

The mathematical form of the kinetics of *irreversible non-modulated unireactant enzymes* (SBO:-0000028), the *Henri-Michaelis-Menten equation* (SBO:0000029), the *Van Slyke and Cullen equation* (SBO:0000030) as well as the *Briggs-Haldane equation* (SBO:0000031) are effectively identical:

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{k_j^{\mathrm{cat}} \cdot [\mathrm{E}]_0 \cdot [S_i]}{K_{ij}^{\mathrm{M}} + [S_i]} \,. \tag{2.17}$$

[24] Enzymatic rate law for simple mixed-type inhibition of irreversible unireactant enzymes
[25] Enzymatic rate law for simple irreversible non-competitive inhibition of unireactant enzymes
[26] Enzymatic rate law for simple uncompetitive inhibition of irreversible unireactant enzymes
[27] Enzymatic rate law for simple competitive inhibition of irreversible unireactant enzymes by one inhibitor
[28] Mixed-type inhibition of irreversible enzymes by mutually exclusive inhibitors
[29] Enzymatic rate law for mixed-type inhibition of irreversible unireactant enzymes by two inhibitors
[30] Enzymatic rate law for non-competitive inhibition of irreversible unireactant enzymes by two exclusively binding inhibitors
[31] Competitive inhibition of irreversible unireactant enzymes by exclusive inhibition
[32] The competitive inhibition of irreversible unireactant enzymes by non-exclusive non-cooperative inhibitors
[33] Enzymatic rate law for competitive inhibition of irreversible unireactant enzymes by one inhibitor

The lattermost one is almost the same as the other three except that the constant K_{ij}^{M} is renamed to K_{ij}^{S} (SBO:0000372[34]). The difference in these equations can be explained by different modeling assumptions, in particular the denotation of K_{ij}^{M}:

$$K_{ij}^{\mathrm{M}} = \frac{k_{-1}}{k_1} \qquad \text{Michaelis-Menten, rapid equilibrium} \tag{2.18}$$

$$K_{ij}^{\mathrm{M}} = \frac{k_{-1} + k_2}{k_1} \qquad \text{Briggs-Haldane, quasi-steady-state} \tag{2.19}$$

$$K_{ij}^{\mathrm{S}} = \frac{k_1}{k_1} \qquad \text{Van Slyke and Cullen, irreversible substrate binding} \tag{2.20}$$

with the underlying reaction equation

$$\mathrm{E} + \mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{EA} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}\,. \tag{2.21}$$

2.1.3 Irreversible non-modulated non-interacting reactant enzymes

Irreversible enzyme-catalyzed reactions with more than one substrate can alternatively be modeled using the following equation if there is no modulator:

$$v_j(\vec{\mathrm{S}}, \vec{p}) = [E]_0 k_{+j}^{\mathrm{p}} \prod_{i=1}^{n} \frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}} + [\mathrm{S}_i]}\,. \tag{2.22}$$

The parameter k_{+j}^{p} (in units per `time` or `size` per `time` if the enzyme is given in concentration units) denotes the *catalytic rate constant* and can be found with the SBO term identifier SBO:00000025. In contrast to the formulas for reversible reactions, the number of products does not matter for rate laws of irreversible reactions. Fig. 2.3 depicts an example of a compatible process diagram. Two substrate molecules react to one product. Eq. (2.23) gives the generated rate law for this example.

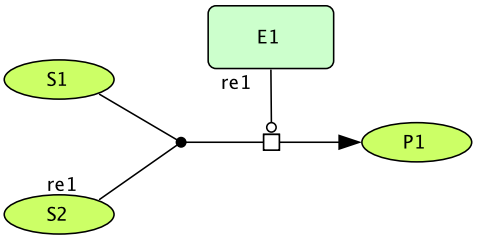


Figure 2.3: Example of an irreversible non-modulated non-interacting bi-reactant enzyme-catalyzed reaction

$$v_{\mathtt{re1}} = \frac{[\mathtt{E1}] \cdot \mathtt{kcat\_re1\_s4} \cdot \frac{[\mathtt{S1}]}{\mathtt{kmc\_re1\_s1\_s4}} \cdot \frac{[\mathtt{S2}]}{\mathtt{kmc\_re1\_s2\_s4}}}{\left(1 + \frac{[\mathtt{S1}]}{\mathtt{kmc\_re1\_s1\_s4}}\right) \cdot \left(1 + \frac{[\mathtt{S2}]}{\mathtt{kmc\_re1\_s2\_s4}}\right)} \tag{2.23}$$

SBMLsqueezer offers the user the choice of selecting this equation (SBO:0000150[35]) whenever the aforementioned conditions are fulfilled. Eq. (2.22) also covers the special cases SBO:0000028[36] (with all of its sub-categories), SBO:0000151[37], and SBO:0000152[38] for one, two, or three substrate molecules, respectively. This rate law is a special case of convenience kinetics with distinct reactants, each with stoichiometry one and no modulation at all.

[34] Michaelis constant in irreversible situation
[35] Enzymatic rate law for irreversible non-modulated non-interacting reactant enzymes
[36] Enzymatic rate law for irreversible non-modulated non-interacting unireactant enzymes
[37] Enzymatic rate law for irreversible non-modulated non-interacting bi-reactant enzymes
[38] Enzymatic rate law for irreversible non-modulated non-interacting tri-reactant enzymes

2.1.4 The generalized Hill equation for uni-uni reactions

Hill (1910) derives a purely empirical equation that describes the cooperative effects of the binding of oxygen to hemoglobin. Mathematically, Hill's equation takes the following form (SBO:0000195[39]):

$$v_j(\mathrm{S}_i, \vec{p}) = \frac{V_j[\mathrm{S}_i]^{h_j}}{K_j + [\mathrm{S}_i]^{h_j}} \tag{2.24}$$

with the *limiting rate* V_j (SBO:0000186), the pseudo-dissociation constant K_j (sometimes referred to as the *half saturation constant*, e.g., by Heinrich and Schuster (1996, p. 23), SBO:0000194), and the *Hill coefficient* h_j (

Eq. (2.24) does not take reverse reactions into account, which are, however, of great importance in the case of systems with multiple enzyme-catalyzed reactions. Cornish-Bowden (2004, p. 314) therefore generalizes this equation to produce a reversible form that can be applied to multi-enzyme systems and also includes the effects of modifier species M:

$$v_j(\mathrm{S},\mathrm{P},\mathrm{M}, \vec{p}) = \frac{\frac{V_j[\mathrm{S}]}{k_{\mathrm{S}j}}\left(1 - \frac{[\mathrm{P}]}{K_j[\mathrm{S}]}\right)\left(\frac{[\mathrm{S}]}{k_{\mathrm{S}j}} + \frac{[\mathrm{P}]}{k_{\mathrm{P}j}}\right)^{h_j-1}}{\frac{\left(k_j^{\mathrm{M}}\right)^{h_j} + [\mathrm{M}]^{h_j}}{\left(k_j^{\mathrm{M}}\right)^{h_j} + \beta_j[\mathrm{M}]^{h_j}} + \left(\frac{[\mathrm{S}]}{k_j^{\mathrm{S}}} + \frac{[\mathrm{P}]}{k_{\mathrm{P}j}}\right)^{h_j}} \tag{2.25}$$

with the *limiting rate* of the forward reaction V_j and the constants $k_{\mathrm{S}j}$, $k_{\mathrm{P}j}$, and $k_{\mathrm{M}j}$ describing the substrate S, product P, and modifier concentrations giving $\frac{1}{2} V_j$. K_j denotes the *equilibrium constant* and h_j is the *Hill coefficient*. The modifier M acts either as an activator for $\beta_j > 1$ or as an inhibitor if $\beta_j < 1$. For $\beta_j = 1$ the modification term vanishes. However, one problem with this equation is the dependence between some of its parameters. The SBO does currently not contain an entry for Eq. (2.25).

As in the Michaelis-Menten equation (see Sec. 2.1.2 on page 10), the limiting rate V_j may be replaced by the product $k_j^{\mathrm{cat}} \cdot [\mathrm{E}]_0$ if the reaction equation explicitly contains an enzyme. The units of k_j^{cat} and V_j follow the same definition as for the Michaelis-Menten equation. The parameters k_j^{S}, k_j^{P}, and k_j^{M} have the same units than the substrate, the product, and the modifier. The parameters K_j and β_j are dimensionless quantities.

The following equation shows an example for a generated equation by SBMLsqueezer:

$$v_{\mathtt{re1}} = \frac{\frac{\mathtt{vmaf\_re1}\cdot[\mathtt{s1}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{ksp\_re1}} \cdot \left(1 - \frac{[\mathtt{s2}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{keq\_re1}\cdot[\mathtt{s1}]\cdot\mathrm{vol}(\mathtt{c1})}\right) \cdot \left(\frac{[\mathtt{s1}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{ksp\_re1}} + \frac{[\mathtt{s2}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{ksp\_re1}}\right)^{\mathtt{hic\_re1}-1}}{\frac{\mathtt{kmc\_re1\_s4}^{\mathtt{hic\_re1}} + ([\mathtt{s4}]\cdot\mathrm{vol}(\mathtt{c1}))^{\mathtt{hic\_re1}}}{\mathtt{kmc\_re1\_s4}^{\mathtt{hic\_re1}} + \mathtt{beta\_re1}\cdot([\mathtt{s4}]\cdot\mathrm{vol}(\mathtt{c1}))^{\mathtt{hic\_re1}}} + \left(\frac{[\mathtt{s1}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{ksp\_re1}} + \frac{[\mathtt{s2}]\cdot\mathrm{vol}(\mathtt{c1})}{\mathtt{ksp\_re1}}\right)^{\mathtt{hic\_re1}}} . \tag{2.26}$$

Here, all species concentrations are brought to units of substance by multiplication with the volume of their surrounding compartment. The role of modifier S_4 cannot be determined from this equation directly, but depends on the value of parameter β_j. If the role of the modifier is specified in the SBML file, SBMLsqueezer automatically sets the value of this parameter to -1 multiplied with the default value in case of an inhibitor and to the default value in case of an activator.

2.1.5 Bi-uni enzyme reactions

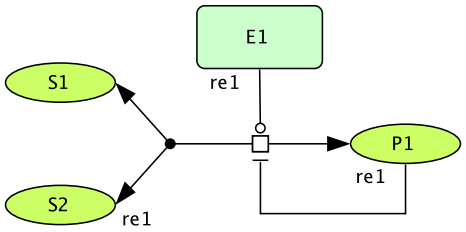
In some cases a single enzyme reacts with two reactants. Depending on the sequence in which the reactants bind to the enzyme, we can basically distinguish two different reaction mechanisms. Additionally, convenience kinetics constitutes a third alternative when no information about the mechanism

[39] Hill-type rate law, microscopic form

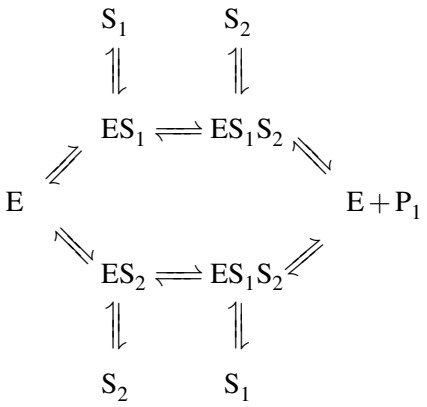
is available. For irreversible bi-uni enzyme reactions without modulation, Eq. (2.22) on page 13 gives an additional modeling alternative. A special case of this bi-uni reaction emerges if there is one reactant species that has a stoichiometric coefficient of two. Fig. 2.4a shows a possible graphical representation of this type of reaction. For both mechanisms we also apply the prefactor defined by convenience kinetics in Eq. (2.1) on page 7. The SBO defines the general category *enzymatic rate law for multireactant enzymes* for these reactions.

Random Order Mechanism

The reaction scheme of this mechanism is presented in Fig. 2.4b. For the sake of simplicity the inhibition mechanism is omitted from this scheme. Both substrates bind in arbitrary sequence to the enzyme. The general formula for this mechanism is given in Eq. (2.27), and its irreversible form is



(a) Bi-uni enzyme-catalyzed reaction



(b) Reaction scheme

Figure 2.4: Bi-uni random order enzyme reaction mechanism

shown in Eq. (2.28). The automatically generated equation to Fig. 2.4a with respect to this mechanism can be found in Eq. (2.29) and its corresponding irreversible form in Eq. (2.30). For a derivation of the latter formula see Section A.2 on page 38.

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \frac{\frac{k_{+j}^{\text{cat}}[E]_0[S_1][S_2]}{k_{j,S_1}^{I} K_{j,S_2}^{M}} - \frac{k_{-j}^{\text{cat}}[E]_0[P_1]}{K_{j,P_1}^{M}}}{1 + \frac{[S_1]}{k_{j,S_1}^{I}} + \frac{[S_2]}{k_{j,S_2}^{I}} + \frac{[S_1][S_2]}{K_{j,S_2}^{M} k_{j,S_1}^{I}} + \frac{[P_1]}{K_{j,P_1}^{M}}} \tag{2.27}$$

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \frac{k_{+j}^{\text{cat}}[E]_0[S_1][S_2]}{k_{j,S_1}^{I} K_{j,S_2}^{M} + K_{j,S_2}^{M}[S_1] + K_{j,S_1}^{M}[S_2] + [S_1][S_2]} \tag{2.28}$$

$$\begin{aligned} v_{\texttt{re1}} = & \frac{\texttt{kic\_re1\_s3}}{\texttt{kic\_re1\_s3} + [\texttt{P1}]} \\ & \cdot \frac{\frac{\texttt{kcrf\_re1\_s4}}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} \cdot [\texttt{E1}] \cdot [\texttt{S1}] \cdot [\texttt{S2}] - \frac{\texttt{kcrr\_re1\_s4}}{\texttt{kmc\_re1\_s3\_s4}} \cdot [\texttt{E1}] \cdot [\texttt{P1}]}{1 + \frac{[\texttt{P1}]}{\texttt{kmc\_re1\_s3\_s4}} + \frac{[\texttt{S1}]}{\texttt{kic\_re1\_s1\_s4}} + \frac{[\texttt{S1}] \cdot [\texttt{S2}]}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} + \frac{[\texttt{S2}]}{\texttt{kic\_re1\_s2\_s4}}} \end{aligned} \tag{2.29}$$

$$v_{\texttt{re1}} = \frac{\texttt{kic\_re1\_s3}}{\texttt{kic\_re1\_s3} + [\texttt{P1}]} \cdot \frac{\texttt{kcat\_re1\_s4} \cdot [\texttt{E1}] \cdot [\texttt{S2}] \cdot [\texttt{S1}]}{\mathscr{D}}, \tag{2.30}$$

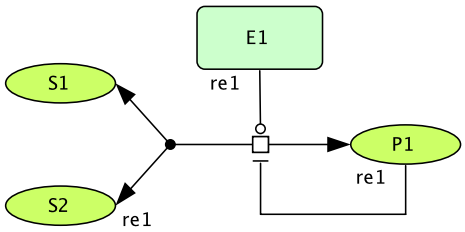
where

$$\mathscr{D} = \texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4} + [\mathrm{S1}] \cdot [\mathrm{S2}] + \texttt{kmc\_re1\_s2\_s4} \cdot [\mathrm{S1}] + \texttt{kmc\_re1\_s1\_s4} \cdot [\mathrm{S2}] \,. \quad (2.31)$$

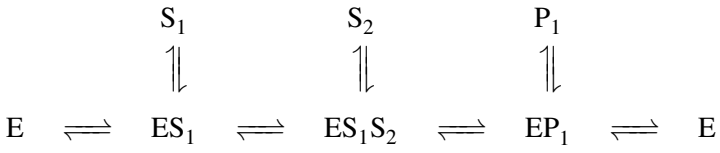
Currently, no definition of the random order mechanism exists in the SBO.

