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Curation of a CHO DG44 Genome Scale Model and Application to Support Cell Culture Development Process

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Introduction

CHO cells have become the favorite expression system for large scale production of complex biopharmaceuticals. However, industrial strategies for upstream process development are based on empirical results, due to a lack of fundamental understanding of intracellular activities. Genome scale models of CHO cells have been reconstructed to provide an economical way of analyzing and interpreting large-omics datasets, since they add cellular context to the data.

Objective

A consensus CHO DG44 genome scale model was manually curated to eliminate problematic fluxes mainly observed in amino acid metabolism and cofactor utilization. The curated model predicted growth rate of 3 industrial cell lines and flux distributions at different days of the process. The objective is to use the model as a tool for identification of metabolic bottlenecks in low producers, and for suggestion of strategies for host and process improvement.

Method

Cell culture parameters

- CHO DG44 cell lines producing mAb1, mAb2 or mAb3 (respectively cell line 1, 2 and 3) were cultivated in 2L stirred tank glass bioreactors in independent fed batch processes.
- Daily sampling for measurement of cell density, cell viability, metabolite concentration (glucose, lactate, glutamate, glutamine, ammonium, amino acid and monoclonal antibody).

Modelling procedure

- Reference of the original model :
Hefzi, Ang et al. 2016. A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism. Cell Syst 3(5):434-443 e438.
- Mathematical optimization with parsimonious Flux Balance Analysis:
Nathan E. Lewis et al. Omic data from evolved E. coli are consistent with computed optimal growth from genome-scale models. Mol Syst Biol. 2010; 6: 390.
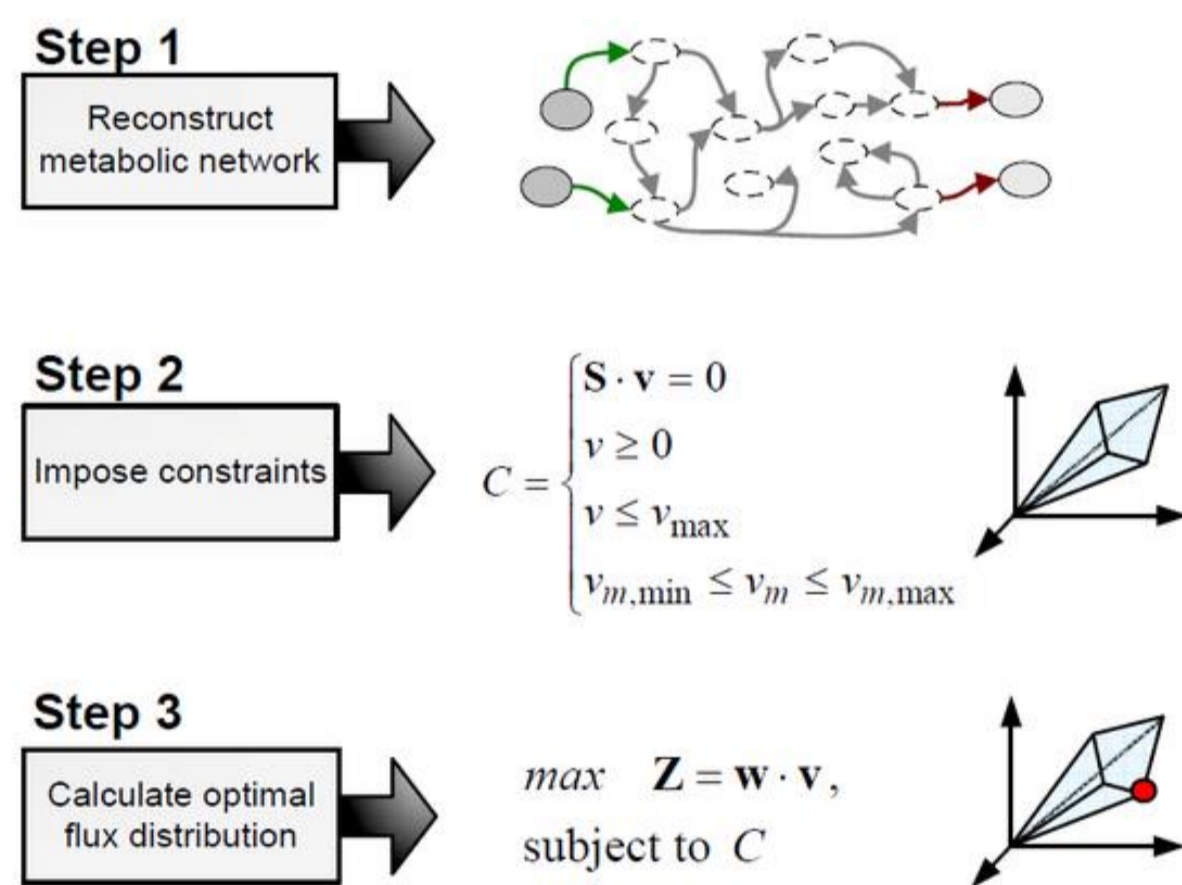
Step 1 Reconstruct metabolic network

