Prediction of Microbial Growth Rate versus Biomass Yield by a Metabolic Network with Kinetic Parameters

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Prediction of Microbial Growth Rate versus Biomass Yield by a Metabolic Network with Kinetic Parameters

Research Thesis

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Computer Science

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Submitted to the Senate of
the Technion - Israel Institute of Technology
Nisan 5771 Haifa May 2011
The research thesis was done under the supervision of Dr. Tomer Shlomi in the Computer Science Department.

Many thanks to Dr. Tomer Shlomi for his vast support, help and encouragement, that made this work possible.

The generous financial support of the Technion is gratefully acknowledged.
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Abstract

Identifying the factors that determine microbial growth rate under various environmental and genetic conditions is a major challenge of systems biology. While current genome-scale metabolic modeling approaches enable to successfully predict a variety of metabolic phenotypes, including maximal biomass yield, the prediction of actual growth rate is a long standing goal that is currently beyond reach. This gap stems from strictly relying on data regarding enzyme stoichiometry and directionality, without accounting for enzyme kinetic considerations. Here, we present a novel constraint-based modeling method, MetabOlic Modeling with ENzyme kineTics (MOMENT), which predicts cellular metabolic state and growth rate by utilizing prior data on enzyme turnover rates and molecular weights. Both enzyme turnover rates and molecular weights are shown to be significantly correlated with measured metabolic flux under various conditions, testifying for the importance of these parameters in metabolic flux analysis. Extending upon previous attempts to utilize kinetic data in genome-scale metabolic modeling, our approach takes into account enzyme concentration requirements for catalyzing metabolic flux, considering isozymes, protein complexes, and multi-functional enzymes. Applied to the prediction of growth rate of *E. coli* across a set of 24 different media, MOMENT’s predictions are shown to significantly correlate with measurements, while existing state-of-the art metabolic modeling approaches fail to do so. MOMENT is further shown to significantly improve the prediction accuracy of various metabolic phenotypes in *E. coli*, including intracellular flux rates and differential gene expression levels across growth rates.
# Abbreviations and Notations

| Abbreviation | Definition                                      |
|--------------|-------------------------------------------------|
| kcat         | - Enzyme turnover number                        |
| Mw           | - Molecular weight                              |
| Mv           | - Molecular Volume                              |
| CBM          | - Constraint-based modeling                     |
| FBA          | - Flux balance analysis                         |
| FBA\text{wMC} | - Flux balance analysis with molecular crowding |
| MOMENT       | - MetabOlic Modeling with ENzyme kinetIcs       |
1 Introduction

1.1 Modeling Cellular Metabolism

Metabolism is the set of biochemical reactions occurring in living organisms to maintain life. These reactions allow cells to perform their vital functions such as harvesting energy while breaking down organic matter and constructing cells’ components, allowing the cells to grow and reproduce. The field of biotechnology engineering has much to gain from metabolic knowledge due to the role of the metabolic process in the production of industrially important nutrients (Durot, Bourguignon et al. 2009).

Chemical reactions convert several metabolites (organic molecules) called reactants (or substrate) into one or several other metabolites. The majority of the biochemical reactions are catalyzed by specific proteins called enzymes. The collection of these reactions forms a complex metabolic network involving thousands of interconnected reactions catalyzed by different enzymes, which require a system-level approach in order to analyze them (Price, Reed et al. 2004). Up to date, more than 50 metabolic reconstructions have been published (Duarte, Herrgard et al. 2004; Feist and Palsson 2008), spanning over the three domains of life: Eukaryote, Bacteria, and Archaea. These include, mammalian such as a mouse and a human, the model plant Arabidopsis, dozens of bacterial and yeast species, various pathogens, and industrially relevant organisms. Since the rate of model reconstructions has lagged far behind the rate of genome sequencing, recent attempts have been made to develop computational methods to automatically reconstruct metabolic network models for additional organisms. For example, a recently presented computational framework for network reconstruction called Model SEED (Henry, DeJongh et al.)
integrates existing methods in an automatic pipeline for nearly every step in the model's reconstruction process. Model SEED was already applied to produce functioning models for a diverse set of 140 organisms across 14 bacterial subdivisions.

