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Modular rate laws for enzymatic reactions: thermodynamics, elasticities and implementation

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ABSTRACT

Motivation: Standard rate laws are a key requisite for systematically turning metabolic networks into kinetic models. They should provide simple, general and biochemically plausible formulae for reaction velocities and reaction elasticities. At the same time, they need to respect thermodynamic relations between the kinetic constants and the metabolic fluxes and concentrations.

Results: We present a family of reversible rate laws for reactions with arbitrary stoichiometries and various types of regulation, including mass–action, Michaelis–Menten and uni–uni reversible Hill kinetics as special cases. With a thermodynamically safe parameterization of these rate laws, parameter sets obtained by model fitting, sampling or optimization are guaranteed to lead to consistent chemical equilibrium states. A reformulation using saturation values yields simple formulae for rates and elasticities, which can be easily adjusted to the given stationary flux distributions. Furthermore, this formulation highlights the role of chemical potential differences as thermodynamic driving forces. We compare the modular rate laws to the thermodynamic–kinetic modelling formalism and discuss a simplified rate law in which the reaction rate directly depends on the reaction affinity. For automatic handling of modular rate laws, we propose a standard syntax and semantic annotations for the Systems Biology Markup Language.

Availability: An online tool for inserting the rate laws into SBML models is freely available at www.semanticsbml.org

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Supplementary Information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Once a metabolic network is available as a stoichiometric matrix or in standard formats such as Systems Biology Markup Language (SBML; Hucka *et al.*, 2003), its potential dynamics can be studied by various kinds of mathematical analysis. Constraint-based methods such as flux balance analysis (Edwards and Palsson, 2000; Price *et al.*, 2004) help to predict the possible metabolic fluxes; energy balance analysis (Beard *et al.*, 2002; 2004), network-embedded thermodynamic (NET) analysis (Kümmel *et al.*, 2006) or related methods (Hoppe *et al.*, 2007) also account for the thermodynamic forces driving the fluxes. Finally, given a stationary flux distribution, the potential dynamic behaviour close to this steady state can be explored by random sampling of reaction elasticities (Steuer *et al.*, 2006; Wang *et al.*, 2004).

Precise dynamic simulations, in contrast, require quantitative rate laws for the enzymatic reactions. The catalytic mechanisms of key enzymes have been investigated in great detail and described by mathematical formulae (for an overview, see Cornish-Bowden, 2004; Segel, 1993). But many kinetic equations are still unknown and have to be substituted by standard rate laws (Heijnen, 2005) such as mass–action kinetics, power laws (Savageau, 1970), reversible Hill kinetics (Hofmeyr and Cornish-Bowden, 1997), lin-log kinetics (Visser and Heijnen, 2003), convenience kinetics (Liebermeister and Klipp, 2006a), generic rate equations (Lee *et al.*, 2007) or TKM rate laws (Ederer and Gilles, 2007). Many mechanistically inspired rate laws share the form (Hofmeyr, 1995)

$$v_r = u_r f_r \frac{T_r}{D_r + D_r^{\mathrm{reg}}}, \tag{1}$$

$$\text{with} \quad T_r = k_r^+ \prod_i \left(\frac{c_i}{k_{ri}^{\mathrm{M}}}\right)^{m_{ri}^+} - k_r^- \prod_i \left(\frac{c_i}{k_{ri}^{\mathrm{M}}}\right)^{m_{ri}^-}. \tag{2}$$

The numerator T_r contains turnover rates $k_r^{\pm}$ (in s^{-1}), metabolite concentrations c_i (in mM), reactant constants k_{ri}^{M} (in mM) and substrate and product molecularities $m_{ri}^{\pm}$. It resembles a reversible mass–action rate law, but in Equation (1), it is multiplied by the enzyme amount u_r and a factor f_r for enzyme regulation. The denominator D_r is a polynomial of scaled concentrations, e.g. $D_r = 1 + c_1/k_{r1}^{\mathrm{M}} + c_2/k_{r2}^{\mathrm{M}} + \ldots$. The individual denominator terms correspond to different binding states of the enzyme, which effectively reduce the enzyme amount available for catalysis. Terms arising from the specific enzyme regulation (see Cornish-Bowden, 2004) are separately listed in the term D_r^{reg}.

Depending on the reaction stoichiometry, on the enzymatic mechanism and on allosteric regulation, the terms f_r, T_r, D_r and D_r^{reg} may assume various mathematical forms. In this article, we propose several variants for each of them, which can be combined in a modular manner (see Fig. 1). The resulting rate laws represent a good compromise between biochemical detail (description of enzyme saturation, activation and inhibition, correct thermodynamic properties, and interpretable parameters) and mathematical abstraction (simple and standardized formulae for various reaction stoichiometries and types of allosteric regulation) and provide a good way to parameterize metabolic network models.

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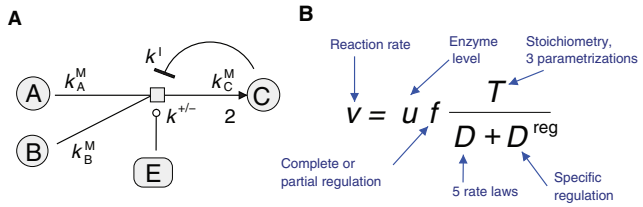

Fig. 1. Reaction scheme and modular rate law. (**A**) Reaction A+B↔ 2 C with enzyme E and product inhibition shown as compound/reaction network. The scheme can be translated into a modular rate law with turnover rates $k^{\pm}$, reactant constants k^{M} and an inhibition constant k^{I}. (**B**) Modular rate laws share the form Equation (1). The terms f [Equation (12)], T [Equation (10)], D [Equation 11] and D^{reg} [Equation (13)] depend on reaction stoichiometry, rate law, allosteric regulation and on the preferred model parameterization.

