Metabolic oscillations in budding yeast described by a dynamical model

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Abstract

The budding yeast *Saccharomyces cerevisiae*, grown under the right conditions in continuous culture, shows spontaneous metabolic oscillations. The oscillations affect not only gas exchange rates and metabolite concentrations, but also the expression of a large fraction of genes. I integrated time-dependent metabolite concentrations, oxygen uptake rates, carbon dioxide and ethanol excretion rates, and thermodynamic data into a large dynamic model of yeast metabolism. A linearised kinetic model was fitted to the data, assuming that the oscillations in metabolism are entirely driven by the time-dependent, experimentally know oxygen uptake. To fit a model of this size to periodic time series data, a number of tricks had to be used: (i) the model variables were determined in a stepwise procedure, starting from a stationary flux distribution and ending with the reaction elasticities, which describe the dynamics around this reference state; (ii) the elasticities themselves were derived from thermodynamic forces and saturation values in order to guarantee thermodynamically feasible reaction kinetics; (iii) and the periodic dynamics was not simulated by numerical integration in time, but by a Fourier synthesis based on periodic response coefficients, which can be directly computed from the reaction elasticities; (iv) the reference state was required to remain dynamically stable, which puts strong constraints on the possible reaction elasticities. The model parameters (thermodynamic forces and saturation values) were then fitted in such a way that their values, by construction, correspond to thermodynamically consistent kinetic rate laws. The model predicts metabolic fluxes, concentrations of non-measurable metabolites, and local metabolite concentrations in cell compartments (cytosol and mitochondria) along the metabolic cycle. Computer animations of experimental data and simulation results can be found on `www.metabolic-economics.de/yeast-metabolic-oscillations/`.

Keywords: Metabolic oscillation, budding yeast, dynamical model, model fit, reaction elasticity

1 Introduction

Metabolic oscillations in yeast have been studied for many years, with a focus on transcriptional and metabolic changes [1, 2, 3], types of oscillatory dynamics [4, 5, 6], and the accompanying changes in DNA structure [7, 8, 9]. During the metabolic oscillations, cells alternate between an oxidative state with higher respiration activity and a reductive state in which biosynthesis tends to become more active. That metabolic pathways change their activity along the metabolic cycle is supported both by metabolomics and gene expression data [10]. However, the pathway fluxes, which would be the ultimate criterion for "activity", cannot be easily measured. In order to infer the non-measurable, time-dependent metabolite concentrations and metabolic fluxes from available data, I developed a dynamic model of metabolism in yeast that can fill these holes. The model was developed in close collaboration with Prof. Douglas Murray (Institute for Advanced Bioscience, Keio University), who also provided most of the experimental data. Even though some metabolite concentrations can be measured – those were used to fit the model – a reconstruction of metabolite curves is important for two reasons: for some metabolites, no measurements are available; and for some metabolites, it is important to determine their concentrations within

cell compartments (cytosol and mitochondria), a piece of information distinction that cannot be extracted from metabolomics data alone.

A main model assumption is that metabolism responds "passively" to peridic changes in oxygen uptake, at a constant uptake of glucose (these uptake rates agree with experimental observations). In particular, I assumed that all enzyme levels remain constant in time. The latter assumption was made because the changes in mRNA levels, even though they are pervasive, are not very strong in amplitude. Given the short oscillation period (a bit less than an hour), these expression changes would translate into very small changes in protein levels. Since no information about regulated protein modifications or protein degradation were available, constant enzyme levels were assumed. Therefore, the only source of oscillations in the model is the predefined, periodically varying oxygen uptake flux. From this uptake reaction, oscillations in fluxes and metabolite levels percolate into the metabolic network like waves, where cofactors and allosteric regulation can also couple distant parts of the network.

This report starts with a brief description of the data used and of how the model was built and fitted to data. Then, simulation results are compared to experimental data. On the website `www.metabolic-economics.de/yeast-metabolic-oscillations/`, computer animations of experimental data and simulation results are shown. There, the fluxes are also compared to time-dependent gene expression data obtained from a similar experimental set-up [10], and to gene expression data measured in a different experimental set-up that gives rise to much slower oscillations [2], in which periodic enzyme levels could have a much larger effect on the metabolic dynamics.

2 Model construction

The objective of this work was to construct a metabolic of yeast that reproduces the measured metabolite profiles and exchange fluxes during metabolic oscillations in growth on glucose and to predict non-measurable quantities from this model. Different types of data were used: (i) time-dependent exchange rates (oxygen, carbon dioxide, ethanol) in yeast continuous cultures, provided by D. Murray; (ii) time-dependent absolute metabolite concentrations in the same experiment, also provided by D. Murray; (iii) standard Gibbs free energies of reactions, provided by Dr. E. Noor (computed using the component contribution method [11]). Some of the data are shown in Figures 1 and 2.

The metabolic network (Figure 3) is a modified version of the yeast metabolic network from Jol et *al.* (2012) [12], with slight modifications by my collaborators S. Hoffmann, W. Gottstein, and D. Murray. The model in Systems Biology Markup Language (SBML) format can be downloaded from `www.metabolic-economics.de/yeast-metabolic-oscillations/`. To construct and fit a dynamic model, I made the following model assumptions:

1. **Aim of model construction** We aim at constructing a linearised model that is thermodynamically consistent and that, with a given time-dependent oxygen uptake rate, reproduces the main experimental facts (CO_2 and ethanol secretion rates, mean metabolite levels, time-dependent metabolite levels, heat production, and respiratory quotient (CO_2 secretion divided by O_2 uptake).

