Metabolic Engineering 11 (2009) 87–100

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Translating biochemical network models between different kinetic formats

Frieder Hadlich [a,*], Stephan Noack [b], Wolfgang Wiechert [a]

[a] Department of Simulation, Institute of Systems Engineering FB 11/12, University of Siegen, Paul-Bonatz-Street 9-11, 57068 Siegen, Germany
[b] Research Centre Jülich GmbH, Institute of Biotechnology 2, Leo-Brandt-Street, 52428 Jülich, Germany

ARTICLE INFO

Article history:
Received 24 January 2008
Received in revised form
29 August 2008
Accepted 7 October 2008
Available online 22 October 2008

Keywords:
Biochemical network modelling
Alternative kinetic formats
Model prediction
Model simplification

ABSTRACT

Mechanistic biochemical network models describe the dynamics of intracellular metabolite pools in terms of substance concentrations, stoichiometry and reaction kinetics. Data from stimulus response experiments are currently the most informative source for *in-vivo* parameter estimation in such models. However, only a part of the parameters of classical enzyme kinetic models can usually be estimated from typical stimulus response data. For this reason, several alternative kinetic formats using different "languages" (e.g. linear, power laws, linlog, generic and convenience) have been proposed to reduce the model complexity. The present contribution takes a rigorous "multi-lingual" approach to data evaluation by translating biochemical network models from one kinetic format into another. For this purpose, a new high-performance algorithm has been developed and tested. Starting with a given model, it replaces as many kinetic terms as possible by alternative expressions while still reproducing the experimental data. Application of the algorithm to a published model for *Escherichia coli*'s sugar metabolism demonstrates the power of the new method. It is shown that model translation is a powerful tool to investigate the information content of stimulus response data and the predictive power of models. Moreover, the local and global approximation capabilities of the models are elucidated and some pitfalls of traditional single model approaches to data evaluation are revealed.

1. Introduction

1.1. Stimulus response experiments

Mechanistic models describing the dynamic behaviour of metabolic networks are an essential tool for obtaining a deeper understanding of cellular functions (Heinrich and Schuster, 1996; Wiechert, 2002). They describe the network in terms of substance concentrations, flow balances and reaction kinetics. Although such models have a long tradition (Domach et al., 1984; Rizzi et al., 1997) and new models are published with increasing frequency (Hucka et al., 2003; Snoep et al., 2005), there is an ongoing discussion on their validity, different ways to gather the required data and the estimation of parameters from experiments (Heijnen, 2005; Rizzi et al., 1997; Wiechert and Takors, 2004). One promising approach is given by stimulus response experiments (SRE) (Theobald et al., 1997) producing highly informative transient data of intracellular metabolite concentrations.

A typical metabolic SRE starts with a steady state of a microbial culture in which metabolite pool sizes and metabolic fluxes are constant over time. In this reference state, metabolism is perturbed by a sudden change of some extracellular concentration, preferably the limiting substrate. The generation of informative measurement data for such experiments require a rapid sampling, a rapid inactivation of metabolic activity and a determination of intracellular metabolite concentrations on a quantitative scale. Several recent developments in this field are described in Buziol et al. (2002), Schaefer et al. (1999) and Visser et al. (2002).

A SRE produces a large number of time-resolved concentration data. A typical experiment measures the concentrations of metabolites with up to several samples per second over a total time spread of 10 s up to minutes. Depending on the total number of measurable metabolite pools, this gives rise to several thousand available data items (Wahl et al., 2006). Although, at first glance, this data set seems to contain a huge amount of information on kinetic parameters and intracellular regulation mechanisms, the actual information yield is rather limited for the following reasons:

1. Intracellular metabolite pool size measurements are still very noisy (Bolten et al., 2007). This measurement uncertainty poses principle limitations on the available information.
2. A high sampling rate can also offer too much information (oversampling). This means that—even under the assumption that no measurement errors are present—time-series data can

* Corresponding author. Fax: +49 271 740 2756.
E-mail address: frieder.hadlich@uni-siegen.de (F. Hadlich).

1096-7176/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymben.2008.10.002

Nomenclature

α_i	ith parameter
$\boldsymbol{\alpha}$	vector of parameters
$\tilde{\boldsymbol{\alpha}}$	vector of parameters in an alternative model
a_i	concentration of ith activating metabolite
$\mathbf{N}$	stoichiometric matrix
d_i	concentration of ith inhibiting metabolite
s_i	concentration of ith extracellular metabolite
$\mathbf{s}$	vector of extracellular metabolite concentrations
t_i	ith discrete time step of time vector t
v	reaction rate
v_i	reaction rate at time t_i
$v_{i,j}$	jth reaction rate at time t_i
$\tilde{v}$	approximate reaction rate
$\mathbf{v}$	vector of reaction rates
$\mathbf{v}_i$	vector of all reaction rates at time t_i
x_i	concentration of ith intracellular metabolite
$\mathbf{x}$	vector of intracellular metabolite concentrations
$\mathbf{x}^0$	vector of intracellular metabolite concentrations at steady state
$\mathbf{x}_i$	vector of intracellular metabolite concentrations at time t_i
$\tilde{\mathbf{x}}_i$	vector of approximate intracellular metabolite concentrations at time t_i
z^0	steady-state value of metabolite or reaction z

be interpolated from neighboured samples. Consequently, the data set has an inherent redundancy.

3. Although all intracellular pools are excited by a SRE, this usually happens in a coordinated way. By the activity of intracellular regulation mechanisms, or by rapidly equilibrating reactions, many pool concentrations are kept within certain limits (Riela et al., 2000; Visser et al., 2000) or stay correlated with each other. Consequently, the experiment can never explore the whole feasible concentration range of a metabolic system.

All these effects pose principle limitations on parameter identifiability of mechanistic models. These limitations are explored in the present contribution by using a new method. The concepts of model translation and perturbation of reaction steps from their reference state play a central role in this investigation.

1.2. Mechanistic models

The dynamic behaviour of biochemical networks is usually described by a standard mass balance model of type

$$\dot{\mathbf{x}} = \mathbf{N} \cdot \mathbf{v}(\mathbf{x}, \mathbf{s}, \boldsymbol{\alpha}) \tag{1}$$

where $\mathbf{x}$ is the vector of all intracellular metabolite concentrations, $\mathbf{N}$ the stoichiometric matrix, $\mathbf{v}$ the vector of all reaction rates, $\mathbf{s}$ the vector of external concentrations and $\boldsymbol{\alpha}$ the vector of reaction kinetic parameters (Heinrich and Schuster, 1996). For the central metabolic pathways, the stoichiometry is well known. For this reason, $\mathbf{N}$ is a fixed matrix in the following.

The situation is quite different when the structure of the used reaction kinetic expressions $\mathbf{v_i}(\mathbf{x},\mathbf{s},\boldsymbol{\alpha})$ has to be determined. There are several reasons why the best choice of kinetic terms is questionable (Heijnen, 2005; Wiechert and Takors, 2004):

1. It is doubtful whether the enzyme kinetic formulas derived in classical enzyme kinetic theory can be transferred from a test tube to the intracellular environment. This is due to the fact that the physico-chemical parameters of the intracellular milieu with respect to molecular crowding, channelling, pH-values or ionic strength are rarely available (Agius and Sherratt, 1996; Kresnowati et al., 2008).
2. Even if the structure of a kinetic expression is fixed, it is obvious that the kinetic parameters might be different in *in-vitro* and *in-vivo* intracellular environments. Particularly, a large number of substances that are only partly known might effect the operation of an enzyme *in-vivo* (i.e. inhibitor or activator). Only a few of them have been characterized under *in-vitro* conditions (Ewings and Doelle, 1980). At least the $v_{\max}$ values can never be obtained from data bases because they depend on the enzyme expression levels.
3. Even if the set of enzyme effectors is fixed to those known from literature, the application of classical enzyme kinetic theory to reactions with many substrates and effectors produces a number of kinetic parameters that grows faster than the number of effectors. As an example, the phosphotransferase glucose uptake system (*Pts*) in *Escherichia coli* is given (Chassagnole et al., 2002):

$$v_{\mathrm{Pts}} = \frac{v_{\max}\mathrm{GLUC\ PEP/PYR}}{(k_{\mathrm{a1}} + k_{\mathrm{a2}}\ \mathrm{PEP/PYR} + k_{\mathrm{a3}}\mathrm{GLUC} + \mathrm{GLUC\ PEP/PYR})(1 + G6P^n/k_{\mathrm{G6P}})}$$

This structurally complicated equation includes six kinetic parameters ($v_{\max}$, k_{a1}, k_{a2}, k_{a3}, k_{G6P} and n) and four metabolite pools (GLUC, PEP, PYR and G6P), thus, containing more parameters than reactants.

The practical consequence is that the information needed to determine the parameters of complex models with many metabolite interactions will not be available from a limited set of measurement information. In fact, fitting complex metabolic models usually produces a large number of poorly determined or highly correlated parameters. It has been reported in Degenring et al. (2004) that even from thousands of data items only about 40% of the 122 parameters in a model could be determined with reasonable statistical confidence. Consequently, using a fitted model for the prediction of cellular behaviour can result in highly uncertain predictions if the information supporting this prediction is not contained in the original experimental data set. This is a fundamental limitation for the predictive power of models.

1.3. Model predictions

A standard way to quantify the uncertainty of a model prediction is to perform a rigorous calculation of the error transduction from the original measurement data set to the predicted values. The statistical tools to carry out this procedure are well documented in literature (Seber and Wild, 1989; Wiechert and Takors, 2004) and shall not be discussed in detail here.

However, the classical statistical methodology—particularly in the context of model simplifications—has a major drawback that is illustrated in Fig. 1a. Here, a Michaelis–Menten kinetic $v = v_{\max}S/(S+k_m)$ is fitted to some fictive concentration–flux measurement data. Obviously, from this data, the k_m value is poorly determined because no concentration data in the range of k_m are available. For this reason, the Michaelis–Menten equation makes insufficient predictions in the k_m range as shown in Fig. 1a by the grey prediction error tube.