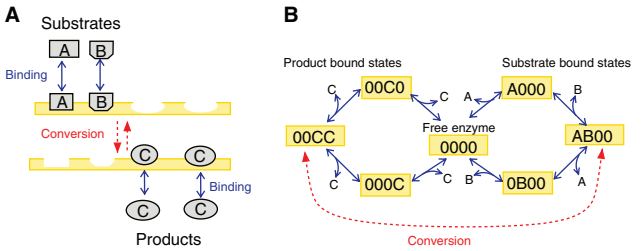

Fig. 2. Enzyme mechanism behind the CM rate law. (**A**) Substrate and product molecules in the reaction A + B ↔ 2C bind rapidly, independently, and in a random order. Substrates and products cannot bind at the same time and are interconverted in a slow reversible step (dotted). (**B**) Binding states of the enzyme. Transitions between the states arise from fast reversible binding (solid arrows) and slow reversible conversion (dotted arrow). Only the states AB00 (both substrate molecules bound) and 00CC (both product molecules bound) are involved in the conversion and contribute terms to the numerator T, while all seven binding states give rise to terms in the denominator D.

2 METHODS

2.1 Five rate laws

In this article, we shall discuss five modular rate laws (called 'common', 'direct binding', 'simultaneous binding', 'power-law' and 'force-dependent'), characterized by different formulae of their denominators. The *common modular* (CM) rate law is a generalized form of the reversible Michaelis–Menten kinetics that applies to any reaction stoichiometry. For a non-regulated reaction A+B ↔ 2 C with concentrations a, b and c, the rate (in mol/s) reads

$$v = u\frac{k^{+}a'b' - k^{-}c'^{2}}{(1+a')(1+b')+(1+c')^{2}-1} \tag{3}$$

with the abbreviations $a' = a/k_A^M$, $b' = b/k_B^M$ and $c' = c/k_C^M$, reactant constants k_A^M, k_B^M, and k_C^M (mM), and turnover rates $k^{\pm}$ (s^{-1}). The enzyme level u is given as an amount (mmol). It can also be a concentration (mM), but then the rate has to be multiplied by the compartment volume (l).

The rate law can be obtained from the random-order enzyme mechanism shown in Figure 2. Each term in the denominator $D = 1+a'+b'+a'b'+c'+c'+c'^{2}$ represents one binding state of the enzyme. The k^{M} values are dissociation constants, just like the K_M values in Michaelis–Menten kinetics, and define a scale for high and low concentrations of a reactant. The CM rate law resembles the convenience kinetics (Liebermeister and Klipp, 2006a), with a slight difference for molecularities $m^{\pm} \neq 1$: in the convenience kinetics, in Equation (3), the denominator term for the product C would read $1+c'+c'^{2}$ instead of $1+2c'+c'^{2}$. The reason is that in the CM rate law, the first C molecule can bind to either of the two binding sites, while in the convenience kinetics, the sites are bound in a fixed order.

The other four rate have simpler denominators. By omitting some of the terms, we obtain the *direct binding modular* (DM) rate law

$$v = u\frac{k^{+}a'b' - k^{-}c'^{2}}{1+a'b'+c'^{2}} = u\frac{k^{+}ab/k^{M+} - k^{-}c^{2}/k^{M-}}{1+ab/k^{M+}+c^{2}/k^{M-}} \tag{4}$$

in which the k^{M} values can be joined to parameters $k^{M+} = k_A^M k_B^M$ and $k^{M-} = (k_C^M)^2$. In contrast to the CM rate law, the denominator contains just the substrate and product terms of the highest order. With the same choice of parameters, the reaction rate is higher than in the CM rate law, especially at low concentrations where the lower-order terms dominate. The *simultaneous binding modular* (SM) rate law

$$v = u\frac{k^{+}a'b' - k^{-}c'^{2}}{(1+a')(1+b')(1+c')^{2}}, \tag{5}$$

on the other hand, contains additional denominator terms, so the reaction rate is lower, especially at high concentrations.

By setting the denominator to a constant value of 1, we obtain the *power-law modular* (PM) rate law

$$v = u\frac{k^{+}a'b' - k^{-}c'^{2}}{1} \tag{6}$$

which, in this case, is just a mass–action kinetics $v = u[k^{+\prime}ab - k^{-\prime}c^{2}]$. Finally, we introduce the *force-dependent modular* (FM) rate law

$$v = u\frac{k^{+}a'b' - k^{-}c'^{2}}{\sqrt{a'b'(c')^{2}}} \tag{7}$$

as an illustration case for the role of thermodynamics (details below).

