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Rites of passage: requirements and standards for building kinetic models of metabolic phenotypes

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The overarching ambition of kinetic metabolic modeling is to capture the dynamic behavior of metabolism to such an extent that systems and synthetic biology strategies can reliably be tested *in silico*. The lack of kinetic data hampers the development of kinetic models, and most of the current models use *ad hoc* reduced stoichiometry or oversimplified kinetic rate expressions, which may limit their predictive strength. There is a need to introduce the community-level standards that will organize and accelerate the future developments in this area. We introduce here a set of requirements that will ensure the model quality, we examine the current kinetic models with respect to these requirements, and we propose a general workflow for constructing models that satisfy these requirements.

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Introduction

Mathematical modeling is an essential tool for understanding and explaining complex behavior and properties of living organisms. In recent years, the prevalent frameworks for modeling metabolic pathways were constraint-based approaches that make use of network stoichiometry to characterize the intracellular fluxes at steady state [1–3]. While proving their utility in studies of cellular physiology and metabolic engineering [4,5], the stoichiometric models lack information about metabolic regulation and enzyme kinetics. Therefore, these static descriptions cannot be used for predicting the complex dynamic responses to environmental and genetic perturbations, or, for example, for studying dynamic transitions of the metabolism [6•,7] or oscillatory phenomena [8].

Kinetic models couple dynamics of metabolic concentrations and fluxes to enzyme concentrations and they allow us to take into consideration regulation at the enzyme and post-translational level [9]. Although the potential of kinetic models compared to their stoichiometric counterparts is promising, it comes at a price. Kinetic models are typically built in a bottom-up manner, wherein for each reaction a kinetic rate expression along with corresponding parameter values is required. This results in model structures with large number of parameters. Due to the absence of experimental assays that could provide the required extent of measurements for the rigorous parameterization of these models, researchers incorporate the needed information from different sources: (i) literature; (ii) databases such as Brenda [10]; or (iii) they perform experimental measurements themselves [11,12•,13]. Whenever the model parameters are not experimentally measured, parametric estimation methods [14] or Monte Carlo methods are used [15••,16–19]. In the latter, the parameters are characterized within well-defined bounds that are consistent with the studied conditions and physicochemical laws. The available experimental values of kinetic parameters are often uncertain due to measurement and estimation errors, and variations stemming from different experimental conditions and set-ups [20]. As a consequence, many existing kinetic models are of a limited scope, often with *ad hoc* stoichiometry, they cover one or a few metabolic pathways, and frequently they neglect the whole network dynamics as observed in [9,21].

Recent efforts have been made toward building genome-scale kinetic models [6•,15••,17,22–25]. In the quest for models with a large-scale or genome-scale scope, one must ensure that the increased size and scope is attained without sacrificing the consistency with physicochemical laws and the necessary mechanistic details. As the activity in kinetic modeling is expected to grow intensively in the coming years, there is a need for establishing community-level standards. The objective of this paper is to review the current state of kinetic modeling and propose a set of requirements that every kinetic model should satisfy. We expect that standardized kinetic models will facilitate future community efforts in model building where knowledge from different sources and research groups is incorporated as advocated in [9].

Issues in building kinetic models

When building kinetic models we are given a set of observations and we seek to identify the set of kinetic

parameters that best describe the observations. In metabolic kinetic models we usually start with a set of metabolic fluxes and concentrations, and we assume that the stoichiometry and the thermodynamic properties of the reactions in the metabolic network are known. The basic problem then is to identify kinetic model(s) that consistently describe the experimental observations. We discuss here the main issues in building kinetic models.

Uncertainty

Uncertainty is recognized in the literature as the main challenge in kinetic modeling of biological systems [9,16,20–22,26,27]. The dynamic behavior of metabolism is a result of complex interactions of metabolite concentrations, through kinetics and thermodynamics, and uncertainty in these interactions propagate to the structure and parameters of the kinetic models.