2. **Constant protein levels** An analysis of gene expression amplitudes suggested that the changes in enzyme levels are probably small. Therefore, all enzyme levels are assumed to be constant in the model. Allosteric regulation of enzyme activities was still considered.

3. **Dynamic response to oxygen uptake rate** We assume that oxygen uptake is limited by non-metabolic processes (possibly involving structural changes of the mitochondrial membrane. Since these processes are outside the scope of the model, we treat the time-dependent oxygen uptake rate as an experimental fact and impose it onto the model as a constraint.

(a) Gas exchange rates

(b) Concentration changes between oxidative and reductive phase

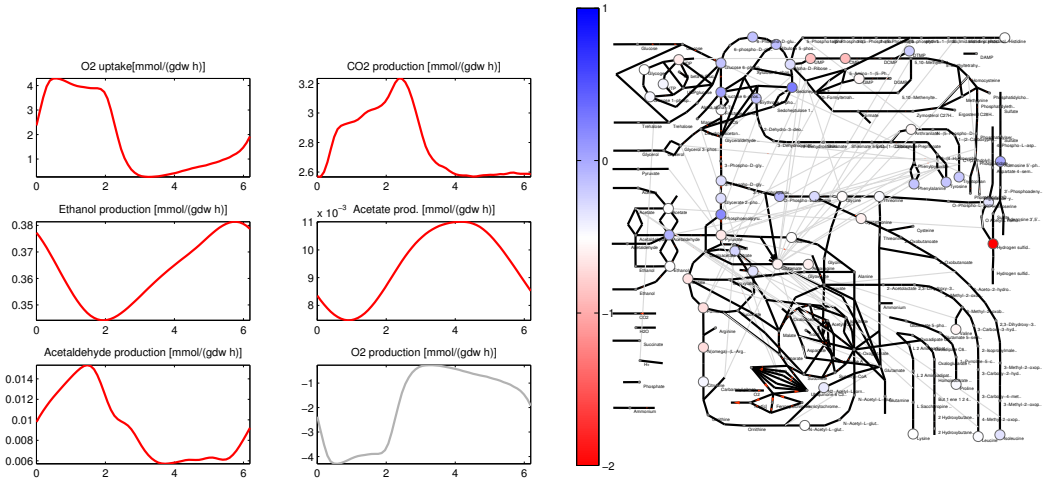


Figure 1: Measured gas exchange rates and changes in metabolite concentrations in yeast during the metabolic cycle (experimental data provided by D. Murray). (a) Measured exchange rates. The oxygen curve (grey) is shown for comparison. (b) Measured concentration changes between oxidative and reductive phase. Relative concentrations are shown in colours (blue: higher in oxidative phase, pink: higher in reductive phase). Highly connected metabolites (e.g., cofactors) are included in the network, but not shown in the graphics. Some metabolites exist both in cytosol and mitochondria; their data values are shown twice and refer to the over concentration in the cell. Some reactions were omitted for clarity.

Due to the large model size and the type of data used (periodic metabolite concentrations), fitting a kinetic model directly to the data would be difficult. Instead, to obtain a viable model, a number of tricks had to be used: the model variables were determined in a stepwise procedure, starting from a stationary flux distribution and ending with the reaction elasticities, which describe the dynamics around this reference state; the elasticities themselves were derived from thermodynamic forces and saturation values in order to guarantee thermodynamically feasible reaction kinetics; and the periodic dynamics was not simulated by numerical integration in time, but by a Fourier synthesis based on periodic response coefficients, which can be directly computed from the reaction elasticities. To allow for one reaction to be externally controlled (namely the oxygen uptake reaction), a new type of "restricted" control coefficients had to be defined. (iv) the reference state was required to remain dynamically stable, which puts strong constraints on the possible reaction elasticities. (v) Finally, to handle cell compartments with different volumes (75 and 25 percent of the cell volume, respectively, for cytosol and mitochondria), reaction fluxes were defined as numbers of reaction events (in moles) per time and cell volume; concentrations were not defined as local concentrations in the cell compartments, but as amounts (in moles) *in the cell compartments*, but *divided by the cell volume*. Therefore, the stoichiometric matrix keeps a simple form; however, the compartment volumes enter the formulae for computing control and response coefficients.

All this resulted in a new procedure for model building. The guiding thought was to determine the values of different types of variables step by step: first a time-averaged flux distribution; then a corresponding set of equilibrium constants and stationary metabolite levels; and then, reaction elasticities defining the dynamics of the model around this metabolic reference state. Altogether, the model was constructed and fitted in the following way:

1. **Compounds, reactions, and network structure** The lists of metabolites and reactions, defining a stoichiometric matrix, were taken from a published model of yeast metabolism [13], with previous modifications by S. Hoffmann, W. Gottstein, and D. Murray. Extracellular compounds were treated as external (i.e., with concentration curves being predefined, not determined by rate equations). A number of known allosteric interactions