2.2 General form of the modular rate laws

We can rewrite all modular rate laws in a general notation that covers various stoichiometries, rate laws and types of allosteric regulation; for a list of symbols, see Table 1. A biochemical network with reaction rates v_r and concentrations c_i is described by the stoichiometric matrix $N = (n_{ir})$. The index i runs over all compounds with variable or fixed concentrations (called 'internal' or 'external' compounds, respectively). To define the rate laws, we introduce structure numbers $m_{ri}^{\pm}$ and $w_{ri}^{\pm}$ describing how many molecules of compound i are apparently involved in a reaction r as substrates (m_{ri}^{+}), products (m_{ri}^{-}), activators (w_{ri}^{+}) and inhibitors (w_{ri}^{-}). In contrast to the stoichiometric coefficients, which are used in the balance equation $dc_i/dt = \sum_r n_{ir} v_r(c)$ for the internal metabolites, the structure numbers $m_{ri}^{\pm}$ denote the apparent molecularities within the kinetic laws. Both are closely related. By default, all relevant regulation numbers $w_{ri}^{\pm}$ are set to a value of 1 and the molecularities $m_{ri}^{\pm}$ are given by the (absolute) stoichiometric coefficients. But in general, we may choose arbitrary positive numbers $w_{ri}^{\pm}$ and set

$$\begin{aligned} m_{ri}^{+} &= h_r |n_{ir}| \quad \text{if } n_{ir} < 0, \quad 0 \quad \text{otherwise} \\ m_{ri}^{-} &= h_r |n_{ir}| \quad \text{if } n_{ir} > 0, \quad 0 \quad \text{otherwise} \end{aligned} \tag{8}$$

with positive cooperativity factors h_r. We can always replace $h_r n_{ir} = m_{ri}^{-} - m_{ri}^{+}$ and for convenience, we define $m_{ri} = m_{ri}^{+} + m_{ri}^{-}$. The cooperativity factor h_r yields sigmoidal rate laws similar to reversible Hill kinetics (Hofmeyr and Cornish-Bowden, 1997) and provides some freedom to rescale the sum formulae. For a reaction 0.5 A → B, for instance, we are not obliged to use a rate law $v \sim k^{+}a^{1/2} - k^{-}b$, but can choose $v \sim k^{+}a - k^{-}b^{2}$ instead. But in any case, the structure numbers $m_{ri}^{\pm}$ need to have the form (8) because otherwise, the rate laws would be thermodynamically inconsistent (see Supplementary Material). Next, we introduce the saturation values

$$\begin{aligned} \alpha_{ri}^{X} &= \frac{1}{1+c_i/k_{ri}^{X}} \qquad \beta_{ri}^{X} = \frac{c_i/k_{ri}^{X}}{1+c_i/k_{ri}^{X}} \\ \theta_r^{\pm} &= \prod_i \left(\frac{c_i}{k_{ri}^{M}}\right)^{m_{ri}^{\pm}} \quad \psi_r^{\pm} = \prod_i \left(1+\frac{c_i}{k_{ri}^{M}}\right)^{m_{ri}^{\pm}}, \end{aligned} \tag{9}$$

where k_{ri}^{X} is a proxy for k_{ri}^{M}, k_{ri}^{A} or k_{ri}^{I} values. The value β_{ri}^{X} quantifies how strongly enzyme r is saturated with respect to compound i and ranges from $\beta_{ri}^{X} = 0$ (linear range) to $\beta_{ri}^{X} = 1$ (full saturation). Given a set of α_{ri}^{X} values, all

Table 1. Table of symbols used in this article.

c_i	Metabolite concentration	f_r	Regulation prefactor [Equation (12)]
v_r	Reaction rate	T_r	Rate law numerator [Equations (2), (10) and (26)]
n_{ir}	Stoichiometric coefficient	D_r	Rate law denominator [Equation (11)]
$m_{ri}^{\pm}$	Structure number [Equation (8)]	D_r^{reg}	Specific regulation [Equation (13)]
h_r	Cooperativity factor	$k_r^{\pm}$	Turnover rate [Equation (25)]
$w_{ri}^{\pm}$, $w_{ri}^{\pm *}$	Regulation number	k_{ri}^{M}	Reactant constant
$v_r^{\pm}$	Forward and reverse rate	k_{ri}^{A}	Activation constant
ζ_r	Rate ratio [Equation (20)]	k_{ri}^{I}	Inhibition constant
μ_i	Chemical potential	k_r^{V}	Velocity constant $\sqrt{k_r^+ k_r^-}$
A_r	Reaction affinity [Equation (18)]	$\alpha_{ri}^{\mathrm{X}}, \beta_{ri}^{\mathrm{X}}$	Saturation values [Equation (9)]
k_r^{eq}	Equilibrium constant [Equation (16)]	$\theta_r^{\pm}, \psi_r^{\pm}$	Saturation values [Equation (9)]
q_r^{ma}	Mass–action ratio	$\rho_{ri}^{\mathrm{A}}, \rho_{ri}^{\mathrm{I}}$	Relative basal rates [Equation (12)]

Superscripts M, A and I are related to reactant, activation or inhibition arrows in the compound/reaction network; X represents either of them. Subscripts r and i refer to reactions and compounds, respectively. A detailed table can be found in the Supplementary Material.

other saturation values are determined by $\beta_{ri}^{\mathrm{X}} = 1 - \alpha_{ri}^{\mathrm{X}}$, $\theta_r^{\mathrm{X}} = \prod_i (1/\alpha_{ri}^{\mathrm{X}} - 1)$ and $\psi_r^{\mathrm{X}} = \prod_i 1/\alpha_{ri}^{\mathrm{X}}$.