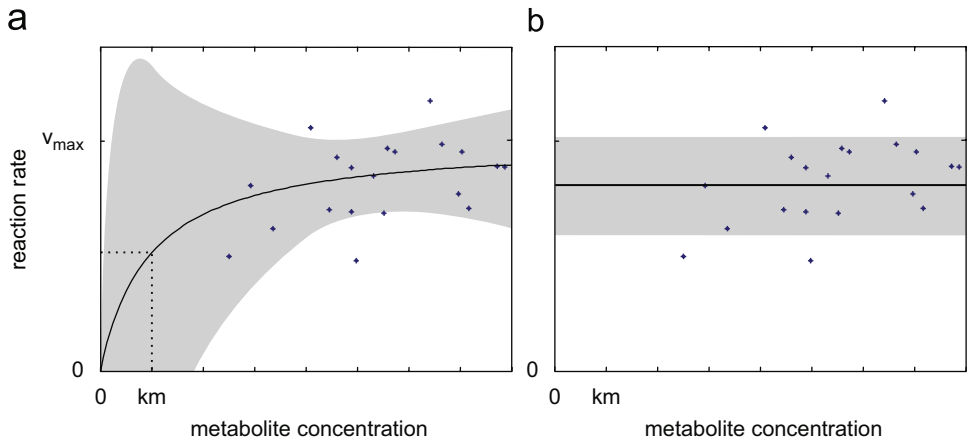

Fig. 1. The paradox of precise prediction by a wrong model illustrated with fictive data: (a) Parameter fit of the correct Michaelis–Menten kinetic. (b) Fit of a wrong (simplified) model with constant reaction rate to the same data. The corresponding statistical prediction quality is indicated by the shaded confidence regions. Obviously, the wrong model has a much better prediction in the range of small concentration values.

An alternative model might be given by simplifying the Michaelis–Menten kinetic to a constant velocity (valid only in case of substrate saturation), i.e. $v = v_{max}$. The new model has only one single parameter and is fitted to the same data in Fig. 1b. Obviously, the less complex model still provides a sufficient fit to the available data and the single parameter v_{max} can be determined with an even higher precision than in the Michaelis–Menten case. Consequently, the prediction precision (here: the precision of v_{max}) is high even in the range of k_m, but nevertheless, the model is completely wrong for low concentrations.

Clearly, an unjustified extrapolation is the reason for this poor performance. Whenever the reaction kinetic is only locally perturbed around a reference state S_0 (in the example $S_0 \ll k_m$) both models agree whereas a non-local perturbation leads to doubtful predictions. In principle, there is no way to circumvent this problem: without having informative data, a prediction must be based on experienced guesses, thus introducing apparently precise new information.

This simple example shows that an excellent fit, well-determined parameters and precise predictions are no cure against using wrong models or critical extrapolations. In particular, model simplification (like that in Fig. 1) is always a risky operation. The present paper introduces a new methodology to reveal such pitfalls. It is based on the systematic fitting of many alternative but reasonable models to the same data set. If two different but plausible models agree in their reproduction of the measured data but disagree in the prediction, this is a clear indication that the predictions are doubtful.

2. Model translation

2.1. Alternative kinetic formats

As a consequence of the limited information available from SREs, several alternative kinetic formats have been suggested in the literature. All of them try to represent reaction kinetic expressions in a certain generic form that can be applied to all cases. Using alternative formats tendentially reduces the number of parameters and simplifies the algebraic treatment of models (Visser and Heijnen, 2003; Voit, 2000). The alternative terms are motivated by approximation concepts, thermodynamic analogies or assumptions on enzyme mechanisms.

Several of these approximate kinetic formats have been compared quite recently, particularly in the context of SRE (Heijnen, 2005). The most promising variants and two new approaches are considered here. In each of the following cases, x_i^0, s_j^0 and v^0 denote the known stationary reference state at the beginning of the SRE. The terms are formulated in such a way that the reference state is obviously reproduced by the model (i.e. $v(x_1^0, x_2^0, \ldots, s_1^0, s_2^0, \ldots) = v^0$):

1. Classical linearization of the model around the reference state by a first-order Taylor-series yields a purely linear model of type:

$$v = v^0 + \sum_{i=1}^{m}(\alpha_i(x_i - x_i^0)) + \sum_{j=1}^{n}(\alpha_{j+m}(s_j - s_j^0)) \tag{2}$$

2. Power-law GMA representation obtained by linearization on a log scale (Savageau and Voit, 1982):

$$v = v^0 \prod_{i=1}^{m}\left(\frac{x_i}{x_i^0}\right)^{\alpha_i} \prod_{j=1}^{n}\left(\frac{s_j}{s_j^0}\right)^{\alpha_{j+m}} \tag{3}$$

3. Linlog approximation in analogy to thermodynamic formalisms (Visser et al., 2004):

$$v = v^0 + \sum_{i=1}^{m}\left(\alpha_i \ln\frac{x_i}{x_i^0}\right) + \sum_{j=1}^{n}\left(\alpha_{j+m} \ln\frac{s_j}{s_j^0}\right) \tag{4}$$

4. Generic kinetic formulas—as a newly introduced approach—are composed from saturation and inhibition terms in a multiplicative way. Essentially, a non-competitive interaction of activators $a_1,\ldots,a_m$ and inhibitors $d_1,\ldots,d_n$ (which must be extracted from $\mathbf{x}$ and $\mathbf{s}$) is assumed:

$$v = v^0 \left[\prod_{i=1}^{m}\left(\frac{1}{1+\alpha_i}\right)\prod_{j=1}^{n}\left(\frac{\alpha_j}{1+\alpha_j}\right)\right]^{-1} \prod_{i=1}^{m}\underbrace{\left(\frac{a_i/a_i^0}{a_i/a_i^0+\alpha_i}\right)}_{\text{saturation}} \prod_{j=1}^{n}\underbrace{\left(\frac{\alpha_{j+m}}{d_j/d_j^0+\alpha_{j+m}}\right)}_{\text{inhibition}} \tag{5}$$

   To accomplish this, the effectors of a reaction must be classified into activating and inhibiting substances. The inverted term in front is responsible for proper normalization.
5. Convenience kinetics as another new approach proposed in Liebermeister and Klipp (2006). This kinetic is build on the assumption of a random binding of all educts $e_1,\ldots,e_m$ and a random unbinding of all products $p_1,\ldots,p_n$ (which are extracted

from **x** and **s**) with equal rate constants:

$$v = \frac{k_+ \prod_{i=1}^{m} \left(\frac{e_i}{\alpha_i}\right)^{N_{e_i,v}} - k_- \prod_{j=1}^{n} \left(\frac{p_j}{\alpha_{j+m}}\right)^{N_{p_j,v}}}{\prod_{i=1}^{m} \left[\sum_{s=0}^{N_{e_i,v}} \left(\frac{e_i}{\alpha_i}\right)^s\right] + \prod_{j=1}^{n} \left[\sum_{t=0}^{N_{p_j,v}} \left(\frac{p_j}{\alpha_{j+m}}\right)^t\right] - 1} \tag{6}$$

Here, one parameter (i.e. k_+) is determined from the reference state:

$$k_+ = \frac{\left\langle \prod_{i=1}^{m} \left[\sum_{s=0}^{N_{e_i,v}} \left(\frac{e_i^0}{\alpha_i}\right)^s\right] + \prod_{j=1}^{n} \left[\sum_{t=0}^{N_{p_j,v}} \left(\frac{p_j^0}{\alpha_{j+m}}\right)^t\right] - 1 \right\rangle v^0 + k_- \prod_{j=1}^{n} \left(\frac{p_j^0}{\alpha_{j+m}}\right)^{N_{p_j,v}}}{\prod_{i=1}^{m} \left(\frac{e_i^0}{\alpha_i}\right)^{N_{e_j,v}}}$$

Additionally, effectors can be included as multiplicative generic terms completely analogous to the generic approach in Eq. (5). As opposed to the other approximating formats which have exactly the same number of parameters than there are concentration variables, a convenience kinetic needs two more parameters (k_+ and k_-) in each situation including the mass balance values $\boldsymbol{N}_{\text{reactant,flux}}$ of the stoichiometric matrix **N**.

Application of the approximate formats to influxes or effluxes of a metabolic model requires further measures: because fluxes over the system boundary must be unidirectional, backfluxes are switched off in this case. Especially the backflux parameter k_- is eliminated, thus the number of parameters is further reduced.

The relationship between kinetic parameters and elasticities is given in Table 1 for all kinetic formats but convenience. The elasticities of convenience kinetics depend on all pools and all parameters of the inspected reactions and cannot be easily written.

2.2. General model translation approach

In this contribution, the validity and predictive power of mechanistic models are investigated by an automatic model translation approach. It starts with a given mechanistic model that already fits the data of a SRE. Typically, this will be a classical biochemical network model with enzyme kinetic expressions for the reaction rates, henceforth called the original model. It has been explained in several other publications (Rizzi et al., 1997; Wahl et al., 2006) how such models can be systematically built up from measured data. Particularly, the fitted model has to reproduce the reference state.

Choosing one of the alternative kinetic formats, it is now tried to replace any single reaction kinetic term of the original model by its alternative. Roughly speaking, the enzyme kinetic model is translated into another language. Here, the requirement is that the alternative model reproduces the measured data as well as the original model. For this purpose, the initial model is translated reaction step by reaction step until no more substitutions of kinetic terms are possible without sacrificing the data reproduction quality. Finally, some reactions might resist all translation attempts, thus indicating that the corresponding kinetic terms are strongly constrained by the data. The translation procedure is carried out for all alternative kinetic formats described above.

The following sections are arranged as follows: firstly, the model translation approach is formalized and its difference to former approaches is explained. Particularly, an established local approximation approach is replaced by a more powerful global approximation. As a consequence, the translation of a whole model requires high computational efforts. The implemented high-performance search algorithm is shortly described. Subsequently, two example networks from literature are used to analyse model translations to different alternative formats. This will pinpoint a couple of problems, which have a general importance for mechanistic modelling.