- Uncertainty in kinetic properties of enzymes
  We can distinguish two types of uncertainty in kinetic properties of enzymes: (i) *structural uncertainty* is associated with the missing information concerning kinetic mechanisms; (ii) *quantitative uncertainty* refers to the inconsistency about the values of kinetic parameters in the data [16].
  While the databases that collect and organize the information about kinetic parameters are growing in size [10,28], the available kinetic data are not standardized, and the reported values of kinetic parameters often range within several orders of magnitude. Furthermore, factors that impact the values of kinetic parameters such as temperature or pH are frequently not reported. An additional question is if the values of the kinetic parameters that are quantified *in vitro* and for each enzyme separately can represent well the behavior of a multitude of enzymes interacting in a crowded *in vivo* environment [11,22]
- Uncertainty in metabolic fluxes
  Despite the availability of abundant fluxomics data, the complex topology of metabolic networks prohibits determination of the exact values and directionality of intracellular metabolic fluxes [29,30]. This translates into the existence of multiple alternative flux profiles that are consistent with the measured data but with uncertainty in determining a *unique* flux profile.
- Uncertainty in metabolite concentration levels and thermodynamic properties
  The introduction of thermodynamics-based constraints in the context of flux balance analysis allows integration of metabolomics data through coupling of the directionality of fluxes with metabolite concentrations [17,29–31]. The thermodynamic properties of many reactions are not measured, instead, they are estimated using group contribution methods [32]. These estimates contain both measurement and estimation errors and together with uncertainties in metabolite concentration measurements they can affect the conclusions about cellular physiology.

Size and content of metabolic networks

As the main purpose of the models is the understanding of system-wide properties, we need large models in order to capture the interactions determining the behavior of the system as a whole. The size of a model introduces a trade-off between the accuracy of the models that comes from the description of all possible and important interactions and the number of unknown and uncertain parameters. There are issues to be considered when large-scale and genome-scale kinetic models are constructed.

- Large number of unknown parameters, sloppiness and overfitting
  As the size of the metabolic network increases, the portion of available kinetic parameters is rapidly decreasing. Consequently, a large number of parameters have to be quantified using parameter estimation techniques [14]. However, due to a large number of parameters, the uncertainty in available data, and the intrinsic sloppiness of parametric models in systems biology [33,34] it is impossible to compute unique parameter values. When the number of parameters is large relative to the number of observations, the obtained models tend to describe measurement errors rather than functional relationships within the modeled process (overfitting). As a result, poor predictions are obtained when these models are validated against independent data sets.
- Issues with parameter estimation methods
  Parameter estimation methods use optimization procedures to obtain the values of parameters. Depending on the underlying formulation, network structure and employed optimization technique parameter estimation might become computationally intractable for large metabolic networks [35].
- Issues with Monte Carlo methods
  In Monte Carlo methods, the admissible parameter space is constrained with physicochemical and thermodynamic laws along with the constraints obtained from available measurements, and then a population of alternative parameter sets is drawn from such a reduced solution space [15••,16–19,23,25,36]. Sampling of such space is a computationally daunting task for large metabolic networks. Another important challenge is that an efficient sampling necessitates well-defined bounds on kinetic parameters such as Michaelis constants, and these bounds are rarely known. To address these issues a new tailor-made formulation and a new sampling technique were proposed [22].
- Stiffness of metabolism dynamics
  Large-scale and genome-scale kinetic models of metabolism are stiff systems of ordinary differential equations (ODE) since they span over metabolic reactions with a wide range of rate dynamics. The

stiffness of these systems and the intrinsic nonlinearities of the kinetic rate expressions will require advanced computational tools for robust simulation of these models [37].

Standard requirements in kinetic modeling

The structure and the complexity of a kinetic model should be adjusted to the modeling goal, to the characteristics of the organism and to the physiological conditions of the system under study. However, there is a set of conditions that a kinetic model must follow in order to preserve most of the prior knowledge about biochemistry and cellular physiology.

Consistent pathway/network stoichiometry

The lack of knowledge about kinetic parameters and the difficulties in parameter estimation for large-scale networks have led to kinetic models that are constrained to few pathways, often with low level of detail both in stoichiometry and in kinetics. As discussed in [38••], by disregarding certain parts of the highly interconnected network of metabolism one potentially neglects dynamics that is crucial for the behavior of the whole system. Moreover, ignoring so-called 'small metabolites' has important consequences on modeling conclusions, as demonstrated by the example of phosphate [39,40]. Thus, while balancing the trade-off between model complexity and its predictive capabilities the elemental and charge balance must be preserved irrespective of the model size.