Step 2 Impose constraints
$$C = \begin{cases} \mathbf{S} \cdot \mathbf{v} = 0 \\ v \geq 0 \\ v \leq v_{max} \\ v_{m,min} \leq v_m \leq v_{m,max} \end{cases}$$

Step 3 Calculate optimal flux distribution
$$max \quad \mathbf{Z} = \mathbf{w} \cdot \mathbf{v},$$
subject to C

Figure 1. Procedure to develop a flux balance analysis model.
From: PhD thesis. F Llaneras. UPV. 2010

Calculation of extracellular fluxes

- Substrate concentrations at each day of the cell culture were transformed into rates in mmol of product per gDW of cells per hour.
- Experimental production and consumption rates calculated were smoothed using a kernel smoother statistical function, in order to avoid irregular data points and noisy observations obtained from experimental results.
- Cell dry weight used: 330 pg (average from literature).
- Stoichiometric coefficients for antibody production in model were adapted for each mAb produced.

Conclusion

This study allowed to identify the most important corrections needed to obtain reliable predictions with the CHO DG44 model. We show that the curated model can be used to predict metabolic flux distribution of 3 recombinant protein production cell lines. The next step is to use the model as a tool to identify suitable strategies for re-routing metabolic fluxes toward the production of the protein of interest.

Results

1. Manual curation of the model

- FBA analysis showed thermodynamically infeasible internal cycles, i.e., metabolic "perpetual motion machines" that do not occur in biological reality.
- These irregularities were corrected manually with 3 types of modifications: removal, addition of metabolic reactions, and change of reaction parameters.
- A total number of 601 reactions were modified.

