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Model reduction of genome-scale metabolic models as a basis for targeted kinetic models

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ABSTRACT

Constraint-based, genome-scale metabolic models are an essential tool to guide metabolic engineering. However, they lack the detail and time dimension that kinetic models with enzyme dynamics offer. Model reduction can be used to bridge the gap between the two methods and allow for the integration of kinetic models into the Design-Built-Test-Learn cycle. Here we show that these reduced size models can be representative of the dynamics of the original model and demonstrate the automated generation and parameterisation of such models. Using these minimal models of metabolism could allow for further exploration of dynamic responses in metabolic networks.

1. Introduction

Computational models of metabolism have increasingly become research aids in the fields of biotechnology and medicine. Initially, these models were bottom-up models of biochemical reactions, where detailed descriptions of the enzyme kinetics are combined into a mechanistic model. However, other than in a few cases and for very small networks, the dynamic data needed to test these models and validate subsequent predictions are not available. Recently, the rise of "omics" and other technological breakthroughs have made extensive amounts of data available that can support genome-scale, constraint-based models and statistical analysis. These high-level models, despite their broad coverage, however, lack dynamic information compared to mechanistic models. In this work we show how automated model reduction can be used to bridge high- and low-level approaches, in order to rationally engineer metabolic systems within the framework of the Design-Build-Test-Learn (DBTL) cycle.

The Design-Build-Test-Learn cycle is a systematic engineering approach that intertwines experiments and computational models (Carbonell et al., 2018). Model-guided experimental design provides better understanding of the underlying system and thus, enables the system to be more precisely engineered for a practical application, such as maximising the yield of a existing or novel production pathway, re-directing metabolic routes or uncoupling growth and production (Liu et al., 2015). The flexibility of an iterative process is important as biological systems are often ill-characterised, and previously unknown or immeasurable interactions can become increasingly more salient as one approaches different limits of the system. Therefore, the ability to iterate quickly and to progressively integrate new data as it becomes available is essential, making both experimental (Hamedi Rad et al., 2019) and computational automation cornerstones of the DBTL cycle (Carbonell et al., 2018).

Metabolic models based on constraint-based optimisation principles, such as flux balance analysis (FBA) fulfil the quick iteration requirement of the DBTL cycle (Nielsen, 2017) and have been used extensively to design (Aslan et al., 2020) or rationally engineer metabolic systems (Brunk et al., 2016). However, once a pathway has been chosen and implemented additional factors become important, such as pathway thermodynamics (Noor et al., 2014), toxicity of intermediates (Jan et al., 2017), or product inhibition and other types of regulation (Link et al., 2015).

Several methods have extended the constraint-based framework to include factors such as thermodynamics (Henry et al., 2007), regulation (Reed, 2012) or enzyme limitations (Mori et al., 2016), in addition to methods that try to reduce the solution space with omics data such as transcriptomics (Banos et al., 2017), proteomics or metabolomics (Yizhak et al., 2010). However, all these methods retain the steady state assumption and do not account for metabolite concentrations explicitly,

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leading to a gap of descriptive and predictive power of the models. Dynamic Flux Balance Analysis takes a step in this direction and limits the steady-state assumption to internal metabolites only, simulating the dynamics of the external concentrations explicitly (Øyås and Stelling, 2018). As external concentrations are often measured more easily than the concentration of metabolites inside the cell, this method is often a good match for the available data. Nonetheless, as internal concentrations are not simulated, factors such as regulation or accumulation of intermediates are still difficult to include.

Kinetic or dynamic metabolic models are based on ordinary differential equations that describe the underlying enzyme kinetics and thereby extend the scope and depth of constraint-based models. These models offer the advantage of the explicit inclusion of metabolite concentrations and the possibilities to include other dynamic processes such as genetic or metabolic regulation. Furthermore, kinetic models are able to simulate time trajectories out of steady state, giving them a wider range of applicability than flux-based models. For example, Petelenz-Kurdziel et al. (2013) model the dynamic response to osmotic shock in yeast with the HOG signalling cascade and glycerol production, whereas van Heerden et al. (2014) show how dynamics are essential to explain yeast glycolysis and O'Brien et al. (2019) use a dynamic model of glucose metabolism to find sets of enzyme modifications that minimize lactate production during the Warburg effect.

Kinetic models are thus a strong supplement to constraint-based metabolic models, but suffer several of their own limitations preventing their widespread use (Strutz et al., 2019). Dynamic models generally include large numbers of unknown parameters, such as enzyme binding or catalytic constants, many of which cannot be measured directly and require dedicated experiments that are not easily performed in high-throughput. Metabolomics and other omics data are more readily available, but are often ill-suited to the task of estimating dynamics (Tummler and Klipp, 2018). Instead, kinetic models are parameterised by computationally fitting parameters to large data sets using time-intensive optimisation procedures, which generally do not scale well as the search space becomes exponentially larger with an increasing number of parameters. For these reasons kinetic models have yet not been fully integrated into the DBTL framework (St John and Bomble, 2019), despite smaller kinetic models having provided valuable understanding of metabolic systems.

Still, recent advances in metabolomics and fluxomics have greatly increased the feasibility of the large-scale data collection that is required to parameterise dynamic metabolic models (Link et al., 2015; Park et al., 2016; Markley et al., 2017; Yurkovich and Palsson, 2018). Furthermore, more and more of these techniques are used to clarify the complex web of metabolic regulation that drives core metabolism (Gerosa et al., 2015; Hackett et al., 2016; Piazza et al., 2018). Since metabolic or genetic regulation is straightforward to integrate into dynamic models, but hard to integrate into constraint-based models (MachadoMarkus, 2014), this shows additional promise for the usage of dynamic models. In addition, parameter estimation techniques for large-scale metabolic models have been improved. In particular, methods based on multi-start optimisation (Villaverde et al., 2012; Penas et al., 2017) in combination with adjoint sensitivities (Fröhlich et al., 2017a) have shown to be suitable for parameter estimation of large dynamic models in biology (Villaverde et al., 2018; Fröhlich et al., 2018), although numerical issues remain for these methods (Kapfer et al., 2019). Despite these advances, parameter estimation for large models such as the pan-cancer model by Fröhlich et al. (2018) (>4000 parameters, >2600 reactions) still requires thousands of CPU hours to converge (Fröhlich et al., 2018; Leonard et al., 2020). Furthermore, ensemble modelling techniques have seen use as an alternative to full-scale parameter parameter estimation, for example, in the ORACLE framework (Miskovic and Hatzimanikatis, 2010; Chakrabarti et al., 2013; Andreozzi et al., 2016). Instead of performing an optimisation step to fit parameter estimates, these methods generate many equally feasible versions of the same model with different parameters and study the properties of this ensemble as a whole.