Ordered Mechanism

Fig. 2.5b depicts the reaction scheme of the ordered bi-uni mechanism. Note that in this reaction mechanism the sequence in which the species react is fixed. Eq. (2.32) gives the general formula of



(a) Bi-uni enzyme-catalyzed reaction



(b) Reaction scheme

Figure 2.5: Reaction scheme of the ordered bi-uni mechanism

this mechanism and Eq. (2.33) shows its corresponding irreversible version. Eqs. (2.34) to (2.35) on this page show both equations generated automatically with respect to Fig. 2.5a.

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{\frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2}} - \frac{k^{\mathrm{cat}}_{-j}[\mathrm{E}]_0[\mathrm{P}_1]}{K^{\mathrm{M}}_{j,\mathrm{P}_1}}}{1 + \frac{[\mathrm{S}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2}} + \frac{[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2][\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} k^{\mathrm{I}}_{j,\mathrm{P}_1}} + \frac{[\mathrm{P}_1]}{K^{\mathrm{M}}_{j,\mathrm{P}_1}}} \quad (2.32)$$

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} + K^{\mathrm{M}}_{j,\mathrm{S}_2}[\mathrm{S}_1] + K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2] + [\mathrm{S}_1][\mathrm{S}_2]} \quad (2.33)$$

$$v_{\texttt{re1}} = \frac{\texttt{kic\_re1\_s3}}{\texttt{kic\_re1\_s3} + [\mathrm{P1}]} \cdot \frac{\frac{\texttt{kcrf\_re1\_s4}}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} \cdot [\mathrm{E1}] \cdot [\mathrm{S1}] \cdot [\mathrm{S2}] - \frac{\texttt{kcrr\_re1\_s4}}{\texttt{kmc\_re1\_s3\_s4}} \cdot [\mathrm{E1}] \cdot [\mathrm{P1}]}{\mathscr{D}_1} \quad (2.34)$$

$$v_{\texttt{re1}} = \frac{\texttt{kic\_re1\_s3}}{\texttt{kic\_re1\_s3} + [\mathrm{P1}]} \cdot \frac{\texttt{kcat\_re1\_s4} \cdot [\mathrm{E1}] \cdot [\mathrm{S1}] \cdot [\mathrm{S2}]}{\mathscr{D}_2} \,, \quad (2.35)$$

where

$$\mathscr{D}_1 = 1 + \frac{\texttt{kmc\_re1\_s1\_s4} \cdot [\mathrm{S2}]}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} + \frac{[\mathrm{S1}]}{\texttt{kic\_re1\_s1\_s4}} + \frac{[\mathrm{S1}] \cdot [\mathrm{S2}]}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} + \frac{\texttt{kmc\_re1\_s1\_s4} \cdot [\mathrm{P1}] \cdot [\mathrm{S2}]}{\texttt{kic\_re1\_s1\_s4} \cdot \texttt{kic\_re1\_s3\_s4} \cdot \texttt{kmc\_re1\_s2\_s4}} + \frac{[\mathrm{P1}]}{\texttt{kmc\_re1\_s3\_s4}} \quad (2.36)$$

and

$$\mathscr{D}_2 = \texttt{kic\_re1\_s1\_s4} \cdot \texttt{kmc\_re1\_s2\_s4} + [\texttt{S1}] \cdot [\texttt{S2}] + \texttt{kmc\_re1\_s2\_s4} \cdot [\texttt{S1}] + \texttt{kmc\_re1\_s1\_s4} \cdot [\texttt{S2}] \,. \quad (2.37)$$

For the derivation of this formula see Section A.1 on page 34. The SBO term for the *ordered Bi-Uni mechanism rate law* is SBO:0000434.

2.1.6 Bi-bi enzyme reactions

Another special case covered by SBMLsqueezer is that of enzyme-catalyzed reactions with two substrates and two products. As was the case for the bi-uni reactions we can observe the possible mechanisms random order, ordered and, if no information about the mechanism is available, convenience kinetics. Additionally, a fourth reaction scheme can be applied: the ping-pong mechanism, that also has a fixed sequence in which all participating molecules bind to the enzyme. Furthermore, irreversible reactions without modulation can also be described by Eq. (2.22) on page 13. Fig. 2.6 illustrates one example of a process diagram for bi-bi reactions. As in the case of bi-uni reactions, it is also possible that only one substrate with stoichiometry coefficient two occurs. Here, there might also be just one product with stoichiometry coefficient two. In all following reaction schemes the mechanisms for inhibition are omitted due to the fact that modulation is included according to Eq. (2.1) on page 7.

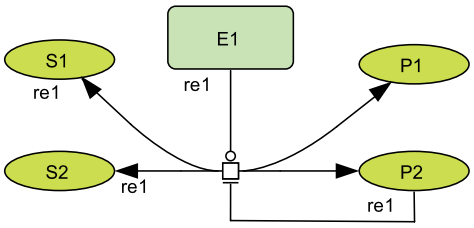


Figure 2.6: An example of a reversible bi-bi enzyme reaction. In this example, the product P_1 interferes with the reaction in a feedback inhibition mechanism.

Random Order Mechanism

The general reaction scheme of the random order mechanism for bi-bi reactions is given in Fig. 2.7 on the following page. The sequence in which the reactants bind to the enzyme and the products leave the enzyme complex is arbitrary. Eq. (2.38) models the reversible reaction with this rapid-equilibrium random order ternary-complex mechanism (Cornish-Bowden, 2004, p. 169) whereas the irreversible alternative is given by Eq. (2.39). The automatically derived equation for the example in Fig. 2.6 is shown in Eq. (2.40) on the following page with its corresponding irreversible form in Eq. (2.41) on the next page.

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \frac{\frac{k^{\text{cat}}_{+j}[E]_0[S_1][S_2]}{k^{\text{I}}_{j,S_1}K^{\text{M}}_{j,S_2}} - \frac{k^{\text{cat}}_{-j}[E]_0[P_1][P_2]}{K^{\text{M}}_{j,P_1}k^{\text{I}}_{j,P_2}}}{1 + \frac{[S_1]}{k^{\text{I}}_{j,S_1}} + \frac{[S_2]}{k^{\text{I}}_{j,S_2}} + \frac{[S_1][S_2]}{K^{\text{M}}_{j,S_2}k^{\text{I}}_{j,S_1}} + \frac{[P_1]}{k^{\text{I}}_{j,P_1}} + \frac{[P_2]}{k^{\text{I}}_{j,P_2}} + \frac{[P_1][P_2]}{k^{\text{I}}_{j,P_2}K^{\text{M}}_{j,P_1}}} \quad (2.38)$$

$$v_j(\vec{S},\vec{p}) = F_j(\vec{S},\vec{p}) \cdot \frac{k^{\text{cat}}_{+j}[E]_0[S_1][S_2]}{k^{\text{I}}_{j,S_1}K^{\text{M}}_{j,S_2} + K^{\text{M}}_{j,S_2}[S_1] + K^{\text{M}}_{j,S_1}[S_2] + [S_1][S_2]} \quad (2.39)$$

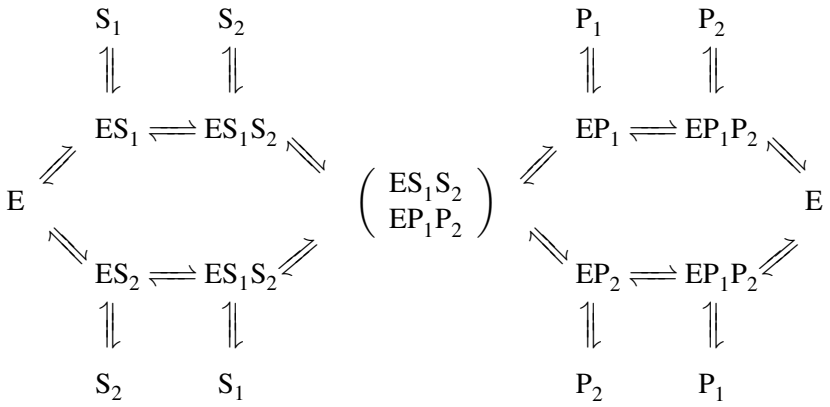


Figure 2.7: Reaction scheme of the random order bi-bi mechanism

$$v_{\mathtt{re1}} = \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathtt{P2}]} \cdot \frac{\frac{\mathtt{kcrf\_re1\_E1}}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \cdot [\mathtt{E1}] \cdot [\mathtt{S2}] \cdot [\mathtt{S1}] - \frac{\mathtt{kcrr\_re1\_E1}}{\mathtt{kic\_re1\_P1\_E1} \cdot \mathtt{kmc\_re1\_P2\_E1}} \cdot [\mathtt{E1}] \cdot [\mathtt{P2}] \cdot [\mathtt{P1}]}{\mathscr{D}_1} \tag{2.40}$$

$$v_{\mathtt{re1}} = \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathtt{P2}]} \cdot \frac{\mathtt{kcat\_re1\_E1} \cdot [\mathtt{E1}] \cdot [\mathtt{S1}] \cdot [\mathtt{S2}]}{\mathscr{D}_2}\,, \tag{2.41}$$

where

$$\mathscr{D}_1 = 1 + \frac{[\mathtt{S2}] \cdot [\mathtt{S1}]}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} + \frac{[\mathtt{S2}]}{\mathtt{kic\_re1\_S2\_E1}} + \frac{[\mathtt{P2}] \cdot [\mathtt{P1}]}{\mathtt{kic\_re1\_P1\_E1} \cdot \mathtt{kmc\_re1\_P2\_E1}} + \frac{[\mathtt{S1}]}{\mathtt{kic\_re1\_S1\_E1}} + \frac{[\mathtt{P1}]}{\mathtt{kic\_re1\_P1\_E1}} + \frac{[\mathtt{P2}]}{\mathtt{kic\_re1\_P2\_E1}} \tag{2.42}$$

and

$$\mathscr{D}_2 = \mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1} + [\mathtt{S2}] \cdot [\mathtt{S1}] + \mathtt{kmc\_re1\_S1\_E1} \cdot [\mathtt{S2}] + \mathtt{kmc\_re1\_S2\_E1} \cdot [\mathtt{S1}]\,. \tag{2.43}$$

Due to the following relations among the Michaelis and inhibition constants, the constants $K^{\mathrm{M}}_{j,\mathrm{S}_1}$ and $K^{\mathrm{M}}_{j,\mathrm{P}_2}$ do not appear explicitly in Eq. (2.38) on the preceding page:

$$K^{\mathrm{M}}_{j,\mathrm{S}_1} k^{\mathrm{I}}_{j,\mathrm{S}_2} = k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} \tag{2.44}$$

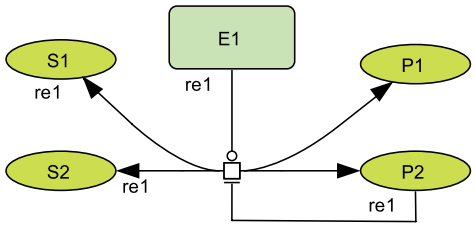
$$K^{\mathrm{M}}_{j,\mathrm{P}_1} k^{\mathrm{I}}_{j,\mathrm{S}_2} = k^{\mathrm{I}}_{j,\mathrm{P}_1} K^{\mathrm{M}}_{j,\mathrm{P}_2}\,. \tag{2.45}$$

At the time of writing, the SBO does not include a definition of the random order mechanism for bi-bi reactions.

Ordered Mechanism

Fig. 2.8b on the next page presents the reaction scheme for the ordered bi-bi mechanism, which is also called the compulsory-order ternary-complex mechanism (Cornish-Bowden, 2004, p. 166-168). As

in the bi-uni case (Section 2.1.5 on page 16), the sequence, in which all reactants bind to the enzyme, is fixed. Furthermore, the products also leave the enzyme complex in a defined sequence. A special case of this reaction is given when there is just one reactant or just one product with the stoichiometry of two.



(a) Example of a bi-bi enzyme reaction

$$\begin{array}{ccccccccccc} & & & S_1 & & S_2 & & P_1 & & P_2 & \\ & & & \uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & \\ E & \rightleftharpoons & ES_1 & \rightleftharpoons & ES_1S_2 & \rightleftharpoons & EP_1 & \rightleftharpoons & EP_2 & \rightleftharpoons & E \end{array}$$

(b) Reaction scheme

Figure 2.8: Reaction scheme for the ordered bi-bi mechanism

The formula for a reversible reaction is given by Eq. (2.46) whereas the corresponding irreversible form can be found in Eq. (2.47). An example for a generated equation with respect to Fig. 2.6 on page 17 can be found in Eq. (2.48). Since Eq. (2.39) on page 17 and Eq. (2.47) are equivalent, we omit to show an example of an automatically generated irreversible equation.

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{\frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2}} - \frac{k^{\mathrm{cat}}_{-j}[\mathrm{E}]_0[\mathrm{P}_1][\mathrm{P}_2]}{K^{\mathrm{M}}_{j,\mathrm{P}_1} k^{\mathrm{I}}_{j,\mathrm{P}_2}}}{\mathscr{D}_1} \tag{2.46}$$

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} + K^{\mathrm{M}}_{j,\mathrm{S}_2}[\mathrm{S}_1] + K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2] + [\mathrm{S}_1][\mathrm{S}_2]} \tag{2.47}$$

$$\begin{aligned} v_{\mathtt{re1}} = & \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathtt{P2}]} \cdot \frac{\frac{\mathtt{kcrf\_re1\_E1}}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \cdot [\mathtt{E1}] \cdot [\mathtt{S1}] \cdot [\mathtt{S2}]}{\mathscr{D}_{\mathtt{re1}}} \\ & - \frac{\frac{\mathtt{kcrr\_re1\_E1}}{\mathtt{kic\_re1\_P1\_E1} \cdot \mathtt{kmc\_re1\_P2\_E1}} \cdot [\mathtt{E1}] \cdot [\mathtt{P2}] \cdot [\mathtt{P1}]}{\mathscr{D}_{\mathtt{re1}}} \end{aligned} \tag{2.48}$$

where

$$\begin{aligned} \mathscr{D}_1 = 1 + \frac{[\mathrm{S}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2}} + \frac{[\mathrm{S}_1][\mathrm{S}_2]}{K^{\mathrm{M}}_{j,\mathrm{S}_2} k^{\mathrm{I}}_{j,\mathrm{S}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2][\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} k^{\mathrm{I}}_{j,\mathrm{P}_1}} + \frac{[\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{P}_2}} + \frac{K^{\mathrm{M}}_{j,\mathrm{P}_2}[\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{P}_2} K^{\mathrm{M}}_{j,\mathrm{P}_1}} \\ + \frac{K^{\mathrm{M}}_{j,\mathrm{P}_2}[\mathrm{S}_1][\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{P}_1} k^{\mathrm{I}}_{j,\mathrm{P}_2}} + \frac{[\mathrm{P}_1][\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{P}_2} K^{\mathrm{M}}_{j,\mathrm{P}_1}} + \frac{[\mathrm{S}_1][\mathrm{S}_2][\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1} K^{\mathrm{M}}_{j,\mathrm{S}_2} k^{\mathrm{I}}_{j,\mathrm{P}_1}} + \frac{[\mathrm{S}_2][\mathrm{P}_1][\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_2} K^{\mathrm{M}}_{j,\mathrm{P}_1} k^{\mathrm{I}}_{j,\mathrm{P}_2}}, \end{aligned} \tag{2.49}$$

and

$$\begin{aligned}\mathscr{D}_{\mathtt{re1}} = 1 &+ \frac{[\mathtt{P1}]}{\mathtt{kic\_re1\_P1\_E1}} + \frac{[\mathtt{S2}]}{\mathtt{kic\_re1\_S2\_E1}} + \frac{\mathtt{kmc\_re1\_P1\_E1}\cdot[\mathtt{P2}]}{\mathtt{kic\_re1\_P1\_E1}\cdot\mathtt{kmc\_re1\_P2\_E1}} \\ &+ \frac{\mathtt{kmc\_re1\_S2\_E1}\cdot[\mathtt{S1}]}{\mathtt{kic\_re1\_S2\_E1}\cdot\mathtt{kmc\_re1\_S1\_E1}} + \frac{[\mathtt{S2}]\cdot[\mathtt{S1}]}{\mathtt{kic\_re1\_S2\_E1}\cdot\mathtt{kmc\_re1\_S1\_E1}} \\ &+ \frac{\mathtt{kmc\_re1\_S2\_E1}\cdot[\mathtt{P1}]\cdot[\mathtt{S1}]}{\mathtt{kic\_re1\_S2\_E1}\cdot\mathtt{kic\_re1\_P1\_E1}\cdot\mathtt{kmc\_re1\_S1\_E1}} \\ &+ \frac{\mathtt{kmc\_re1\_P1\_E1}\cdot[\mathtt{P2}]\cdot[\mathtt{S2}]}{\mathtt{kic\_re1\_S2\_E1}\cdot\mathtt{kic\_re1\_P1\_E1}\cdot\mathtt{kmc\_re1\_P2\_E1}} \\ &+ \frac{[\mathtt{P2}]\cdot[\mathtt{P1}]}{\mathtt{kmc\_re1\_P2\_E1}\cdot\mathtt{kic\_re1\_P1\_E1}} \\ &+ \frac{[\mathtt{S2}]\cdot[\mathtt{P2}]\cdot[\mathtt{S1}]}{\mathtt{kic\_re1\_S2\_E1}\cdot\mathtt{kic\_re1\_P2\_E1}\cdot\mathtt{kmc\_re1\_S1\_E1}} \\ &+ \frac{[\mathtt{S1}]\cdot[\mathtt{P2}]\cdot[\mathtt{P1}]}{\mathtt{kic\_re1\_S1\_E1}\cdot\mathtt{kic\_re1\_P1\_E1}\cdot\mathtt{kmc\_re1\_P2\_E1}}\,. \end{aligned} \tag{2.50}$$

The SBO term for this equation is *Ordered Bi-Bi mechanism rate law* (SBO:0000433).