One of the most important questions in metabolic modeling, and especially in building kinetic models of metabolism, is how the model size impacts its quality. While small models might have provided some insights [41], the bias introduced by the *ad hoc* choice of model topology and size can always contaminate the results and limit their predictive strength as well as the reliability of the conclusions. Physiologically relevant kinetic models should be built on a scaffold of **context specific stoichiometric models** with the same stoichiometric detail and consistency.

A critical quality check for a kinetic model is how well it represents the stoichiometric model it has used as a scaffold and how well it accounts for those parts of the stoichiometric models that the modeler has chosen to omit from the stoichiometric description. Most of the current kinetic models use the stoichiometry without accounting consistently for the many reactions around the pathways of interest [38••,42–44]. One could argue that such models simulate the dynamics of mutant organisms with the knockouts of the reactions omitted from their model.

Researchers should consider an elementally and charge balanced stoichiometry that focuses on the studied metabolic pathway(s), and that is consistent with the genome-scale metabolic reconstruction (GEM) it was derived from. Along this direction, our group has developed an algorithm capable of generating consistently reduced stoichiometric models from GEMs [unpublished work].

Metabolic pathways of eukaryotic cells involve reactions within and between more than one compartment. Although researchers are tempted to neglect compartments, it was shown that removal of compartment information had significant effects on energy-related pathways due to disruption of concentration gradients between compartments [45,46].

Consistency with physicochemical laws and experimental data

Kinetic models have to be consistent with the observed flux and metabolite measurements. While the consistency with stoichiometric models will guarantee elemental balance, we must ensure the thermodynamic consistency of the integrated metabolomics data. In this way, model uncertainty is reduced and reaction directionalities that are not consistent with the observed physiology are discarded [29]. Respecting the conservation of the physicochemical laws is also critical for the performance of the models. Palsson and Lee have demonstrated that neglecting electro-neutrality and osmotic balances for the case of a red blood cell model can lead to erroneous interpretation of the studied physiology [47].

Appropriate kinetic descriptions and regulation

The purpose of the model, its complexity, and prior knowledge and experience of the researchers determine the choice of kinetic rate laws, and the mechanistic regulatory details. Simplified rate expressions are more frequently encountered as they require fewer parameters. However, kinetic models with oversimplified rate laws and neglected allosteric regulations can have limited predictive capabilities [41,48]. We have recently shown for a specific concentration and flux profile in aerobically grown *Escherichia coli*, if all reactions are modeled with the mass-action kinetics that does not account for the enzyme saturation, it is hardly possible to find parameters that can describe that physiology. However, when we considered kinetic models with detailed mechanistic rate laws we found a large population of models that could describe the observed physiology [15••]. These studies suggest that the foremost requirement is to use kinetic rate laws that are able to model enzyme saturations. The second important consideration is to model regulation at the enzyme level whenever possible [11,41].

Analyses of a large class of biological nonlinear systems have shown that the system behavior is determined by only a reduced set of parameters, which depends on the particular state of the system. Therefore, one can model in detail only the kinetics of reactions that determine the

system behavior and use simple approximate kinetic laws for the remaining reactions [6•,9]. While this idea is appealing, we have to know in advance which reactions are important for a particular physiological condition. Therefore, we suggest using an efficient method to generate a representative class of nonlinear models, next perform such dynamic model reduction, and then generate a larger population of reduced models, which can be kinetic models with stoichiometric, flux-balanced, subsystems.

Such kinetic models that couple kinetic and stoichiometric models have appeared recently [25]. However, they are based on rather strong and potentially misleading assumptions because they use an *ad hoc* reduction of the kinetic scale without prior knowledge about the steady-state and dynamic properties of the modeled system. As we discussed earlier, one must first construct a detailed mechanistic model, show that some parts of the model operate near steady state (quasi-steady state), and then to model these parts by stoichiometry only. Therefore, hybrid models that combine subsystems of kinetic models with subsystems of stoichiometric models should be used with caution and must follow a rigorous model reduction procedure.