Traditional metabolic network modeling techniques involved the reconstruction of kinetic models based on detailed knowledge on enzyme kinetic parameters for all enzymes in a certain system. These kinetic models were limited to small-scale systems due to lack of sufficient data on kinetic constants and the highly complex nature of these models. An alternative approach called Constraint-Based Modeling (CBM) predicts cellular metabolic phenotypes in microorganisms on a genome-scale by relying solely on simple physical-chemical constraints (without requiring enzyme kinetic data) (Price, Papin et al. 2003). This modeling approach, described in the next section, is the main focus of the metabolic study presented in this thesis. CBM is very commonly used for the prediction of metabolic phenotype prediction (Guldberg, Rey et al. 1998), metabolic engineering (Pharkya, Burgard et al. 2004), network evolution studies (Fong, Joyce et al. 2005), and biomedical applications (Apic, Ignjatovic et al. 2005).
1.2 Constraint-Based Modeling

The nature of a metabolic network with its large scale and high interconnectivity requires a way to interpret this information in a systematic, computational manner. One common way to interpret this data in order to predict cellular metabolic phenotypes is Constraint-Based Modeling (CBM). CBM imposes a set of simple physical-chemical constraints on the possible metabolic behaviors and allows for the filtering of behaviors that are not biologically feasible.

CBM searches for a flux distribution, or a flux vector, (denoted here as $v \in R^m$) describing the flux rate (in units of mmol/g_DW/h) for each reaction in the model. This flux vector should satisfy various constraints represented as a set of linear equations as following:

1. Mass balance – assuming the cell metabolism is in a steady state, meaning there is no accumulation or depletion of metabolites within the metabolic network, or in other words, the production rate and the consumption rate of each metabolite in the network are equal. Relying on the stoichiometric matrix $S \ (N \times M)$ which is composed of $N$ metabolites and $M$ reactions ($S_{ij}$ corresponds to the stoichiometric coefficient of metabolite $i$ in reaction $j$) (Figure 1), the mass balance constraint can be enforced by the equation:

$$ (i) \quad S \cdot v = 0 $$

The set of flux distributions satisfying this constraint spans a sub-space of $R^m$. 
Figure 1 – Formal representation of a metabolic network model: A schematic illustration of a metabolic network. The nodes represent metabolites and the edges represent the reactions. The stoichiometric matrix (S) contains the same information in a mathematical form: for example, in the figure, the entries show that in reaction (r) 1, one molecule each of red and green combine (are consumed) to form one molecule of red–green product. Figure from (Breitling, Vitkup et al. 2008).

2. Thermodynamic constraints – the directionality for many reactions is known due to thermodynamic consideration, therefore, the rate of the corresponding reactions is restricted.

3. Enzymatic rates – The rate of some enzyme can be estimated based on physiological data or experimental measurements, effecting the flux rate that can be carried by the corresponding reactions. The enzymes maximal rates are enforced by defining an upper bound on the reaction flux. These constraints as well as the above thermodynamic constraints are enforced based on the linear constraints:
(ii) \[ v_{lb} \leq v \leq v_{ub} \]

where \( v_{lb} \) and \( v_{ub} \) represent the lower and upper bounds vectors, respectively.

**Figure 2 - The conceptual basis of constraint-based modeling:** With no constraints, the flux distribution of a biological network may lie at any point in a solution space. When mass balance constraints imposed by the stoichiometric matrix \( S \) (labeled 1) and capacity constraints imposed by the lower and upper bounds \( (a_i, b_i) \) (labeled 2) are applied to a network, it defines an allowable solution space. The network may acquire any flux distribution within this space, but points outside this space are denied by the constraints. Through optimization of an objective function, optimization methods can identify a single optimal flux distribution that lies on the edge of the allowable solution space. Figure from (Orth, Thiele et al.).

The set of linear equations is used in CBM to define the feasible metabolic collection of flux distribution, allowing an efficient exploration of the solution space via standard linear programming (LP) solvers. The analysis of a convex solution space obtained with the constraints described above is commonly done in two major approaches: (i) Characterizing the solution space – since CBM does not strive to find a single solution but rather finds a collection of feasible flux distributions, several methods were developed to characterize this solution space. For example Flux Variability Analysis (FVA) is used to determine the possible flux range of each reaction within the solution space by formulating an LP problem in order to minimize
and maximize the flux through each reaction independently (Mahadevan and Schilling 2003). (ii) Optimization methods – looking for an optimal flux distribution under different optimization criteria. For example, Flux Balance Analysis (FBA) is the most widely studied CBM method (Varma and Palsson 1994; Kauffman, Prakash et al. 2003) which searches for the steady state flux distribution that maximizes a certain linear objective function (Figure 2). In microorganisms, FBA assumes maximum biomass production rate as the optimization criterion.
1.3 The Prediction of Biomass Yield and Growth Rate