We now define the modular rate laws by formula (1), where the numerator of Equation (2) is written in the compact form

$$T_r = k_r^+ \theta_r^+ - k_r^- \theta_r^- \tag{10}$$

and the possible denominators read

$$\begin{aligned} &\text{CM: } D_r = \psi_r^+ + \psi_r^- - 1 \\ &\text{DM: } D_r = \theta_r^+ + \theta_r^- + 1 \\ &\text{SM: } D_r = \psi_r^+ \psi_r^- \\ &\text{PM: } D_r = 1 \\ &\text{FM: } D_r = \sqrt{\theta_r^+ \theta_r^-}\,. \end{aligned} \tag{11}$$

Rate laws for unspecific enzymes can be constructed by combining denominators from the different catalysed reactions (see Supplementary Material). Allosteric regulation is implemented by the terms

$$f_r = \prod_j \left(\rho_{rj}^{\mathrm{A}} + [1 - \rho_{rj}^{\mathrm{A}}]\beta_{rj}^{\mathrm{A}}\right)^{w_{rj}^+} \prod_l \left(\rho_{rl}^{\mathrm{I}} + [1 - \rho_{rl}^{\mathrm{I}}]\alpha_{rl}^{\mathrm{I}}\right)^{w_{rl}^-}. \tag{12}$$

The relative basal rates ρ_{rj}^{A} can vary between 0 and 1 and represent the rate without activator divided by the rate at saturating activator levels. Relative basal rates ρ_{rl}^{I} for inhibitors are defined accordingly. If a basal rate is set to zero, the regulation is called *complete* activation or inhibition (or in more familiar terms, essential activation and non-competitive inhibition). Otherwise, we obtain *partial* activation or inhibition, which are also called stimulation and hyperbolic inhibition. In addition, we consider another type of regulation, which we call *specific* activation or inhibition. Regulation arrows of this type are listed separately in regulation matrices w_{ri}^{+*} and w_{ri}^{-*} and lead to the additive term

$$D_r^{\mathrm{reg}} = \sum_i (k_{ri}^{\mathrm{A}}/c_i)^{w_{ri}^{+*}} + \sum_i (c_i/k_{ri}^{\mathrm{I}})^{w_{ri}^{-*}} \tag{13}$$

in the denominator of the rate law. In the case of inhibitors, this formula describes the well-known competitive inhibition.

2.3 Thermodynamic properties

The laws of thermodynamics link the reaction rates tightly to thermodynamic forces and to the molar Gibbs free energies of the compounds involved, called chemical potentials μ_i. In an ideal mixture, the chemical potentials are linked to the concentrations c_i (measured in units of a standard concentration, usually 1 mM) *via*

$$\mu_i = \mu_i^{(0)} + RT \ln c_i \tag{14}$$

with the absolute temperature T (K) and Boltzmann's gas constant R. A central concept in reaction thermodynamics is the chemical equilibrium state, in which the total Gibbs free energy is minimal and all net reaction rates vanish. The mass–action ratios $q_r^{\mathrm{ma}} = \prod_i (c_i)^{n_{ir}}$ in equilibrium follow from thermodynamic properties of the molecules. They are identical for all equilibrium states and, therefore, called equilibrium constants $k_r^{\mathrm{eq}} = \prod_i (c_i^{\mathrm{eq}})^{n_{ir}}$.

A kinetic model that does not show consistent equilibrium states runs the risk of describing a *perpetuum mobile*. We can test it by numerical simulations (see Supplementary Material); but thermodynamical consistency can be directly ensured when choosing the kinetic constants. First, the numerator of [Equation (2)] has to vanish for any choice of equilibrium concentrations c_i^{eq}. With structure numbers given by Equation (8), this leads to a Haldane relationship of the form

$$\left(k_r^{\mathrm{eq}}\right)^{h_r} = \frac{k_r^+}{k_r^-} \prod_i \left(k_{ri}^{\mathrm{M}}\right)^{h_r n_{ir}}, \tag{15}$$

linking the kinetic constants to the equilibrium constants. The equilibrium constants themselves depend on the standard chemical potentials $\mu_i^{(0)}$ *via*

$$\ln k_r^{\mathrm{eq}} = -\frac{1}{RT} \sum_i n_{ir} \mu_i^{(0)}. \tag{16}$$

If we consider an entire reaction network, Equation (16) implies Wegscheider conditions for the equilibrium constants

$$K^{\mathrm{T}} \ln k^{\mathrm{eq}} = 0, \tag{17}$$

where K is a right nullspace matrix of the stoichiometric matrix N describing all (internal and external) metabolites. The columns of K represent cyclic stationary fluxes without the net production of metabolites.

Out of equilibrium, all chemical reactions are driven by the chemical potentials μ_i or more precisely, by their negative balances

$$A_r = -\sum_i n_{ir} \mu_i \tag{18}$$

called reaction affinities (kJ/mol). The second law of thermodynamic requires that chemical reactions consume Gibbs free energy, so reaction rates and affinities must share the same sign:

$$v_r \neq 0 \Rightarrow \mathrm{sign}(v_r) = \mathrm{sign}(A_r). \tag{19}$$

A vanishing reaction rate implies that either there is no driving force ($A_r = 0$, chemical equilibrium) or the reaction is kinetically blocked, e.g. because there is no enzyme ($u_r = 0$, but $A \neq 0$). Given a metabolic network, feasible fluxes v_r and reaction affinities A_r respecting condition (19) can be determined by energy balance analysis or NET analysis.