Despite the increased availability of data and improved methods for model parameterisation, genome-scale dynamic models are still a long way off. However, whole-genome model resolution is most likely not needed for most purposes. For instance, in the optimisation of a production pathway, the focus is often on the reaction steps directly leading to the target product, and the relevant precursors, co-factors or toxic by-products. Different areas of metabolism, however, may be of lesser interest for several reasons. First, pathways can be genetically inactive or their activity tightly controlled and can thus be assumed more or less constant. Second, reactions might simply be outside of the scope of the current research, because of practical limitations, such as difficulty in measuring important metabolites or the inability to make changes in their respective pathways.

For dynamic metabolic models, model reduction can generally take place at two stages in the modelling process. First, an existing dynamic model can be reduced *"a posteriori"* using several methods, such as lumping, time-scale separation or variable elimination (Gerdtzen et al., 2004; Apri et al., 2014; Rao et al., 2014; Prescott and Papachristodoulou, 2014; Gupta et al., 2016; Snowden et al., 2017). However, this requires an existing dynamic model of sufficient quality which is subsequently reduced in complexity. In our case, however, a high-quality model does not exist as a starting point, so we are forced to look at *"a priori"* methods that can reduce the scope of a model before having to fully specify and parameterise the model. Whereas the first group of methods utilises mathematical simplifications that are not necessarily specific to any model, this second group of methods includes prior biological knowledge instead to guide the extent of the reduction. Model reduction methods have been proposed for constraint-based metabolic models, based on either contextualisation of the model (see a review by Opdam et al. (2017)) or on the preservation of selected flux behaviour and a core set of metabolites and reactions. This would make it possible to use the more scalable, constraint-based representation of the model to reduce the scope of the model, and thus the complexity, before incorporating dynamics.

Integrating automated model reductions methods in the DBTL cycle by starting from genome-scale metabolic reconstructions offers additional advantages. First of all, the constraint-based model and the reduced size dynamic model complement each other, allowing the user to take advantage of the strengths of different modelling approaches when appropriate. For example, the initial choice of pathway or knock-out targets can be predicted using constraint-based methods, while predicting optimal enzyme ratios or regulatory interactions is more suitable to a dynamic metabolic model. By basing the dynamic model on a reduced subset of the constraint-based model, knowledge obtained from either model can be used to further improve or constrain the other model. Furthermore, the processes of producing and using genome-scale metabolic models has greatly matured and an abundance of tools and existing curated models are available (Heirendt et al., 2019). Finally, genome-scale metabolic reconstructions are often improved over time as sequence annotation improves or new metabolic functions are discovered. By creating dynamic models from these genome-scale models through a standardised workflow, these improvements are straightforwardly integrated.

Several other approaches have been used to generate large-scale kinetic models of metabolism, as reviewed by Srinivasan et al. (2015) and Saa and Nielsen (2017). However, none of these frameworks uses systematic model reduction of constraint-based genome-scale models as the starting point for the creation of dynamic metabolic models, which could be a promising approach due to the modularity of metabolism. In addition, these frameworks make other trade-offs in order to be able to scale to larger models, such as reducing the complexity of the rate law and dynamics, limiting their validity to a region close to equilibrium or sacrificing their ability to simulate dynamic time-series.

In this work, we investigate constraint-based model reduction methods to create dynamic versions of reduced genome-scale models in order to automatically include kinetic models in the DBTL cycle. We

assess whether these *"a priori"* methods can be used to create reduced models that preserve important metabolic characteristics, and whether these methods lead to accurate and reduced dynamic metabolic models. Finally, we show how ensemble based methods can be used to estimate the quality of a reduced models even in the absence of a genome-scale kinetic model.

2. Methods

2.1. Overview of model reduction

To create the dynamic metabolic models, we used our previous work (Smith et al., 2018) as a starting point. This computational pipeline (Fig. 1, new additions are highlighted in yellow) automatically translates constraint-based models into kinetic ODE models suitable for dynamic simulation using modular rate laws (Liebermeister et al., 2010). Specifically, the common modular rate law (Liebermeister et al., 2010) is used for all enzymatic reactions, while the simplified power-law modular rate law, equivalent to mass action kinetics, is used for reactions representing entire biological processes such as biomass synthesis and export reactions. In order to avoid reactions without products or metabolites breaking thermodynamic consistency, metabolite pools for these reactions are added to the model. Furthermore, since reactions in a genome-scale metabolic model are often simplified versions of multiple biochemical reaction steps, they can often contain unrealistic stoichiometries leading to third or higher order reactions. Therefore, for reactions exceeding third order, stoichiometric coefficients are scaled by a reaction specific cooperativity factor (Liebermeister et al., 2010) resulting in a third order rate law.

2.2. Models

A small test model (17 metabolites and 16 reactions) was produced as an example (Fig. 2). Four more distinct models were created and used for additional verification. Models were designed to represent network structures and features commonly found in biological systems, the exact network structures can be found in Supplementary A. In addition we used the *E. coli* core model of Orth et al. (2010).

2.3. Model reduction

Three methods were tested for model reduction, namely, FastCore (Vlassis et al., 2014), NetworkReducer (Erdrich et al., 2015), and minNW (Röhl and Alexander, 2017). Although the three methods use different algorithms, they all allow the user to select an essential subset of reactions and metabolites. In addition, both NetworkReducer and minNW allow the user to mark certain flux behaviour as essential. Details of the reactions, metabolites and behaviour selected as essential and the reduced models can be found in Supplementary A.