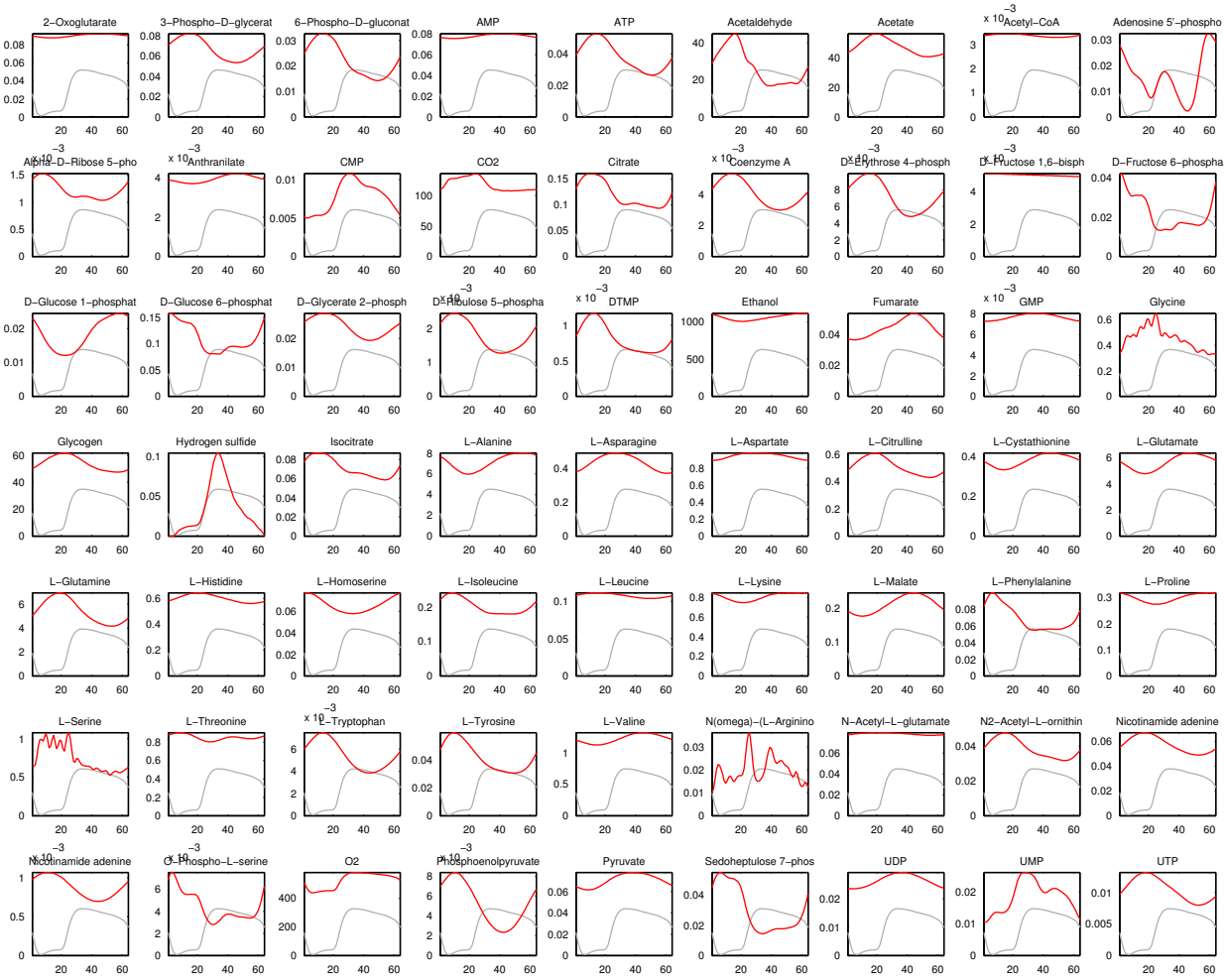


Figure 2: Temporal metabolite profiles (experimental data provided by D. Murray). The time axis (x-axis) shows one oscillation period. Oxygen curves (grey) are shown for comparison. Only metabolites appearing in the model are shown.

were added to the model. The network structure is shown in Figure 3.

2. **Exchange rate data: conversion and mapping** Measured time series of uptake and secretion fluxes (for oxygen, carbon dioxide, and ethanol), as well as heat production, were obtained from D. Murray (64 time points, standardised to oscillation phase angles) and were converted into units of mM/s (referring to concentrations inside the cell).

3. **Concentration data: conversion and mapping** Measured time series of metabolite concentrations were obtained from D. Murray. The data were smoothed and interpolated, resulting in time series with 64 equally spaced time points, roughly standardised to oscillation phase angles. The data were then converted to mM (in the cell volume) and mapped onto the model.

4. **Time-average fluxes: calculation by Flux Balance Analysis** Time-averaged fluxes were computed by flux balance analysis. The uptake and secretion rates (oxygen, carbon dioxide, ethanol) were roughly fixed to their (time-averaged) experimental values. Fluxes were predicted in two steps, by applying a normal FBA (to determine the maximal possible biomass production rate), followed by a minimal-flux FBA (where this biomass production rate was used as a constraint and the sum of absolute fluxes was minimised).