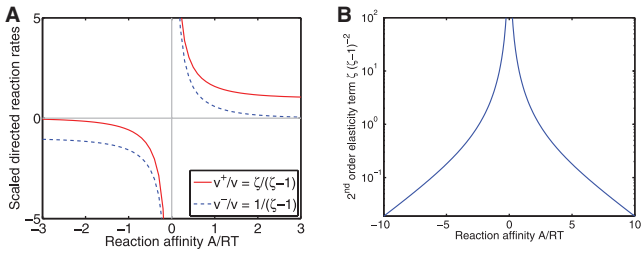

Fig. 3. Impact of the reaction affinities on reaction rates and elasticities. (**A**) The ratio $\zeta_r = v_r^+/v_r^-$ can be computed from the reaction affinity A_r by Equation (21). The plot shows the ratios $v_r^+/v_r = \zeta/(\zeta-1)$ and $v_r^-/v_r = 1/(\zeta-1)$ as functions of the reaction affinity A_r with cooperativity factor $h_r = 1$. These ratios play an important role for the first-order elasticities. (**B**) The term $(v_r^+ v_r^-)/v^2 = \zeta_r/(\zeta_r-1)^2$ appears in the second-order elasticities. It is shown on log-scale as a function of the reaction affinity A_r, again with $h_r = 1$.

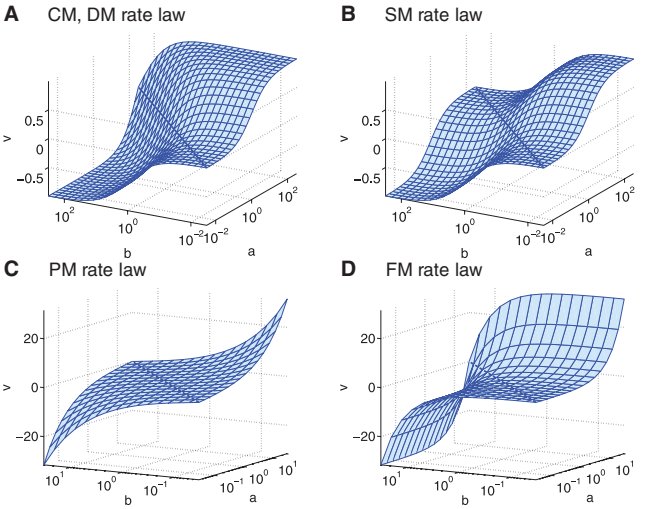

Fig. 4. Concentration dependence of modular rate laws. The rate v (vertical axis) of reaction A ↔ B depends on the substrate and product concentrations a and b (horizontal axes). All parameters k^{eq}, k_A^M, k_B^M, and $k^\pm$ are set to values of 1. (**A**) For this unimolecular reaction, the CM rate law and the DM rate law are identical. The reactant constants k^M denote the concentrations that would lead to half-saturation, defining a scale along the concentration axes. The turnover rates $k^\pm$ determine the maximal forward and backward reaction rates. Due to the numerator Eq. (2), the reaction rate vanishes at chemical equilibrium states, satisfying $b/a = k^{eq} = 1$ (dashed line). (**B**) SM rate law. (**C**) PM rate law. (**D**) FM rate law.

2.4 Velocity ratios and reaction affinities

The reaction rate Equation (1) can be written as a difference $v_r = v_r^+ - v_r^-$ of forward and backward rates with the ratio

$$\zeta_r = \frac{v_r^+}{v_r^-} = \frac{k_r^+}{k_r^-} \prod_i \left(\frac{c_i}{k_{ri}^M} \right)^{-h_r n_{ir}} . \tag{20}$$

By using the Haldane relationship (15) and the chemical potentials [Equation (14)] for ideal mixtures, we can rewrite it as

$$\begin{aligned} \zeta_r &= (k^{eq})^{h_r} \prod_i (c_i)^{-h_r n_{ir}} = \left(\frac{k_r^{eq}}{q_r^{ma}} \right)^{h_r} \\ &= e^{-h_r \sum_i n_{ir} \ln\left(\frac{c_i}{c_i^{eq}}\right)} = e^{h_r A_r / RT} \end{aligned} \tag{21}$$

with the mass–action ratio $q_r^{ma} = \prod_i (c_i)^{n_{ir}}$. We obtain the two important relations

$$A_r = RT \ln \frac{k_r^{eq}}{q_r^{ma}}, \quad h_r A_r = RT \ln \frac{v_r^+}{v_r^-} \tag{22}$$

between reaction affinities A_r, equilibrium constants k_r^{eq}, mass–action ratios q_r^{ma} and reaction rates. If the net velocities v_r and the ratios ζ_r in a metabolic state are known [possibly *via* A_r and Equation (21)], the forward and backward velocities can be computed by $v_r^+ = \frac{\zeta_r}{\zeta_r - 1} v_r$ and $v_r^- = \frac{1}{\zeta_r - 1} v_r$ (shown in Fig. 3A) and the sum of forward and backward velocities reads

$$v_r^* = v_r^+ + v_r^- = \frac{\zeta_r + 1}{\zeta_r - 1} v_r = \coth\left(\frac{h_r A_r}{2RT} \right) v_r . \tag{23}$$

3 RESULTS

3.1 Properties of the saturable rate laws

The five modular rate laws, shown in Figure 4, have particular strengths and weaknesses. While the CM rate law is biochemically most plausible, the DM, SM and PM rate laws can be obtained by ignoring (DM and PM rate laws) or adding (SM rate law) some of the binding states and, therefore, some of the denominator terms. As a consequence, these rate laws represent upper (DM and PM rate laws) and lower (SM rate law) bounds of the CM rate law. This simplification can reduce the number of parameters. In the DM enzyme mechanism, the substrate molecules bind in a single step, so their k^M values can be combined into a single parameter k^{M+}, and likewise for the reaction products. In the PM rate law, the k^{M+} values can further be merged with the catalytic constants $k^\pm$. The SM rate law, on the other hand, can be factorized into

$$v_r = u_r \left[k_r^+ \prod_i (\beta_{ri}^M)^{m_{ri}^+} (\alpha_{ri}^M)^{m_{ri}^-} - k_r^- \prod_i (\alpha_{ri}^M)^{m_{ri}^+} (\beta_{ri}^M)^{m_{ri}^-} \right], \tag{24}$$

which leads to simple formulae for the reaction elasticities. However, its enzyme mechanism assumes a simultaneous binding of all substrates and products, which is biochemically arguable and would block the enzyme at high reactant concentrations.