2. Modeling industrial processes

- In order to validate the manual curation performed, the model was tested for simulation of known cell culture processes for 3 cell lines.
- The maximal bound for external nutrients rates was set equal to the experimental flux.
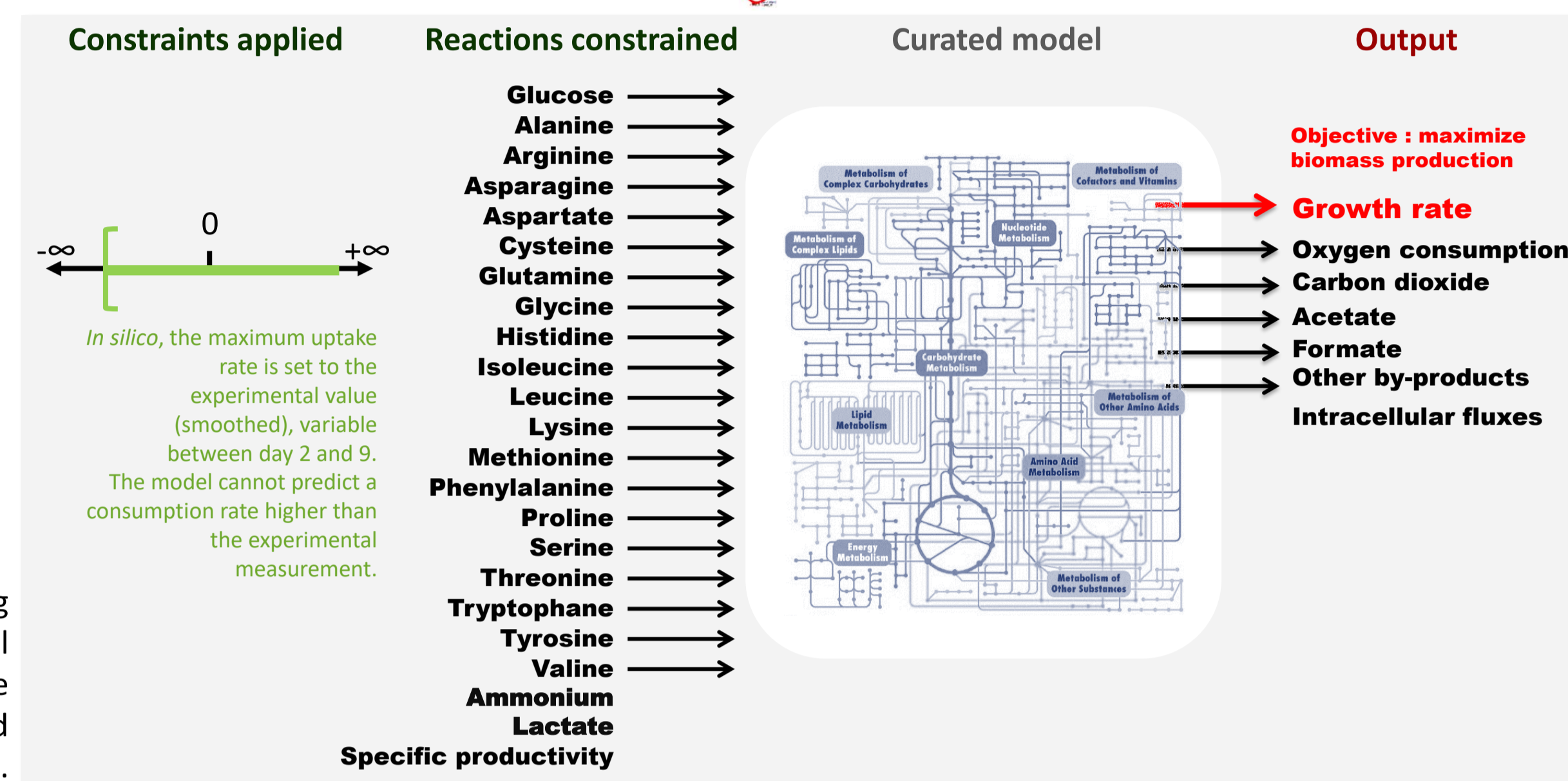- $R^2 > 0.75$ for 96% of reactions.
- $p > 0.05$ for 84% of reactions.