2.4. Generation of parameter sets reproducing flux profile of the constraint-based model

In order to match the simulations of the dynamic model closely to the FBA simulation of the constraint-based model the dynamic model is based upon, parameters are generated in such a way as to guarantee the same flux in the steady-state. In brief, parameter balancing (Lubitz et al., 2010; Lubitz and Liebermeister, 2019) was used to establish a base set of parameters using prior information for approximate ranges of chemical potentials and steady state metabolite concentrations. Resulting chemical potentials and concentrations were optimised within their uncertainty ranges to lead to reaction fluxes of the same sign as the flux profile from the constraint-based model. These optimised parameters were balanced again and a parameter set (including steady-state metabolite concentrations) was selected from the resulting distribution. Finally, reaction velocity constants were scaled to match the flux profile of the constraint-based model. Full details of the procedure can be found in Supplementary A.

2.5. Simulation and data generation

In order to be able to control the exact quality of the data used for parameter estimation of the dynamic reduced models, data were simulated using the dynamic full model and the AMICI toolbox (Fröhlich et al., 2017a). Six simulations were performed that consider one or more of the following scenarios. These scenarios represent experiments impacting metabolites marked as conserved in the reduction step (see Supplementary A for details):

- The starting concentration is increased (Batch).
- After a given time-period, a concentration is increased by a set amount (Pulse).
- A constant additional influx of a metabolite is applied (Feed).

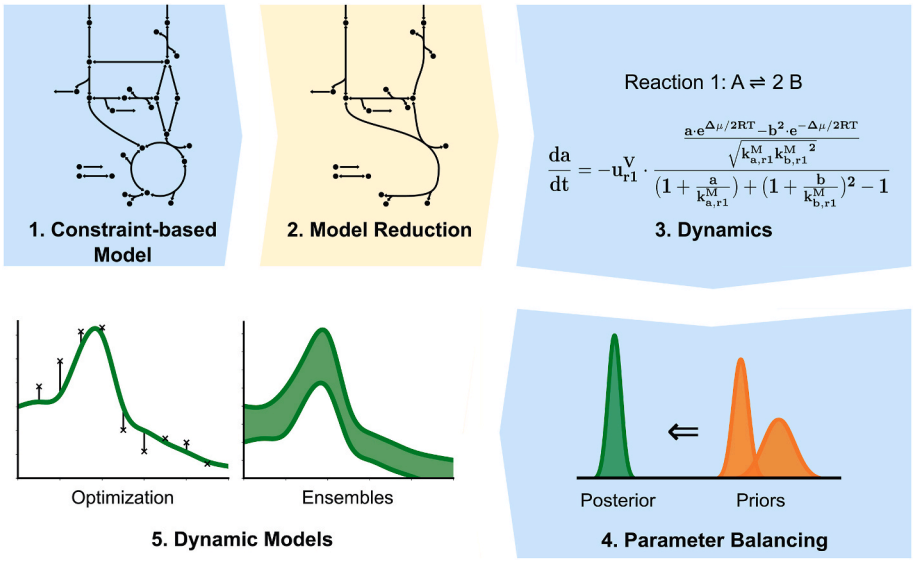

Fig. 1. Overview of the integration of reduced kinetic models in the DBTL cycle. The constraint-based model (1) is reduced based on behaviour, reactions and metabolites marked as essential (2). Based on standardised rate laws and regulatory interactions, a dynamic ODE model is created (3). Existing parameters, such as enzyme kinetic rates (u^V), Michaelis-Menten constants (k^M), standard chemical potentials (μ'°) and steady-state concentrations are integrated using parameter balancing (4) to serve as a starting point for parameter estimation or ensemble modelling (5), in combination with additional data such as (time-course) metabolomics and fluxomics. Both the constraint-based model and the derived dynamic model can then be used for hypothesis generation or experimental design.

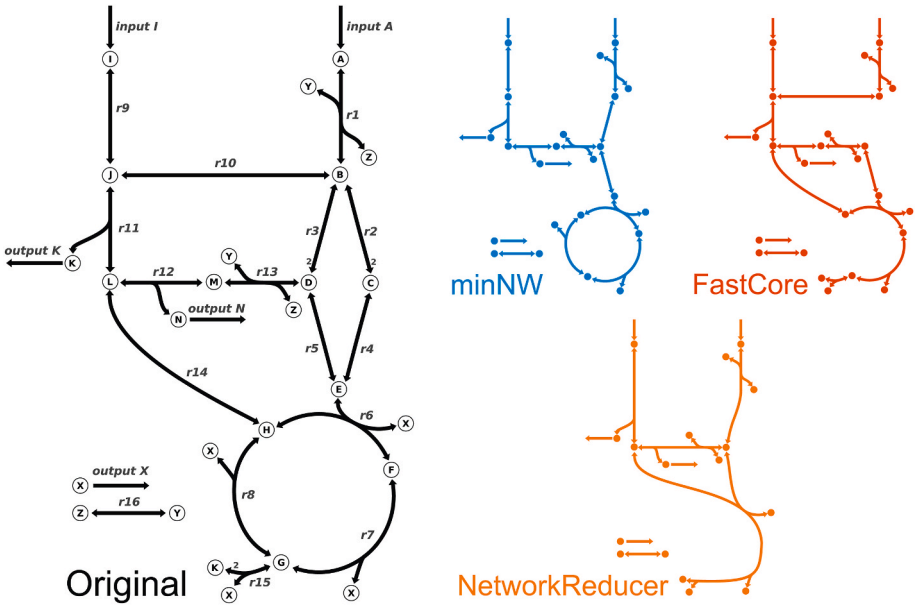

Fig. 2. Test metabolic model and reductions. The test model (left) was designed in such a way as to contain common elements of metabolism, such as co-factors (*x* and *y*), branched pathways (*r2-r5*) and cycles (*r6-r8*). The reduced models are show on the right, as reduced by minNW (blue), FastCore (red) and NetworkReducer (orange).

2.6. Parameter estimation

Parameters were estimated for each of the models using PESTO (Paul et al., 2018) and AMICI (Fröhlich et al., 2017a) for simulation of the trajectories and sensitivity analysis. We used multi-start local optimisation (`fmincon`, 100 starts, 1000 maximum iterations), with a log-likelihood objective function as described in Fröhlich et al. (2017b). The reference data for the optimisation procedure consisted of time-courses of selected metabolite concentrations (*A, I, D, X, Y* and *Z*) for the six different simulated scenarios.

2.7. Model comparison

In order to compare the full and reduced dynamic models, two tests were performed. First, the dynamic behaviour of the models was compared using a second "test" set of three additional computational experiments, independent of the data set used to optimise the reduced models. These test experiments were simulated for each model and concentration profiles of metabolites were compared using the root mean square error (RMSE) for all metabolites occurring in the reduced model.