5. **Intracellular concentrations: adjustment by thermodynamic constraints** Time-averaged intracellular concentrations for the model were computed based on (time-averaged) measured metabolite concentrations, on

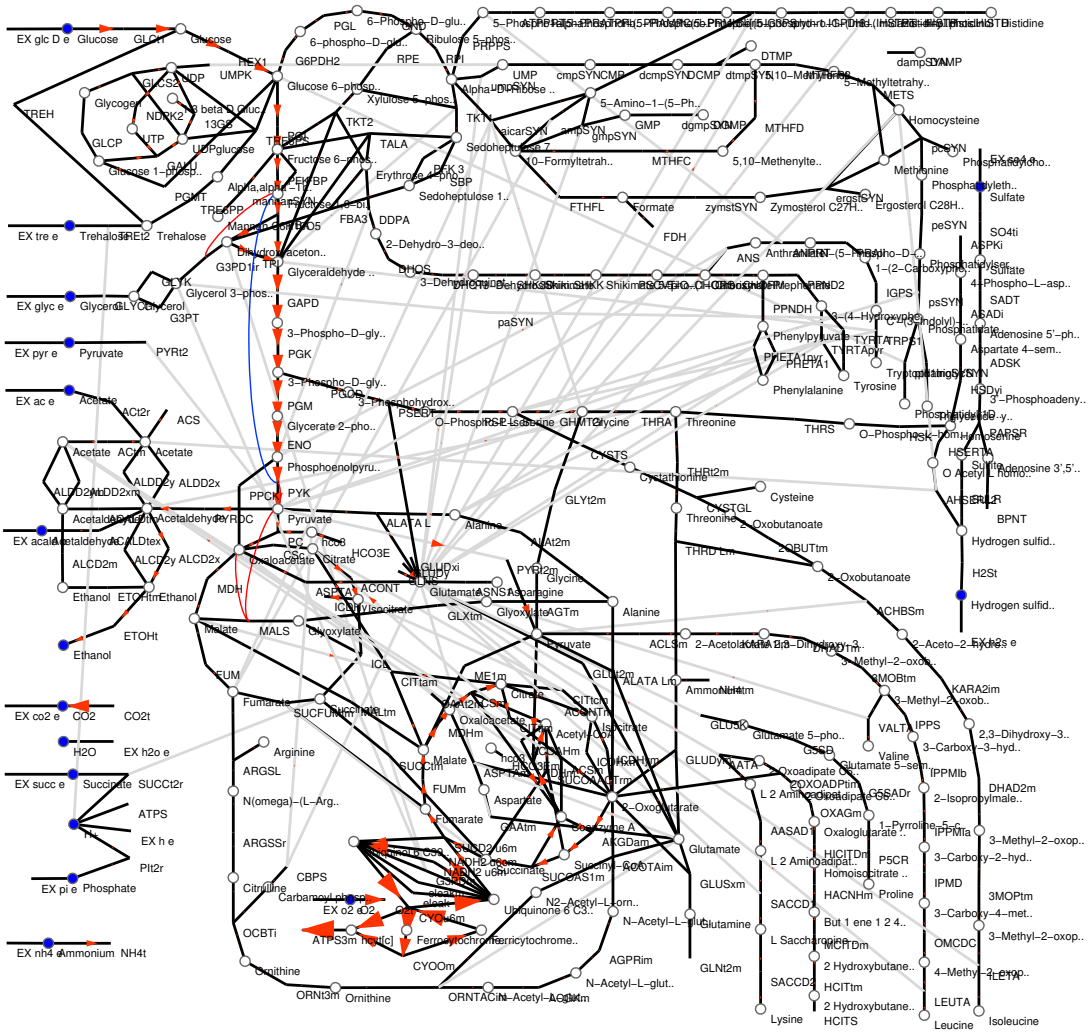


Figure 3: Model structure. Static metabolic fluxes were determined by flux balance analysis, using the time-averaged measured exchange rates as a constraint. Allosteric activation and inhibition is shown by blue and red arcs, respectively.

the previously computed flux directions, and on standard Gibbs free energies of formation. Standard reaction Gibbs free energies of for the model were computed by E. Noor (ETH Zürich) using the component contribution method [11]. Based on these Gibbs energies and on the measured metabolite concentrations, thermodynamic parameter balancing [14] was used to obtain a consistent sets of Gibbs energies and concentrations, resembling these input data and in agreement with the flux directions.

6. **Reaction elasticities: structure of the elasticity matrix** In the model, we first consider, as a reference state, a steady state with the time-average fluxes and compound concentrations computed so far. To describe a dynamics around this reference state, we define the reaction elasticities. We first assume that the structure of the elasticity matrix follows the stoichiometric matrix (with positive substrate elasticties and negative product elasticities) and add further entries to describe allosteric regulation. The actual elasticity values were determined in several steps. A first version of the elasticity matrix was obtained by a simple heuristics, assuming approximately half-saturated enzymes in all cases. These values were then refined by fitting the model to metabolite curves, as described below. Importantly, to ensure a thermodynamically feasible model, the reactions elasticities were not treated as independent model parameters; instead, their values were derived from thermodynamic driving forces and saturation values, which can be independently varied without violating any constraints [15, 16], and which were used as model parameters to be fitted.

7. **Response to static changes and sine-wave oscillations** To simulate metabolite curves (which was also necessary to fit the model parameters), the dynamical properties of the model were analysed. The dynamic response to oscillating inputs can be characterised by spectral response coefficients (computed from the Jacobian, which follows from stoichiometric matrix and elasticity matrix).

8. **Dynamic response to predefined time-dependent oxygen uptake** To simulate the response to non-sine-wave perturbations (most importantly, the predefined oxygen uptake time curve), we Fourier-transform the input time series, multiply with the frequency-dependent spectral response matrices, and apply the reverse Fourier transformation. This has several advantages over time-simulations: (i) long simulation runs due to a slow initial relaxation are avoided. (ii) with a restriction to low-frequency components, fast noise is automatically suppressed.