Figure 2. Initial network map of flux distribution after applying FBA. 68% of the total active reactions have an infeasible high rate.
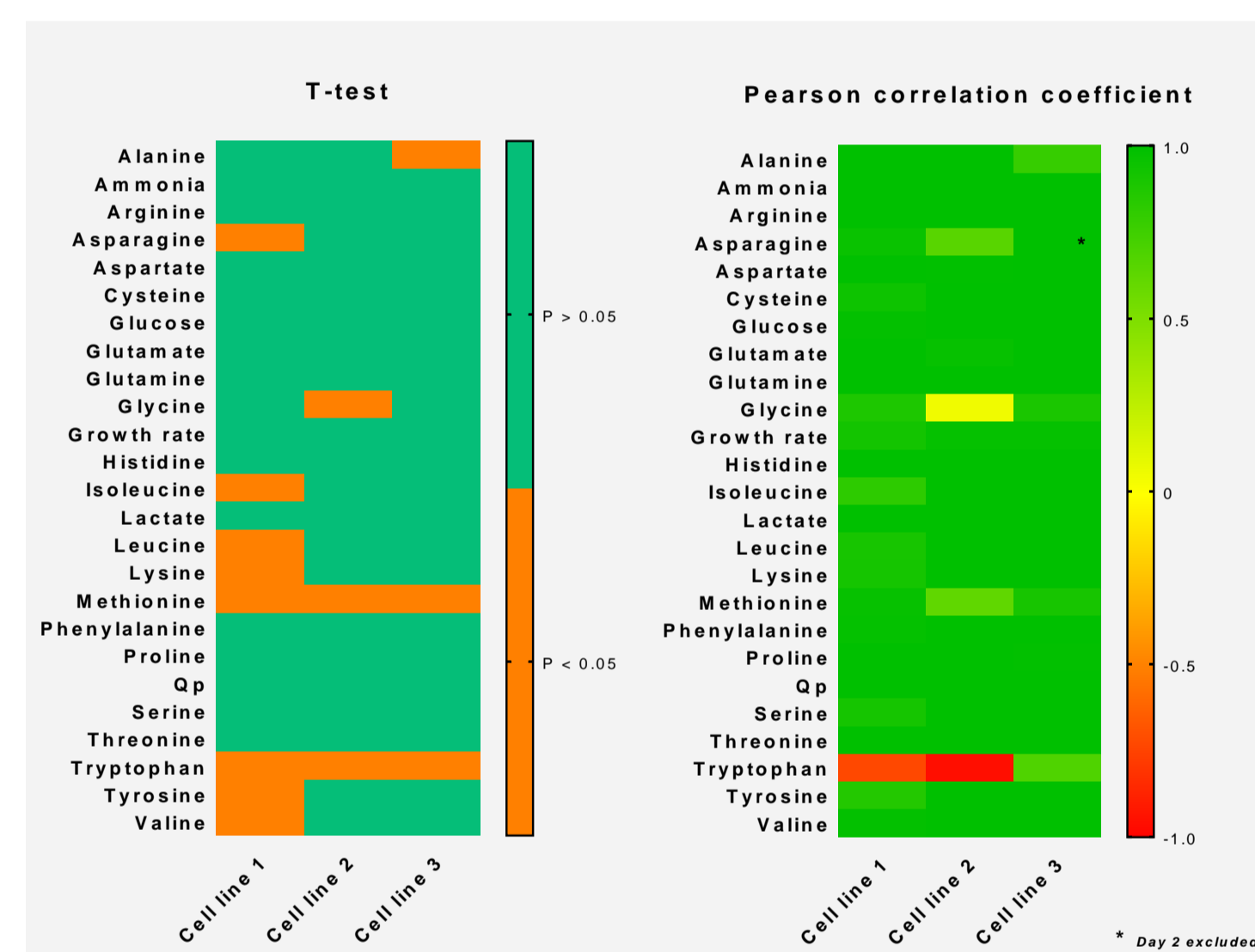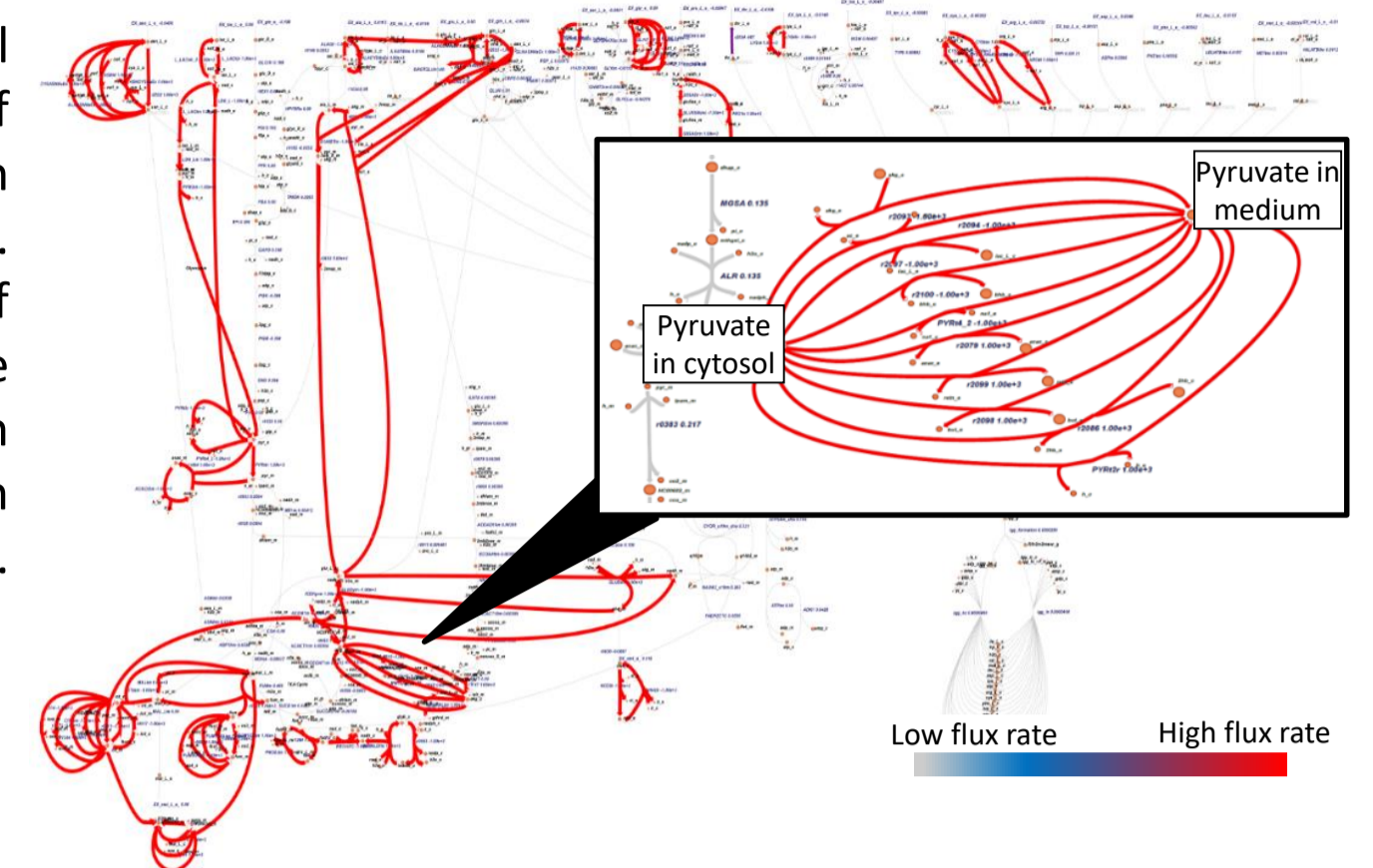
Figure 3. Modeling approach for cell growth, metabolite production and consumption rates.