Ping-Pong Mechanism

A special case of the ordered mechanism is the ping-pong reaction, whose scheme is presented in Fig. 2.9. The reactants bind in a fixed sequence, and the products leave the enzyme complex in a specific succession. However, during the reaction, the enzyme passes through different states so that it can only react with the next reactant or set the next product free. This is why the mechanism is also called a substituted-enzyme mechanism. No corresponding bi-uni reaction exists because it would formally be equal to the ordered bi-uni mechanism. Eq. (2.51) gives the general formula for

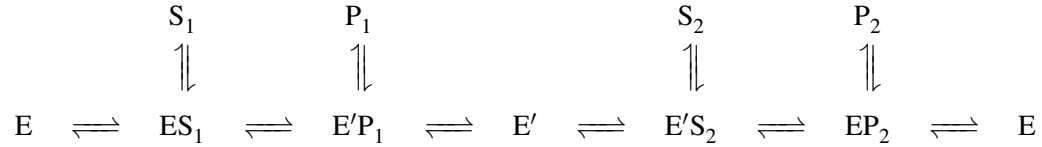


Figure 2.9: Reaction scheme of the ping-pong bi-bi mechanism

this particular mechanism, and its corresponding irreversible form is shown in Eq. (2.52) (Cornish-Bowden, 2004, p. 169-171). Eq. (2.53) on the facing page was generated by SBMLsqueezer with respect to the example in Fig. 2.6 on page 17.

$$\begin{aligned} v_j(\vec{\mathrm{S}},\vec{p}) =& F_j(\vec{\mathrm{S}},\vec{p}) \\ &\cdot \frac{\frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}K^{\mathrm{M}}_{j,\mathrm{S}_2}} - \frac{k^{\mathrm{cat}}_{-j}[\mathrm{E}]_0[\mathrm{P}_1][\mathrm{P}_2]}{K^{\mathrm{M}}_{j,\mathrm{P}_2}k^{\mathrm{I}}_{j,\mathrm{P}_1}}}{\frac{[\mathrm{S}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}K^{\mathrm{M}}_{j,\mathrm{S}_2}} + \frac{[\mathrm{S}_1][\mathrm{S}_2]}{K^{\mathrm{M}}_{j,\mathrm{S}_2}k^{\mathrm{I}}_{j,\mathrm{S}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2][\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}K^{\mathrm{M}}_{j,\mathrm{S}_2}k^{\mathrm{I}}_{j,\mathrm{P}_2}} + \frac{[\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{P}_1}} + \frac{K^{\mathrm{M}}_{j,\mathrm{P}_1}[\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{P}_1}K^{\mathrm{M}}_{j,\mathrm{P}_2}} + \frac{[\mathrm{S}_1][\mathrm{P}_1]}{k^{\mathrm{I}}_{j,\mathrm{S}_1}k^{\mathrm{I}}_{j,\mathrm{P}_1}} + \frac{[\mathrm{P}_1][\mathrm{P}_2]}{k^{\mathrm{I}}_{j,\mathrm{P}_1}K^{\mathrm{M}}_{j,\mathrm{P}_2}}} \end{aligned} \tag{2.51}$$

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot \frac{k^{\mathrm{cat}}_{+j}[\mathrm{E}]_0[\mathrm{S}_1][\mathrm{S}_2]}{K^{\mathrm{M}}_{j,\mathrm{S}_2}[\mathrm{S}_1] + K^{\mathrm{M}}_{j,\mathrm{S}_1}[\mathrm{S}_2] + [\mathrm{S}_1][\mathrm{S}_2]} \tag{2.52}$$

$$v_{\mathtt{re1}} = \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathrm{P2}]} \cdot \frac{\frac{\mathtt{kcrf\_re1\_E1}}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \cdot [\mathrm{E1}] \cdot [\mathrm{S1}] \cdot [\mathrm{S2}] - \frac{\mathtt{kcrr\_re1\_E1}}{\mathtt{kic\_re1\_P2\_E1} \cdot \mathtt{kmc\_re1\_P1\_E1}} \cdot [\mathrm{E1}] \cdot [\mathrm{P2}] \cdot [\mathrm{P1}]}{\mathscr{D}_{\mathtt{re1}}}, \tag{2.53}$$

where

$$\begin{aligned}\mathscr{D}_{\mathtt{re1}} = {} & \frac{[\mathrm{S2}]}{\mathtt{kic\_re1\_S2\_E1}} + \frac{\mathtt{kmc\_re1\_P2\_E1} \cdot [\mathrm{P1}]}{\mathtt{kic\_re1\_P2\_E1} \cdot \mathtt{kmc\_re1\_P1\_E1}} + \frac{\mathtt{kmc\_re1\_S2\_E1} \cdot [\mathrm{S1}]}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \\ & + \frac{[\mathrm{P2}]}{\mathtt{kic\_re1\_P2\_E1}} + \frac{[\mathrm{S2}] \cdot [\mathrm{S1}]}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \\ & + \frac{\mathtt{kmc\_re1\_S2\_E1} \cdot [\mathrm{P1}] \cdot [\mathrm{S1}]}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kic\_re1\_P1\_E1} \cdot \mathtt{kmc\_re1\_S1\_E1}} \\ & + \frac{[\mathrm{S2}] \cdot [\mathrm{P2}]}{\mathtt{kic\_re1\_S2\_E1} \cdot \mathtt{kic\_re1\_P2\_E1}} + \frac{[\mathrm{P2}] \cdot [\mathrm{P1}]}{\mathtt{kic\_re1\_P2\_E1} \cdot \mathtt{kmc\_re1\_P1\_E1}}. \end{aligned} \tag{2.54}$$

Comparing all bi-bi kinetic formulas, one can see that the ordered mechanism is the slowest one because of its large denominator.

The *Ping Pong Bi-Bi mechanism rate law* can be found at SBO:0000436.

2.1.7 Convenience kinetics and thermodynamics

In their original work Liebermeister and Klipp (2006) publish the convenience kinetics in two forms:

$$v_j(\vec{\mathrm{S}}, \vec{p}) = F_j(\vec{\mathrm{S}}, \vec{p}) \cdot [\mathrm{E}_j] \cdot \frac{k^{\mathrm{cat}}_{+j} \prod_i \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^{n^-_{ij}} - k^{\mathrm{cat}}_{-j} \prod_i \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^{n^+_{ij}}}{\prod_i \sum_{m=0}^{n^-_{ij}} \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^m + \prod_i \sum_{m=0}^{n^+_{ij}} \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^m - 1} \tag{2.55}$$

$$v_j(\vec{\mathrm{S}}, \vec{p}) = F_j(\vec{\mathrm{S}}, \vec{p}) \cdot k^{\mathrm{V}}_j \cdot [\mathrm{E}_j] \cdot \frac{\prod_i \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^{n^-_{ij}} \left(k^{\mathrm{G}}_i K^{\mathrm{M}}_{ij}\right)^{-\frac{n_{ij}}{2}} - \prod_i \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^{n^+_{ij}} \left(k^{\mathrm{G}}_i K^{\mathrm{M}}_{ij}\right)^{\frac{n_{ij}}{2}}}{\prod_i \sum_{m=0}^{n^-_{ij}} \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^m + \prod_i \sum_{m=0}^{n^+_{ij}} \left(\frac{[\mathrm{S}_i]}{K^{\mathrm{M}}_{ij}}\right)^m - 1}. \tag{2.56}$$

The prefactor $F_j(\vec{\mathrm{S}}, \vec{p})$ introduces the modifiers for activation or inhibition to the kinetic equation and is defined in Eq. (2.1) on page 7. If the reaction does not explicitly reference an enzymatic catalyst, the parameters $k^{\mathrm{cat}}_{\pm j}$, which have the same units and SBO term definitions than the parameters of the Michaelis-Menten equation (see Section 2.1.2 on page 10), can be replaced by the *limiting rates* $V^{\mathrm{m}}_{\pm j}$ (also defined in Section 2.1.2 on page 10).

Eq. (2.55) can be applied to any enzyme-catalyzed reaction. However, if the stoichiometric matrix $\mathbf{N}$ of the reaction system contains linearly dependent columns, i.e., $\mathbf{N}$ does not have full column rank, then at least one reaction is thermodynamically dependent on another. In this case, choosing the parameters of the equation while ignoring this dependency may fit given measurement data well but will violate the thermodynamic constraints of the system. Hence, Liebermeister and Klipp derived a second form of convenience kinetics, which is shown in Eq. (2.56). The parameters $k^{\mathrm{cat}}_{\pm j}$ are replaced by $\prod_i \left(k^{\mathrm{G}}_i K^{\mathrm{M}}_{ij}\right)^{\mp \frac{n_{ij}}{2}}$ and the whole fraction is multiplied by the additional parameter k^{V}_j. This ensures that all newly introduced parameters are thermodynamically independent. Note that every dimensionless

energy constant k_i^{G} stands for molecule *i* regardless of the respective reaction, whereas every *velocity* k_j^{V} (in per `time` or `size` per `time`, depending on the units of the enzyme) is a parameter for reaction R_j. The Michaelis-analog parameter K_{ij}^{M} depends on both reaction R_j and molecule S_i and thus links both parameters together. This parameter has the same units and SBO annotation than the Michaelis constant described in Section 2.1.2 on page 10. Currently, the SBO does not contain any definition for the parameters k_i^{G} and k_j^{V}. For a complete derivation see the original paper of Liebermeister and Klipp (2006).

Because Eq. (2.56) on the preceding page is more complicated and contains additional parameters, SBMLsqueezer uses the simpler formula whenever applicable. To ensure the thermodynamic correctness of the system, an implementation of the Gaussian algorithm, which computes the rank of a matrix, is invoked. If the stoichiometric matrix of the system has full column rank, there is no need to apply Eq. (2.56) on the previous page. Otherwise SBMLsqueezer will assign every reaction to be modeled using convenience kinetics with Eq. (2.56) on the preceding page.

For all enzyme-catalyzed reactions independent of the mechanism, convenience kinetics may be an appropriate choice if the user lacks detailed biochemical knowledge. As stated before, reactions with more than two substrate molecules are unlikely to take place. SBMLsqueezer will show a warning message for such reactions. This number does not only stand for the number of different reactant species, but rather for the stoichiometry on the left hand side. For instance, the reaction

$$4\,\mathrm{A} \longrightarrow 6\,\mathrm{B} \tag{2.57}$$

will also be considered unrealistic. This warning is, however, user-defined and the equations can still be generated properly pursuant to the particular formula. In the case of the context menu, warnings are shown whenever the number of reacting species exceeds two.

In an application of convenience kinetics to a mixed network together with uni-uni Michaelis-Menten equations, it was shown that convenience kinetics leads to reasonable results when fitted to in vivo data (Dräger *et al.*, 2007a, 2009a).

At the time of writing no form of convenience kinetics is included in the Systems Biology Ontology (SBO, Fig. B.1 on page 43). However, for reactions with multiple reactants the general term SBO:-0000429[40] can be applied.

Thermodynamically dependent form

Eq. (2.55) on the preceding page shows the thermodynamically dependent formula of convenience kinetics for reversible reactions. Eq. (2.58) gives its corresponding irreversible form (Liebermeister and Klipp, 2006). An example of a generated rate law is shown in Eq. (2.59) for the bi-bi reaction presented in Fig. 2.6 on page 17.

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot [\mathrm{E}]_0 \frac{k_{+j}^{\mathrm{cat}} \prod_i \left(\frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}}\right)^{n_{ij}^-}}{\prod_i \sum_{m=0}^{n_{ij}^-} \left(\frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}}\right)^m} \tag{2.58}$$

$$v_{\mathtt{re1}} = \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathrm{P2}]} \cdot [\mathrm{E1}] \cdot \frac{\mathtt{kcat\_re1\_E1} \cdot \frac{[\mathrm{S2}]}{\mathtt{kmc\_re1\_S2\_E1}} \cdot \frac{[\mathrm{S1}]}{\mathtt{kmc\_re1\_S1\_E1}}}{\left(1 + \frac{[\mathrm{S2}]}{\mathtt{kmc\_re1\_S2\_E1}}\right) \cdot \left(1 + \frac{[\mathrm{S1}]}{\mathtt{kmc\_re1\_S1\_E1}}\right)} \tag{2.59}$$

[40] Enzymatic rate law for multi-reactant enzymes

Thermodynamically independent form

As stated at the beginning of this Section (see page 21) if there are linear dependencies within the stoichiometric matrix, SBMLsqueezer applies the thermodynamically independent form of convenience kinetics, which is shown in Eq. (2.56) on page 21 and for its corresponding irreversible form in Eq. (2.60) (Liebermeister and Klipp, 2006). Eq. (2.62) shows the generated independent form of irreversible version of the reaction example depicted in Fig. 2.6 on page 17.

$$v_j(\vec{\mathrm{S}},\vec{p}) = F_j(\vec{\mathrm{S}},\vec{p}) \cdot [\mathrm{E}] \cdot k_j^{\mathrm{V}} \frac{\prod_i \left(\frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}}\right)^{n_{ij}^-} \left(k_i^{\mathrm{G}} K_{ij}^{\mathrm{M}}\right)^{-\frac{n_{ij}}{2}}}{\prod_i \sum_{m=0}^{n_{ij}^-} \left(\frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}}\right)^{m}} \tag{2.60}$$

$$v_{\mathtt{re1}} = \frac{\mathtt{kic\_re1\_P2}}{\mathtt{kic\_re1\_P2} + [\mathtt{P2}]} \cdot [\mathtt{E1}] \cdot \mathtt{kcrg\_re1\_E1} \cdot \frac{\frac{[\mathtt{S2}]}{\mathtt{kmc\_re1\_S2\_E1}} \cdot \frac{[\mathtt{S1}]}{\mathtt{kmc\_re1\_S1\_E1}} \cdot \mathscr{R}}{\left(1 + \frac{[\mathtt{S2}]}{\mathtt{kmc\_re1\_S2\_E1}}\right) \cdot \left(1 + \frac{[\mathtt{S1}]}{\mathtt{kmc\_re1\_S1\_E1}}\right)} \tag{2.61}$$

(2.62)

where

$$\mathscr{R} = \sqrt{\frac{\mathtt{kG\_S2} \cdot \mathtt{kmc\_re1\_S2\_E1} \cdot \mathtt{kG\_S1} \cdot \mathtt{kmc\_re1\_S1\_E1}}{\mathtt{kG\_P2} \cdot \mathtt{kmc\_re1\_P2\_E1} \cdot \mathtt{kG\_P1} \cdot \mathtt{kmc\_re1\_P1\_E1}}} \,. \tag{2.63}$$

2.1.8 Modular rate laws for enzymatic reactions

In 2010, Liebermeister *et al.* propose a set of five modular rat laws (*Power-law modular rate law (PM)*, *Common modular rate law (CM)*, *Direct binding modular rate law (DM)*, *Simultaneous binding modular rate law (SM)*, and *Force-dependent modular rate law (FM)*) as an advancement of the convenience kinetics. These rate laws include as special cases mass-action, Michaelis-Menten, and uni-uni reversible Hill kinetics.