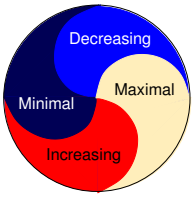
9. **Reaction elasticities: parameter fit based on concentration profiles** The values in the elasticity matrix were further optimised by fitting the simulated metabolite time curves to experimental data. To do so, the thermodynamic driving forces and saturation values, which together determine the reaction elasticities, were iteratively optimised by a greedy optimisation method. (i) As a first fit, we considered a stationary response to increased oxygen uptake, and fitted the resulting concentration changes to observed concentration changes between the reductive and the oxidative phase. (ii) Then, we fitted the temporal variation profiles of metabolites (for each metabolite, scaled by its mean concentration) to the corresponding data. Since the data refer to whole-cell concentrations, and not to the concentrations in cytosol or mitochondria, the concentrations obtained from the model were converted into predicted cellular concentrations.

3 Model results

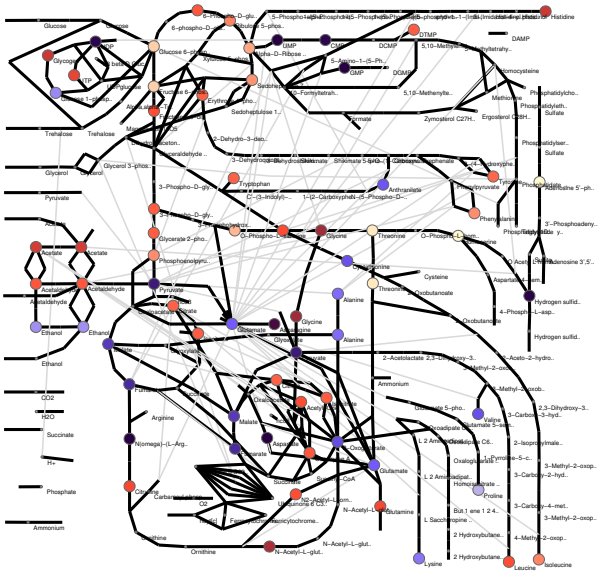
Simulation results are shown in this section. Computer animations of experimental data and simulation results can be found on `www.metabolic-economics.de/yeast-metabolic-oscillations/`.

3.1 Simulated response to harmonic perturbations in oxygen level

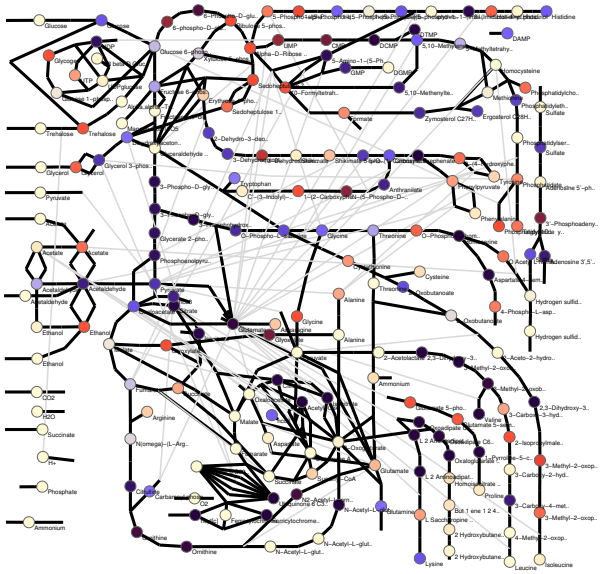
Before fitting the model precisely to metabolite time series, I started with simpler fits and predictions in which all curves were approximated by phase shifted sine curves. Each such curve can be characterised by a single complex-valued amplitude (or, equivalently, by a real-valued amplitude and a phase angle). With this simplification, the goodness of fit can be computed much faster. Probably, the simplification also helps the fitting algorithm to fit the overall shape of many metabolite curves, instead of getting stuck in a state where some curves are fitted very precisely, while other show no good fit at all. Results from the fitted model (fitted and measured metabolite phase angles, and predicted phase angles of internal fluxes) are shown in Figure 4. The colour code for phase angles is shown in the inset Figure.