Table 1
Link of reaction kinetic elasticity and its respective kinetic parameter α_i in different approximate kinetic formats.

Kinetic type	Elasticity	
Linear	$E^0_{x_i} = \frac{x_i^0 \alpha_i}{v^0}$	$E^0_{s_j} = \frac{s_j^0 \alpha_j}{v^0}$
Power law	$E^0_{x_i} = \alpha_i$	$E^0_{s_j} = \alpha_j$
Linlog	$E^0_{x_i} = \frac{\alpha_i}{v^0}$	$E^0_{s_j} = \frac{\alpha_j}{v^0}$
Generic	$E^0_{a_i} = \frac{\alpha_i}{\alpha_i + 1}$	$E^0_{d_j} = -\frac{1}{\alpha_j + 1}$

2.3. Extending the data set

The model translation procedure intentionally starts with a classical enzyme kinetic model because the large number of parameters in such models increases their flexibility to fit the given data. On the other hand, as explained before, this type of model will usually be overparameterized. For model simplification, the availability of an already fitted model offers a big advantage over the information available from the raw data alone: in this situation, not only the concentration time courses are available but also the time courses of the (model) reaction rates (cf. Fig. 2).

More formally, if the metabolic system is sampled at discrete times t_i, $i = 1,\ldots,K$, the available data based on a simulation with a general model from Eq. (1) are given, on the one hand, by the noise-free, time-resolved concentrations (Fig. 2a bottom):

$$\mathbf{x}_i = \mathbf{x}(t_i), \quad i = 1,\ldots,K \tag{7}$$

and on the other hand, by the corresponding time-resolved flux data (Fig. 2a middle):

$$\mathbf{v}_i = \mathbf{v}_i(t_i) = \mathbf{v}(\mathbf{x}(t_i), \boldsymbol{\alpha}), \quad i = 1,\ldots,K \tag{8}$$

The latter data, of course, are not available from a measured data set alone. A new relation, which is of central importance for the present approach, is now obtained by the elimination of the time stamps from the dynamic data in Eqs. (2) and (3):

$$\mathbf{v}_i = \mathbf{v}(\mathbf{x}_i, \boldsymbol{\alpha}), \quad i = 1,\ldots,K \tag{9}$$

By using this relation, kinetic terms can be individually fitted to the respective concentration–flux data, which is a time-independent problem.

Taking this approach it is possible to consider each single reaction in isolation from the rest of the network (cf. Fig. 2a top). Clearly, the reference state ($\mathbf{x}^0$,$\mathbf{v}^0$) is reproduced in each concentration–rate diagram. The other data then reveal whether the reaction step is locally or non-locally perturbed with respect to the deviation of concentrations from the reference state. This surplus of information not only reduces the further computational effort but also gives more intuitive insight into the nature and behaviour of the system.

2.4. Approximation concepts

These considerations give rise to the following approximation concepts, which are ordered by increasing potential of data reproduction.

(a) *Local Taylor approximation* (*LTA*): Based on the known reference state, vector $\mathbf{x}^0$ alternative kinetic formats $\tilde{v}(\mathbf{x}, \tilde{\boldsymbol{\alpha}})$ can be used to approximate the original kinetics $v(\mathbf{x},\boldsymbol{\alpha})$ similarly to a

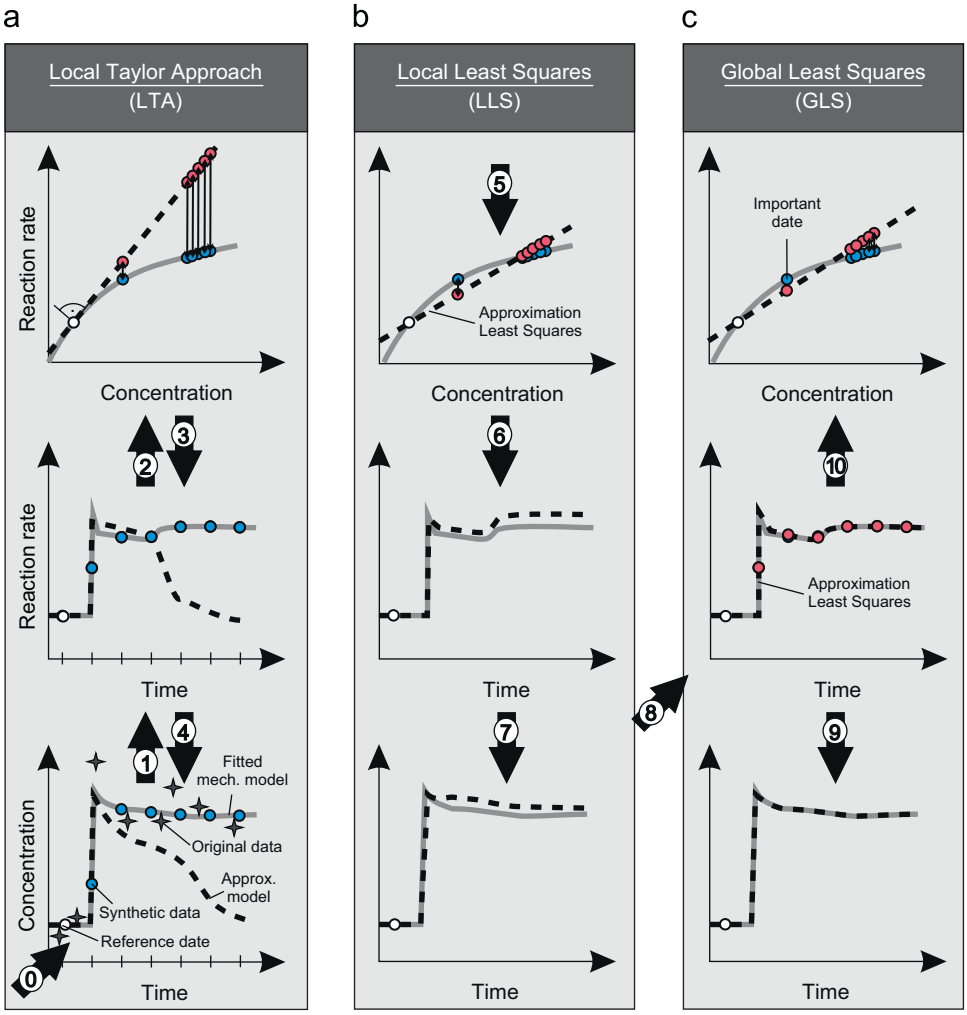

Fig. 2. General procedure (a–c) for substituting one single reaction kinetic in a model. The rows show the data from the viewpoints of concentration time courses (bottom), reaction rate time courses (middle) and rate versus concentration (top). The columns show the three stages of the algorithm. (0) Concentration measurements (stars) given from an experiment are simulated using a model with only mechanistic kinetics (solid line). Synthetic (reference) measurements are produced by sampling the simulated concentration course. (1) Knowing the concentration data and the reaction kinetic from a single kinetic it is now possible to sample the reaction rates at the same times. (2) This gives rise to a time-independent concentration versus reaction rate plot (solid line). Here, the mechanistic kinetic is approximated by a linear kinetic using a Taylor approximation (dotted line top) in the reference state (white point). (3,4) The simulation runs of the approximate model (dotted line) do not follow any more the original run. (5) LLS approach is used to refit the single alternative kinetic given by LTA to the reference data. (6,7) The results of LLS curve are closer to the original model data. (8) Finally, all kinetic parameters of the coupled model are refitted to the reference data. (9) The outcome of this is a well-fitted concentration time course (10) while using a not perfectly fitting alternative kinetic.

Taylor series, i.e. it is required:

$$\tilde{v}(\mathbf{x}^0,\tilde{\boldsymbol{\alpha}}) = v(\mathbf{x}^0,\boldsymbol{\alpha}) \quad \text{and} \quad \frac{d}{dx}\tilde{v}(\mathbf{x}^0,\tilde{\boldsymbol{\alpha}}) = \frac{d}{dx}v(\mathbf{x}^0,\boldsymbol{\alpha}) \tag{10}$$

Note that $v(\mathbf{x},\boldsymbol{\alpha})$ need not be linear (as in the case of a classical Taylor series) to fulfil these relations. Eq. (10) now has to be solved for the alternative kinetic parameters $\tilde{\boldsymbol{\alpha}}$. This concept, essentially, is applied in Heijnen (2005) where alternative kinetic formats are parameterized by elasticity coefficients.

(b) *Local least squares* (*LLS*): Unfortunately, the data might not be concentrated around the reference state (cf. Fig. 2a). In this situation, the Taylor approach is unable to produce the best approximation result w.r.t. the underlying data set. Thus, an alternative approximation concept based on the experimental data is given by the constrained least squares minimization:

$$\sum_i [v_i(\mathbf{x}^0,\boldsymbol{\alpha}) - \tilde{v}_i(\mathbf{x}^0,\tilde{\boldsymbol{\alpha}})]^2 \rightarrow \min_{\substack{\tilde{\boldsymbol{\alpha}} \\ v_i(\mathbf{x}^0)=\tilde{v}_i(\mathbf{x}^0)}} \tag{11}$$

The difference is shown in Fig. 2b for a simple linear approximation approach. Obviously, the substituted kinetic of the LLS approximation has different elasticities in the reference state than the original model. This is not a contradiction because the original model was obtained by parameter fitting and, thus, the elasticities (in contrast to the rather well-determined reference state) are subject to uncertainties.