Figure 4. Predicted rates compared to experimental data with linear correlation and independant two-sample T-test.
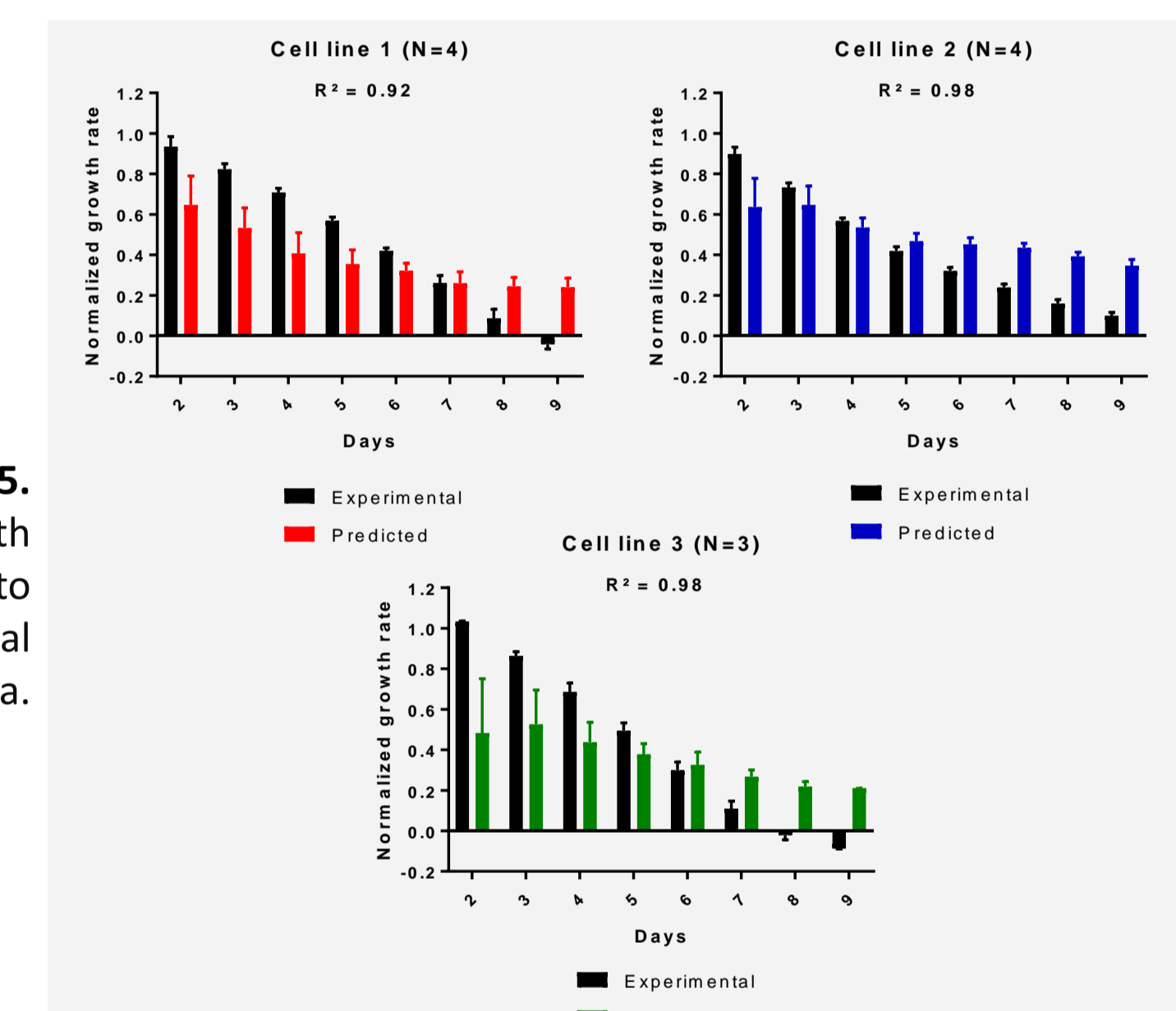
Figure 5. Predicted growth rates compared to experimental data.
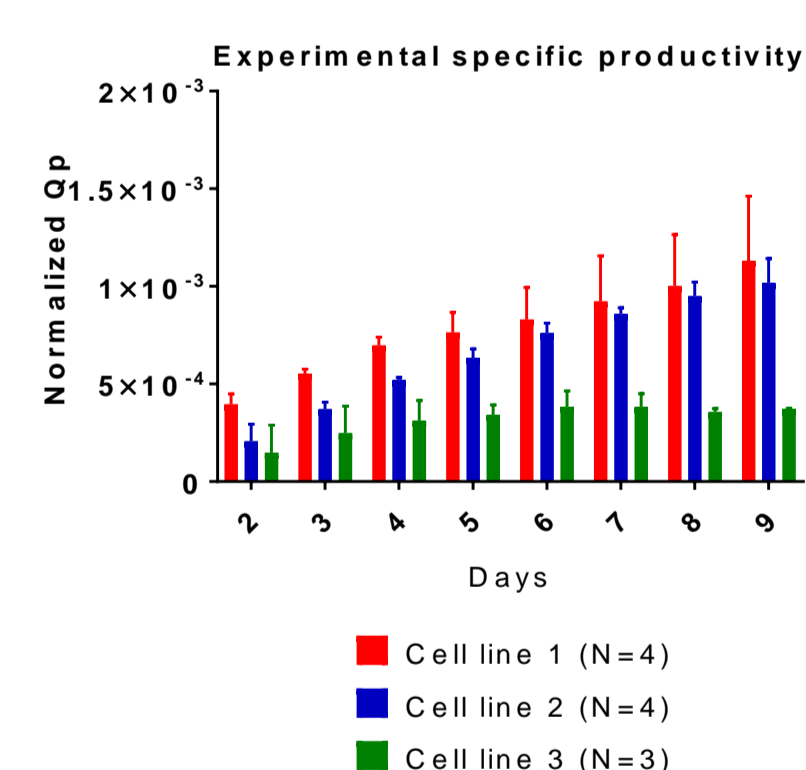
3. Analysis of intracellular fluxes

Figure 6. Predicted intracellular flux rates for each cell line between day 2 and 9.