Second, all parameters sets resulting from each run in the multi-start optimisation procedure were compared to the parameter sets resulting from the re-optimised original model. Only parameters present in both the full and the reduced model were compared. Parametric sensitivity was calculated for the final parameter set of each of the optimisation runs of the multi-start optimisation procedure, using the absolute forward sensitivity as calculated by AMICI and averaged over all experiments and time-points. Both the parameter distributions and the parameter sensitivity distributions were trimmed to retain only the 5th to 95th percentile in order to remove outliers. To compare the overlap between the distribution of parameters resulting from the multi-start optimisation of the reduced and original models, the Jaccard index was calculated for each parameter by calculating the number of samples in the intersection of the two trimmed distributions divided by the number of samples in the union.

2.8. Model comparison utilising ensemble simulation

Ensemble simulation was used to compare the *E. coli* core model and its reduced versions. Parameter sets (10 000) and initial metabolite concentrations were randomly sampled using Latin hypercube sampling from the multivariate-normal parameter distribution resulting from the parameter balancing step. A reference steady state reaction flux profile was simulated using parsimonious FBA (Lewis et al., 2010) on the *E. coli* core constraint-based model. Reaction rate constants were then scaled to correspond to the simulated reaction fluxes, as described in Section 2.1. Due to the balancing step involving the thermodynamic properties of the underlying system, the parameter and concentration set is guaranteed to be in steady state, equivalent to the steady state from the FBA solution, eliminating the need for simulation of the ODE system (see Supplementary A for details). Local steady-state metabolite and flux control coefficients with respect to all parameters were calculated for each model in the ensemble (Burns et al., 1985). The resulting distributions were compared between version of the full and reduced models using the trimmed Jaccard index as described in the previous section.

2.9. Model reduction using time-scale separation

In this work we have focussed on reducing model size prior to performing parameter estimation using (simulated) experimental data for different conditions. Another approach to model reduction is to simplify the network post-optimisation, splitting reactions and fluxes into slow and fast networks. This method is referred to as time-scale separation. As a comparison with our pre-optimisation reductions, we take the reference model of our test metabolic system (that was used to create the synthetic data) and look to remove the fastest fluxes from the network. In this way, we assume that the full model has been accurately optimised in relation to the experimental data. To reduce the network size we follow the approach of Gerdtzen et al. (2004) and Gupta et al. (2016). A complete description of the process can be found in Supplementary A.

2.10. Availability

Analysis and generation of the models and parameters was done using Python (version 3.7, Python Software Foundation). Simulation

and parameter estimation was performed in MATLAB (version R2017b, The Mathworks, Inc.). All code is available open-source at https://gitlab.com/wurssb/model-reduction-for-targeted-kinetic-models.

3. Results

In order to test whether the dynamic version of the reduced model reproduces the dynamics of the full model, we require a model that is both small enough to simulate dynamically without running into numerical issues, and large enough to be reduced by a significant amount. For this purpose, we created a small test model of 17 metabolites and 16 reactions (Fig. 2). In addition we use the *E. coli* core model (Orth et al., 2010), which contains 95 reactions and 72 metabolites and covers central carbon metabolism. Using these models as a base, we create a dynamic model using a similar process as in our previous work (Smith et al., 2018). In addition, the constraint-based model is reduced using several methods and these reduced models will subsequently be used to create dynamic models. Simulated data of the dynamic full model is used to fit and compare the reduced models (Fig. 3). The *E. coli* core model is reduced and verified using ensemble simulation instead of parameter estimation.

The test model is designed to include common features of metabolic networks such as cofactors, branched pathways and cycles. Different reductions are obtained by all three methods (Fig. 2). Some reactions, such as the branch to metabolite *C* by reactions *r2* and *r4* are removed in all models, which is to be expected as the branch is fully redundant in the network. The network reduced by FastCore (red), shows the least reductions, rerouting all uptake flux through intermediate *J* using reaction *r10* and breaking up the cycle by removing reaction *r8*. minNW, on the other hand, keeps the cycle as is, but removes the redundant conversions *r10* and *r14* between the two main uptake pathways. Finally, NetworkReducer combines parts of the reduction of both the other methods, but further compresses all reactions into only four main steps (ignoring exchange reactions): uptake of the two input metabolites, interconversion between the two inputs, and a single reaction representing all products of the metabolic cycle in one.

All three reduced models were optimised using time-course data of a subset of the metabolites (*A, I, D, X, Y* and *Z*) resulting from six computational experiments. A limited set of the metabolites was chosen for the simulated data set, reflecting that often only a subset of the metabolites can be directly measured. When examining the results of the parameter estimation, by looking at simulations of each experiment for each of the optimisation traces (Fig. 4), it shows that even in the optimal case with the exact model structure, there is a large variety in the final fit of each run despite an approximate convergence of the error metric during optimisation (Supplementary A). This can be an indication of a complex fitness landscape featuring many local optima (as defined by our objective function), as well as potential non-identifiability of the parameters - issues that are commonly encountered in dynamic metabolic models. The development of faster and more powerful parameter estimation methods may alleviate these problems in the future.

For the reduced models, we see similar results for the minNW and FastCore reduced networks with a qualitatively similar fit and comparable RMSE. The NetworkReducer model, however, is not able to reproduce the behaviour of the original model, neither quantitatively nor qualitatively. Possibly, this is due to the compressed reactions having large differences in stoichiometries, making this model more non-linear and thus harder to optimise. When comparing the training versus the testing scenarios, the training experiments do not differ much in RMSE from the test experiments, neither in the directly fitted metabolites, nor for the "hidden" metabolites not directly fitted to the generated data, suggesting little to no overfitting. Contrasting the *"a priori"* reduction to an *"a posteriori"* approach (Gerdtzen et al., 2004; Gupta et al., 2016), one finds a significantly smaller RMSE for the observed metabolites in the case of a reduction from 17 to 14 components (Fig. 5). However, when the model is further reduced to 10 components, significant issues arise with the prediction of negative concentrations in a subset of the experimental conditions (see Supplementary A for detailed results).