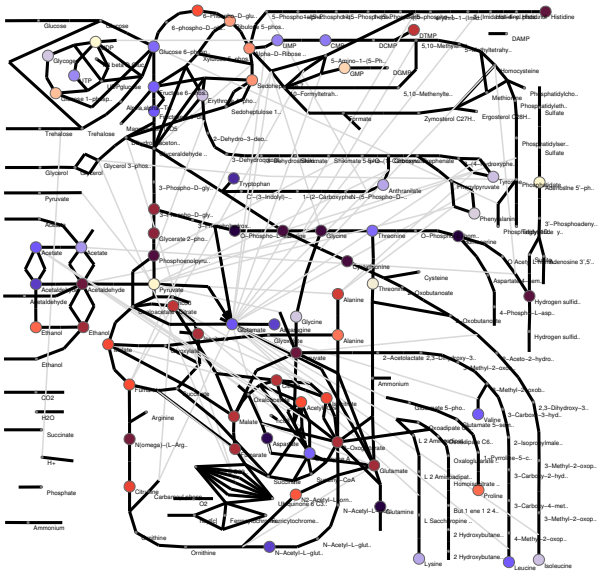
(a) Metabolite phase angles, measured



(b) Metabolite phase angles, predicted



(c) Metabolite phase angles, mismatch



(d) Flux phase angles, predicted

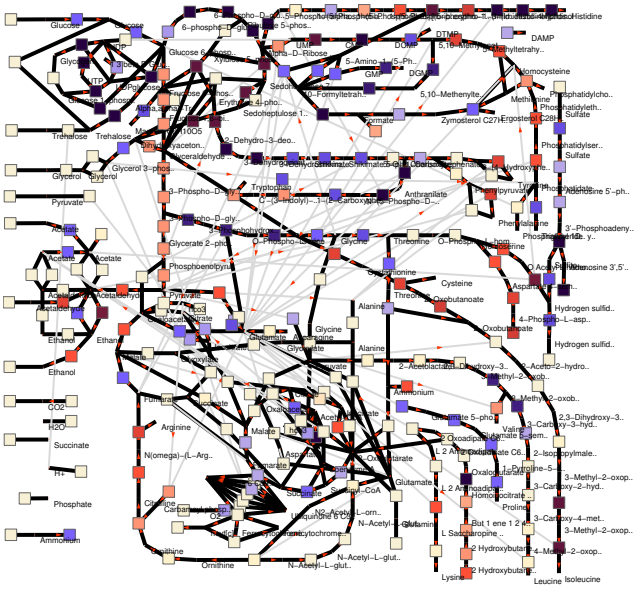


Figure 4: Periodic metabolite concentrations and fluxes caused by a harmonically varying perturbation of oxygen uptake. Phase angles are colour-coded (inset Figure in section 3.1). (a) Measured metabolite phase angles. (b) Predicted metabolite phase angles. (c) Differences between predicted and measured metabolite phase angles. (d) Predicted flux phase angles.

3.2 Metabolic dynamics in response to temporal oxygen uptake

Figure 5 shows simulated metabolic concentrations, assuming a predefined periodic oxygen uptake rate (experimentally measured oxygen uptake curve). The model parameters (reaction elasticities) were fitted to the measured metabolite curves. Measured and fitted metabolite curves are compared in Figure 6. The phase angles of many metabolites were fitted relatively well. However, some phase angles in glycolysis were wrongly predicted.

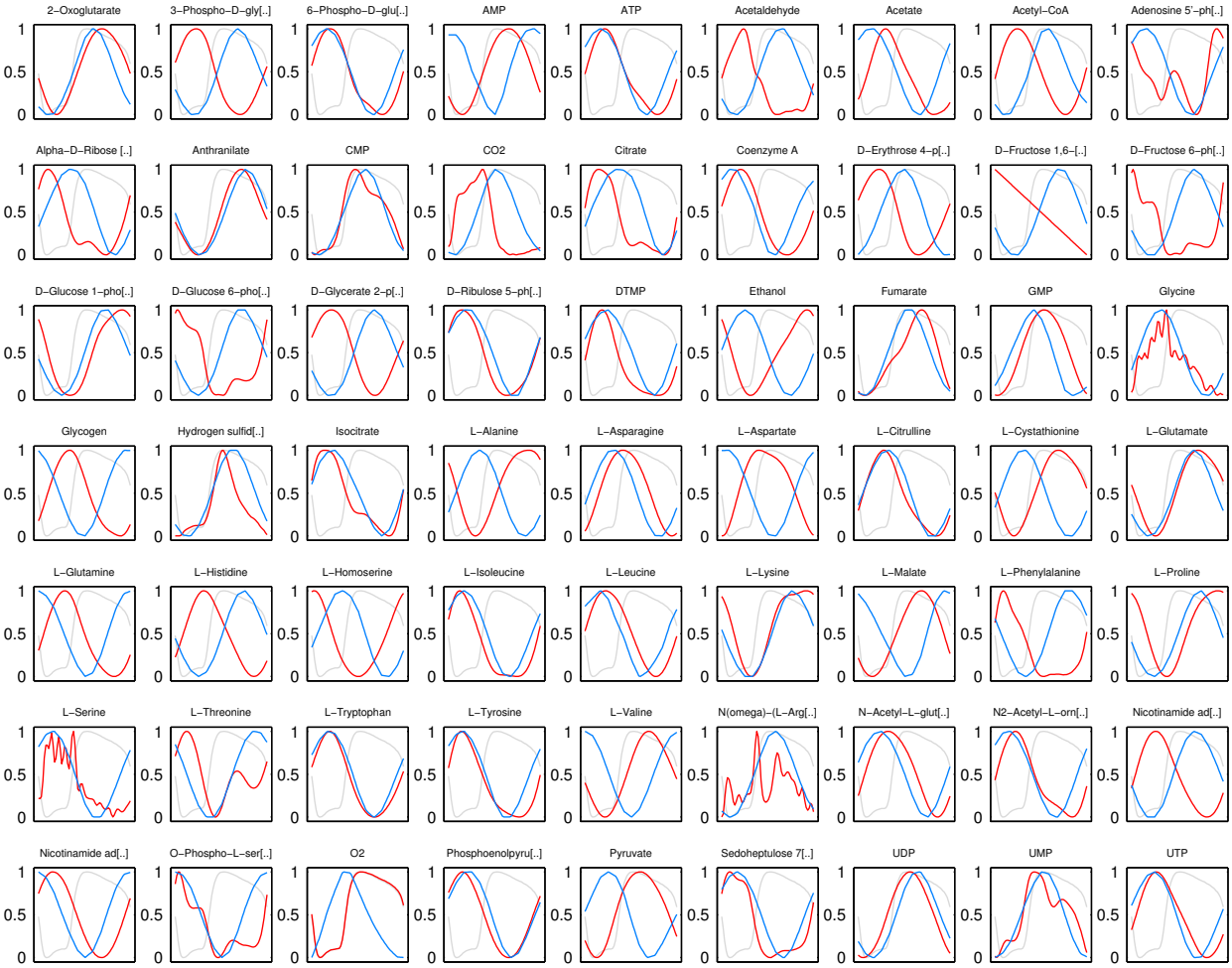
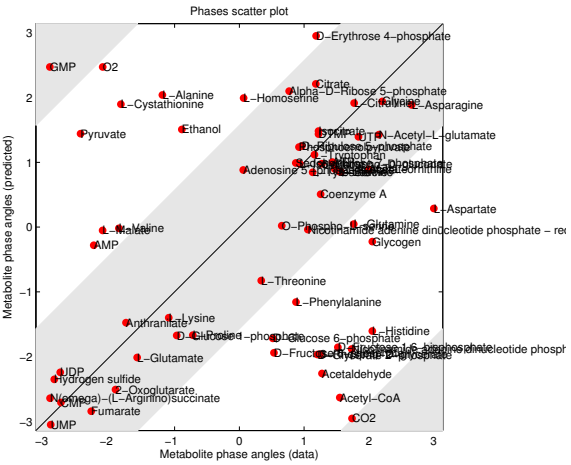