3.2 Thermodynamically safe parameterization

We already saw that the kinetic constants in a metabolic system need to satisfy the Wegscheider conditions (17) and Haldane relationships (15). This may lead to numerous and complicated constraints in parameter fitting and optimization. Luckily, this obstacle can be overcome by a special, thermodynamically safe parameterization using independent parameter sets (Liebermeister and Klipp, 2005, 2006a). First, we introduce the standard chemical potentials $\mu_i^{(0)}$ and the geometric mean values $k_r^V = \sqrt{k_r^+ k_r^-}$ of the turnover rates as independent model parameters. Then, equilibrium constants satisfying the Wegscheider conditions are computed from Equation (16) and turnover rates $k_r^\pm$ satisfying the Haldane relationships (15) are computed by $k_r^\pm = k_r^V (k_r^{eq} \prod_i (k_{ri}^M)^{-n_{ir}})^{\pm h_r/2}$ or

$$\ln k_r^\pm = \ln k_r^V \pm \frac{h_r}{2} \left(\ln k^{eq} - \sum_i n_{ir} k_{ri}^M \right). \tag{25}$$

With Equations (16) and (25), all kinetic constants can be written as linear functions of the independent parameters. This parameterization automatically ensures correct Wegscheider

conditions and Haldane relations, which can greatly simplify parameter fitting and optimization (Liebermeister and Klipp, 2006b).

For practical purposes, the numerator can be parameterized in alternative ways: the explicit version, as in Equation (2), contains the turnover rates $k_r^{\pm}$ as parameters. In the Haldane-compliant version, they are substituted by Equation (25), so the Haldane relationships are automatically satisfied and the equilibrium constants and the k_r^{V} appear as parameters. In the Wegscheider-compliant version, the equilibrium constants are further expressed by the standard chemical potentials *via* Equation (16), so the Wegscheider conditions will also be satisfied. This version yields thermodynamically correct models, but it comes with a higher numerical effort. In practice, one may first employ the Wegscheider-compliant version, estimate the parameters, and then use them to compute parameters for the other versions. An example is given in the Supplementary Material.

3.3 The FM rate law

With the help of Equations (22) and (25), and using the identity $\sqrt{x}-\sqrt{1/x}=2\sinh(\frac{\ln x}{2})$, the numerator (2) can be rewritten as

$$\begin{aligned} T_r &= k_r^{\mathrm{V}} \left(\prod_i \left(\frac{c_i}{k_{ri}^{\mathrm{M}}}\right)^{\frac{m_{ri}}{2}}\right)\left(\left(\frac{k^{\mathrm{eq}}}{q^{\mathrm{ma}}}\right)^{\frac{h_r}{2}} - \left(\frac{k^{\mathrm{eq}}}{q^{\mathrm{ma}}}\right)^{\frac{-h_r}{2}}\right) \\ &= k_r^{\mathrm{V}} \sqrt{\theta^+\theta^-}\, 2\sinh\left(\frac{h_r A_r}{2RT}\right). \end{aligned} \tag{26}$$

With this formula, any modular rate law (1) can be written as a product of positive terms multiplied by the force term $\sinh\left(\frac{h_r A_r}{2RT}\right)$, which depends directly on the reaction affinity. The reaction rate increases with the reaction affinity A_r and has the same sign. However, the numerator cannot be solely written as a function of A_r because of the remaining term $\sqrt{\theta^+\theta^-}$, which also involves the reactant concentrations. But if we repeat the same term in the denominator, it cancels out and we obtain the simple rate law

$$v_r = u_r k_r^{\mathrm{V}} 2\sinh\left(\frac{h_r A_r}{2RT}\right) \tag{27}$$

without any explicit dependence on concentrations or k^{M} values. This is nothing but the FM rate law.

In contrast to the other modular rate laws, the FM rate law lacks the biochemically required denominator summand 1 and is not supported by a biochemical mechanism. Even worse, as one of the substrate or product concentrations goes to zero, the reaction affinity and accordingly the rate becomes infinite—a drawback that is also shared by the lin-log kinetics (Visser and Heijnen, 2003). Despite these shortcomings, the FM rate law is a simple and practical choice because it can easily be adjusted to given stationary flux distributions. If the reaction rates and affinities in a network are given, e.g. from a previous energy balance analysis, the remaining prefactor $u_r k_r^{\mathrm{V}}$ can be computed and the rate law is completely specified.

The direct relationship between rate and affinity makes the FM rate law a nice illustration case for Prigogine's principle of minimal entropy production (Prigogine, 1947). In thermodynamic systems with a linear velocity–force relationship $v_r \sim A_r$, states of minimal entropy production have to be stationary. For metabolic systems, this principle does usually not even apply as an approximation because the fluxes cannot be directly written as a function of the reaction affinities. For the FM rate law, however, this is possible and we obtain a simple criterion for minimal chemical entropy production σ_r^{chem}

$$0 \stackrel{!}{=} \frac{\partial \sigma_r^{\mathrm{chem}}}{\partial \ln c_i} = -RT \sum_r n_{ir} \left[1 + \frac{h_r A_r}{2RT} \coth\left(\frac{h_r A_r}{2RT}\right)\right] v_r. \tag{28}$$

Close to chemical equilibrium ($A_r \approx 0$), the term in square brackets approaches a constant value of 2, so minimal entropy production implies $Nv=0$, i.e. a stationary state. Far from equilibrium, Equation (28) starts to deviate from the steady-state condition. At the same time, also the approximation $\sinh(x) \approx x$ breaks down, so the rate (27) depends nonlinearly on the force and the principle of minimal entropy production ceases to hold.