One important driving force of the development of these new kinetic equations is to ensure thermodynamic correctness and numerical stability of all parameters. In particular, all kinetic constants in these equations satisfy the thermodynamic Wegscheider conditions

$$\mathbf{K}^T \ln \vec{k^{\mathrm{eq}}} = 0 \,, \tag{2.64}$$

with the null-space matrix $\mathbf{K}$ of the stoichiometric matrix $\mathbf{N}$ that describes the reactions of all, i.e., internal and external, metabolites, and the vector of equilibrium constants for all reactions $\vec{k^{\mathrm{eq}}}$. Writing all equilibrium constants in terms of standard chemical potentials $\mu_i^{(0)}$ of the participating species

$$\ln k_j^{\mathrm{eq}} = -\frac{1}{RT} \sum_i n_{ij} \mu_i^{(0)} \tag{2.65}$$

ensures the above conditions. This equation includes the *temperature T* (in Kelvin, see SBO:0000147) and Boltzmann's gas constant $R = 8.31447215\,\mathrm{J} \cdot \mathrm{K}^{-1} \cdot \mathrm{mol}^{-1}$.

Furthermore, all parameters satisfy the Haldane relationships, i.e., a relation interelating phenomenological coefficients such as Michaelis constants or limiting rates with the equilibrium constant of the reaction. For the turnover rates $k_j^{\pm}$, the velocity constants k_j^{V}, the Michaelis constants K_{ij}^{M} and the Hill coefficients h_j this relationship reads

$$k_j^{\pm} = k_j^{\mathrm{V}} \left(k_j^{\mathrm{eq}} \prod_i \left(K_{ij}^{\mathrm{M}}\right)^{-n_{ij}} \right)^{\pm \frac{h_j}{2}} \,. \tag{2.66}$$

The name *modular rate laws* originates from the three different versions, in which each one of these five rate laws can occur. The simplest version is the `cat` mode, in which the rate laws contain the turnover rates $k_j^{\pm}$ as parameters. The `hal` mode ensures the Haldane relationship by replacing these turnover rates with Eq. (2.66) on the preceding page. By expressing the equilibrium constants in the `hal` mode in terms of standard chemical potentials (see Eq. (2.65) on the previous page), the `weg` mode can be obtained.

Hence, for all combinations of parameters the system will still be physically feasible. This can in practice be achieved by at first using the more complex `weg` mode for parameter estimation and then by computing the parameters of the more simple modes from these. The settings of SBMLsqueezer allow the user to select one version of the modular rate laws.

Like the convenience kinetics, the parameters in the modular rate laws exhibit analogy to parameters in traditional rate laws. Therefore, these equations are designed to be applied in large-scale modeling with a minimum of human intervention where parameter values may originate from literature mining, fitting, or sampling. All rate laws are derived from the common assumption that all substrate molecules bind cooperatively to the enzyme and include several types of enzyme regulation and can only be applied to reversible reactions. In the following all five modular rate laws will be introduced.

The SBO contains all modular rate laws in the subgraph *modular rate law* (SBO:0000527).

Power-law modular rate law

This equation is equivalent to a mass-action rate law with appropriate parameter settings. Its numerator can be chosen from one of the following three versions:

$$\mathrm{cat}_j(\vec{\mathrm{S}},\vec{p}) = k_j^+ \prod_i \left(\frac{[\mathrm{S}_j]}{K_{ij}^{\mathrm{M}}}\right)^{h_j \cdot n_{ij}^-} - k_j^- \prod_i \left(\frac{[\mathrm{S}_j]}{K_{ij}^{\mathrm{M}}}\right)^{h_j \cdot n_{ij}^+} \tag{2.67}$$

$$\mathrm{hal}_j(\vec{\mathrm{S}},\vec{p}) = k_r^{\mathrm{V}} \cdot \frac{\sqrt{\left(k_j^{\mathrm{eq}}\right)^{h_j}} \prod_i [\mathrm{S}_i]^{h_j \cdot n_{ij}^-} - \sqrt{\left(k_j^{\mathrm{eq}}\right)^{-h_j}} \prod_i [\mathrm{S}_i]^{h_j \cdot n_{ij}^+}}{\sqrt{\prod_i \left(K_{ij}^{\mathrm{M}}\right)^{h_j \cdot |n_{ij}|}}} \tag{2.68}$$

$$\mathrm{weg}_j(\vec{\mathrm{S}},\vec{p}) = k_j^{\mathrm{V}} \cdot \frac{\exp\left(-\frac{h_j \sum_i n_{ij} \mu_i^{(0)}}{2RT}\right) \prod_i [\mathrm{S}_i]^{h_j \cdot n_{ij}^-} - \exp\left(\frac{h_j \sum_i n_{ij} \mu_i^{(0)}}{2RT}\right) \prod_i [\mathrm{S}_i]^{h_j \cdot n_{ij}^+}}{\sqrt{\prod_i \left(K_{ij}^{\mathrm{M}}\right)^{h_j \cdot |n_{ij}|}}} \,. \tag{2.69}$$

In case of absence of specific modulators the denominator $\Theta_j(\vec{\mathrm{S}},\vec{p})$ of the *power-law modular rate law* reads:

$$\Theta_j(\vec{\mathrm{S}},\vec{p}) = 1 \tag{2.70}$$

The denominator of the rate law can be extended by adding effects of specific modifiers:

$$f_j^{\mathrm{si}}(\vec{\mathrm{S}},\vec{p}) = \sum_i \left(\frac{[\mathrm{S}_i]}{k_{ji}^{\mathrm{I}}}\right)^{w_{ji}^{-*}} \tag{2.71}$$

$$f_j^{\mathrm{sa}}(\vec{\mathrm{S}},\vec{p}) = \sum_i \left(\frac{k_{ji}^{\mathrm{A}}}{[\mathrm{S}_i]}\right)^{w_{ji}^{+*}} \tag{2.72}$$

with the specific inhibition function $f_j^{\mathrm{si}}(\vec{\mathrm{S}},\vec{p})$, the inhibitory constant k_{ji}^{I}, the specific activation function $f_j^{\mathrm{sa}}(\vec{\mathrm{S}},\vec{p})$, the activation constant k_{ji}^{A}, and the element $w_{ji}^{\pm *}$ from the modulation matrix $\mathbf{W}$ that is marked with an asterisk to distinguish specific modulation from other types of modulation, which can be included by multiplying the following prefactors with the rate law:

$$f_j^{\mathrm{ca}}(\vec{\mathrm{S}},\vec{p}) = \prod_i \left(\frac{[\mathrm{S}_i]}{[\mathrm{S}_i] + k_{ji}^{\mathrm{A}}} \right)^{w_{ji}^{+}} \tag{2.73}$$

$$f_j^{\mathrm{ci}}(\vec{\mathrm{S}},\vec{p}) = \prod_i \left(\frac{k_{ji}^{\mathrm{I}}}{[\mathrm{S}_i] + k_{ji}^{\mathrm{I}}} \right)^{w_{ji}^{-}} \tag{2.74}$$

$$f_j^{\mathrm{nca}}(\vec{\mathrm{S}},\vec{p}) = \prod_i \left(\rho_{ji}^{\mathrm{A}} + \left(1 - \rho_{ji}^{\mathrm{A}}\right) \frac{[\mathrm{S}_i]}{[\mathrm{S}_i] + k_{ji}^{\mathrm{A}}} \right)^{w_{ji}^{+}} \tag{2.75}$$

$$f_j^{\mathrm{nci}}(\vec{\mathrm{S}},\vec{p}) = \prod_i \left(\rho_{ji}^{\mathrm{I}} + \left(1 - \rho_{ji}^{\mathrm{I}}\right) \frac{k_{ji}^{\mathrm{I}}}{[\mathrm{S}_i] + k_{ji}^{\mathrm{I}}} \right)^{w_{ji}^{-}} , \tag{2.76}$$

where f_j^{ca} and f_j^{nca} denote the function for complete and non-competitive activation, whereas f_j^{ci} and f^{nci} fulfill the purpose of complete and non-competitive inhibition. Hence, the complete rate law for the *power-law modular rate law* reads

$$v_j(\vec{\mathrm{S}},\vec{p}) = f_j^{\mathrm{ca}}(\vec{\mathrm{S}},\vec{p}) \cdot f_j^{\mathrm{nca}}(\vec{\mathrm{S}},\vec{p}) \cdot f_j^{\mathrm{ci}}(\vec{\mathrm{S}},\vec{p}) \cdot f_j^{\mathrm{nci}}(\vec{\mathrm{S}},\vec{p}) \cdot [\mathrm{E}_0] \cdot \frac{\mathrm{num}_j(\vec{\mathrm{S}},\vec{p})}{\Theta_j(\vec{\mathrm{S}},\vec{p}) + f_j^{\mathrm{sa}}(\vec{\mathrm{S}},\vec{p}) + f_j^{\mathrm{si}}(\vec{\mathrm{S}},\vec{p})} \tag{2.77}$$

with $\mathrm{num}_j(\vec{\mathrm{S}},\vec{p})$ being one of the three functions cat_j, hal_j, or weg_j (see Eqs. (2.67) to (2.69) on the facing page). The PM rate law and all following kinetic equations only differ in the denominator Θ_j. Hence, all following rate laws constitute special cases of the PM rate law. Due to the high number of possible combinations, we omit examples of kinetic equations generated by SBMLsqueezer and refer to Table A.5 in the supplementary material to the original work of Liebermeister *et al.* (2010), where all parameter values are explained in detail.

The power-law modular rate law can be annotated with SBO term SBO:0000531.

Common modular rate law

The denominator of this generalized Michaelis-Menten rate law reads

$$\Theta_j(\vec{\mathrm{S}},\vec{p}) = \prod_i \left(1 + \frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}} \right)^{h_j \cdot n_{ij}^{-}} + \prod_i \left(1 + \frac{[\mathrm{S}_i]}{K_{ij}^{\mathrm{M}}} \right)^{h_j \cdot n_{ij}^{+}} - 1 \,. \tag{2.78}$$

In contrast to the Michaelis-Menten equation, this generalized rate law can be applied to any reversible enzyme-catalyzed reaction, irrespective of its stoichiometry. The assumption underlying this rate law is that a fast equilibrium state of binding and unbinding of the substrates exists and that the enzyme cannot bind substrates and products at the same time.

The SBO-term for this rate law is SBO:0000528.

Direct binding modular rate law

For the direct binding modular rate law function Θ_j takes the following form:

$$\Theta_j(\vec{S},\vec{p}) = 1 + \prod_i \left(\frac{[S_i]}{K_{ij}^M}\right)^{h_j \cdot n_{ij}^-} + \prod_i \left(\frac{[S_i]}{K_{ij}^M}\right)^{h_j \cdot n_{ij}^+} . \tag{2.79}$$

This rate law simplifies the common modular rate law based on the assumption that the substrates bind in one single step.

To annotate this rate law with an SBO term, the identifier SBO:0000529 can be used.

Simultaneous binding modular rate law

The denominator of this rate law reads

$$\Theta_j(\vec{S},\vec{p}) = \prod_i \left(1 + \frac{[S_i]}{K_{ij}^M}\right)^{h_j \cdot |n_{ij}|} . \tag{2.80}$$

In this rate law, virtually the product is "bound as a substrate" to the enzyme during the conversion process. Hence the number of interconversion steps increases in comparison to all other rate laws leading to a slower velocity of the reaction.

The simultaneous binding modular rate law has the SBO term identifier SBO:0000530.

Force-dependent modular rate law

For this rate law the denominator is defined as

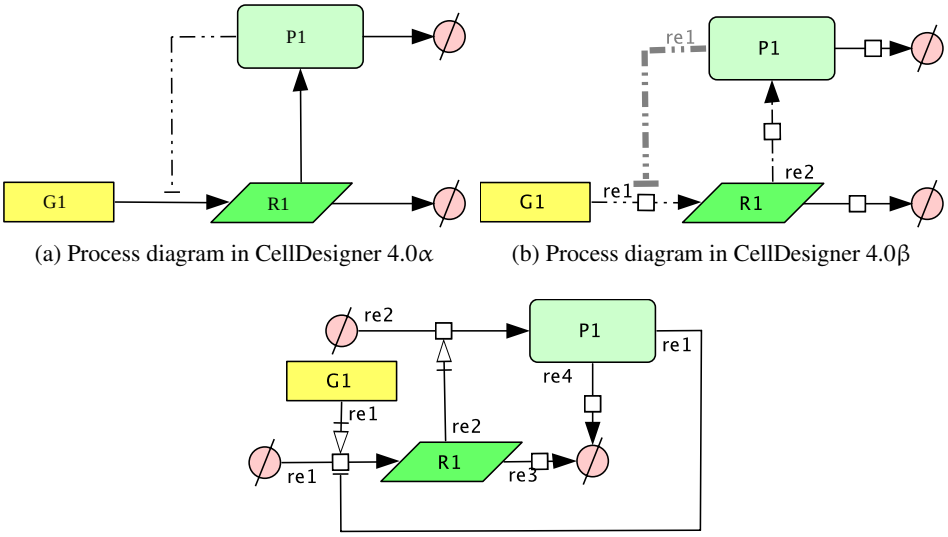
$$\Theta_j(\vec{S},\vec{p}) = \sqrt{\prod_i \left(\frac{[S_i]}{K_{ij}^M}\right)^{h_j \cdot |n_{ij}|}} . \tag{2.81}$$

This rate law yields a compromise of the saturation behavior of all other modular rate laws and the (multi-) linear behavior of mass-action kinetics.

This rate law can be annotated with SBO term identifier SBO:0000532.

2.2 Kinetic equations for gene-regulatory reactions

Gene regulation can also be modeled using CellDesigner. Fig. 2.10 on the next page depicts three different versions of an example process considered gene regulation. Gene G_1 is expressed and the RNA molecule R_1 assembles. This process is (transcriptionally) inhibited by the translation product, protein P_1. Note that the concentration of gene G_1 remains unchanged during this process as the transcription does not change the state of the gene. SBMLsqueezer recognizes mistakes within the SBML file and sets the boundary conditions of genes to `true`. Furthermore, SBMLsqueezer will show warnings if a transcription is, for instance, *translationally* activated. Since the release of CellDesigner 4.0β there have been two special arrows for the transitions described here (Fig. 2.10b on the facing page): *transcription* and *translation*. To ensure backwards compability, SBMLsqueezer supports simple state transitions (Fig. 2.10a on the next page), even between genes and RNA as well as between RNA and proteins. However, if transcription and translation arrows are used, SBMLsqueezer will show a warning message if these are mixed up. CellDesigner 4.1β still provides these specific arrows but users should now express gene-regulatory processes in terms of triggers, stimulators and inhibitors (Fig. 2.10c on the facing page) as defined in the SBGN specification (Le Novére *et al.*, 2008, p. 65).