(c) *Global least squares* (*GLS*): Even if all reaction kinetics are well reproduced in the respective concentration–rate diagrams, the ultimate fitting criterion must be the reproduction of the original experimental time–concentration data set. In this context, the LLS concept is still a local one because it does not account for the effect of exchanging a kinetic term of one single reaction step on the overall dynamic behaviour of the system in Eq. (1). In fact, the exchange of a single kinetic term, although locally well fitting to the concentration–flux data, can lead to perturbations of the whole dynamic system which by the time may become amplified by the coordinated action of all other kinetic terms. Consequently,

the ultimate criterion to identify the feasibility of a model simplification in the context of sampled data is given by

$$\frac{1}{K\dim\mathbf{x}}\sum_{i=1}^{K}\left\|\mathbf{x}_i(\boldsymbol{\alpha})-\tilde{\mathbf{x}}_i(\tilde{\boldsymbol{\alpha}})\right\|_{\Sigma}^{2}\rightarrow\min_{\tilde{\boldsymbol{\alpha}}}=\mathrm{GLS}(\tilde{\boldsymbol{\alpha}}) \tag{12}$$

Due to the assumption that the original model fits well, $\mathbf{x}_i$ can be taken here instead of the measured data. $\tilde{\mathbf{x}}_i$ denotes the output of the translated model, $K\dim\mathbf{x}$ is the number of data, and $\|.\|_{\Sigma}^{2}$ denotes a weighted Euclidian norm with measurement standard deviations collected in the covariance matrix Σ. The weighting is chosen so that a value of $\mathrm{GLS}(\tilde{\boldsymbol{\alpha}})\approx 1$ means that the translated model prediction on an average deviates from the original model within the order of the respective measurement standard deviation.

Fig. 2a–c shows the results of a typical kinetic substitution with LTA, LLS and GLS in contrast: the LTA and LLS approaches cannot reproduce the full reaction time course, while the GLS approach successfully approximates the given reaction run. Roughly speaking, a poor representation of one reaction step can be compensated by adjusting the parameters of other steps. This is the distinguishing feature of the global over the local concepts.

One reason why all three concepts are discussed here is the computational efficiency: approximation effort dramatically increases from Eqs. (10) to (12). Whereas LTA can be analytically computed, LLS already needs a rather simple parameter fit and GLS is burdened with the full complexity of fitting an ordinary differential equation system to the data.

A three-step approach now significantly reduces the overall effort to solve the most relevant GLS problem (Fig. 2): the solution of LTA is taken as an initial value for the solution of LLS which in terms serves as an initial value for GLS. Only in few cases, a restart of parameter fitting with other initial values was necessary. More details on the algorithm are given in the following section.

2.5. Automatic model translation

Based on the solution of the GLS problem for a single kinetics substitution, a fully automatic algorithm for the translation of full biochemical network models to an alternative kinetic format was developed. This algorithm has to solve a complex search problem as follows.

For every original enzyme kinetic expression, there is given exactly one alternative using a fixed format (i.e. linear, linlog, power law, etc.). Consequently, because either the original kinetic or its alternative is considered for each step, any (partially) translated model can be described by a binary number $\mathbf{i}=(i_1,\ldots,i_n)$, $i_j\in\{0,1\}$. This gives rise to a family of models parameterized by $\mathbf{i}$ (Haunschild et al., 2005):

$$\dot{\tilde{\mathbf{x}}}^{\mathbf{i}}=\mathbf{N}\cdot\tilde{\mathbf{v}}^{\mathbf{i}}(\tilde{\mathbf{x}}^{\mathbf{i}},\mathbf{s},\tilde{\boldsymbol{\alpha}}^{\mathbf{i}}),\quad i=(i_1,\ldots,i_n),\quad i_j\in\{0,1\} \tag{13}$$

Clearly, the original model is described by the binary number $\mathbf{i}=(0,\ldots,0)$ which means that no kinetic substitutions are involved. The fully translated model (if this is possible) would be $\mathbf{i}=(1,\ldots,1)$.

The space of all created models must now be explored with respect to their ability for data reproduction. This is done by fitting their parameters in the sense of GLS. To this end a threshold ε for the least squares functional (12) is chosen to decide if a model with substituted kinetics can reproduce the original data sufficiently well. Tendentially, the model fit becomes worse with each new substitution by simplified kinetic terms. However, there might still be many models fulfilling the threshold criterion. In this case, the models with the largest number of substitutions are selected.

More formally spoken, in contrast to the optimization goal of Haunschild et al. (2006) given by

$$\min_{\mathbf{i}}\mathrm{GLS}_{\mathbf{i}} \tag{14}$$

which looks for the best fitting model in the whole model family the wanted model now is found by solving the constrained optimization problem

$$\max_{\mathbf{i}=(i_1,\ldots,i_n)\atop \mathrm{GLS}_{\mathbf{i}}<\varepsilon} i_1+\cdots+i_n \tag{15}$$

Note that $i_1+\cdots+i_n$ is the number of substitutions in model $\mathbf{i}$. If there are still different optimal candidates, the candidate with the lowest GLS is taken.

Because the model space has 2^n models, it can be extremely large in practice. For this reason, the optimization problem (15) is solved by a newly developed search heuristics that need not explore the whole model family. The algorithm makes use of the LTA and LLS criteria for finding good initial values (Fig. 2). The global parameter fitting process (GLS) for one model is then managed by the MMT2 tool (Wahl et al., 2006).

Doing substitutions, it is (heuristically) assumed that the quality criterion decreases with a growing number of substitutions due to model simplification, i.e. it holds for two models $\mathbf{i}$, $\mathbf{i}'$:

$$\mathbf{i}<\mathbf{i}'\Rightarrow\mathrm{GLS}_{\mathbf{i}'}<\mathrm{GLS}_{\mathbf{i}}$$

where the inequality $\mathbf{i}\leqslant\mathbf{i}'$ must hold component-wise. Consequently, if a partially translated model would not meet the requirement of data reproduction it makes no sense to try any further substitution. The whole procedure is carried out with parallel computations on a high-performance compute cluster with 300 processors. The technical details of the algorithm are described in Appendix A.

3. Results and discussion

3.1. Test cases

The model translation algorithm has been tested with two different models taken from literature. The corresponding enzyme kinetic types as well as the number of their parameters are given in Table 2.

The first model (Fig. 3) is a small hypothetical model with eight reactions published in Mendes and Kell (1998) which was discussed in Visser and Heijnen (2003) to illustrate the power of the linlog approach. Essentially, the LTA criterion was already sufficient to substitute reaction kinetic terms of the original model by linlog expressions. Since it works well in the original publication, it is not surprising that with the new method, the linlog approach is also able to substitute any single kinetic expression in the model. This makes the first model a good test case for the implemented algorithm. Because the product pools P_1 and P_2 are assumed to be constant, a parameter reduction in the kinetics of effluxes R_5 and R_8 from four down to two parameters was performed (cf. Section 2.1). Here, the translation process is based on 234 measurements synthesized from the models simulation run by the use of 55 kinetic parameters.

The second model (Fig. 4) with 27 reactions, taken from Chassagnole et al. (2002), is a much bigger one. It describes glycolysis and pentose-phosphate-pathway dynamics in *E. coli* and was obtained by evaluating a SRE. Few alterations have been made to use the model in the present context. Precisely, conservation relations have been introduced for (a) ATP, ADP, AMP, (b) NAD, NADH and (c) NADP, NADPH. These relations allow computing ADP, NAD and NADP from their respective energy charged forms. Although the 113 kinetic parameters from

Table 2
Reaction kinetic expressions from the tested models and their number of parameters (see Chassagnole et al., 2002; Visser and Heijnen, 2003 for more details).

Kinetic type	Reversible	Parameters	Reactants	Modifiers	Model reaction
Michaelis–Menten	–	2	1	–	G3pdh, R_5, R_8, Rppk, Sersynth, Synth1, Synth2
	–	4	1	1	Pepcx
	X	4	2	–	Eno, Pglumu, Pgm, Tis
	–	5	3	1	G6pdh
	–	5	3	2	Pgdh
	X	6	4	–	Gapdh, Pgk
	X	6	2	1	Pgi
Allosteric regulation	–	8	4	2	Pk
Transfer equation	–	6	4	–	Pts
Allosteric enzyme	–	11	3	3	Pfk
Ordered bi-bi	X	10	4	–	R_2, R_4, R_7
Ordered uni-bi	X	7	3	–	Aldo
Hill equation	–	3	1	–	Pdh
	–	5	2	–	Dahps
	–	7	2	1	R_1, R_3, R_6
Allosteric activation	–	5	2	1	G1pat
Mass action	X	2	2	–	R5pi, Ru5p
	X	2	4	–	Ta, Tka, Tkb

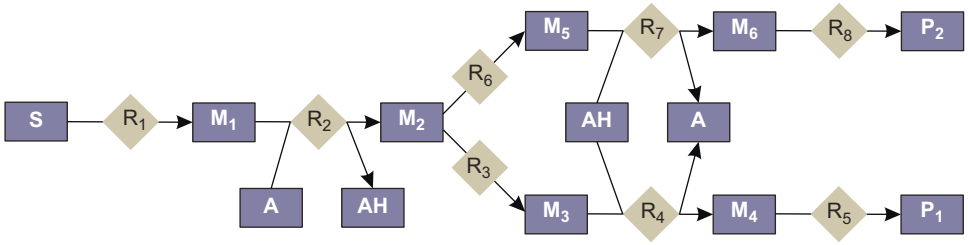

Fig. 3. Simple metabolic model taken from Visser and Heijnen (2003) containing 10 pools combined by eight reactions.

Chassagnole et al. (2002) have been left unchanged the simulation output only slightly changes. All in all, 1085 measurements are sampled from the models simulation run for the automated translation.

3.2. Translated models

The complete model translation procedure was performed for both test examples and the five different alternative formats from Eqs. (2) to (6). The best models for each approach obtained with the threshold $\varepsilon = 1$ (i.e. data reproduction within about one measurement standard deviation) are summarized in Tables 3 and 4 (see GLS columns).

For the simple example, there were exactly 36 model translation trials necessary for convenience and linlog kinetic substitutions whereas most effort was needed by the generic approach with the study of more than 100 model variants. Best results are obtained with convenience and linlog approaches. They permit for a complete substitution of classical equations and need 23 (linlog) and 29 (convenience) parameters. Whereas the generic approach still allows for six substitutions, power law and linear kinetics perform rather poorly (five and four substitutions). Interestingly, all simple Michaelis–Menten kinetics could be replaced by all tested alternatives. This already indicates that these reactions are rather locally perturbed by the SRE or alternatively they do not directly influence other reactions than effluxes.