Kinetic models of metabolism

Current scope and consistency in kinetic models

The majority of kinetic modeling studies are focused on *Saccharomyces cerevisiae* [6•,8,11,13,17,25,49,50] and *E. coli* [7,15••,19,23,36,38••,51,52••,53–56], and models for other organisms have appeared (*Bacillus subtilis* [44], *Plasmodium falciparum* [12•], *Plasmodium knowlesi* [43], *Zymomonas mobilis* [42], *Lactococcus lactis* [18,34,39], *Streptococcus pyogenes* [39], *Clostridium acetobutylicum* [57], *Trypanosoma brucei* [40], CHO cells [50], human cell [58,59]). Most of these models are focused around glycolysis [8,11,12•,13,39,42] or in combination with either pentose phosphate pathway and/or citric acid cycle [7,25,40,51,52••,53,55–57] (Table 1). Some models are focused around a specific pathway of interest, for example Entner–Doudoroff pathway [42], phospholipid [43] or riboflavin [44] synthesis, and only a few models have a broader scope [6•,15••,17,23,36,59] (Table 1). In most cases, the reactions and the topology of the metabolic networks are selected *ad hoc*. Only in few cases kinetic models are derived systematically from reference models [6•,15••,17,23,36], and a subset of these are consistent with the properties of the original models [15••,17] (Table 1).

There are attempts to reduce complexity either by combining kinetic and stoichiometric models [25,43], or by considering the concentrations of co-factors and small molecules such as CO_2 and phosphate as constant in the rate expressions [54,58]. In some studies these molecules are completely overlooked in kinetic descriptions [50,52••,57]. Another attempt for complexity reduction in eukaryotes is by neglecting the reactions and species across and within compartments. Indeed, only few of the models [17,40,58,59] used to study eukaryote organisms include compartments for the different

Table 1

Summary of the recent studies in kinetic modeling

		Model topology						
		Ad hoc selection	Systematically derived	Consistently reduced	Accounts for small molecules		Includes compartments*	
					Yes	No	Yes	No
Pathway coverage	Glycolysis	[8]^{y}, [11]^{y}, [12•]^{pf}, [13]^{y}, [18]^{ll}, [34]^{ll}, [39]^{ll}, [39]^{sp}, [42]^{zm}, [54]^{e}			[39]^{ll}, [39]^{sp}	[8]^{y}, [11]^{y}, [12•]^{pf}, [13]^{y}, [18]^{ll}, [34]^{ll}, [42]^{zm}, [54]^{e}		[8]^{y}, [11]^{y}, [12•]^{pf}, [13]^{y}
	Glycolysis + PPP/TCA	[7]^{e}, [19]^{e}, [25]^{y}, [25]^{e}, [40]^{tb}, [50]^{y}, [50]^{CHO}, [51]^{e}, [52••]^{e}, [53]^{e}, [55]^{e}, [56]^{e}, [57]^{ca}, [58]^{hc}			[58]^{hc}	[7]^{e}, [19]^{e}, [25]^{y}, [25]^{e}, [40]^{tb}, [50]^{y}, [50]^{CHO}, [51]^{e}, [52••]^{e}, [53]^{e}, [55]^{e}, [56]^{e}, [57]^{ca}	[40]^{tb}, [50]^{y}, [50]^{CHO}, [58]^{hc}	[25]^{y}
	Specific pathway	[34]^{ll}, [38••]^{e}, [42]^{zm}, [43]^{pk}, [44]^{bs}, [49]^{y}				[34]^{ll}, [38••]^{e}, [42]^{zm}, [43]^{pk}, [44]^{bs}, [49]^{y}		[43]^{pk}, [49]^{y}
	Broader scope	[59]^{hc}	[6•]^{y}, [17]^{y}, [15••]^{e}, [23]^{e}, [36]^{e}	[15••]^{e}, [17]^{y}	[6•]^{y}, [15••]^{e}, [17]^{y}	[23]^{e}, [36]^{e}	[17]^{y}, [59]^{hc}	[6•]^{y}

* Only for eukaryotic cells.
e: *E. coli*; y: Yeast; bs: *B. subtilis*; pf: *P. falciparum*; pk: *P. knowlesi*; zm: *Z. mobilis*; ll: *L. lactis*; sp: *S. pyogenes*; ca: *C. acetobutylicum*; tb: *T. brucei*; CHO: Chinese hamster ovary cells; hc: human cells.

organelles. However, all these attempts reduce complexity at the cost of elemental balances and thermodynamic consistency, and only a few kinetic modeling approaches provide thermodynamically consistent models [6•,15••,17,23,36,59].