(a) Process diagram in CellDesigner 4.0α

(b) Process diagram in CellDesigner 4.0β

(c) Process diagram in CellDesigner 4.1β

Figure 2.10: An example of gene expression regulation in CellDesigner notation

2.2.1 Various versions of the Hill equation

Hill (1910) derived an empirical equation to describe the sigmoid function of O_2 saturation on hemoglobin (see Sec. 2.1.4 on page 14):

$$v_j(S_i, \vec{p}) = V_j^{\mathrm{m}} \cdot \frac{[S_i]^{h_{ij}}}{[S_i]^{h_{ij}} + \left(K_j^{\mathrm{S}}\right)^{h_{ij}}} \,. \tag{2.82}$$

As a major advantage, this equation is able to describe cooperatively effects, which is important if the binding of a ligand on a macromolecule highly depends on the number of ligands already bound to the macromolecule. The binding of O_2 on hemoglobin is one example for such a cooperative process. The dimensionless *Hill coefficient* h_{ij} (SBO:0000190) quantifies this effect. It describes the fraction of macromolecules that are saturated with ligands as a function of the concentration of the ligands. A coefficient of one corresponds to an independent binding of all ligands. If $h_{ij} > 1$ the binding of ligands enhances the binding of additional ligands (positive cooperatively). If ligands compete for free binding places, a negative cooperatively can be expressed with $h_{ij} < 1$. However, this coefficient does not correspond to the number of free binding sites in the macromolecule because the equation was derived in a purely empirical way (Cornish-Bowden, 2004, p. 244).

Although Hill's equation was originally derived for metabolic systems, many publications suggest variants of it to model gene-regulatory networks. For this purpose, Hinze *et al.* (2007) and Radde

(2007); Radde and Kaderali (2007) apply the following differential equations:

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{S}_i] &= V_i^{\mathrm{m}} \cdot h(A_1, K^{\mathrm{S}}_{iA_1}, h_{iA_1}) \cdot \ldots \cdot h(A_{|\vec{A}|}, K^{\mathrm{S}}_{iA_{|\vec{A}|}}, h_{iA_{|\vec{A}|}}) \cdot \\ &\quad (1 - h(I_1, K^{\mathrm{S}}_{iI_1}, h_{iI_1}) \cdot \ldots \cdot h(I_{|\vec{I}|}, K^{\mathrm{S}}_{iI_{|\vec{I}|}}, h_{iI_{|\vec{I}|}})) - \gamma_i \cdot [\mathrm{S}_i] \\ &= V_i^{\mathrm{m}} \cdot \prod_{j=1}^{|\vec{A}|} h(A_j, K^{\mathrm{S}}_{ij}, h_{ij}) \cdot \left(1 - \prod_{k=1}^{|\vec{I}|} h(I_k, K^{\mathrm{S}}_{ik}, h_{ik})\right) - \gamma_i \cdot [\mathrm{S}_i]\end{aligned} \tag{2.83}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{S}_i] = s_i + \sum_{j=1}^{J} k_{ij} \cdot h(\mathrm{S}_j, K^{\mathrm{S}}_{ij}, h_{ij}) - \gamma_i \cdot [\mathrm{S}_i]\,, \tag{2.84}$$

where h denotes the sigmoid regulation function

$$h(\mathrm{S}_j, K^{\mathrm{S}}_{i\mathrm{S}_j}, h_{ij}) = \frac{[\mathrm{S}_j]^{h_{ij}}}{[\mathrm{S}_j]^{h_{ij}} + \left(K^{\mathrm{S}}_{i\mathrm{S}_j}\right)^{h_{ij}}} \tag{2.85}$$

with the Hill coefficient h_{ij}, the vector of activators $\vec{A}$ and the vector of inhibitors $\vec{I}$. The constant $K^{\mathrm{S}}_{i\mathrm{S}_j}$ (in `substance` per `size` or `substance`, depending on the units of the corresponding species S_i) denotes the *half saturation constant* according to Heinrich and Schuster (1996, p. 23) or the *Hill constant* as it is defined in SBO:0000191. The parameter V_j^{m} is equivalent to the *limiting rate* in the Michaelis-Menten equation (Cornish-Bowden, 2004, see Section 2.1.2 on page 10). The parameter k_{ij} was introduced by Radde and Kaderali (2007) to express the maximal influence of the gene product j on the formation of gene product i, but is effectively equivalent to the limiting rate V_j^{m}. The degradation rate γ_i fulfills the same role as the the *first order rate constant* in mass-action kinetics (see Eq. (2.5) to (2.6) on page 8). The Eqs. (2.83) to (2.84) on this page express the temporal changes of gene product S_i influenced by activators and repressors and include a term for the degeneration of the gene product S_i. In these equations K^{S}_{ij} denotes the minimum concentration that is required for regulator j to have a significant influence on gene i. To be applicable to a reaction network as shown in Fig. 2.10 on the previous page, the degeneration processes have to be separated from the formation of the gene products in two distinct reactions.

SBMLsqueezer offers the general Hill equation according to Hinze *et al.* (2007) as a kinetic law for the formation of gene products as given in Eq. (2.86). The formula for the translation example in Fig. 2.10c on the previous page can be found in Eq. (2.87) and the rate of transcription generated according to Fig. 2.10c on the previous page is given by Eq. (2.88). Note that the exponents $w^{\pm}_{ji}$ are defined according to the modulation matrices $\mathbf{W}^{\pm}$ in Section 2.1.7 on page 21. This leads to the following form of the Hill-Hinze equation:

$$v_j(\vec{\mathrm{S}}, \vec{p}) = v_j^{\mathrm{m}} \prod_i \left(\frac{[\mathrm{S}_i]^{h_{ij}}}{[\mathrm{S}_i]^{h_{ij}} + \left(K^{\mathrm{S}}_{ij}\right)^{h_{ij}}}\right)^{w^{+}_{ji}} \left(1 - \prod_i \left(\frac{[\mathrm{S}_i]^{h_{ij}}}{[\mathrm{S}_i]^{h_{ij}} + \left(K^{\mathrm{S}}_{ij}\right)^{h_{ij}}}\right)^{w^{-}_{ji}}\right) \tag{2.86}$$

$$\begin{aligned}v_{\mathtt{re1}} = &\mathtt{vmax\_re1} \cdot \frac{[\mathtt{G1}]^{\mathtt{hic\_re1\_s1}}}{[\mathtt{G1}]^{\mathtt{hic\_re1\_s1}} + \mathtt{ksp\_re1\_s1}^{\mathtt{hic\_re1\_s1}}} \\ &\cdot \left(1 - \frac{[\mathtt{P1}]^{\mathtt{hic\_re1\_s3}}}{[\mathtt{P1}]^{\mathtt{hic\_re1\_s3}} + \mathtt{ksp\_re1\_s3}^{\mathtt{hic\_re1\_s3}}}\right)\end{aligned} \tag{2.87}$$

$$v_{\mathtt{re2}} = \mathtt{vmax\_re2} \cdot \frac{[\mathtt{R1}]^{\mathtt{hic\_re2\_s2}}}{[\mathtt{R1}]^{\mathtt{hic\_re2\_s2}} + \mathtt{ksp\_re2\_s2}^{\mathtt{hic\_re2\_s2}}} \tag{2.88}$$

Furthermore, SBMLsqueezer provides the variant of the Hill equation suggested by Radde and Kaderali for transcription reactions in the following form:

$$v_j(\vec{\mathrm{S}}, \vec{p}) = s_j + \sum_{i=1} k_{ij} \cdot h(\mathrm{S}_j, K_{ij}^{\mathrm{S}}, h_{ij}) \,. \tag{2.89}$$

The SBO defines three basic forms of the Hill equation (SBO:0000192[41], SBO:0000195[42] and SBO:0000198[43]). The form described here is the general microscopic form (SBO:0000195) where no inhibition is involved; see Fig. B.1 on page 43 for details. Both other types are special cases of this formula for appropriate parameter settings. If a gene regulation or translation reaction without an assigned activator or inhibitor occurs, Eq. (2.87) on the preceding page formally becomes a zeroth order mass-action equation (Section 2.1.1 on page 8).

2.2.2 Additive network models

Additive network models describe gene-regulatory networks with ordinary differential equations, based on a weighted sum of inputs. Many different versions of such equations have been suggested. Often, the inputs are classified as gene products and external factors. The equations may be linear or non-linear in additional factors or functions. Additional constants can be added to describe degradation rates. Sometimes different equations are used to describe transcription and translation processes.

The weights provide information on the relationship between genes and the inputs and are gathered in a weighting matrix. A weight of zero means that no interaction takes place. A positive or negative weight corresponds to stimulation or suppression. The absolute value of the weight corresponds to the strength of the interaction.

Besides a few variations, all additive models can be written in terms of a general differential equation (2.90):

$$\begin{aligned} \frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{S}_i] &= \underbrace{m_i \cdot g\left(\sum_{j=1}^{J} w_{ij}[\mathrm{S}_j] + \sum_{k=1}^{K} v_{ik} u_k(t) + b_i\right)}_{\text{synthesis}} - \underbrace{\sum_{j=1}^{J} \lambda_{ij}[\mathrm{S}_j]}_{\text{degradation}} \\ &= m_i \cdot g\left(w(\vec{\mathrm{S}}) + v(\vec{u}(t)) + b_i\right) - \lambda(\vec{\mathrm{S}}) \,, \end{aligned} \tag{2.90}$$

where the factor m_i (in `substance` per `time`) denotes the transcription rate of gene i which is multiplied with the monotone regulation function g (activation function). Often, g has a sigmoid shape and can hence approximate saturation and activation effects. The parameter b_j gives the basal expression level of gene i. The weight w_{ij} determines the strength of the influence of gene product j on the expression of gene i. Analogously, v_{ij} describes the influences of external input j, such as chemicals, temperature or the degradation of nutrients, on the expression of gene i. J gives the number of genes and K the number of external inputs. Depending on the mathematical form of function g, the parameters b_j, v_{ij}, and w_{ij} can have various different units. In some variants of the additive models, g contains an exponential function, which takes as an argument a term that depends on a molecule count or a concentration of gene products. From the perspective of unit consistency this can be problematic because such a function can hardly be interpreted in terms of its physical meaning. Function $u_k(t)$

[41] Hill-type rate law, generalised form
[42] Hill-type rate law, microscopic form
[43] Hill-type rate law, reduced form

yields the evolution of input k depending on time t. In this equation, the j^{th} gene product influences the degradation of the i^{th} gene product with strength λ_{ij}.

Table 2.1 on the facing page provides an overview of many versions of additive kinetic equations supported by SBMLsqueezer, where we denote

$$w(\vec{\mathrm{S}}) := \sum_{j=1}^{J} w_{ij}[\mathrm{S}_j] \tag{2.91}$$

the synthesis velocity of the i^{th} gene product that is influenced by gene products only,

$$v(\vec{u}(t)) := \sum_{k=1}^{K} v_{ik} u_k(t) \tag{2.92}$$

the synthesis rate of the i^{th} gene product that is only influenced by external factors, and

$$\lambda(\vec{\mathrm{S}}) := \sum_{j=1}^{J} \lambda_{ij}[\mathrm{S}_j] \tag{2.93}$$

degradation velocity of the i^{th} gene product. Since in SBML degradation and synthesis of gene products are two distinct reactions, the degradation term has to be created in a separate step.

At the moment, none of the additive kinetic equations and none of their parameters described here are included in the SBO.

2.2.3 S-systems

Saturation or *Synergy* systems describe complex biological and biochemical phenomena that converge to a limit state or to a state of saturation. Due to their relatively high number of parameters S-systems exhibit a high degree of freedom leading to a very flexible behavior. Hence, S-systems fit most phenomena of reaction kinetics. S-systems are constructed by a set of non-linear first-order ordinary differential equations, consisting of a synthesis and a degradation term. Originally derived by Savageau (1969a,b, 1970) as power-law approximation for biochemical systems (Biochemical Systems Theory, BST), Irvine and Savageau (1990) and subsequently many others suggest the application of these equations for models of gene-regulatory networks (Spieth *et al.*, 2004; Tournier, 2005; Spieth *et al.*, 2006; Hecker *et al.*, 2009). The mathematical form of S-systems looks similar to a mass-action equation without the prefactor F_j (cf. Eq. (2.5) on page 8):

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{S}_i] = \underbrace{\alpha_i \cdot \prod_{j=1}^{J} [\mathrm{S}_j]^{\mathscr{G}_{ij}}}_{\text{synthesis}} - \underbrace{\beta_i \cdot \prod_{j=1}^{J} [\mathrm{S}_j]^{\mathscr{H}_{ij}}}_{\text{degradation}} . \tag{2.94}$$

The kinetic exponents $\mathscr{G}_{ij}$ and $\mathscr{H}_{ij}$ constitute the most important difference. In contrast to the values from the stoichiometric matrix $n_{ij}^{\pm}$, which define the structure of the reaction system and therefore cannot be changed, the dimensionless kinetic orders $\mathscr{G}_{ij} \in \mathbb{R}$ and $\mathscr{H}_{ij} \in \mathbb{R}$ belong to the set of model parameters. The parameters α_i and β_i fulfill the same purpose as the forward and reverse rate constants in the mass-action kinetics and can therefore be annotated with the same SBO term and units (see Eq. (2.5) to (2.6) on page 8).

Thomas *et al.* (2004, 2007) derive a variant of S-systems specifically for gene-regulatory processes. To this end, this formula distinguishes between transcription and translation. Note that the Eq. (2.95)

Citation	m_i	$g(z)$	$w(\vec{S})$	$v(\vec{u}(t))$	b_i	Remarks
Chen *et al.* (1999)	1	z	0	$v(\vec{u}(t))$	0	external factors: proteins in case of transcription and mRNA for translation
D'haeseleer *et al.* (1999)	1	z	$w(\vec{S})$	$v(\vec{u}(t))$	$b_i + T_i$	external factors: kainate (salt of the kainic acid) T_i bias difference between tissue types
Weaver *et al.* (1999); Spieth *et al.* (2006)	m_i	$\frac{1}{1+\exp(-\alpha_i z+\beta_i)}$	$w(\vec{S})$	0	0	α_i and β_i are gene-specific constants
D'haeseleer *et al.* (2000)	1	z	$w(\vec{S})$	0	b_i	
Vohradský (2001); Vu and Vohradský (2007)	m_i	$\frac{1}{1+\exp(-z)}$	$w(\vec{S})$	0	b_i	
Tegnér *et al.* (2003)	1	z	$\sum_{j=1}^{J} w_{ij}([S_j]-a_j)$	0	P_i	P_i perturbation ramp function, a_i average activity of gene i
Guthke *et al.* (2005); Schmidt-Heck *et al.* (2005); Zellmer *et al.* (2005); Schmidt-Heck *et al.* (2006); Guthke *et al.* (2007); Hecker *et al.* (2009)	1	z	$w(\vec{S})$	$\vec{v}_i \cdot h(t)$	0	$\vec{v}_i$ external stimulus response vector, step function $h(t) = \begin{cases} 0 & \text{if } t < 0 \\ 1 & \text{else} \end{cases}$
Marbach *et al.* (2007)	m_i	$\frac{1}{1+\exp(-z)}$	$w(\vec{S})$	0	0	
Töpfer *et al.* (2007)	1	z	$w(\vec{S})$	$v(\vec{u}(t))$	0	linear modell of the *NetGenerator*
Töpfer *et al.* (2007)	m_i	$\frac{1}{1+\exp(-z)}$	$\sum_{j=1, j\neq i}^{J} w_{ij}[S_j]$	$v(\vec{u}(t))$	b_i	Non-linear modell of the *NetGenerator*. This equation adds the summand $w_{ii}[S_i]$ to the rate law v_i.
Wu *et al.* (2008)	1	$\frac{1}{1+\exp(-\alpha_i z+\beta_i)}$	$w([S_j])$	0	b_i	α_i and β_i gene-specific constants
Marbach *et al.* (2009a,b)	m_i	$\frac{1}{1+\exp(-z)}$	$\sum_{j=1}^{J} w_{ij}\log([S_j])$	0	b_i	

Table 2.1: Overview of variants of additive network models

for transcription depends on the concentration of translation products, i.e., proteins, and Eq. (2.96) describing translation takes the amount of transcription products, i.e., mRNA molecules, as input variables:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{R}_i] \quad = \quad V_{sr,i} - V_{dr,i} \quad = \quad \alpha_i^{\mathrm{TR}} \prod_{j=1}^{J} [\mathrm{P}_j]^{\mathscr{G}_{ij}} - \beta_i^{\mathrm{TR}} [\mathrm{R}_i] \tag{2.95}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{P}_i] \quad = \quad V_{sp,i} - V_{dp,i} \quad = \quad \alpha_i^{TL} [\mathrm{R}_i] - \beta_i^{TL} [\mathrm{P}_i] \,. \tag{2.96}$$

Here, the transcription rate $V_{sr,i}$ and the translation rate $V_{sp,i}$ depend on $[\mathrm{P}_i]$, the concentration of protein i, and $[\mathrm{R}_i]$, the concentration of mRNA i at time t, whereas the degradation rates $V_{dr,i}$ for mRNA molecule i and $V_{dp,i}$ for protein i just depend on the respective molecule itself. Finally, α_i^{TR} and α_i^{TL} denote the transcription and translation velocity, and β_i^{TR} and β_i^{TL} are the first-order degradation rates of mRNA i and protein i. The units of α_i^{TR} can be derived by the following formula:

$$\mathrm{unit}\left(\alpha_i^{\mathrm{TR}}\right) = \frac{\texttt{substance}}{\texttt{time} \cdot \prod_{j=1}^{J} \mathrm{unit}(p_j)^{\mathscr{G}_{ij}}} \tag{2.97}$$

and the units of α_i^{TL}, β_i^{TR}, and β_i^{TL} are $\texttt{time}^{-1}$.