For the large model the automatic translation process required the generation of several hundred model variants in each case. Notice that this is much less than the total number of $2^{27} = 134$ million models. In total, about 2 million single simulation runs have been started for the parameter fits.

Again, some of the equations, mostly primitive Michaelis–Menten kinetics, are substitutable by all studied approximating methods while others (i.e. *Aldo*) are almost not replaceable. Here, the linlog approach allows the highest number of kinetic substitutions (25), i.e. only two non-substitutable equations are remaining. It is followed by linear (23), generic (20), convenience (20) and power laws (16).

Obviously, convenience kinetics require comparatively many parameters in both test cases while performing rather well w.r.t. the number of substitutions. The high number of parameters stems from the convenience equation (Eq. (6)) itself. Here, unlike other approaches one additional parameter (i.e. k_+) is necessary for the kinetic calculation.

Interestingly, the generic approach allows the substitution of four more kinetic terms than the power-law approach in the complex model but, nevertheless, has a higher number of parameters. The reason is that the reference kinetics can be

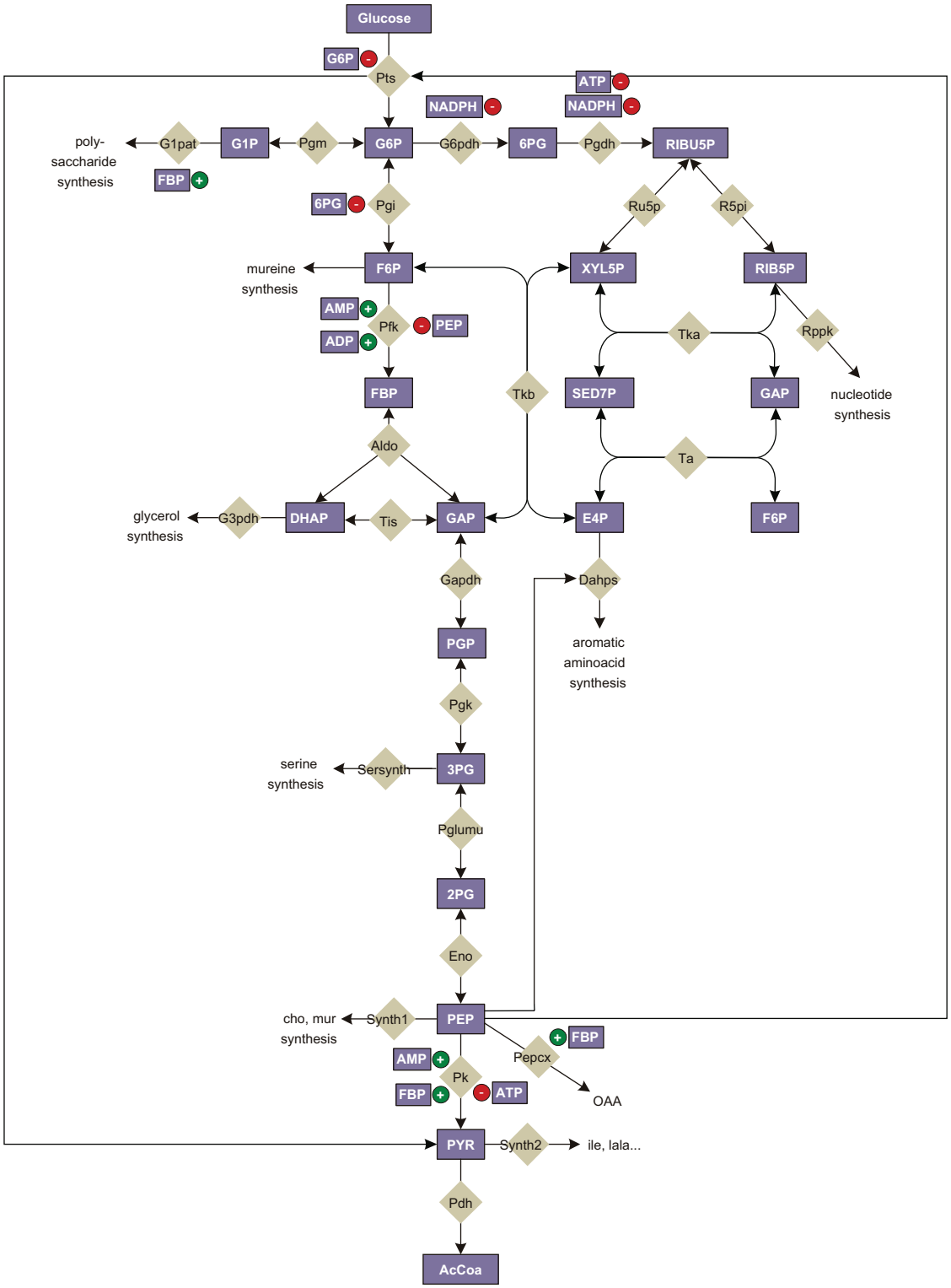

Fig. 4. Complex metabolic model from Chassagnole et al. (2002) with 27 reactions and 35 pools.

substituted with different success regarding the number of required kinetic parameters (see Table 2): most kinetic approximations reduce the number of model parameters (e.g. approximation of *Pfk* needs six instead of 11 parameters), others require the same number (e.g. *Pgdh* with five parameters) but some require even more (e.g. *Ta* with four instead of two parameters). However, this is rather an exception.

3.3. Analysing single kinetic substitutions

So far the final results of the complete model translation procedure have been discussed. Additional insight can be achieved when only one single kinetic from the original model is substituted while all others are left untouched. In each case, the model is refitted by using the GLS criterion from Eq. (12) with

Table 3
Results of multiple kinetic substitutions in the simple model (Visser and Heijnen, 2003).

	Convenience		Generic		Linear		Linlog		Power law	
	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS
R_1 (7)	X	X			X	X	X	X		
R_2 (10)		X	X				X	X	X	
R_3 (7)	X	X	X	X			X	X	X	X
R_4 (10)	X	X	X	X			X	X		
R_5 (2)	X	X	X	X	X	X	X	X	X	X
R_6 (7)	X	X	X	X	X	X	X	X		X
R_7 (10)	X	X	X	X				X		X
R_8 (2)	X	X	X	X	X	X	X	X	X	X
Σ subst.	7	8	7	6	4	4	7	8	4	5
Σ param.	–	29	–	33	–	45	–	23	–	39

Each kinetic, given by its abbreviated enzyme name with its number of parameters (given in brackets), is marked only if its substitution is possible by an approximate kinetic in the simplified model for the discussed constraints.

Table 4
Results of multiple kinetic substitutions in the complex model (Chassagnole et al., 2002).

	Convenience		Generic		Linear		Linlog		Power law	
	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS	GLS_1	GLS
Aldo (7)	X	X								
Dahps (5)	X	X	X	X	X	X	X	X	X	X
Eno (4)	X	X	X	X	X	X	X	X		
G1pat (5)	X	X	X	X	X	X	X	X	X	X
G3pdh (2)	X	X	X	X			X	X	X	X
G6pdh (5)	X	X	X	X	X	X	X	X		
Gapdh (6)	X	X			X	X	X	X		
Pdh (3)	X				X	X	X	X	X	X
Pepcx (4)		X			X	X				
Pfk (11)					X	X	X	X		
Pgdh (5)	X	X	X	X	X	X	X	X		
Pgi (6)	X				X	X	X	X	X	X
Pgk (6)			X	X			X	X		
Pglumu (4)	X	X	X	X	X	X	X	X		
Pgm (4)	X	X	X	X	X	X	X	X	X	X
Pk (8)	X	X	X		X	X	X	X	X	X
Pts (6)	X	X	X	X	X	X	X	X	X	X
R5pi (2)			X	X	X	X	X	X	X	X
Rppk (2)	X	X	X	X	X	X	X	X	X	X
Ru5p (2)			X	X	X	X	X	X	X	X
Sersynth (2)	X	X	X	X	X	X	X	X	X	X
Synth1 (2)	X	X	X	X	X	X	X	X	X	X
Synth2 (2)	X	X	X	X	X	X	X	X	X	X
Ta (2)	X	X	X	X	X	X	X	X	X	X
Tis (4)	X				X	X	X	X	X	X
Tka (2)	X	X	X	X			X	X	X	
Tkb (2)	X	X	X	X	X	X	X	X		
Σ Subst.	22	20	21	20	23	23	25	25	17	16
Σ Param.	–	102	–	96	–	81	–	82	–	92

Each kinetic, given by its abbreviated enzyme name with its number of parameters (given in brackets), is marked only if its substitution is possible by an approximate kinetic in the simplified model for the discussed constraints.

threshold $\varepsilon = 1$. The results are summarized in the GLS_1 columns of Tables 3 and 4. The comparison between the GLS column (best performing model with maximal number of substitutions) and the GLS_1 column (one single substitution in the original model) gives an interesting characterization of the role of single kinetics in the whole concert:

- Clearly, when substitutions with simpler terms are done, it becomes more and more difficult to reproduce the original data. But success will also depend on the chosen combination of substituted kinetics, i.e. one substitution might hinder others to perform well. Consequently, it can happen that a kinetic is substitutable in one step (GLS_1) but is not substituted in the best performing model. This situation occurs most frequently for the generic and power-law approaches.
- It also happens (convenience and linlog within the simple model) that a term cannot be substituted in one step (GLS_1) but is well translatable in the multiple substituted model. This again indicates that in the concert of all substituted kinetics the roles of kinetic terms might change so that the coordinated action of substituted terms permit for other substitutions than the same steps have in the original model. However, this phenomenon never occurred in the large model.
- Nevertheless, in the vast majority of cases the substitutable terms in columns GLS and GLS_1 agree. This suggests that the importance of one single kinetic for data description is in most cases rather defined by intrinsic features of this kinetic term.