Looking at the distribution for each parameter at the end of each run of the multi-start optimisation, we observe that most parameters were not well identified, however, this is the case for both the full model and the reduced models. Most parameters and the sensitivities of the metabolites towards the parameters (Fig. 6) have a large overlap in their optimised distributions when compared to the distributions resulting from the reference optimisation of the original full-sized model. The Jaccard index, or the fraction of the intersection of two distributions relative to the union, is higher on average for both the minNW and Fastcore reduced models than for the NetworkReducer based model (0.83 ± 0.14 and 0.86 ± 0.15 versus 0.62 ± 0.23, averaged over all parameters).

To further analyse whether the differences between methods depend on the model structure, four more models with different structures were tested. All four models show results similar to the test model, with the RMSE obtained by the best reduction method being in the same range as the original model. In general, the FastCore method performs well in all cases (Fig. 7), with the minNW reduced networks fitting less well in two of the cases ("EDEMP" and "Cycles") and similar in the other two cases ("Branched" and "Co-factors"). For all models, networks reduced by NetworkReducer were small with strongly lumped reactions and produced simulations with the worst match to the reference data. Detailed results per model are shown in Supplementary A.

For the larger test models based on the *E. coli* core model, parameter estimation was less feasible due to challenges in the numerical

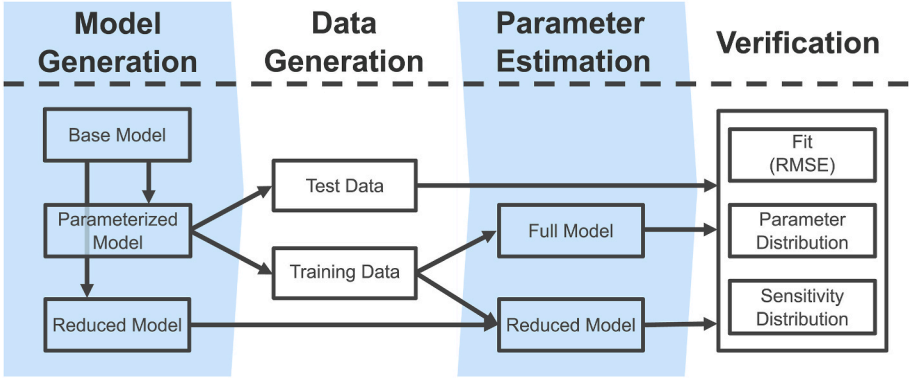

Fig. 3. Overview of the verification process. Starting from the base model, a reference model with known parameters is generated to simulate the test and training data. The training data is subsequently used to optimise kinetic versions of both the original and the reduced models. Finally, using the testing data and the re-optimised original model as a reference, the reduced model is analysed.

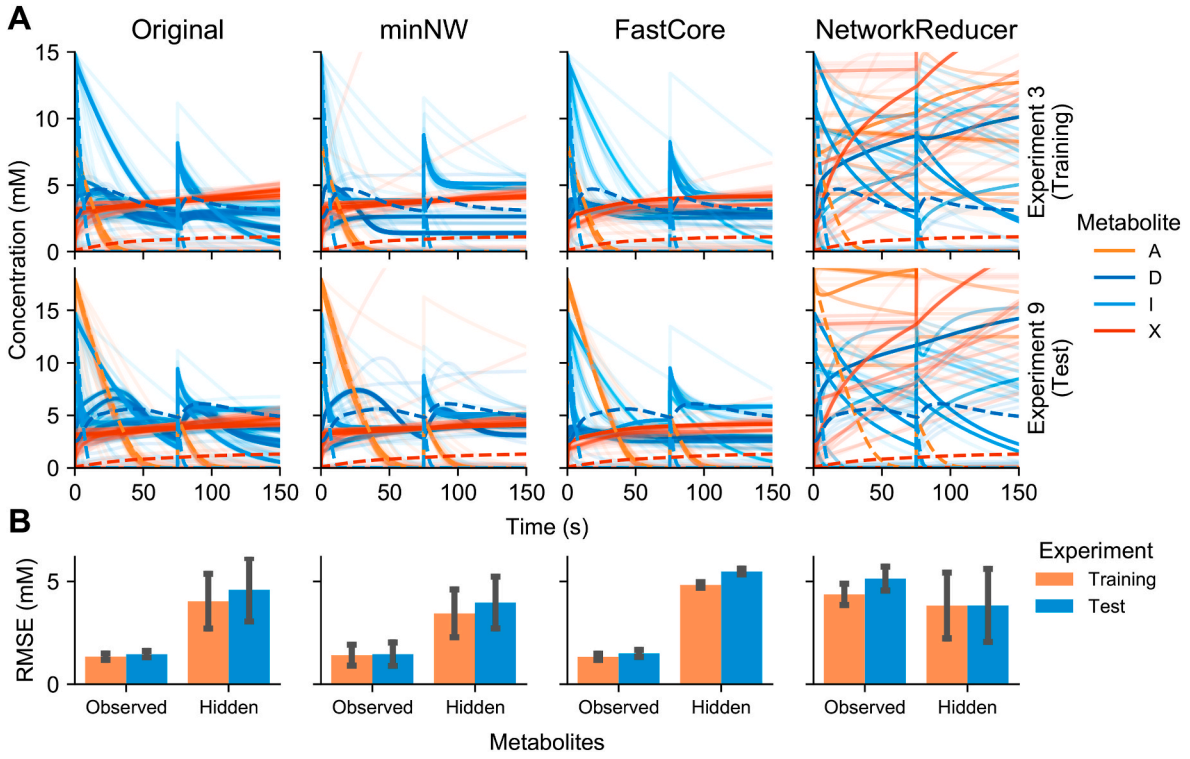

Fig. 4. Reduced models reproduce dynamic behaviour. (A) Simulations of the original and reduced models, fitted with the synthetic data set (dashed lines). Each trajectory represents one of the multi-start optimisation outcomes. On the top row one of the data sets (experiment 2) used for fitting is shown, while on the bottom row one of the test data sets (experiment 8) is shown. (B) Root Mean Square Error (RMSE) for the different models, separated by the training and test data sets, and the observed (i.e. used for fitting) and hidden metabolites. Note that the observed states are conserved in each of the models, while the hidden states number differently depending on the model reduction output. Error bars show the standard deviation of the RMSE over the different runs of the multi-start optimisation procedure.