Currently, the SBO does neither contain any power-law approximations nor any other variant of S-systems presented in this section.

When using S-systems to model gene-regulatory networks, it is useful to have an idea of how these are derived, because this approximation is only valid in the proximity of a defined operation point. Here we briefly demonstrate the origin of S-systems according to (Irvine and Savageau, 1990) and the power-law approximation (Heinrich and Schuster, 1996, p. 36-38).

The mass-action rate law (cf. Eq. (2.5) on page 8) constitutes the mathematical basis of the power-law approximation. The rate law consists of a synthesis term from which a degradation term is subtracted:

$$v_j = v_j^+(\vec{\mathrm{S}}) - v_j^+(\vec{\mathrm{S}}) \,. \tag{2.98}$$

Here, $\vec{\mathrm{S}}$ denotes the vector of species concentrations. Rewriting this equation in terms of logarithmic concentrations yields:

$$v_j = v_j^+(\ln \vec{\mathrm{S}}) - v_j^+(\ln \vec{\mathrm{S}}) \,. \tag{2.99}$$

An operation point must be chosen, for instance, the equilibrium point of the system. Subsequently, we linearized the logarithms of the forward and reverse reaction velocities around the selected operation point $\vec{\mathrm{S}}^0$:

$$\ln v_j^{\pm}(\ln \vec{\mathrm{S}}) = \ln v_j^{\pm}(\ln \vec{\mathrm{S}}^0) + \sum_{i=1}^{n} \left. \frac{\partial \ln v_j^{\pm}}{\partial \ln S_i} \right|_{\vec{\mathrm{S}}^0} (\ln S_i - \ln S_i^0) \,. \tag{2.100}$$

With the abbreviations

$$\ln v_j^+(\ln \vec{\mathrm{S}}^0) - \sum_i \frac{\partial \ln v_j^+}{\partial \ln S_i} \ln S_i^0 = \ln \alpha_j \tag{2.101}$$

$$\ln v_j^-(\ln \vec{S}^0) - \sum_i \frac{\partial \ln v_j^+ -}{\partial \ln S_i} \ln S_i^0 = \ln \beta_j \tag{2.102}$$

$$\left.\frac{\partial \ln v_j^+}{\partial \ln S_i}\right|_{\vec{S}^0} = \mathscr{G}_{ij} \tag{2.103}$$

$$\left.\frac{\partial \ln v_j^-}{\partial \ln S_i}\right|_{\vec{S}^0} = \mathscr{H}_{ij} \tag{2.104}$$

Eq. (2.100) on the facing page can be rewritten to:

$$\ln v_j^+(\ln \vec{S}) = \ln \alpha_j + \sum_i \mathscr{G}_{ij} \ln S_i \tag{2.105}$$

$$\ln v_j^-(\ln \vec{S}) = \ln \beta_j + \sum_i \mathscr{H}_{ij} \ln S_i \tag{2.106}$$

Transforming these equations back into Cartesian coordinates yields

$$v_j(\vec{S}, \vec{p}) = \alpha_j \prod_i S_i^{\mathscr{G}_{ij}} - \beta_j \prod_i S_i^{\mathscr{H}_{ij}} \,. \tag{2.107}$$

Hence, the power-law approximation are a linearization of the mass action rate law in logarithmic space. A directly linearized rate law of reaction R_j contains $n+1$ parameters, whereas the power law approximation comprises $2(n+1)$ parameters, leading to a high flexibility of the resulting kinetic equation.

An important aspect when working with this equation is the effect of inhibitors. It can be seen in Eq. (2.107) that the exponent of an inhibitor must be negative. Hence, for very small concentrations of inhibitor molecules, i.e., $[\mathrm{I}] \to 0$, $v_j \to \infty$, which actually implies an activation. Some models using this approach therefore lead to significantly enhanced concentrations. In contrast to this inhibition mechanism, regular enzyme-kinetic approaches cannot approach infinity even if the inhibitor concentration becomes very small. Replacing known mechanisms, such as Michaelis-Menten equations with a power law approximations therefore means that knowledge about the actual reaction mechanism get lost for the sake of a higher degree of freedom of the kinetic equation.

2.2.4 H-systems

Hadeler (2003) defines the H-systems, which have been successfully applied for the modeling of gene-regulatory networks (Spieth *et al.*, 2006; Hecker *et al.*, 2009). Eq. (2.108) shows the mathematical structure of this rate law:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{S}_i] = b_i + \sum_k b_{ik}[\mathrm{S}_k] + [\mathrm{S}_i] \sum_k a_{ik}[\mathrm{S}_k] \,. \tag{2.108}$$

The parameters b_{ik} and a_{ik} weight the k^{th} influence on the expression of gene i. Their units can be derived as follows

$$\mathrm{unit}(a_{ik}) = \frac{\texttt{substance}}{\texttt{time} \cdot \mathrm{unit}(\mathrm{S}_i) \cdot \mathrm{unit}(\mathrm{S}_k)} \tag{2.109}$$

$$\mathrm{unit}(b_{ik}) = \frac{\texttt{substance}}{\texttt{time} \cdot \mathrm{unit}(\mathrm{S}_k)} \,. \tag{2.110}$$

The parameter b_i (in `substance` per `time`) denotes the basal transcription rate of gene i.

The SBO does currently not include H-systems or any one of the parameters contained therein.

A Derivation of predefined kinetic equations for bi-uni reactions

This section shows the derivation of rate laws for the random order and the ordered bi-uni mechanisms using the method of King and Altman (see Bisswanger, 2000, p. 126-135 and Cornish-Bowden, 2004, p. 91-99). This derivation is necessary, since for this special case the common literature does not provide appropriate equations to be used as a pre-computed formula in SBMLsqueezer (Segel, 1993; Bisswanger, 2000; Cornish-Bowden, 2004).

The King-Altman method provides an algorithm to create rate laws even for complex enzyme-catalyzed reaction mechanisms according to the quasi-steady-state approximation (Bisswanger, 2000; Cornish-Bowden, 2004). The algorithm roughly consists of five steps that will be explained in detail in the remainder of this section.

A.1 Ordered bi-uni mechanism

Firstly, we derive the rate law for the ordered bi-uni mechanism. Note that the sequence, in which the reactants bind to the enzyme molecule, is fixed (Fig. 2.5b on page 16).

First step

A polygon, whose arcs and vertices reflect the reaction mechanism, is charted (Fig. A.1). Every vertex symbolizes one of the forms of the enzyme during the reaction. The edges mirror the transition between these forms. All charted transitions have to be first-order reactions. Second-order reactions must be given in pseudo-first-order form. The arrows, which constitute the edges of the diagram, are labeled with the rate constants, which are multiplied with entering ligands if necessary, for the corresponding transition.

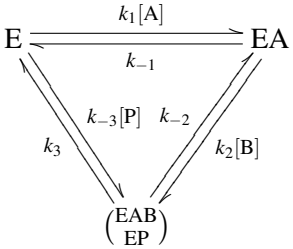


Figure A.1: Underlying reaction scheme for the ordered bi-uni mechanism

The master pattern drafts the reaction scheme as a rough structure. In this case we obtain a triangle (Fig. A.2 on the next page).

Second step

Next, we construct all possible substructures of Fig. A.2 on the facing page that

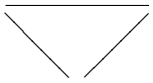


Figure A.2: Master pattern of the ordered bi-uni mechanism

1. contain only edges of the master pattern
2. connect all enzyme states and
3. do not contain closed loops.

Figure A.3: All valid sub-structures of the master pattern

Each of these patterns contains exactly one edge fewer than the master pattern. We obtain three structures, each with two edges (Fig. A.3).

Third step

In this step every single enzyme state is marked one time within each pattern and directed arcs replace the edges, each pointing towards the highlighted enzyme state. According to the resulting pattern, we determine an equation for the relative amount of each highlighted enzyme state (Fig. A.4 on the following page).

The denominator $\mathscr{D}$ equals the sum of all numerator terms of the equations in Fig. A.4 on the next page and reads

$$\begin{aligned}\mathscr{D} = k_{-1}k_{-2} + k_2k_3[\mathrm{B}] + k_{-1}k_3 + k_1k_2[\mathrm{A}][\mathrm{B}] + k_{-2}k_{-3}[\mathrm{B}][\mathrm{P}] \\ + k_{-1}k_{-3}[\mathrm{P}] + k_1k_2[\mathrm{A}] + k_{-2}k_{-3}[\mathrm{P}] + k_1k_3[\mathrm{A}]\,. \quad \text{(A.4)}\end{aligned}$$

Fourth step

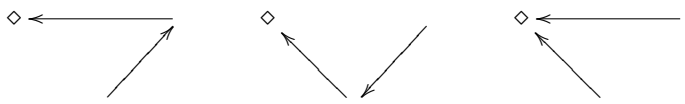
We now write the denominator as the product of coefficients in a way that all constants are ordered with respect to their concentration terms:

$$\mathscr{D} = \mathscr{D}_0 + [\mathrm{A}]\mathscr{D}_1 + [\mathrm{B}]\mathscr{D}_2 + [\mathrm{A}][\mathrm{B}]\mathscr{D}_3 + [\mathrm{B}][\mathrm{P}]\mathscr{D}_4 + [\mathrm{P}]\mathscr{D}_5 \quad \text{(A.5)}$$

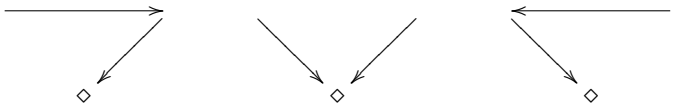
where

$$\mathscr{D}_0 = k_{-1}(k_{-2} + k_3) \quad \text{(A.6)}$$
$$\mathscr{D}_1 = k_1(k_{-2} + k_3) \quad \text{(A.7)}$$
$$\mathscr{D}_2 = k_2 + k_3 \quad \text{(A.8)}$$
$$\mathscr{D}_3 = k_1 + k_2 \quad \text{(A.9)}$$
$$\mathscr{D}_4 = k_2 + k_{-3} \quad \text{(A.10)}$$
$$\mathscr{D}_5 = k_{-3}(k_{-1} + k_{-2})\,. \quad \text{(A.11)}$$

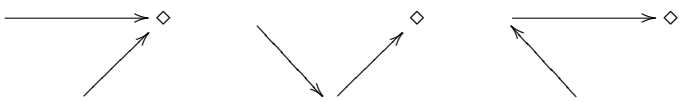
The rate law is then given as the sum of the rates to form a particular product decremented by the rates that reduce this product. In this ordered bi-uni mechanism there is only one step, in which P is



$$\frac{[\mathrm{E}]}{[\mathrm{E}]_0} = \frac{k_{-1}k_{-2} + k_2k_3[\mathrm{B}] + k_{-1}k_3}{\mathscr{D}} \tag{A.1}$$



$$\frac{[\mathrm{EAB}]}{[\mathrm{E}]_0} = \frac{k_1k_2[\mathrm{A}][\mathrm{B}] + k_{-2}k_{-3}[\mathrm{B}][\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}]}{\mathscr{D}} \tag{A.2}$$



$$\frac{[\mathrm{EB}]}{[\mathrm{E}]_0} = \frac{k_1k_2[\mathrm{A}] + k_{-2}k_{-3}[\mathrm{P}] + k_1k_3[\mathrm{A}]}{\mathscr{D}} \tag{A.3}$$

Figure A.4: Sub-patterns with their respective equations

produced. Hence, there is only one way to consume P again and the formula reads:

$$\begin{aligned} v = \frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} =& k_3[\mathrm{EAB}] - k_{-3}[\mathrm{E}][\mathrm{P}] \\ =& [\mathrm{E}]_0 \frac{k_1k_2k_3[\mathrm{A}][\mathrm{B}] + k_2k_3k_{-3}[\mathrm{B}][\mathrm{P}] + k_{-1}k_3k_{-3}[\mathrm{P}]}{\mathscr{D}} \\ &+ \frac{-k_{-1}k_{-2}k_{-3}[\mathrm{P}] - k_2k_3k_{-3}[\mathrm{B}][\mathrm{P}] - k_{-1}k_3k_{-3}[\mathrm{P}]}{\mathscr{D}} \\ =& [\mathrm{E}]_0 \frac{k_1k_2k_3[\mathrm{A}][\mathrm{B}] - k_{-1}k_{-2}k_{-3}[\mathrm{P}]}{\mathscr{D}} \,. \end{aligned} \tag{A.12}$$

Fifth step

The kinetic parameters are defined based on the coefficients determined in the fourth step. The Michaelis constants K_i^{M} are defined as the ratio of all constants of the substrate or product formation rate, minus the constants of the product or substrate formation rate, and the coefficient of the

rates of all substrates or products.

$$K_{\mathrm{A}}^{\mathrm{M}} = \frac{\mathscr{D}_2}{\mathscr{D}_3} = \frac{k_2 k_3}{k_1 k_2} = \frac{k_3}{k_1} \tag{A.13}$$

$$K_{\mathrm{B}}^{\mathrm{M}} = \frac{\mathscr{D}_1}{\mathscr{D}_3} = \frac{k_1(k_{-2}+k_3)}{k_1 k_2} = \frac{k_{-2}+k_3}{k_2} \tag{A.14}$$

$$K_{\mathrm{P}}^{\mathrm{M}} = \frac{\mathscr{D}_0}{\mathscr{D}_5} = \frac{k_{-1}(k_{-2}+k_3)}{k_{-3}(k_{-1}+K_{-2})} \tag{A.15}$$

The limiting rates for the forward and reverse reaction, V_+^{m} and V_-^{m}, are the quotient of the respective numerator coefficient and the coefficient of all substrates or products, respectively.

$$V_+^{\mathrm{m}} = \frac{\text{numerator}_1}{\text{coefficient of all substrates}} = [\mathrm{E}]_0 \frac{k_1 k_2 k_3}{k_1 k_2} \tag{A.16}$$

$$V_-^{\mathrm{m}} = \frac{\text{numerator}_2}{\text{coefficient of all products}} = [\mathrm{E}]_0 \frac{k_{-1} k_{-2} k_{-3}}{k_{-3}(k_{-1}+k_{-2})} \tag{A.17}$$

