Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Information

An entropy-like index of bifurcational robustness for metabolic systems

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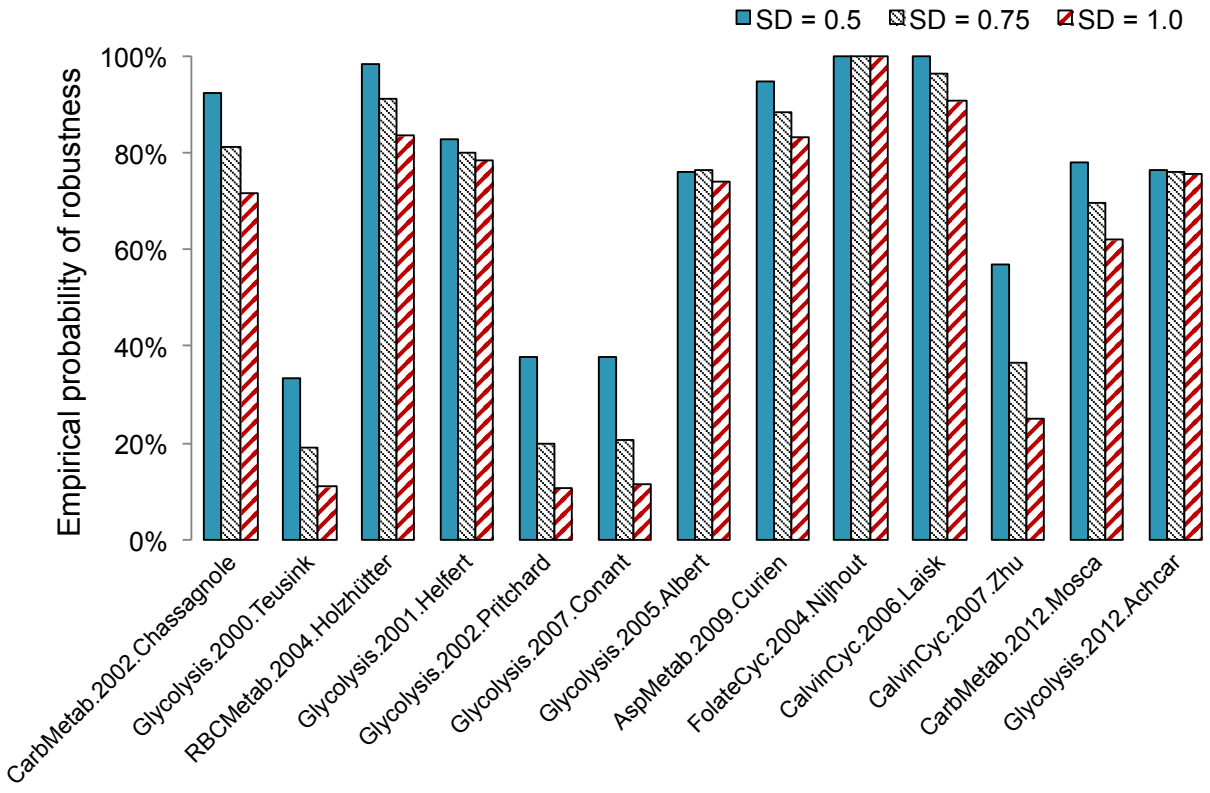
Table of Contents

Simulation of natural perturbation

To characterize the robustness of each model *in silico*, we performed 10,000 Monte Carlo simulations where the expression level of each enzyme, which is reflected in V_{max}, was subject to a random change. Starting from the default steady state, each perturbed model was simulated for sufficiently long to determine if a steady state (which need not be the default steady state) was reached (Supplementary Figure 1A). For a given model, we calculated the *in silico* probability of steady-state retention as the fraction of 10,000 simulations that reached a steady state. For simplicity, we investigated only perturbations in $V_{\max}$ and did not involve other kinetic parameters such as $K_{M,}$ which could be similarly investigated.