Methods for determining the experimentally unavailable parameters

Parameter values that are not available from experimental measurements have to be provided in the rate expressions. Many parameter estimation methodologies have been developed to address this problem ranging from the straightforward identification used in the tendency modeling approach [37] to the sophisticated Cooperative Enhanced Scatter Search (CeSS), which is a tailor-made development for parameter estimation of large scale models [60]. Typically these techniques perform sensitivity analysis [7,56] using time-series data, and they are adapted to overcome difficulties related with the absence of observations and large number of parameters [49,51].

An alternative to parameter estimation techniques is Monte Carlo kinetic modeling. These techniques can handle uncertainties by generating alternative sets of parameters that can describe the observations [15••, 16–19,23,25,36]. Moreover, these approaches have the potential to generate models scalable to the genome-scale size with a judiciously constrained solution space and with an adequate formulation of parameter sampling. What is critical in developing efficient Monte Carlo kinetic modeling methods is the sequence of integration of data coming from different sources. From our experience in developing the ORACLE framework [15••,16,17] it is important to first include thermodynamics and to integrate the metabolomics data that constrains the concentrations independently of kinetics. Another important aspect is to reformulate the original problem of sampling kinetic parameters into an equivalent problem of sampling enzyme saturations. This way, it is possible to draw samples from the well-bounded space of enzyme saturations and then to back-calculate the corresponding values of kinetic parameters.

A general workflow for building kinetic models

From the above-discussed specifications for kinetic models and taking into consideration the features of the currently existing methods, we propose here the essential steps that a procedure for generation of consistent large-scale and genome-scale kinetic models should follow (Figure 1).

Define the scope of the model and the level of details

The scope of the model and how detailed it will be should depend primarily on the purpose of the model. At this step, the modeler should decide about the extent of modeled metabolic pathways. For instance, a model can describe a set of reactions, a pathway such as glycolysis or amino-acid synthesis, a subsystem such as mitochondrial pathways or a whole metabolic network. The level of details will depend on the phenomenon to be studied. For instance, a detailed large-scale kinetic model is necessary when studying potential targets for metabolic engineering interventions. In contrast, to analyze certain process conditions it is likely that a simple, coarsely grained model would do the job.

Define stoichiometry

Based on the decisions made in the previous step and on the GEM of the studied organism we define the network structure of the model following the requirements we outlined in Section 'Consistent pathway/network stoichiometry' in this article.

Assign kinetic mechanisms and parameterize models

For each of the reactions within the model structure we assign kinetic rate expressions as recommended in Section 'Appropriate kinetic descriptions and regulation'. With this and the previous step the model structure is determined.

Input data

In this step we input the available experimental data. The exact way of incorporating the data will depend on the modeling approach. As discussed previously, the sequence of data incorporation might play significant role on the performance of the modeling approach.

Determine parameters

With the quantitative and structural information obtained in the previous steps we determine the parameters. The computational methods used for the parameter identification will depend on the available experimental data as well as on the type of network structure of the model and rate expressions used for the reactions.