After some conversions these kinetic equations can be combined as follows:

$$[\mathrm{E}]_0 k_{-1} k_{-2} k_{-3} = V_+^{\mathrm{m}} k_1 k_2 \tag{A.18}$$

and

$$[\mathrm{E}][\mathrm{E}]_0 k_1 k_2 k_3 = V_-^{\mathrm{m}} k_{-3}(k_{-1}+k_{-2}). \tag{A.19}$$

Applying some more conversions and aggregation, the rate law then reads

$$v = \frac{V_+^{\mathrm{m}} k_1 k_2 [\mathrm{A}][\mathrm{B}] - V_-^{\mathrm{m}} k_{-3}(k_{-1}+k_{-2})[\mathrm{P}]}{\mathscr{D}} \tag{A.20}$$

$$= \frac{\frac{V_+^{\mathrm{m}} k_1 k_2 [\mathrm{A}][\mathrm{B}] - V_-^{\mathrm{m}} k_{-3}(k_{-1}+k_{-2})[\mathrm{P}]}{k_{-1}(k_{-2}+k_3)}}{\frac{\mathscr{D}}{k_{-1}(k_{-2}+k_3)}} \tag{A.21}$$

$$= \frac{\frac{V_+^{\mathrm{m}}[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} - \frac{V_-^{\mathrm{m}}[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}}{1 + \frac{[\mathrm{A}]}{k_{\mathrm{A}}^{\mathrm{I}}} + \frac{K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{A}}^{\mathrm{I}}} + \frac{K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}][\mathrm{P}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{P}}^{\mathrm{I}}} + \frac{[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}}, \tag{A.22}$$

where we define the following constants:

$$k_{\mathrm{P}}^{\mathrm{I}} = \frac{k_{-3}}{k_3} \tag{A.23}$$

$$k_{\mathrm{A}}^{\mathrm{I}} = \frac{k_{-1}}{k_1}\,. \tag{A.24}$$

To accommodate this equation with the uni-uni Michaelis-Menten equation, we set $V_+^{\mathrm{m}} = k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0$ and $V_-^{\mathrm{m}} = k_{-j}^{\mathrm{cat}}[\mathrm{E}]_0$. This leads us to the following equation for a reversible ordered bi-uni mechanism:

$$v = \frac{\frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} - \frac{k_{-j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}}{1 + \frac{[\mathrm{A}]}{k_{\mathrm{A}}^{\mathrm{I}}} + \frac{K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{A}}^{\mathrm{I}}} + \frac{K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}][\mathrm{P}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{P}}^{\mathrm{I}}} + \frac{[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}}. \tag{A.25}$$

For the irreversible ordered bi-uni mechanism the formula for the rate law reads

$$v = \frac{\frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}}}{1 + \frac{[\mathrm{A}]}{k_{\mathrm{A}}^{\mathrm{I}}} + \frac{K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{A}}^{\mathrm{I}}}} = \frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}} + K_{\mathrm{B}}^{\mathrm{M}}[\mathrm{A}] + K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}] + [\mathrm{A}][\mathrm{B}]}\,. \tag{A.26}$$

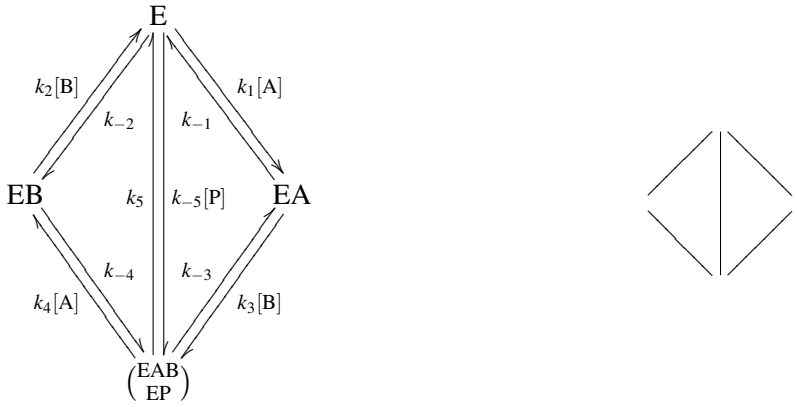
Eq. (A.26) on the preceding page is identical to the rate law defined by SBO:0000432, the *irreversible Michaelis Menten rate law for two substrates*.

A.2 Random order bi-uni mechanism

The random order mechanism is characterized by its arbitrary sequence, in which the reactants bind to the enzyme. The binding of every substrate is carried out independently of the others (Fig. 2.4a on page 15). This must also be reflected in the corresponding rate laws.

To derive appropriate pre-computed formulas for this mechanism, we again apply the five steps of the King-Altman method (Bisswanger, 2000, p. 126-131). Here, we only briefly summarize the steps and omit a detailed explanation.

First step



(a) Polygon showing the structure

(b) Master pattern

Figure A.5: Structure and master pattern of the bi-uni random mechanism

We chart the master pattern of the mechanism from the polygon that belongs to this particular reaction scheme (Fig. A.5).

Second step

Next we construct all possible substructures, each with exactly one edge fewer than the master pattern (Fig. A.6 on the next page).

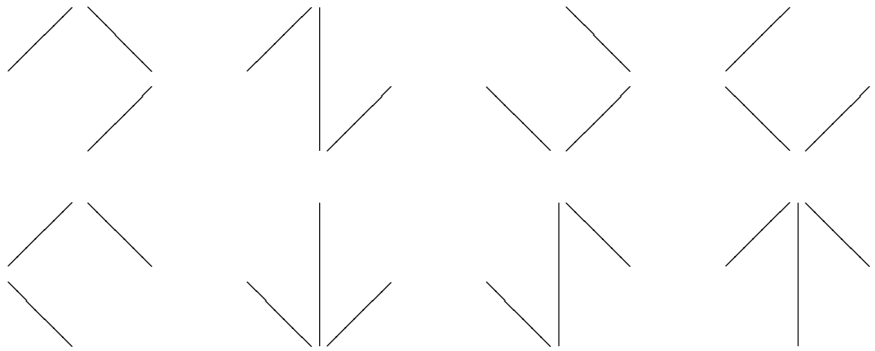


Figure A.6: All valid sub-structures derived from the master pattern

Third step

In this step, we derive the equations for every form of the enzyme attributable to the respective sub-patterns.

$$\frac{[\mathrm{E}]}{[\mathrm{E}]_0} = \frac{k_{-2}k_{-1}k_{-3} + k_{-1}k_{-3}k_4[\mathrm{A}] + k_{-2}k_{-4}k_3[\mathrm{B}] + k_{-4}k_{-2}k_{-1}}{\mathscr{D}} + \frac{k_5k_4k_3[\mathrm{A}][\mathrm{B}] + k_{-1}k_{-2}k_5 + k_{-2}k_3k_5[\mathrm{B}] + k_{-1}k_4k_5[\mathrm{A}]}{\mathscr{D}} \tag{A.27}$$

$$\frac{[\mathrm{E}][\mathrm{A}][\mathrm{B}]}{[\mathrm{E}]_0} = \frac{k_1k_{-2}k_3[\mathrm{A}][\mathrm{B}] + k_1k_3k_4[\mathrm{A}]^2[\mathrm{B}] + k_2k_3k_4[\mathrm{A}][\mathrm{B}]^2 + k_{-1}k_2k_4[\mathrm{A}][\mathrm{B}]}{\mathscr{D}} + \frac{k_3k_4k_{-5}[\mathrm{A}][\mathrm{B}][\mathrm{P}] + k_{-1}k_{-2}k_{-5}[\mathrm{P}] + k_{-2}k_3k_{-5}[\mathrm{B}][\mathrm{P}] + k_{-1}k_4k_{-5}[\mathrm{A}][\mathrm{P}]}{\mathscr{D}} \tag{A.28}$$

$$\frac{[\mathrm{E}][\mathrm{B}]}{[\mathrm{E}]_0} = \frac{k_{-1}k_2k_{-3}[\mathrm{B}] + k_1k_3k_{-4}[\mathrm{A}][\mathrm{B}] + k_2k_3k_{-4}[\mathrm{B}]^2 + k_{-1}k_2k_{-4}[\mathrm{B}]}{\mathscr{D}} + \frac{k_3k_{-4}k_{-5}[\mathrm{P}][\mathrm{B}] + k_{-1}k_2k_5[\mathrm{B}] + k_2k_3k_5[\mathrm{B}]^2 + k_{-1}k_{-4}k_{-5}[\mathrm{P}]}{\mathscr{D}} \tag{A.29}$$

$$\frac{[\mathrm{E}][\mathrm{A}]}{[\mathrm{E}]_0} = \frac{k_1k_{-2}k_{-3}[\mathrm{A}] + k_1k_{-3}k_4[\mathrm{A}]^2 + k_2k_{-3}k_4[\mathrm{A}][\mathrm{B}] + k_1k_{-2}k_{-4}[\mathrm{A}]}{\mathscr{D}} + \frac{k_{-3}k_4k_{-5}[\mathrm{A}][\mathrm{P}] + k_1k_{-2}k_5[\mathrm{A}] + k_{-2}k_{-3}k_{-5}[\mathrm{P}] + k_1k_4k_5[\mathrm{A}]^2}{\mathscr{D}} \tag{A.30}$$

Fourth step

The denominator and the preliminary kinetic equation can now be written as:

$$\begin{aligned}\mathscr{D} =&(k_{-1}k_{-2}k_{-3}+k_{-1}k_{-2}k_{-4}+k_{-1}k_{-2}k_5)\\ &+[\mathrm{A}](k_{-1}k_4k_5+k_1k_{-2}k_{-3}+k_1k_{-2}k_{-4}+k_1k_{-2}k_5+k_{-1}k_{-3}k_4)\\ &+[\mathrm{A}]^2(k_1k_{-3}k_4+k_1k_4k_5)+[\mathrm{A}][\mathrm{B}](k_3k_4k_5+k_1k_{-2}k_3+k_1k_3k_{-4}+k_2k_{-3}k_4+k_{-1}k_2k_4)\\ &+[\mathrm{A}]^2[\mathrm{B}](k_1k_3k_4)+[\mathrm{B}](k_{-2}k_3k_{-4}+k_{-2}k_3k_5+k_{-1}k_2k_{-3}+k_{-1}k_2k_{-4}+k_{-1}k_2k_5)\\ &+[\mathrm{B}]^2(k_2k_3k_{-4}+k_2k_3k_5)+[\mathrm{A}][\mathrm{B}]^2(k_2k_3k_4)+[\mathrm{P}](k_{-1}k_{-2}k_{-5}+k_{-1}k_{-4}k_{-5}+k_{-2}k_{-3}k_{-5})\\ &+[\mathrm{A}][\mathrm{P}](k_{-3}k_4k_{-5}+k_{-1}k_4k_{-5})+[\mathrm{A}][\mathrm{B}][\mathrm{P}](k_3k_4k_{-5})+[\mathrm{B}][\mathrm{P}](k_3k_{-4}k_{-5}+k_{-2}k_3k_{-5})\\ =&\mathscr{D}_0+[\mathrm{A}]\mathscr{D}_1+[\mathrm{A}]^2\mathscr{D}_2+[\mathrm{A}][\mathrm{B}]\mathscr{D}_3+[\mathrm{A}]^2[\mathrm{B}]\mathscr{D}_4+[\mathrm{B}]\mathscr{D}_5+[\mathrm{B}]^2\mathscr{D}_6+\\ &[\mathrm{A}][\mathrm{B}]^2\mathscr{D}_7+[\mathrm{P}]\mathscr{D}_8+[\mathrm{A}][\mathrm{P}]\mathscr{D}_9+[\mathrm{A}][\mathrm{B}][\mathrm{P}]\mathscr{D}_{10}+[\mathrm{B}][\mathrm{P}]\mathscr{D}_{11}\end{aligned} \tag{A.31}$$

$$\begin{aligned}v=\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t}=&k_5[\mathrm{EAB}]-k_{-5}[\mathrm{E}][\mathrm{P}]\\ =&[\mathrm{E}]_0\frac{(k_1k_{-2}k_3+k_{-1}k_2k_4)k_5[\mathrm{A}][\mathrm{B}]+k_1k_3k_4k_5[\mathrm{A}]^2[\mathrm{B}]+k_2k_3k_4k_5[\mathrm{A}][\mathrm{B}]^2}{\mathscr{D}}\\ &-\frac{(k_{-1}k_{-2}+k_{-1}k_{-2}k_{-3})k_{-5}[\mathrm{P}]+k_{-1}k_{-3}k_4k_{-3}[\mathrm{A}][\mathrm{P}]+k_{-2}k_3k_{-4}k_{-5}[\mathrm{B}][\mathrm{P}]}{\mathscr{D}}\,.\end{aligned} \tag{A.32}$$

Fifth step

To complete the derivation, we have to define the Michaelis constant. According to Segel, the random order bi-uni mechanism does not provide a hyperbole function when no substrate saturation is present (Segel, 1993, p. 646). Thus, a kinetic formula based on this mechanism cannot be linearized. Hence, we may combine several rate constants to Michaelis-like constants similar to what we applied in the derivation for the ordered bi-uni mechanism. However, these constants would not be in accordance with the definition of the Michaelis constant of the uni-uni formula (Segel, 1993, p. 648). Assuming fast steady-states between the ternary and the binary complexes EAB and EP, and also assuming all conversions to be fast, this equation can be simplified as follows: According to Cornish-Bowden, the squared terms in the numerator and denominator as well as the terms [B][P], [A][P] and [A][B][P] then vanish (Cornish-Bowden, 2004, p. 169). This leads to the definition of the dissociation constants $k_{\mathrm{A}}^{\mathrm{I}}$, $k_{\mathrm{B}}^{\mathrm{I}}$ and $k_{\mathrm{P}}^{\mathrm{I}}$ for $K_{\mathrm{A}}^{\mathrm{M}}$, $K_{\mathrm{B}}^{\mathrm{M}}$ and $K_{\mathrm{P}}^{\mathrm{M}}$. Since the sequence, in which the reactants bind to the enzyme, is random, we have $K_{\mathrm{A}}^{\mathrm{M}}k_{\mathrm{B}}^{\mathrm{I}}=k_{\mathrm{A}}^{\mathrm{I}}K_{\mathrm{B}}^{\mathrm{M}}$. Hence, the derived equation is valid for this mechanism and assumes an underlying rapid-equilibrium-random-mechanism. The constants of this equation are defined as follows:

$$K_{\mathrm{A}}^{\mathrm{M}}=\frac{\mathscr{D}_5}{\mathscr{D}_3} \tag{A.33}$$

$$K_{\mathrm{B}}^{\mathrm{M}}=\frac{\mathscr{D}_1}{\mathscr{D}_3} \tag{A.34}$$

$$K_{\mathrm{P}}^{\mathrm{M}}=\frac{\mathscr{D}_0}{\mathscr{D}_8} \tag{A.35}$$

$$k_{\mathrm{A}}^{\mathrm{I}}=\frac{\mathscr{D}_1}{\mathscr{D}_0} \tag{A.36}$$

$$k_{\mathrm{B}}^{\mathrm{I}}=\frac{\mathscr{D}_0}{\mathscr{D}_5}\,. \tag{A.37}$$

And the final equation then reads:

$$v = \frac{\frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}} - \frac{k_{-j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}}{1 + \frac{[\mathrm{A}]}{k_{\mathrm{A}}^{\mathrm{I}}} + \frac{[\mathrm{B}]}{k_{\mathrm{B}}^{\mathrm{I}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{A}}^{\mathrm{I}}} + \frac{[\mathrm{P}]}{K_{\mathrm{P}}^{\mathrm{M}}}} \,. \tag{A.38}$$

For the irreversible random order bi-uni mechanism the above formula can be simplified to

$$v = \frac{\frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}}}}{1 + \frac{[\mathrm{A}]}{k_{\mathrm{A}}^{\mathrm{I}}} + \frac{[\mathrm{B}]}{k_{\mathrm{B}}^{\mathrm{I}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K_{\mathrm{B}}^{\mathrm{M}} k_{\mathrm{A}}^{\mathrm{I}}}} = \frac{k_{+j}^{\mathrm{cat}}[\mathrm{E}]_0[\mathrm{A}][\mathrm{B}]}{k_{\mathrm{A}}^{\mathrm{I}} K_{\mathrm{B}}^{\mathrm{M}} + K_{\mathrm{B}}^{\mathrm{M}}[\mathrm{A}] + K_{\mathrm{A}}^{\mathrm{M}}[\mathrm{B}] + [\mathrm{A}][\mathrm{B}]} \,. \tag{A.39}$$

Note that the equations (A.26) on page 37 and (A.39) are identical and defined by SBO:0000432[1].