3.4 Modular rate laws are equivalent to thermodynamic–kinetic modelling rate laws

The thermodynamic–kinetic modelling (TKM) formalism (Ederer and Gilles, 2007) describes biochemical networks in the language of electrical engineering and highlights the relationship between concentrations and thermodynamic forces. The TKM rate laws read

$$v_r = \kappa_r \left(\prod_i \xi_i^{m_{ri}^+} - \prod_i \xi_i^{m_{ri}^-}\right) \tag{29}$$

with a concentration-dependent conductivity κ_r and thermokinetic potentials $\xi_i = \mathrm{e}^{\mu_i/RT}$. Originally, the $m_{ri}^{\pm}$ values represent the stoichiometric coefficients n_{ir}, but we may also employ Equation (8) instead. In analogy to Equation (26), the TKM rate law can be rewritten as

$$v_r = \kappa_r \prod_i \xi_i^{m_{ri}/2}\, 2\sinh\left(\frac{h_r A_r}{2RT}\right). \tag{30}$$

If we assume an ideal mixture, a direct comparison to Equation (26) shows that modular rate laws are identical to TKM rate laws with concentration-dependent conductivities

$$\kappa_r = \frac{u_r f_r k_r^{\mathrm{V}}}{(D_r + D_r^{\mathrm{reg}}) \prod_i (k_{ri}^{\mathrm{M}} C_i)^{m_{ri}/2}}. \tag{31}$$

The capacity C_i of compound i is defined as $C_i = \xi_i / c_i$ and given by $C_i = \mathrm{e}^{\mu_i^{(0)}/RT}$ for ideal mixtures.

3.5 Reaction elasticities and control coefficients

The dynamics of kinetic models close to a steady state depends on their reaction elasticities, i.e. the sensitivities of the reaction rates to changes of substrate concentrations and other parameters. The scaled elasticities, defined as the partial derivatives

$$E_{c_i}^{v_r} = \frac{\partial \ln |v_r|}{\partial \ln c_i} = \frac{c_i}{v_r} \frac{\partial v_r}{\partial c_i}, \tag{32}$$

are dimensionless and represent an effective reaction order (1 for irreversible mass–action kinetics, 0 in full saturation). They are used to describe the effects of relative changes, for Taylor expansion on a logarithmic scale, and in the context of log-normal parameter distributions (Liebermeister and Klipp, 2005).

Reaction elasticities for metabolite concentrations, enzyme amounts and kinetic constants can be computed from derivatives of the saturation values. The first and second order, scaled and unscaled elasticities for all modular rate laws and different types of regulation are given in the Supplementary Material. If we consider the case of

complete activation or inhibition, the scaled substance elasticities contain three terms

$$E_{c_i}^{v_r} = E_{c_i}^{T_r} - E_{c_i}^{D_r} + E_{c_i}^{f_r} \tag{33}$$

related to thermodynamics, enzyme mechanism and regulation. The thermodynamic term $E_{c_i}^{T_r}$ can be expressed by the ζ_r values (i.e. reaction affinities) or by the forward and backward rates

$$\begin{aligned} E_c^T &= \mathrm{dg}(\zeta - \hat{1})^{-1}\left[\mathrm{dg}(\zeta)M^+ - M^-\right] \\ &= \mathrm{dg}(v)^{-1}\left[\mathrm{dg}(v^+)M^+ - \mathrm{dg}(v^-)M^-\right] \end{aligned} \tag{34}$$

where $\mathrm{dg}(\cdot)$ denotes diagonal matrices and $\hat{1} = (1,1,..)^{\mathrm{T}}$. For reactions close to equilibrium ($A_r \approx 0, v_r \approx 0$), this term becomes infinite, just as expected for scaled elasticities. For an irreversible forward reaction ($|A_r| \to \infty$), in contrast, the thermodynamic term simply reads m_{ri}^+, so a non-regulated PM rate law will show substrate elasticity 1 and product elasticity 0. In general, the elasticities are further shifted by the denominator term

$$\begin{aligned} \text{CM: } E_c^D &= \mathrm{dg}(D)^{-1}\,\beta^{\mathrm{M}} \star [\mathrm{dg}(\psi^+)M^+ + \mathrm{dg}(\psi^-)M^-] \\ \text{DM: } E_c^D &= \mathrm{dg}(D)^{-1}\,[\mathrm{dg}(\theta^+)M^+ + \mathrm{dg}(\theta^-)M^-] \\ \text{SM: } E_c^D &= \beta^{\mathrm{M}} \star M \\ \text{FM: } E_c^D &= \frac{1}{2}M \\ \text{PM: } E_c^D &= 0, \end{aligned} \tag{35}$$

where $A = B \star C$ denotes the elementwise multiplication of two matrices (i.e. $a_{ij} = b_{ij}c_{ij}$). The elasticity matrix of the non-regulated FM rate law can also be written in the simple form

$$E_c^v = E_c^T - E_c^D = -\frac{1}{2}\mathrm{dg}(v)^{-1}\,\mathrm{dg}(v^*)\mathrm{dg}(h)N^{\mathrm{T}} \tag{36}$$

with $v^* = v^+ + v^-$. Complete enzyme activation and inhibition lead to the regulation term

$$E_c^f = \alpha^{\mathrm{A}} \star W^+ - \beta^{\mathrm{I}} \star W^-. \tag{37}$$