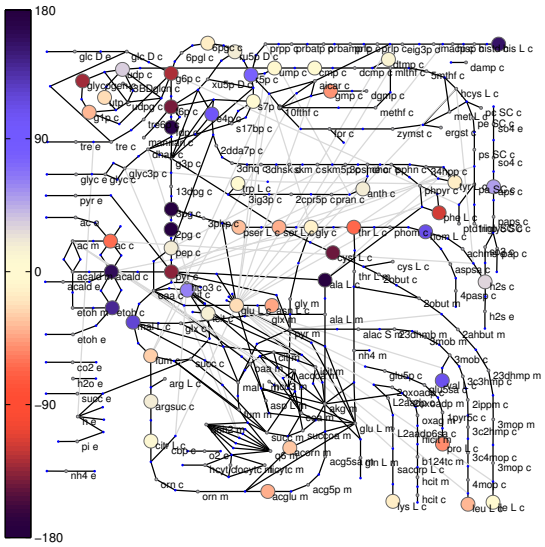
Figure 5: Metabolite concentration curves, measured (red) and simulated (blue). The simulated curves represent model fits, not independent predictions. All curves were shifted and scaled to span the (y-axis) range from 0 to 1.

(a) Concentration curves, measured *vs* fitted

(b) Metabolite phase angles, measured *vs* fitted



(c) Metabolite phase differences on network



(d) Metabolite phase difference, histogram

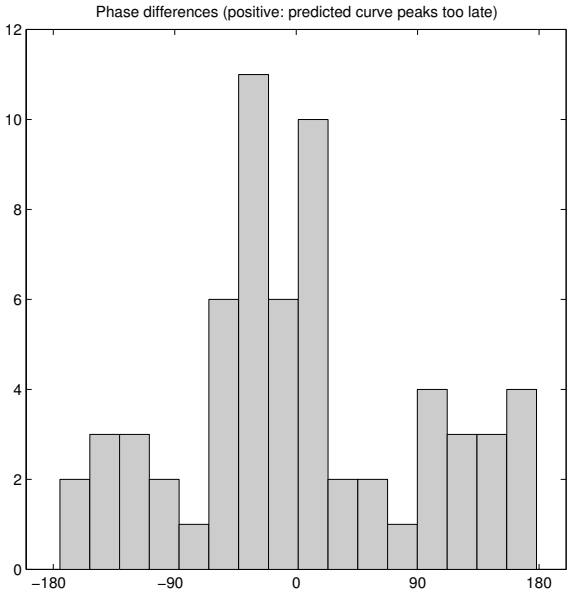


Figure 6: Comparison between measured and simulated metabolite curves. (a) Measured (x-axis) *versus* simulated (y-axis) metabolite time series. Dots close to a rising diagonal indicate good predictions, dots close to a falling diagonal indicate poor predictions. (b) Measured and predicted phase angles of oscillating metabolite levels. Dots in the grey zone indicate good predictions, dots in the white zone indicate poor predictions. Phase angles were determined from concentration curves by taking the first Fourier component. (c) Mismatch between measured and predicted phase angles. Phase mismatches are colour-coded (inset Figure in section 3.1). (d) Histogram of phase mismatches. The peak around the phase angle 0 represents the large number of dots in the grey zone in (b).

3.3 Simulated drop in glucose level

As an independent test, the model was used to predict the metabolic dynamics in another experiment, i.e., data that had not been used during model construction. The simulation concerns a part of the experimental time series in which the cells that underwent a sudden drop in available external glucose. The metabolomics data show how metabolite levels glycolysis are dropping fast, while some of them recover after a while. In the simulations, the model starts in a steady state with standard external glucose concentration. At the beginning of the simulation, this concentration is set to a lower value, causing a drop in internal metabolite levels as seen in the experimental data (Figure 7). The experimentally observed recovery of some metabolite concentrations may be caused by a shift to ethanol consumption, which is not appropriately captured by the model. The oxygen level remained fixed in this simulation.