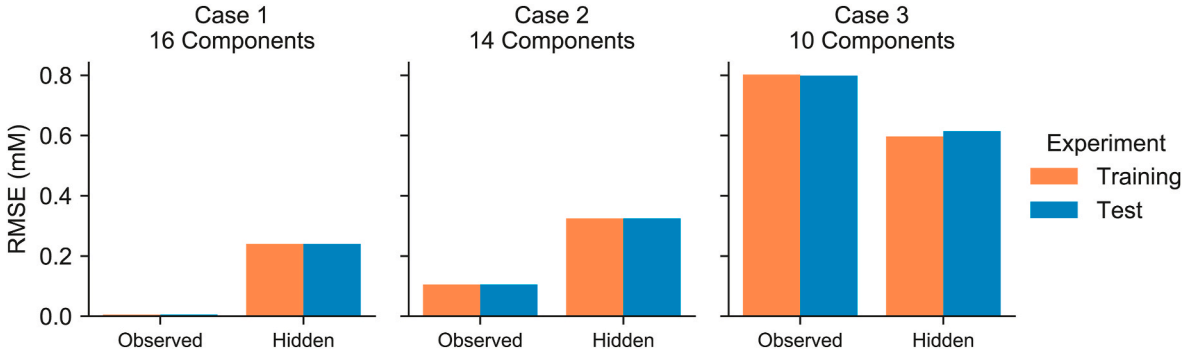

Fig. 5. *"A posteriori"* model reduction using time-scale separation performs better then *"a priori"* model reduction starting from a parameterised full model. Using the fully parametrised test model used to generate the training and testing data, model reduction by time-scale separation was applied. The reduced models show significantly lower average RMSE values then all of the *"a priori"* reduced models. However, for the most reduced case 3, negative concentrations are predicted for some states by the final model (Supplementary A).

integration of the sensitivity functions of large-scale stiff ODE systems, as was also noted by Kapfer et al. (2019). Here, they note that rounding of the stoichiometric coefficients in the case of non-integer values and ensuring positivity of the concentration state vector during integration helps to alleviate the problem of optimisation starting points not being evaluable due to numerical issues in the ODE integration. However, in our case, these methods were still not sufficient to lead to a feasible amount of evaluable starting points in order to perform a reliable parameter estimation. Despite this limitation, with ensemble simulations of parameter sets displaying the same steady-state flux solution as the constraint-based model, we can take a limited look at semi-dynamic properties such as the flux and metabolite control coefficients in the steady state (Fig. 8). Here, we see again that the FastCore and minNW model reductions are more similar in results to the full model than the NetworkReducer based model. Furthermore, there is a large difference between the similarity of the flux control coefficients versus the metabolite control coefficients (0.92 ± 0.23 versus 0.47 ± 0.43, averaged over all control coefficients for all three models). This difference could be due to reactions either being included in the model or removed as a whole, while a metabolites might have some reactions they participate in removed and others conserved, leading to a larger difference in control coefficients.

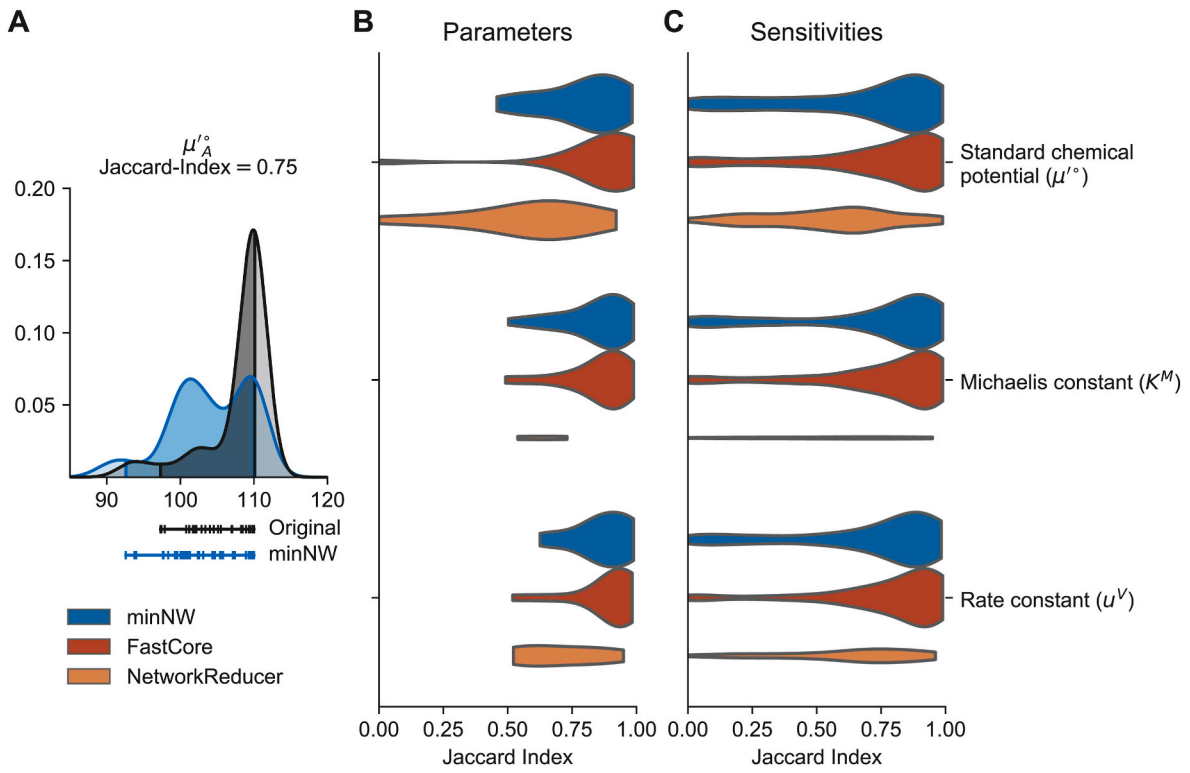

Fig. 6. Estimated parameters show similarity between original and reduced models. (A) A visual example of the Jaccard index calculation. The distribution of the optimised value for each parameter, in this case μ'_A, is taken from the multi-start optimisation runs and trimmed by 5% on both ends. The Jaccard index is then calculated by dividing the number of values in the intersection of the two distributions by the union. (B) The distribution of the Jaccard indices for all of the parameter distributions of the reduced models, each compared against the results of the optimisation of the original model. (C) The distribution of the Jaccard indices of the sensitivity coefficients. Only parameters occurring in both the reduced model and the optimised model were used for the comparison.