The system's Jacobian matrix and the metabolic control and response coefficients (Reder, 1988) can be computed from the reaction elasticities (33) and the stoichiometric matrix. In a system with non-regulated PM rate laws, for instance, the elasticities are given by Eq. (34) and the scaled control matrix reads

$$C^{\mathrm{S}} = -\left(N\left[\mathrm{dg}(v^+)M^+ - \mathrm{dg}(v^-)M^-\right]\right)^{-1} N\,\mathrm{dg}(v), \tag{38}$$

which can be directly computed from the reaction rates and affinities *via* Equation (23). Due to the equivalence of modular and TKM rate laws, Equation (38) also represents the response matrix $R_{\kappa_l}^{\xi_k} = \partial \ln \xi_k / \partial \ln \kappa_l$ of a TKM rate law with constant conductivity κ_r. For systems with other rate laws and regulated enzymes, the terms (35) and (37) have to be factored in.

The second-order elasticities describe the interactive effects of perturbations. In analogy to Equation (33), they can be computed as

$$E_{c_i c_j}^{v_r} = \frac{\partial^2 \ln |v_r|}{\partial \ln c_i \partial \ln c_j} = E_{c_i c_j}^{T_r} - E_{c_i c_j}^{D_r} + E_{c_i c_j}^{f_r}. \tag{39}$$

The thermodynamic term, the kinetic term for the SM rate law, and the term for complete activation and inhibition read

$$\begin{aligned} E_{c_i c_j}^{T_r} &= -\frac{\zeta_r h_r^2 n_{ir} n_{jr}}{(\zeta_r - 1)^2} = -\frac{v_r^+ v_r^-}{v_r^2} h_r^2 n_{ir} n_{jr} \\ E_{c_i c_j}^{D_r} &= \delta_{ij} m_{ri} \gamma_{ri}^{\mathrm{M}} \\ E_{c_i c_j}^{f_r} &= -\delta_{ij}(\gamma_{ri}^{\mathrm{A}} w_{ri}^+ + \gamma_{ri}^{\mathrm{I}} w_{ri}^-) \end{aligned} \tag{40}$$

where $\gamma_{rj}^{\mathrm{X}} = \alpha_{rj}^{\mathrm{X}} \beta_{rj}^{\mathrm{X}}$. The kinetic terms E_{cc}^D for the other rate laws are more complicated and are given in the Supplementary Material.

4 DISCUSSION

The modular rate laws cover all possible stoichiometries and several types of regulation with relatively few kinetic parameters and are, therefore, well suited for translating metabolic networks systematically into kinetic models. The reaction rate is determined by four main factors: the thermodynamic force arising from energies and concentrations of reactants (numerator T), the enzyme level, the enzymatic mechanism (denominator D) and allosteric regulation (f and D^{reg}). All modular rate laws share the same thermodynamic properties and are reversible, which is a precondition for thermodynamically correct models. If thermodynamic correctness is not required, irreversible reactions—implying infinite reaction affinities—can be obtained by omitting the negative term in the numerator. A reformulation based on reaction affinities links the reaction rates to the underlying thermodynamic forces, in close analogy to the thermodynamic–kinetic formalism (Ederer and Gilles, 2007). A prototypic example is the FM rate law, in which the rate solely depends on the reaction affinity; the resulting entropy production density conforms to Prigogine's principle of minimal entropy production.

The reaction affinities also have an impact on the reaction elasticities. Owing to the thermodynamic term, different reaction elasticities are dependent, so independent random sampling as proposed by Steuer *et al.* (2006) is thermodynamically incorrect. However, our formulae pave the way to an alternative, thermodynamically safe sampling scheme: (i) thermodynamically consistent fluxes and reaction affinities A_r are chosen, for instance, by energy balance analysis based on experimental data; (ii) the saturation values β_{ri}^{X} are chosen independently, e.g. from a uniform probability distribution, leading to (iii) thermodynamically correct first- and second-order elasticities. With the PM and FM rate law, the sampling step can be omitted because the elasticities are determined by the reaction rates and affinities alone.

Modular rate laws can be automatically inserted into SBML models using semanticSBML (www.semanticsbml.org) or the tool SBMLsqueezer (Dräger *et al.*, 2008), which also works as a plug-in for CellDesigner. The stoichiometry and regulators are read from the SBML model, while other information, e.g. about the types of regulation, can be given by Systems Biology Ontology (SBO) terms. Further details (such as cooperativity factors) or the type of thermodynamic parameterization needs to be set by the user. To facilitate this process and to establish a consistent syntax for SBML models, we propose a nomenclature and SBO (Le Novère, 2006) annotations for the rate laws and their kinetic parameters in the Supplementary Material. Among other things, these conventions

help to specify quantitative rate laws within the Systems Biology Graphical Notation (SBGN; Novère *et al.*, 2009).

5 CONCLUSIONS

To translate metabolic networks into kinetic models, standard rate laws are needed as substitutes for unknown rate equations. The modular rate laws are less accurate than the detailed kinetic equations, but they are flexible and biochemically plausible, yield simple formulae for reaction rates and elasticities, and can be easily inserted into SBML models. Thermodynamically safe reformulations make it easy to handle the Wegscheider conditions and Haldane relations. They guarantee consistent chemical equilibrium states and clarify the role of thermodynamic forces.

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