Flux Balance Analysis (FBA) is a commonly used CBM approach that enables to predict biomass production yield (in units of gram biomass/gram nutrient) based solely on reactions' stoichiometry and directionality. The prediction of biomass yield works by searching for a feasible flux distribution with maximal flux through the growth reaction, considering an arbitrary upper bound on the uptake rate of the carbon nutrient. The maximal biomass production rate predicted by FBA reflects optimal yield metabolism and is equal to the assumed uptake rate multiplied by maximal biomass yield. The prediction of growth rate by FBA is possible only when experimental measurement of nutrient uptake rates is available and is used to constrain the uptake flux in the model (or alternatively, by multiplying FBA-predicted biomass yield with the measured uptake rate). A major computational challenge is the prediction of growth rate without measurements of nutrient uptake rate, enabling to anticipate growth capabilities under a variety of environmental and genetic conditions. Notably, even in growth conditions where nutrient uptake rates are known, FBA may predict unrealistically high growth rates due to inefficient metabolism, such as that of over-flow metabolism (Vemuri, Altman et al. 2006). Predicting the correct growth rate in such cases is another computational challenge.

FBA with Molecular Crowding (FBAwMC) was previously suggested to address the challenge of predicting growth rates without requiring measured nutrient uptake rates (Beg, Vazquez et al. 2007). This was achieved by utilizing data on enzyme turnover numbers (i.e. kcat) to constrain metabolic flux based on an estimated upper bound on the total cellular volume of metabolic enzymes. It enabled prediction of growth rates of *E.coli* across a limited set of growth media, as well as under conditions of over-flow metabolism (Beg, Vazquez et al. 2007; Vazquez, Beg et al.
2008). A recent study has utilized a variant of this approach to predict inefficient metabolism in cancer cells, in accordance with the Warburg effect (Shlomi, Benyamini et al. 2011).
1.4 MetabOlic Modeling with ENzyme kineTics (MOMENT)

In this thesis, we present a method, MetabOlic Modeling with ENzyme kineTics (MOMENT), for predicting metabolic fluxes and growth rates by accounting for the maximal cellular capacity for metabolic enzymes. Extending upon FBAwMC, MOMENT accurately quantifies the enzymatic requirements for catalyzing each metabolic reaction based on known kinetic constants, accounting for isozymes, protein complexes and multi-functional enzymes. MOMENT is shown to successfully predict growth rates for *E.coli* under a diverse set of growth media without requiring measured nutrient uptake rates, significantly outperforming the prediction accuracy of FBAwMC. Furthermore, MOMENT is shown to markedly improve the prediction performance of various metabolic phenotypes, including metabolic fluxes and expression level of metabolic genes. To support our usage of existing enzyme kinetic data to enhance flux prediction, we begin the analysis by exploring the relation between enzyme kinetic parameters and measured metabolic flux, showing that enzyme turnover numbers and molecular weight are highly correlated with measured fluxes under various conditions.
2 Results

2.1 The Evolution of Enzyme Kinetic Parameters Optimizes Metabolic Flux

To investigate the relation between enzyme kinetic parameters in *E. coli* and typical flux rate across growth conditions, we extracted measurements of both from several databases and the literature available. Data on enzyme kinetic parameters was extracted and filtered from BRENDA (Pharkya, Nikolaev et al. 2003) and SABIO-RK (Wittig, Golebiewski et al. 2006) databases based on Enzyme Commission (EC) numbers and reactant names in the *E.coli* metabolic model by Feist et al (Feist, Henry et al. 2007). Measured turnover rates for mutated enzymes were filtered out. When multiple turnover numbers were available for a certain enzyme, the median value was chosen. The extracted data included enzyme turnover numbers for 251 reactions and binding affinities for 488 metabolites. For reactions and metabolites in *E.coli* for which no kinetic parameters were found, the corresponding parameters from other species were obtained, yielding a total set of 513 turnover numbers and 798 metabolites' binding affinities. Enzyme molecular weights were computed based on genomic sequences, extracted from KEGG (Kanehisa, Goto et al. 2010). Measured metabolic fluxes under various growth rates in glucose minimal media for several dozen enzymes were obtained from Ishii et al. (in glucose-limited chemostat cultures) (Ishii, Nakahigashi et al. 2007) and Schuetz et al. (in unlimited batch growth on glucose under aerobic conditions) (Schuetz, Kuepfer et al. 2007). To expand the measured fluxes to infer global flux distributions, we utilized the genome-scale metabolic network model of *E.coli* by Feist et al.(Feist, Henry et al. 2007), based on standard quadratic programming optimization to fit the predicted fluxes to measured
ones (by minimizing the L2 norm between the measured and predicted flux rates) (Shlomi, Berkman et al. 2005).