3.4. Ranking of kinetic approximations

Assuming that the two examples are representatively chosen, the following conclusions can be drawn:

- In both cases, power laws do not perform as well as other methods. There are two known problems with equations of this type (Heijnen, 2005): first, if the concentration data of an inhibitor run towards zero then the reaction rate has a singularity instead of becoming zero. The second problem is that negative reaction rates of bidirectional steps are not reproducible with power laws. Introducing different forward and backward steps might solve this problem.
- Surprisingly, the linear approach is one of the best methods in the translation of the complex model whereas it rather fails in the translation of the simple model. A possible reason is that the simple model simulation runs more detached from the reference state compared with the complex model. Furthermore, the two models use a different number of parameters which have (of course) a more global model influences based on the model structures and stimulus behaviours.
- Generic and convenience kinetics behave very similar in both examples. This could be expected because they are quite similar and even agree for several simple kinetic terms (like Michaelis–Menten). Although both approaches approximate simply structured reference kinetics in outstanding good manner, like with the power law it is not possible to describe reaction reversibility completely. Interestingly, the most simple mass action kinetics (i.e. *R5pi* and *Ru5p*) are not exchangeable in two cases with convenience. Finally, it must be mentioned that the convenience approach needs one more parameter than its rivals for the substitution of each kinetic.
- Linlog kinetics performs best in both cases. However, even this approach does not allow for a complete translation of the complex model. One known problem of this approach is that for an extremely small concentration of a reacting metabolite, the reaction rate runs towards negative infinity.

3.5. Data reproduction

Fig. 5 shows how well the original model output is reproduced by the best performing simplified models from Table 4. Exemplarily, the concentration time courses of metabolites dihydroxy

acetone phosphate (DHAP) and fructose-6-phosphate (F6P), substrate glucose (GLUC) and product isoleucin (ILE) are shown. Due to the threshold criterion, the alternative models generate results that stay—in average—within typical measurement errors of 10% around the original data.

The model translation approach clearly demonstrates that many different models having the same network structure, and differing only in their kinetic terms can produce almost the same simulation results. Moreover, it should be noticed that all those models with a lower number of kinetic substitutions sometimes better reproduce the data than more reduced ones. Tendentially, power laws perform worst but nevertheless still keep the threshold ($\varepsilon = 1$).

From a statistical perspective, there is no chance to distinguish between the different models solely based on the measured data. Although the number of parameters is different this factor only slightly influences typical statistical model quality criteria like the χ^2 value. Likewise, model selection criteria like the Akaike information criterion (AIC) produces no significant difference as shown in Wahl et al. (2006).

Finally, it should be noticed that the global (GLS) parameter fitting approach taken here turned out to be well justified in retrospective. It helps to re-adjust disoriented parameters of LTA and LLS models. Although in most cases, these starting values proved to be close to the final GLS fit, it frequently happened that GLS led to significant network-wide re-adjustments. In many cases, it even happened that parameters produced by the LLS or LTA methods led to an aborted simulation. An extreme case is given in Fig. 6 illustrating the simulation run of kinetic *Aldo* which aborts using the linear and linlog single translations (GLS_1) of *Aldo* in the large model. Here, the model translation cannot be fixed by fitting the model parameters to data.

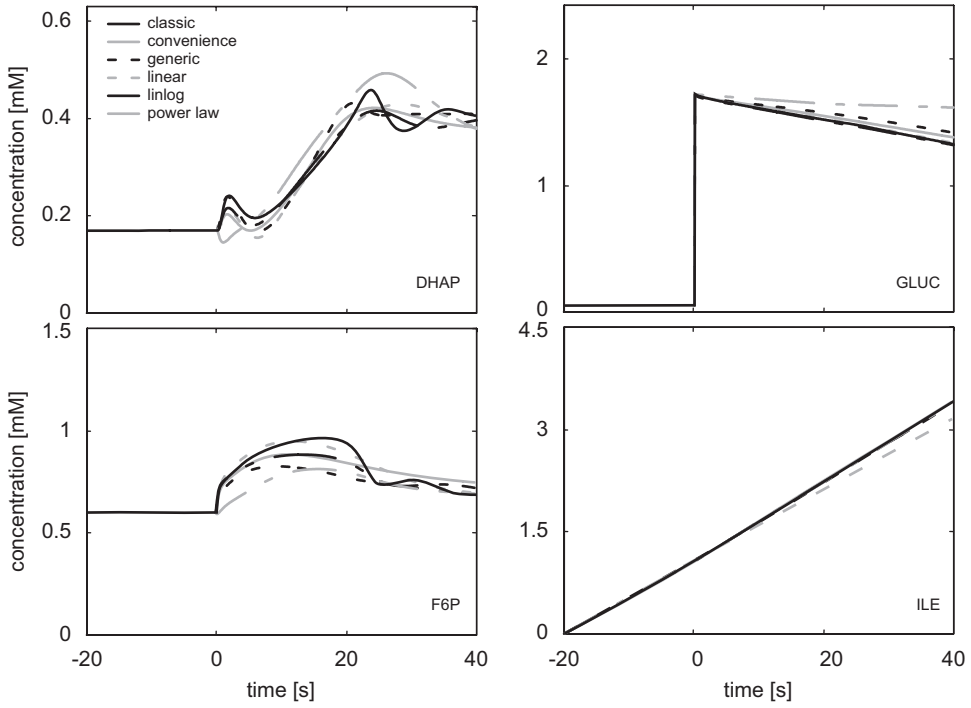

Fig. 5. Simulation results for some exemplary pools of the complex metabolic network model (solid line) in contrast to its GLS approximations.

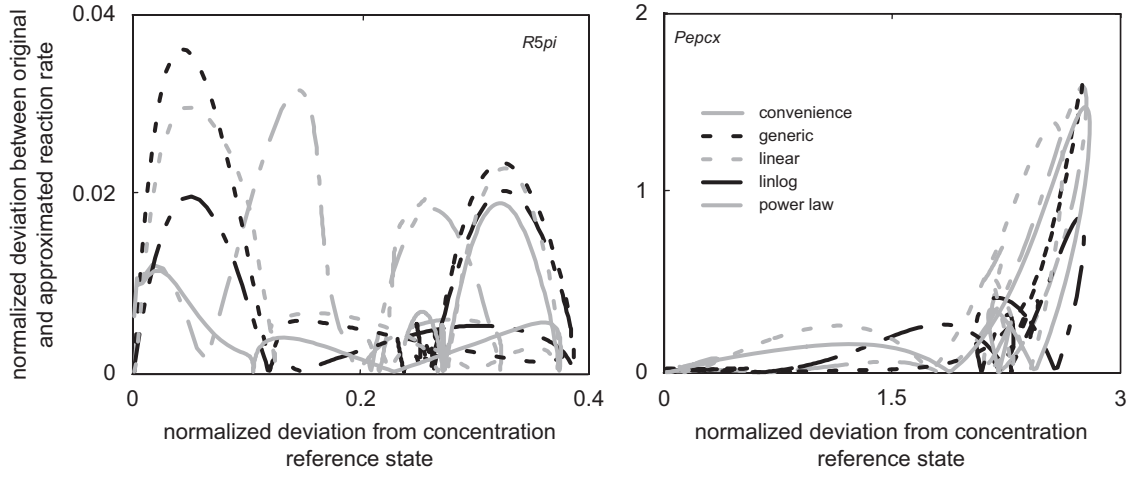

Fig. 6. Classic and approximated (substitution of *Aldo* kinetic) simulation runs of reaction *Aldo*.

3.6. Perturbation from reference state

Clearly, there is no problem for any reasonable kinetic approach to substitute kinetic terms when concentrations are only locally perturbed around the reference state. Consequently, those reaction steps experiencing a larger concentration deviation from the reference tend to be most critical for the substitution process. These deviations are now explored. To this end, the concentration deviations from the reference state are displayed against the reaction velocity difference between the original and the translated model.

Multi-dimensional concentration deviations from the reference state can be measured at any time t_i by the (scaled) distances

$$\Delta \mathbf{x_i} = \frac{1}{\dim \mathbf{x_i}} \left\| \frac{\mathbf{x_i} - \mathbf{x}^0}{\mathbf{x}} \right\| \tag{16}$$

where the vector quotient has to be taken component-wise. This distance is almost the same if the original data $\mathbf{x_i}$ is replaced by its approximation $\tilde{\mathbf{x}}_\mathbf{i}$ because $\mathbf{x_i} \approx \tilde{\mathbf{x}}_\mathbf{i}$ is guaranteed by the threshold criterion. If only the jth reaction rate $v_{i,j}$ is considered at any time t_i, the vector $\mathbf{x_i}$ must be reduced to those entries actually influencing it.

On the other hand—as explained above—the velocities of reaction step j in the original and translated model can be different. For this reason, the corresponding reaction rate difference at time t_i is measured by

$$\Delta v_{i,j} = |v_{i,j} - \tilde{v}_{i,j}| \tag{17}$$

Fig. 6 shows the resulting time-dependent plots of $\Delta v_{i,j}$ over $\Delta \mathbf{x_i}$ for two exemplarily chosen reaction steps and for all maximally translated *E. coli* models (cf. Table 4). It displays the discrepancies between original and translated kinetics relative to the specific part of the concentration space visited by the experiment.

Fig. 7 shows results for the reactions *R5pi* (A) and *Pepcx* (B) for the maximum translations of the *E .coli* model. Obviously, the pools involved in *R5pi* are rather locally perturbed whereas the concentrations involved in *Pepcx* run more far away from the reference steady state. Regarding the different scaling of the velocity discrepancies in Fig. 7, some typical dependencies between a reaction and its participating pools are apparent: the Michaelis–Menten originated kinetics *Pepcx* shows a uniform behaviour for all alternative formats. The approximation is acceptable in close and medium distance from the reference point but has a strong discrepancy far away from the reference. On the other hand, the reaction *R5pi* only slightly deviates from the reference because of its local concentration deviation from the reference state. The flux deviations are almost neglectable (notice the different scales in Fig. 7).