[1] Irreversible Michaelis Menten rate law for two substrates

B Systems biology ontology of mathematical expressions

Figs. B.1 to B.2 on pages 43–44 visualize the Systems Biology Ontology (SBO) subgraph rooted at the term *mathematical expression* (SBO:0000064) at the time of writing[1] (Le Novère, 2006,?). For the sake of a clear arrangement of all currently available rate laws, the subgraph *mathematical expression* is splitted into three distinct figures. This ontology contains many of the rate equations described in the previous sections. For an up-to-date list, we refer to the SBO homepage, which is available at `http://www.ebi.ac.uk/sbo`. SBO

[1] March 31, 2010

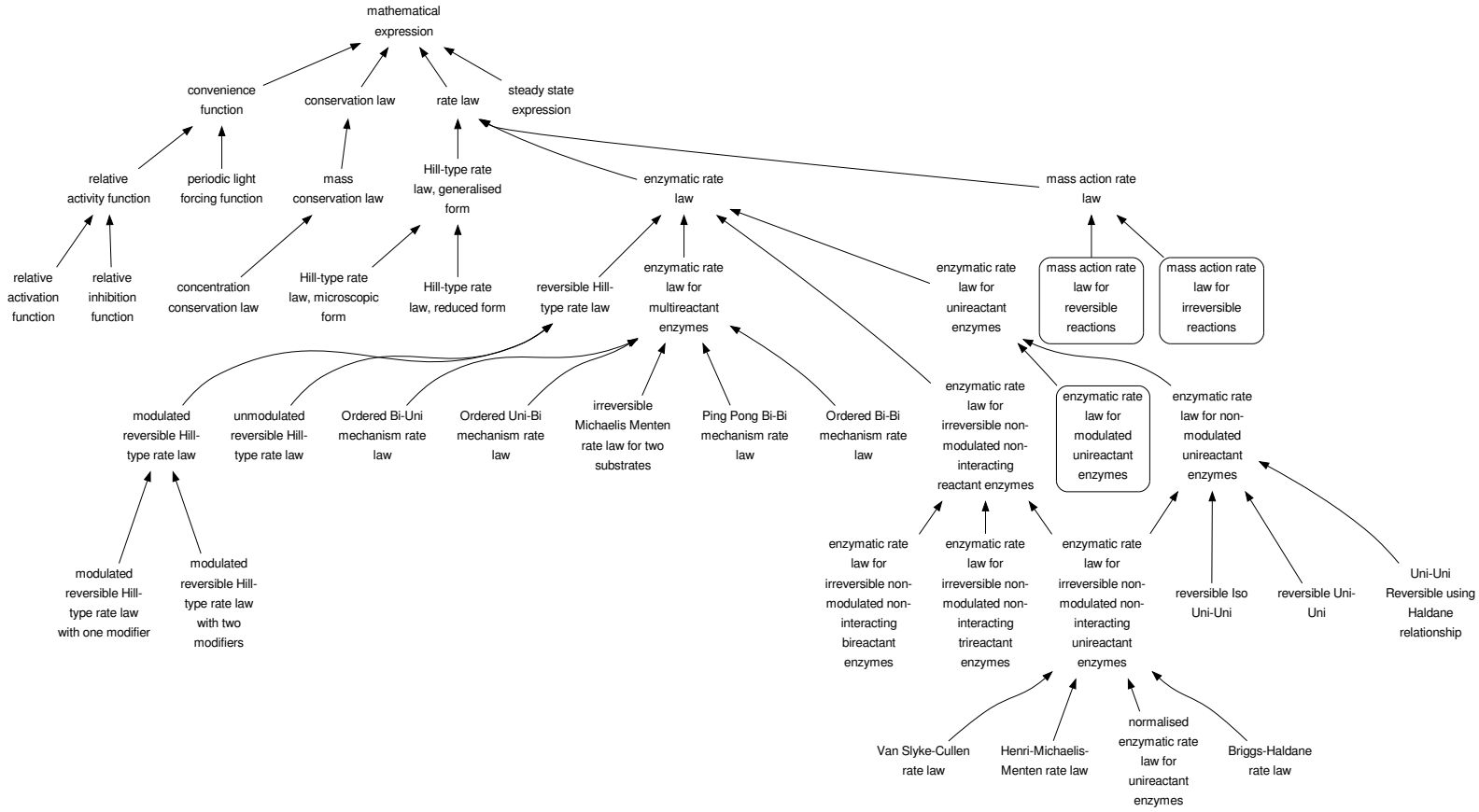


Figure B.1: SBO subgraph of mathematical expressions. The subgraphs rooted at nodes that are surrounded with rounded boxes are omitted from this figure for the sake of a clear arrangement.

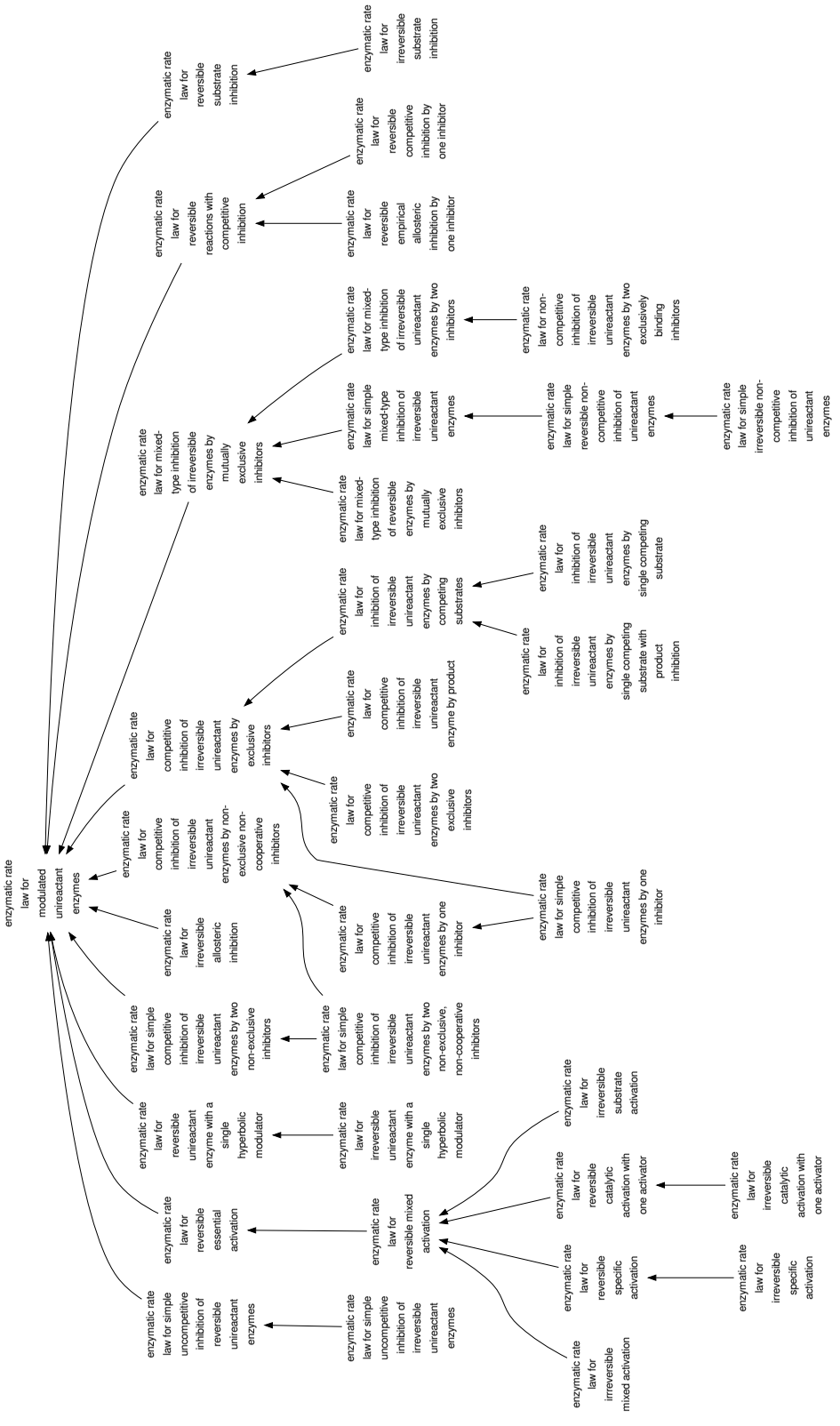


Figure B.2: SBO subgraph of rate laws for enzymatic and nonmodulated unireactant reactions

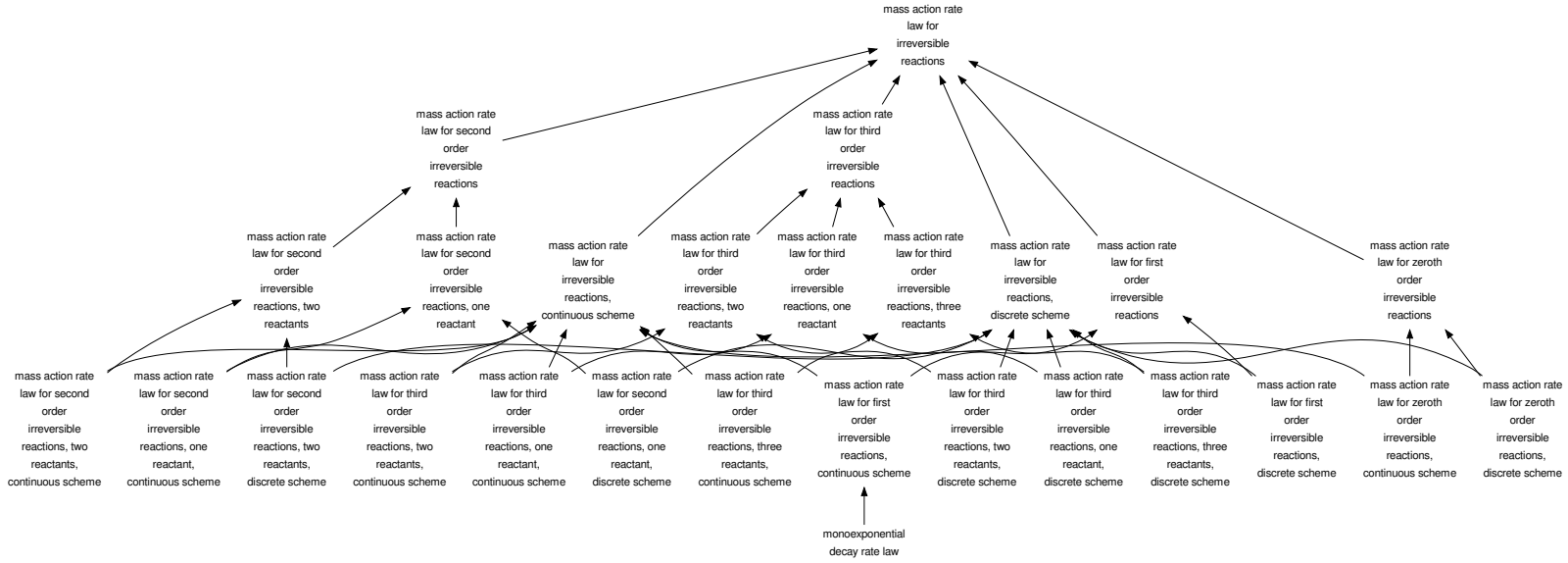


Figure B.3: SBO subgraph of mass-action rate laws for irreversible reactions

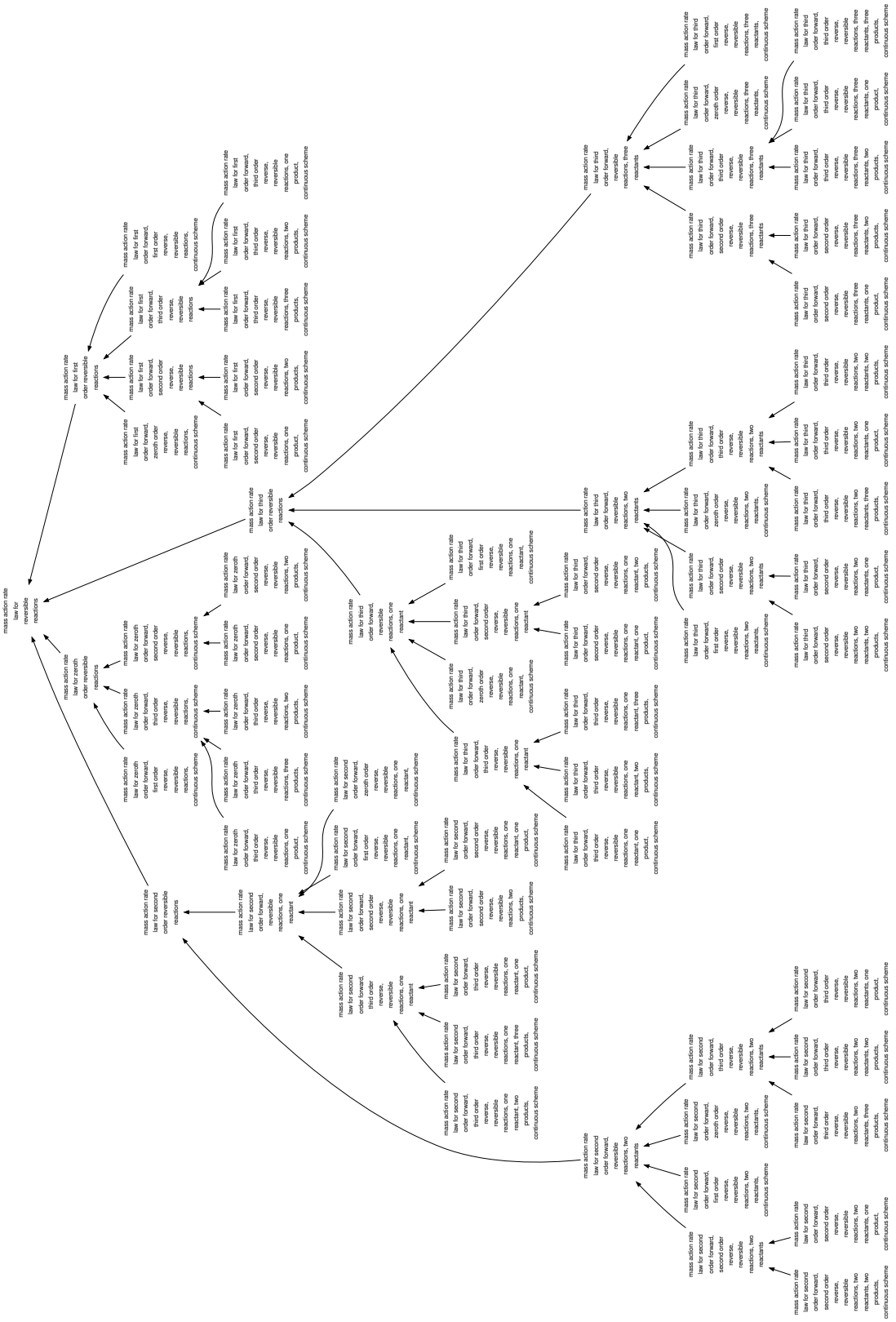


Figure B.4: SBO subgraph of mass-action rate laws for reversible reactions

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