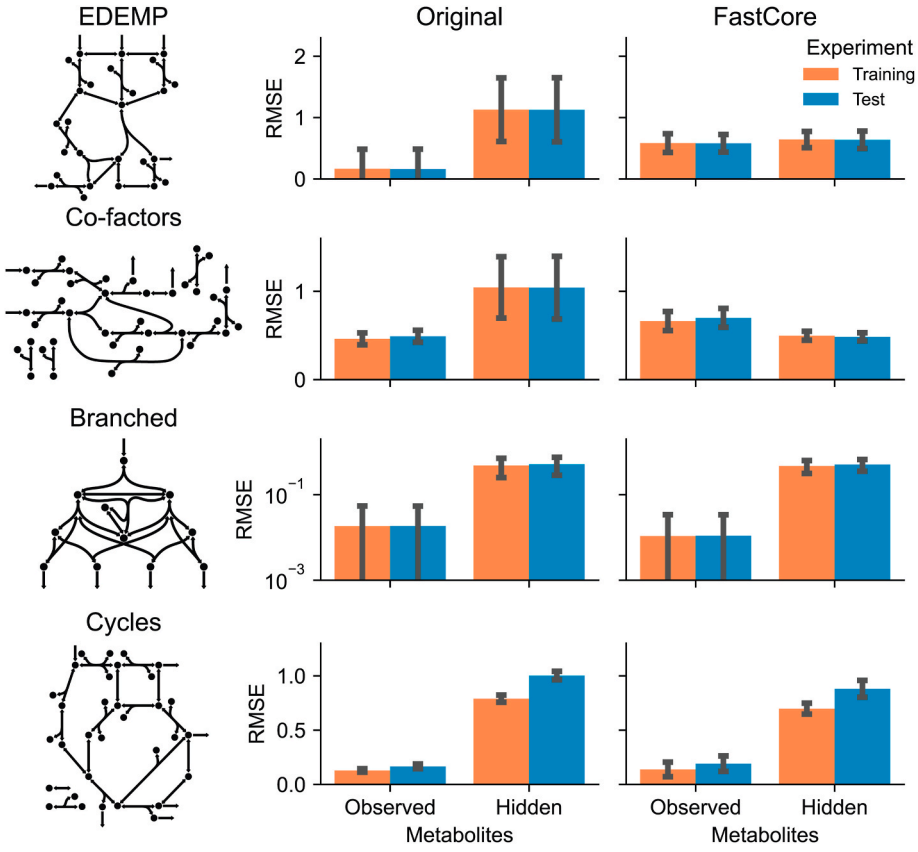

Fig. 7. *"A priori"* model reduction works on different model structures. Root Mean Square Error (RMSE) for four different model structures inspired by biological pathways (left). On the right the RMSE after parameter estimation is shown for the original model structure, and the structure reduced using the FastCore method. The RMSE is further divided into the RMSE for the training and test data, and the observed and hidden metabolites. Note that the observed states are conserved in each of the models, while the hidden states number differently depending on the model reduction output. Error bars show the standard deviation of the RMSE over the different runs of the multi-start optimisation procedure.

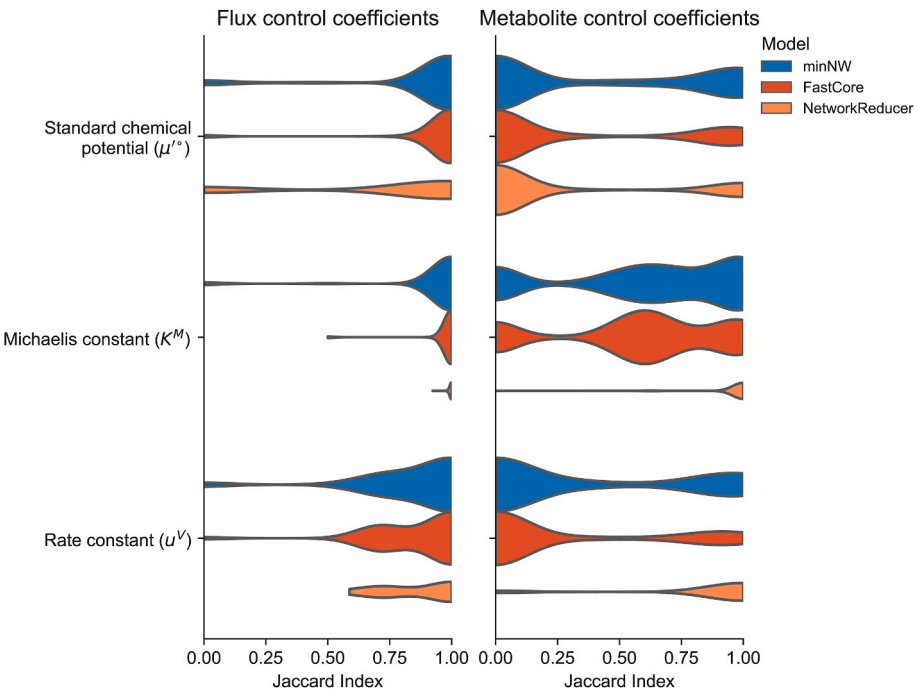

Fig. 8. Flux control coefficients show greater similarity between full and reduced models than metabolite control coefficients. Distribution of the Jaccard index of reaction and metabolite control coefficient distributions for 10 000 randomly sampled parameterisations of kinetic versions of the reduced and full *E. coli* core model displaying the same steady-state flux profile.

4. Discussion

In this work, we compared dynamics produced by models of genome-scale metabolic maps with *"a priori"* reduced systems to see how much temporal dynamics are maintained in these smaller networks. In order to reduce the networks, we used three existing methods to reduce constraint-based metabolic networks. FastCore (Vlassis et al., 2014) is based on building a consistent minimal network around a known core of user-specified active reactions. NetworkReducer (Erdrich et al., 2015) on the other hand, is based on a heuristic process of removing low-variability reactions as determined using Flux Variability Analysis (FVA), unless removing a reaction would disallow a set of user-specified flux constraints infeasible. In addition, it provides an optional lumping step, where linear reactions chains are merged, eliminating intermediates and further reducing the number of reactions in the model. Finally, minNW (Röhl and Alexander, 2017) is based on the same principle as NetworkReducer in that the user specifies a set of flux constraints that must remain feasible, but instead of a heuristic approach it solves a MILP to obtain the minimal sized networks.