Looking more closely to the *Pepcx* plot in Fig. 7, it becomes clear that the rate difference for large concentration deviations is actually up to two times higher than the reference value. It is very instructive to investigate the reasons for this strange finding:

1. A well-known example from approximation theory is the approximate relation

   $f(t) = \varepsilon \sin(\omega t) \approx g(t) = 0$

   Here, f can approximate the zero function g to any desired precision ε. In contrast, the time derivative $f'(t) = \varepsilon\omega \cos(\omega t)$ does not approximate $g'(t) = 0$ at all and, in fact, can have an arbitrarily large deviation from zero. Transferring this result to the general model from Eq. (1), it becomes clear that an excellent reproduction of concentration time courses does not necessarily imply an acceptable approximation of concentration derivatives, i.e. flux balances. Consequently, even the elasticity values can change dramatically from the reference model to its translations. Exemplary elasticities for different translations (LTA, LLS and GLS) of the large model are given in Table 5 for the fluxes *Pts* and *Pgm*. Obviously, the elasticity values agree in most cases but sometimes are completely different. Because elasticities and control coefficients are linked to each other by summation and connectivity theorems (Kholodenko et al., 1998) a change in elasticities also means a change of control coefficients.
2. The deviation plots in Fig. 7 do not contain the information on how long the system stays in a state where rates have large differences. If the original and approximated model disagrees only for a short transient between well-matching states, this will have little influence on the concentration time course.
3. The role of a kinetic term in the whole concert can be quite different. If there is a mechanistic kinetic term that affects many pools it must be reproduced in a perfect manner. Thus, it cannot be substituted because even minimal deviations between the original and the alternative kinetic significantly change the time course of the overall metabolic system.

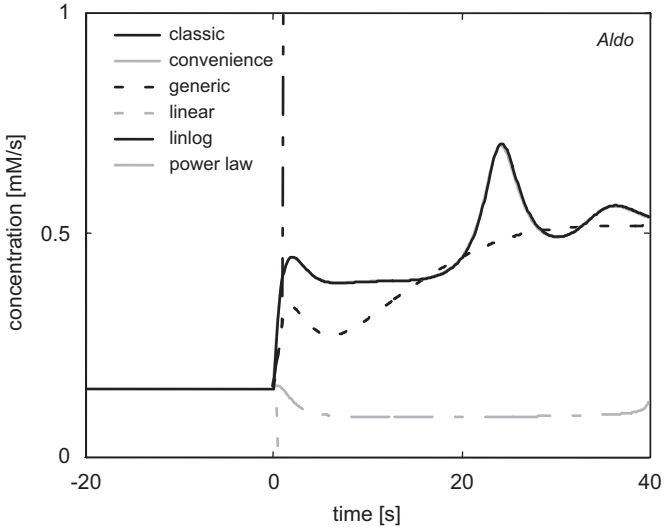

Fig. 7. Discrepancy of a single reaction velocity (*R5pi* (left) and *Pepcx* (right)) versus deviation from reference state in concentration space for the maximal translations of the *E. coli* model (Table 3).

This, finally, leads over to a discussion of the *Aldo* kinetics, which turned out to resist almost all translation attempts (except for the convenience approach). Fig. 7 shows the simulation runs of *Aldo* made with the classical model and the kinetic *Aldo* approximating models. Whereas convenience reproduces the classic simulation run in a perfect way, the generic translated model has small problems in velocity approximation, which still is overbid by power laws having no more similar simulation run. The simulations of linlog and linear translated models collapse after few seconds and, consequently, demonstrate the limited power of these alternatives in the replacement of the *Aldo* kinetic. Other mechanistic kinetics (like these from *Pepcx*, *Pfk* and R_2) must be refitted within tight bounds which can only be managed by few alternative kinetic formats. This suggests that the *Aldo* step plays a central role for the reproduction of the measured data which is also intuitively clear from its central topological role in the network (Fig. 4).

3.7. Model predictions

Another striking difference between the various models is given by their predictions of a new experiment. As an example,

Table 5
Elasticity values after the translation steps LTA, LLS and GLS for the fluxes *Pts* and *Pgm* of the big model.

(Pts)	Elasticity w.r.t. GLUC			Elasticity w.r.t. PEP			Elasticity w.r.t. G6P			Elasticity w.r.t. PYR		
	LTA	LLS	GLS	LTA	LLS	GLS	LTA	LLS	GLS	LTA	LLS	GLS
Linear		0.46	0.40		5.23	3.65		−14.19	−12.36		−4.98	−7.27
PowerLaw		0.25	0.25		0.77	0.77		−0.31	−0.31		0.01	0.01
Linlog	1.00	5.30	5.46	1.00	6.33	5.72	3.58	−22.49	−21.59	−1.00	−14.00	−15.57
Generic		1.00	1.00		0.81	0.63		−0.51	−0.47		1.86	1.85
Convenience		0.72	0.37		−4.70	−6.41		2.03	2.52		−7.70	−8.77

(Pgm)	Elasticity w.r.t. G6P			Elasticity w.r.t. G1P		
	LTA	LLS	GLS	LTA	LLS	GLS
Linear		19.67	19.50		−20.81	−15.70
PowerLaw		6.73	6.73		0.01	0.01
Linlog	22.87	25.61	20.96	−22.85	−25.90	−18.59
Generic		1.00	1.00		0.00	0.00
Convenience		−0.05	1.00		0.05	−1.00

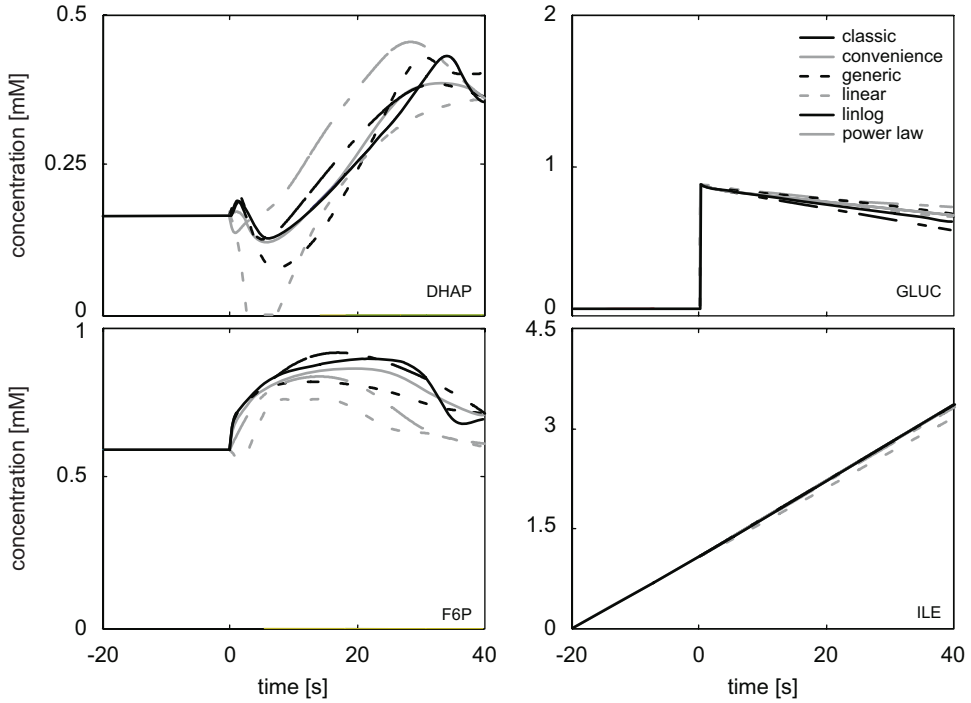

Fig. 8. Predictions of some concentration time courses by different well-fitting maximally translated models. The input signal is changed to a half glucose pulse.

a glucose pulse with the half-substrate amount is imposed on the *E. coli* model. Because the system is less strongly excited it can be expected that the original response is well reproduced by the simplified models. Fig. 8 shows some exemplary concentration time courses for the reference model and its GLS approximations (Table 4). Obviously, the predictions with linlog, generic and power-law kinetics are more or less similar to the reference simulation while the result with linear approach diverges after few seconds (especially in the course of DHAP decreasing to zero). However, in every case, the inter-model deviations are significantly higher than the measurement precision and, thus, it might be possible to distinguish between the models in a future experiment.

4. Conclusions and outlook

Alternative kinetic formats such as power laws, linlog, convenience or generic kinetics have been suggested as unifying frameworks for the simplification of complex biochemical network models. However, the use of simplified kinetic formats may result in a limited predictive power of the model. Here, standard statistical methods to asses the predictive power of a model have strong limitations. Firstly, they rely on the correctness of the current model and, secondly, they are based on a rather local analysis of the model fit.

Consequently, the simultaneous consideration of different well-fitting models can help to obtain more insight into the

information content of the underlying data. In this contribution, the translation of a given biochemical network model to different alternative kinetic formats proved to be a useful tool for testing the validity of simplified models and studying the intrinsic approximation capabilities of alternative kinetic formats. Most important, a network-wide viewpoint can be achieved instead of a focus on single kinetic terms.