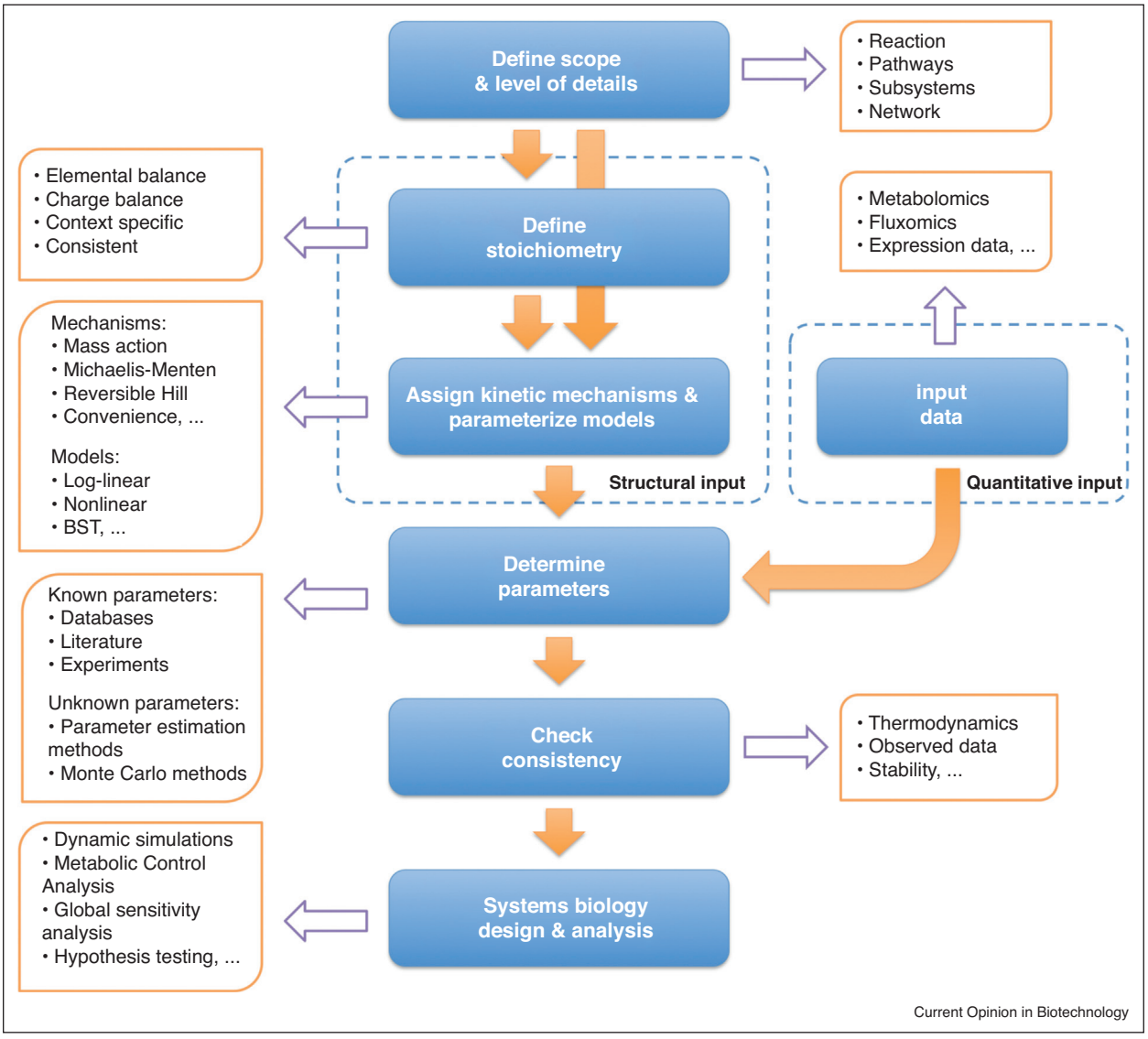
Consistency tests

The consistency of the obtained model(s) with respect to the experimental observations, physicochemical laws, and expert knowledge should be tested (see Section 'Consistency with physicochemical laws and experimental data'). The consistency of the models can be ensured by the way a modeling approach handles the data integration at every stage of model development. An additional test is to verify the local stability around the reference steady state. The model(s) that do not pass the consistency tests should be rejected.

Systems biology design and analysis

We use the kinetic model(s) that satisfy requirements defined in Section Standard requirements in kinetic modeling for the purpose they were built.

Figure 1



The critical steps of the procedure for generating consistent large-scale and genome-scale kinetic models. Depending on the modeling approach some steps will be repeated iteratively until the requirements presented in Section 'Standard Requirements in Kinetic Modeling' are met.

Conclusions

The ultimate goal of kinetic modeling is to procure the models of such a scope and level of details that metabolic engineering and synthetic biology designs and hypotheses can reliably be tested firstly *in silico*. Though the required quality of kinetic models is hard to achieve for every system and study, the efforts in this area have engendered a few promising methodologies that are closing this gap.

The principal challenges in developing large-scale to genome-scale kinetic models remain the uncertainty and the complexity that increase with the size of the networks. There is a clear need for computational tools that will model the uncertainty and that will analyze and reduce the uncertainty propagation in the system. Recognizing this we have recently proposed an approach for characterization and reduction of uncertainty propagation that makes use of the ORACLE framework and machine learning classification techniques [unpublished work]. With this approach, we demonstrate that information about the parameters of a few key enzymes is enough to capture a physiological state, while the values of kinetic parameters in most of the enzymes can vary in a broad range. This is an important finding as it will allow reducing the space of kinetic parameters significantly, and therefore it will enable more comprehensive analyses of the metabolic networks.

We expect that the proposed set of requirements for building kinetic models will streamline the future efforts and accelerate developments in this area in terms of modularity and portability of models between research groups.

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