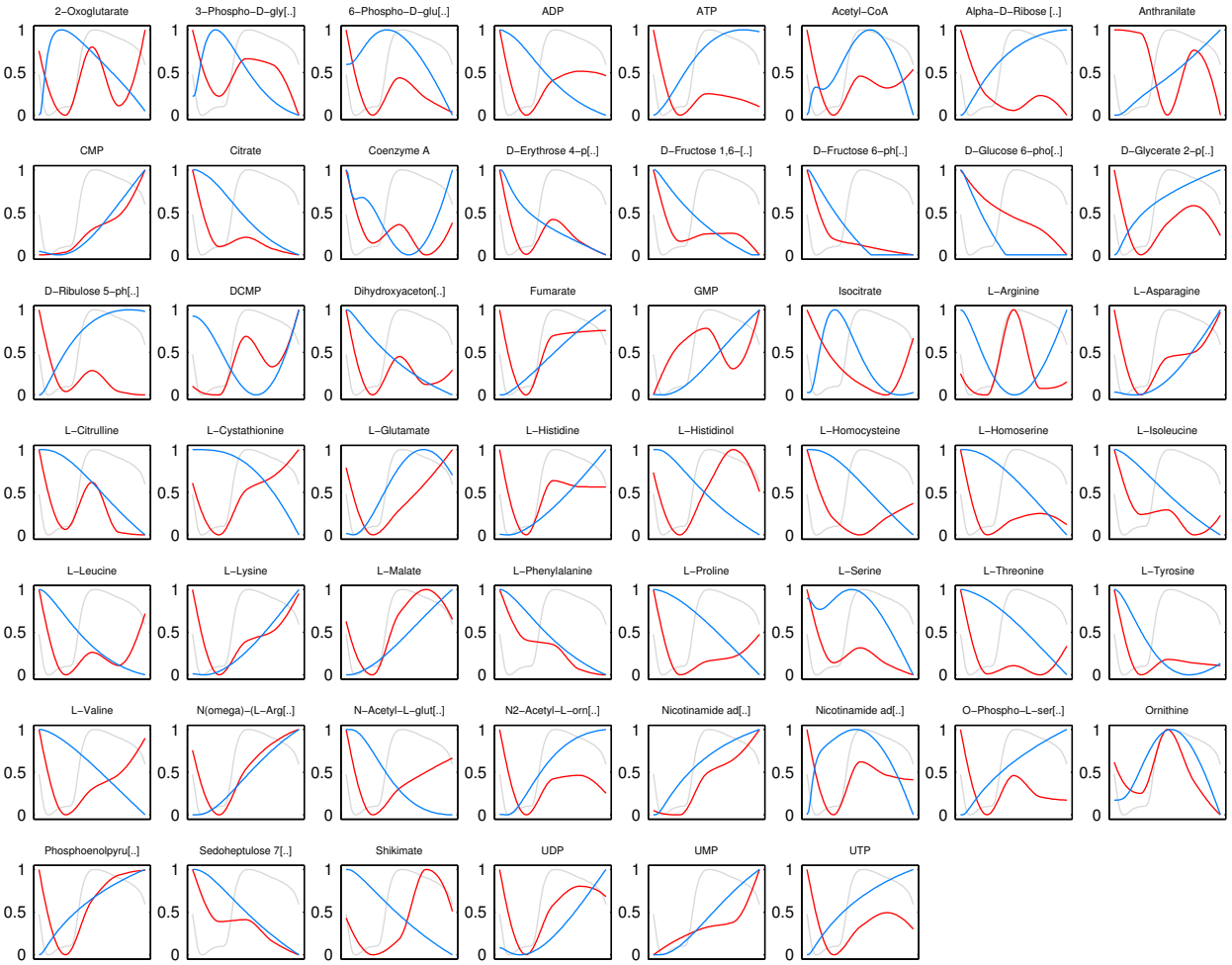


Figure 7: Concentration curves for glucose drop (scaled per metabolite), smoothed measured (red) and simulated (blue). All curves are shifted and scaled to cover the range from 0 to 1.

4 Discussion

It is important to remember that the current model only describes a subsystem of the cell – the metabolic network. The model is not meant to explain the origin of the observed oscillations, but only what shape these oscillations assume in different parts of the network. An explanation of the oscillations themselves would require a whole-cell model that couples metabolism to other processes, for example, to the physiological changes in mitochondrial

structure that may be responsible for the periodic breakdown of respiration and for its subsequent recovery. Also other questions remain untouched: How do cells synchronize each other? Do single cells oscillate also in other experimental conditions, where oscillations are not observed on a cell population level? What are the possible fitness advantages or disadvantages provided by the oscillations? Other types of models would be required to answer such questions.

The results presented here are still preliminary. Some details of the model still need to be improved, and the numerical optimisation used for model fitting may need some improvements as well. At the current stage, however, there are already good matches for many metabolite curves. Despite the large number of model parameters (the number of reaction elasticities is given by the number of reactions, multiplied with the average number of substrates, products, and allosteric regulators per reaction), it is highly unlikely that that this match is a mere result of overfitting because the only dynamical input to the model was the (experimentally determined) time-dependent oxygen uptake rate, the model's network structure restricts the possible shapes and phase shifts of metabolite levels quite strongly, and the fitted reaction elasticities were constrained to yield a dynamically stable state. Whether overfitting occurs, or to what extent, will have to be checked by crossvalidiation.

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