4.1. Comparing different models

The following conclusions can be drawn from the above investigations on realistic example systems:

1. The most efficient model translation can be achieved by a combination of local approximations focused on one single reaction step at a time and a subsequent global refitting of the model (GLS criterion). Approximation errors introduced in one single reaction step can then be compensated by re-adjustment of the parameters in other steps (i.e. by network-wide re-adjustments). For this reason, the new approach rather shows a network-wide picture than just the isolated approximation of each reaction kinetics which proved to be inferior with respect to data reproduction.
2. The rigorous fitting of many models produced a whole family of models which all reproduce the measured data within tight error bounds (i.e. one measurement standard deviation on the average). These models are statistically indistinguishable based on the available data (Wahl et al., 2006), i.e. there is no "best" model. Consequently, only those features which remain unchanged over all well-fitting models have a strong evidence whereas more data are needed to make a distinction in "non preserved" parts of the model (Wahl et al., 2006).
3. Even when the global GLS criterion is used, thus permitting maximal flexibility of the alternative models to fit the data, and even if high-performance computing power is exploited to explore a large part of the search space (as done here), it is not possible to completely translate realistic metabolic models to one single alternative format.
4. The most important experience made is the surprising flexibility of many different models, all sharing the same network structure, to reproduce the same data. Roughly speaking, if a model has sufficiently many parameters, then there are good chances that it can describe the experiment. This, in turn, means that the available data still leave several degrees of freedom for several reaction steps to "cooperate" in such a way that the data are reproduced. Clearly, this is a global aspect and such results cannot be obtained if each kinetic is treated isolated from the network. Even a local network analysis by linearization probably does not yield this result.
5. In contrast, some reaction steps (i.e. *Aldo*) resisted almost all simplification attempts. This fact might not only have intrinsic reasons that can be derived from the reaction kinetic term alone. It is rather the case that these reaction steps have a critical influence on the whole systems behaviour, i.e. a change of these reactions cannot be compensated by a coordinated action of all others. From this viewpoint, the non-substitutable reactions play an "important" role in the whole concert. On the other hand, less important terms with only local influence tolerate much larger deviations.
6. An excellent fit of concentration data does not necessarily mean a good fit of the underlying reaction rates because the latter are related to concentration time derivatives. Moreover, the regions of good fit can be different between the alternatives. Thus, the various approximations achieve their goals in different ways. Particularly, from the concentration versus reaction rate plots, it can be judged where or when the model is not well supported by the data. Looking only at concentration time courses, these features are hard to find. In Bardow et al. (2005), it has been shown that for a chemical reaction system with concentration measurement by Raman spectroscopy all required data can be directly obtained from the signal by estimation.
7. Unlike any single model approach, the generation and comparison of different alternatives of well-fitting models allows to circumvent a classical statistical pitfall, i.e. apparently precise predictions based on wrong models. Clearly, the simpler the fitted models are the more important this problem is. The comparison of different well-fitting models based on alternative kinetic formats showed that the prediction of new experiments indeed produces significantly different results. The reason might be an "oversimplification", an incomplete model or a bad statistical prediction quality. Only the latter can be analysed from a single model viewpoint.
8. Assuming that the chosen models are representative for comparing the different approaches the linlog format performed best, whereas the similar generic and convenience formats show a suboptimal performance. Power laws cannot be recommended. These findings partly coincide with former results based on purely local arguments (Heijnen, 2005).

In summary, this investigation shows that, although some approaches have strong computational advantages, there is no silver bullet of metabolic model formulation. Moreover, there are currently little chances to avoid the situation shown in Fig. 1. Thus, the use of simplified or even non-simplified mechanistic models for a reliable prediction of new experiments must still be critically discussed because the necessary information required to extrapolate even slightly changed conditions might not be contained in the data. Again, this underlines that the region of the metabolic concentration space visited by a single stimulus response experiment is typically too narrow for a complete model validation (Degenring et al., 2004).

4.2. Increasing the amount of information

Several directions for future work can be suggested:

1. Fitting to multiple experiments (Wahl, 2007) or using a BioScope for multiple perturbation (Mashego, 2005) can certainly help to close the information gap. However, this is an extremely laborious task. Moreover, it is not easy to disturb a cellular system in such a way that different regions of the concentration space are visited.
2. An analysis of the concentration space explored by the underlying experiment can help to achieve more insight. Specially tailored nonlinear multi-variate statistical methods have to be developed for this purpose (Gorban et al., 2008).
3. Due to the local nature of kinetic approximations any simplified model should carry some kind of information on its range of validity. This helps to find critical extrapolations when the model is used for predictions.
4. If it is possible to obtain more direct information on the rates (e.g. by metabolic flux analysis) this is an extremely valuable information for model validation (Petersen et al., 2003). If data density is very high, this information can already be obtained directly from the instationary data as could be shown in other disciplines (Bardow et al., 2005).
5. As many additional constraints as possible should be imposed on a network model to exclude models by their intrinsic features. Several efforts have recently been undertaken by

exploiting thermodynamic constraints (Ederer and Gilles, 2007; Liebermeister and Klipp, 2006) or guaranteeing model stability. Moreover, plausible concentration and reaction velocity ranges should be given. However, if all these constraints are assembled, the computational problem of constrained optimization becomes extremely difficult because most constraints are put on the model output and not directly on its parameters.

Acknowledgment

This work was funded by the German Ministry of Education and Research within the SysMAP project (BMBF 0313704).

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ymben.2008.10.002.

References

Agius, L., Sherratt, H.S.A., 1996. Channeling in Intermediary Metabolism. Portland Press Ltd., London.
Bardow, A., et al., 2005. Concentration-dependent diffusion coefficients from a single experiment using model-based Raman spectroscopy. Fluid Phase Equilibr. 228–229, 357–366.
Bolten, C.J., et al., 2007. Sampling for metabolome analysis of microorganisms. Anal. Chem. 79, 3843–3849.
Buziol, S., et al., 2002. New bioreactor-coupled rapid stopped-flow sampling technique for measurements of metabolite dynamics on a subsecond time scale. Biotechnol. Bioeng. 80, 632–636.
Chassagnole, C., et al., 2002. Dynamic modeling of the central carbon metabolism of *Escherichia coli*. Biotechnol. Bioeng. 79, 53–73.
Degenring, D., et al., 2004. Sensitivity analysis for the reduction of complex metabolism models. J. Process Contr. 14, 729–745.
Domach, M.M., Leung, S.K., Cahn, R.E., Cocks, C.G., Shuler, M.L., 1984. Computer model for glucose-limited growth of a single cell of *Eschericia coli* B/r-A. Biotechnol. Bioeng. 26, 203–216.
Ederer, M., Gilles, E.D., 2007. Thermodynamically feasible kinetic models of reaction networks. Biophys. J. 92, 1846–1857.
Ewings, K.N., Doelle, H.W., 1980. Further kinetic characterization of the non-allosteric phosphofructokinase from *Escherichia coli* K-12. Biochim. Biophys. Acta 615, 103–112.
Gorban, A.N., et al., 2008. Principal Manifolds for Data Visualization and Dimension Reduction. Springer, Berlin.
Haunschild, M.D., et al., 2005. Investigating the dynamic behavior of biochemical networks using model families. Bioinformatics 21, 1617–1625.
Haunschild, M.D., et al., 2006. A general framework for large scale model selection. Optim. Methods Softw. 21, 901–917.
Heijnen, J.J., 2005. Approximative kinetic formats used in metabolic network modeling. Biotechnol. Bioeng. 91, 534–545.
Heinrich, R., Schuster, S., 1996. The Regulation of Cellular Systems. Kluwer Academic Publishers, Dordrecht.
Hucka, M., et al., 2003. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19, 524–531.
Kholodenko, B.N., et al., 1998. Control analysis of metabolic systems involving quasi-equilibrium reactions. Biochim. Biophys. Acta 1379, 337–352.
Kresnowati, M.T.A.P., et al., 2008. Quantitative physiological study of the fast dynamics in the intracellular pH of *Saccharomyces cerevisiae* in response to glucose and ethanol pulses. Metab. Eng. 10, 39–54.
Liebermeister, W., Klipp, E., 2006. Bringing metabolic networks to life: convenience rate law and thermodynamic constraints. Theor. Biol. Med. Model. 3, 1–11.
Mashego, M.R., 2005. Robust Experimental Methods to Study In-vivo Pre-steady State Kinetics of Primary Metabolism in *Saccharomyces cerevisiae*. Delft University of Technology, The Netherlands.
Mendes, P., Kell, D., 1998. Non-linear optimization of biochemical pathways: applications to metabolic engineering and parameter estimation. Bioinformatics 14, 869–883.
Petersen, S., et al., 2003. A Multi-scale Approach for the Predictive Modeling of Metabolic Regulation. Metabolic Engineering in the Post Genomic Era. Horizon Scientific Press.
Riela, N.A.W.v., et al., 2000. Dynamic optimal control of homeostasis: an integrative system approach for modeling of the central nitrogen metabolism in *Saccharomyces cerevisiae*. Metab. Eng. 2, 49–86.
Rizzi, M., et al., 1997. In vivo analysis of metabolic dynamics in *Sacharomyces cerevisiae*: II. Mathematical model. Biotechnol. Bioeng. 55, 592–608.
Savageau, M.A., Voit, E.O., 1982. Power-law approach to modeling biological systems. I. Theory. J. Ferment. Technol. 60, 221–228.
Schaefer, U., et al., 1999. Automated sampling device for monitoring intracellular metabolite dynamics. Anal. Biochem. 270, 88–96.
Seber, G.A.F., Wild, C.J., 1989. Nonlinear Regression. Wiley, New York.
Snoep, J.L., et al., 2005. Towards building the silicon cell: A modular approach. Biosystems 83, 207–216.
Theobald, U., et al., 1997. In vivo analysis of metabolic dynamics in *Saccharomyces cerevisiae*: I. Experimental observations. Biotechnol. Bioeng. 55, 305–316.
Visser, D., et al., 2000. Tendency modeling: a new approach to obtain simplified kinetic models of metabolism applied to *Saccharomyces cerevisiae*. Metab. Eng. 2, 252–275.
Visser, D., Heijnen, J.J., 2003. Dynamic simulation and metabolic re-design of a branched pathway using linlog kinetics. Metab. Eng. 5, 164–176.
Visser, D., et al., 2002. Rapid sampling for analysis of in vivo kinetics using the BioScope: a system for continuous-pulse experiments. Biotechnol. Bioeng. 79, 674–681.
Visser, D., et al., 2004. Optimal re-design of primary metabolism in *Escherichia coli* using linlog kinetics. Metab. Eng. 6, 378–390.
Voit, E.O., 2000. Computational Analysis of Biochemical Systems. A Practical Guide for Biochemists and Molecular Biologists. Cambridge University Press, Cambridge.
Wahl, A., 2007. Methoden zur integrierten Analyse metabolischer Netzwerke unter stationären und instationären Bedingungen., Vol. Dissertation. Universität Siegen, Siegen.
Wahl, S.A., et al., 2006. Unravelling the regulatory structure of biochemical networks using stimulus response experiments and large scale model selection. IEE Proc. Syst. Biol. 153, 275–286.
Wiechert, W., 2002. Modeling and simulation: tools for metabolic engineering. J. Biotechnol. 94, 37–63.
Wiechert, W., Takors, R., 2004. Validation of metabolic models: concepts, tools, and problems. Metabolic engineering in the post genomic era. Horizon Biosci.