Supplementary Figure 1. Existing models have robustness problems to different extent. The empirical probability of robustness, calculated as the fraction of 10,000 randomly perturbed models that retain a steady state, is shown for each of the 13 BioModels database models [1-13]. SD, the standard deviation of log fold change. CarbMetab, carbohydrate metabolism; RBCMetab, red blood cell metabolism; AspMetab, aspartate metabolism; FolateCyc, folate cycle; CalvinCyc, calvin cycle.

Each V_{max} was subjected to F-fold changes, where F = $V_{max,new}/V_{max,default}$, and log(F) was sampled from a normal distribution with mean = 0 and standard deviation= 0.5 (Fig. 1B). Apparently, models' robustness varied widely. Some models, such as the folate cycle model[10], are very robust and almost always retain a steady state after perturbation. Others, such as Glycolysis-1[12], Glycolysis-3[11], and Glycolysis-4[4] responded poorly even to moderate perturbations, as only <60% of the models reach any steady state after perturbation. Interestingly, the difference in robustness can be dramatic even between models of the same metabolic pathway (*cf.* Glycolysis-1[12] and Glycolysis-2[6] yeast glycolysis models). This result suggests that not all descriptions of a native metabolic system perform equally well when subjected to perturbation. If one accepts the notion that these native pathways are robust against perturbations, the models did not describe such behavior.

One limitation of the Monte Carlo simulation is its scalability. The numerical integration of ODEs, while accurate, is computationally expensive and not scalable. Such scalability issues are most critical if the robustness calculation is to be coupled to highly iterative algorithms such as the ones used for model building and parameter fitting. For example, if we wish to re-fit the parameters of the non-robust models (*e.g.* Teusink *et al.*'s glycolysis model) so that the revised models could have higher bifurcational robustness, it is imperative that the quantification of robustness is accomplished in a more efficient and scalable way.

Optimized parameter values are biologically meaningful

Figure 4B shows a comparison between the parameter set returned by an optimization considering robustness and the original parameter set used by Teusink *et al.*[12]. In the optimal set only four parameters (K_{glyco}, K_{treha}, V_{pdc}^{m}, and K_{ATP}) required significant modifications; most parameters had little to no effect on the robustness and thus remained unchanged. Interestingly, the significant parameter modifications correspond with the adjustments made by van Heerden *et al.*[14] in their updated version of Teusink *et al.*'s model[12]. For example, both teams identified the need for adjustment in glycogen and trehalose production, the increase in pyruvate decarboxylase activity, and the consideration of phosphate dynamics as important features for

robustness. Overall, we demonstrate that a parameter optimization considering *S* can significantly improve the robustness of a metabolic model without any alteration to the kinetic rate expressions.

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Supplementary Table 1. Abbreviations used in Figure 1C and Figure 4B

Abbreviation	*Enzyme Name/Function*
Glk	Gluco kinase
Pgi	Phosphogluco isomerase
Glyc	Glycogen production
Treha	Trehalose production
Pfk	Phosphofructokinase
Aldo	Fructose-1,6-bisphosphate aldolase
Gapdh	D-glyceraldehyde-3-phosphate dehydrogenase
Pgk	Phosphoglycerate kinase
Pgm	Phophoglycerate mutase
Eno	Phosphopyruvate hydratase
Pyk	Pyruvate kinase
Pdc	Pyruvate decarboxylase
Succ	Succinate production
Glt	Glucose transport
Adh	Alcohol dehydrogenase
G3pdh	Glycerol 3-phosphate dehydrogenase
ATPase	Adenosine triphosphatase

Supplementary Table 2. Abbreviations used in Figure 4B

Abbreviation	*Metabolite Name*
NADH	Reduced nicotinamide adenine dinucleotide
P	High energy phosphate pool (2[*ATP*] + [*ADP*])
ACE	Acetaldehyde
PYR	Pyruvate
PEP	Phosphoenolpyruvate
P2G	2-phospho-D-glycerate
P3G	3-phospho-D-glycerate
BPG	1,3-biphospho-D-glycerate
TRIO	Dihydroxyacetone phosphate + glyceraldehyde-3-phosphate
F16P	Fructose-1-6-biphosphate
F6P	Fructose-6-phosphate
G6P	Glucose-6-phosphate
GLCi	Internal glucose