The reduced models obtained by the FastCore and minNW methods show a reasonable match to the full network, not only in parameter distributions after estimation, but also in first order parametric sensitivities for the small models and control coefficients for the larger *E. coli* core model. For the NetworkReducer method results are less comparable. As this is the only method of the three that implements reaction lumping, this additional step is a likely cause of the difference with the other two methods. As this step is optional, the results could potentially be improved for this method by disabling the lumping step. Of the three reduction methods, FastCore produces the best results on all tested models, followed by minNW. Although the results slightly favour FastCore, we find minNW easier to incorporate in the whole process, as it allows the flexibility of setting conserved reactions, metabolites and behaviour, while at the same time guaranteeing a minimal network after reduction. Overall, the reduced models seem to be able to represent a substantial amount of the behaviour and sensitivity of the full metabolic network, and as such, show potential to be used in the DBTL cycle for methods requiring dynamic ODE models, such as Optimal Experimental Design (OED), sensitivity analysis, or the study of dynamic regulatory elements. While the *"a posteriori"* method using time-scale separation generated models with a significantly lower RMSE then the *"a priori"* methods it required the starting model to be fully parameterised. Thus, *"a priori"* reduction can serve as a worthwhile alternative to existing *"a posteriori"* methods, in the common case that a well-parameterised large-scale model does not exist.

One must exercise caution, however, when methods lump multiple reactions (such as in the NetworkReducer compression step), or when metabolites are involved in multiple reactions that are partially removed (as seen in the *E. coli* core model). Many existing model reduction techniques, including most *"a posteriori"* methods, focus not only on directly removing parameters or variables from the model, but also on lumping together correlated components. This can be extremely effective for reducing the size of the model, such as in the example of the Michaelis-Menten rate law approximation, but a disadvantage is that the final parameters or variables no longer correspond to biological states or parameters, hampering interpretation of the model and experimental validation. To improve further upon these cases, model reduction methods could be re-designed to incorporate the concept of a core group of components that should be left untouched as much as possible, while components further away in the network are allowed to be removed or lumped. This naturally would work well with a reformulation of the reduction to a dual objective optimisation, where the size or complexity of the models is investigated as a trade-off for the predictive power and accuracy. Here we have looked at the predictive power of models in relation to matching dynamics of unmeasured metabolites in the system. However, the use of reduced dynamic metabolic networks allows us to ask questions related to the response of these systems in the face of fluctuating environments and reaction perturbations. Accurate predictions of such experiments would allow one to further differentiate between multiple model candidates when reducing larger systems to their core structures. As the amount of experimental data increases throughout iterations of the DBTL cycle, increasingly complex models can be investigated.

Furthermore, current constraint-based methods are based upon equivalence of optimal target flux solutions, and allow any solution for intermediate fluxes not constrained explicitly. Potentially, this could lead to a less-robust reduction procedure, as the reduced model potentially could have large deviations in the flux profile. Methods such as MOMA (Segrè et al., 2002) could be investigated as an alternative objective function for the reduction, which adds a constraint based on distance to the original flux profile. In addition, next generation constraint-based methods are continuously being improved and offer the opportunity to integrate additional sources of omics data and biochemical constraint such as GECKO (Sánchez et al., 2017), ME (LloydAli et al., 2018), REMI (Pandey et al., 2019), MOMENT (Bekiaris and Klamt, 2020) and RBA (Bulović et al., 2019). Since these methods are based on similar constraint-based formulations, model reduction methods should be adaptable to consider these additional constraints.

Unfortunately, despite advances in numerical optimisation of highly dynamic and large-scale ODE systems, optimisation is still a bottleneck for the size of dynamic metabolic models. It could be questioned whether the solvability issues are inherent to metabolic systems or specific rate-laws, or are a result of unrealistic initial parameterisations. However, as we showed in our *E. coli* core case-study, ensemble modelling can serve as a step in-between constraint-based models and full kinetic models. In our case study, the ensemble model was only constrained by the allowed flux profile, but additional constraints can be used to filter out less realistic models from the ensemble through the use of additional experimental data such as metabolomics, fluxomics or perturbation data. This adds a tool for medium to large scale (<100 reactions) semi-dynamic models while carefully parameterised dynamics models remain a good option for smaller scale interactions (<25 reactions). It must be noted that these size indications further depend on the number of metabolites and parameters in the system. The available data, as well as the topology and the specific dynamics of the system, also play an important role.

5. Conclusion

Targeted reduction of metabolic models can bridge high- and low-level models in systems biology, enabling workflows where large omics data sets are initially combined with statistical or genome-scale constraint-based methods to pinpoint potential regions of interest and model reduction is then used for a complimentary bottom-up investigation of the same phenomenon. This approach allows to go from statistical correlations to the formulation and subsequent verification of a comprehensive mechanistic theory.

Parameter estimation and simulation procedures are still lacking for genome-scale metabolic ODE systems. As an alternative for doing parameter estimation on medium-to large-scale models we describe an ensemble method to generate parameter samples for a fixed flux profile. We show how this method can be used to compare full and reduced version of a medium-scale dynamic metabolic model (*E. coli* core metabolism) without having to explicitly fit the models with experimental data.

In addition, we analysed the potential of automated methods to reduce constraint-based models before parameterisation in order to facilitate iterative implementation of DBTL cycles. Starting from existing genome-scale constraint-based metabolic models, we show how to both generate and utilise dynamic models in the DBTL cycle for medium-scale using ensemble simulation and for small-scale systems using parameter estimation.

We found that *"a priori"* reduced models can show significant overlap with the dynamic properties of the full models, and thus can be used to drive decision making using sensitivity analysis or optimal experimental design methods. By automating the reduction and creation of these models, the iteration time of the DBTL cycle is kept short and targeted models can be created on demand when required for experimental guidance. In the future, *"a priori"* model reduction techniques can be optimised for the purpose of generating representative reduced dynamic models, and to integrate other sources of (omics) data.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymben.2021.01.008.

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