We found that enzymes catalyzing high flux reactions have high turnover number, with highly significant Pearson correlations of 0.45 ($p$-value = 7.8e-5) and 0.46 ($p$-value = 3.6e-5) between turnover rates and fluxes under conditions of low and high growth rates, respectively (Figure 3; considering base 10 log of both fluxes and turnover numbers). These observed correlations suggest that higher selection pressure for enzymatic efficiency (i.e. higher turnover rates) acts on enzymes carrying high flux reactions. Notably, our findings are in agreement with a recent report of central metabolic enzymes having higher turnover rates than secondary metabolic enzymes (Bar-Even, Noor et al. 2011). As enzymes catalyzing high flux reactions are more efficient in terms of having high turnover numbers, one would also expect such enzymes to have higher binding affinities to metabolic substrates. However, testing the correlation between the average binding affinity of metabolites for a certain enzyme and flux rates revealed no significant correlation ($p$-value = 0.24). A statistically significant Pearson correlation of 0.22 ($p$-value = 3.2e-5) was found between metabolic flux rates and enzyme molecular weights, indicating higher flux rates for enzymes with high molecular weights (Figure 3a). Interestingly, a simple regression model utilizing both enzyme turnover and molecular weight provided a Pearson correlation of 0.55 ($p$-value = 8.1e-7) with metabolic flux rates, suggesting that each parameter contributes independently to explaining flux rates (Figure 3a).

While enzyme kinetic parameters are scarcely used by genome-scale metabolic modeling approaches, gene expression data is commonly utilized as the basis for metabolic flux prediction (Akesson, Forster et al. 2004; Becker and Palsson 2008). To evaluate the correlation between gene expression and metabolic flux, in comparison to
that achievable for kinetic parameters, we obtained gene expression for *E.coli* (Dong, Kirchhof et al. 2008) measured under the very same growth conditions as the utilized measured fluxes (cultures of *E. coli* K-12 were grown aerobically at 37°C in triplicate in M63 minimal media with glucose.). we found a significantly lower Pearson correlation of 0.26 between gene expression and metabolic flux than that achieved between enzyme turnover numbers and flux rates. This is a remarkable result considering that both the gene expression and metabolic fluxes were measured under the very same conditions, while the kinetic parameters are constant characteristics of the enzymes across different growth conditions. Adding the gene expression data to the above described regression model provided an insignificant contribution to metabolic flux predictions (Figure 3a). This finding further highlights the importance of utilizing enzyme kinetic data as a prime data source for metabolic flux prediction.

Having shown that enzyme turnover numbers are significantly correlated with measured flux rates under glucose minimal media, we set to examine the correlations obtained under a diverse set of growth media. Towards this end, we applied FBA to predict likely flux distributions (with maximal biomass production yield) under a set of media finding that the average Pearson correlation across these media with the enzyme turnover numbers is 0.46 (Figure 4a). Next, we computed characteristic flux rates throughout all enzymes in the network by averaging their predicted flux across the same set of growth media (while setting the growth rate to experimental measurements described below). Interestingly, we find a Pearson correlation of 0.52 between the characteristic flux rates and the enzyme turnover numbers, which is higher than the average correlation obtained under specific media. This result suggests that enzyme turnover rates may potentially evolve to support efficient metabolism across multiple media. To explore whether metabolism is better tuned for a specific
growth environment, we compared the correlation between predicted fluxes and enzyme turnover numbers achieved for aerobic versus anaerobic conditions (with maximal biomass production yield; Figure 4b). We found that the correlation between predicted fluxes and enzyme turnover numbers is significantly higher in aerobic conditions (paired Wilcoxon test $p$-value = 3e-15), suggesting a potentially stronger selection pressure for efficient metabolism under aerobic conditions.

![Figure 3 - Kinetics parameters correlation with measured reaction flux rates](image)

(a.) Correlations of kinetic parameters and gene expression with measured metabolic flux rates. Measured fluxes in *E.coli* under glucose minimal media in low and high growth rates were taken from Ishii et al (Ishii, Nakahigashi et al. 2007), and Schuetz et al data (Schuetz, Kuepfer et al. 2007), respectively. The ‘+’ signs represents which parameters are used in the linear regression model for predicting the measured fluxes. (b.) Enzyme turnover numbers correlate with measured metabolic flux rates in *E. coli* (both in log$_{10}$ scale); only reactions with turnover numbers measured in *E. coli* were considered. Linear regression line in red.
Figure 4 - *E. coli* enzyme turnover rate correlate with FBA-predicted flux rates across growth media: (a.) Histogram of Pearson correlations between enzyme turnover numbers and predicted flux rates under different single substrate-limited media (in blue). The Pearson correlation between enzyme turnover numbers and the the averaged flux distribution (across media) is $R=0.52$ (shown in green), which is higher than those obtained under the different media. (b.) Pearson correlations between enzyme turnover numbers and predicted fluxes under a set of aerobic versus anaerobic media with different carbon sources. As shown, under most growth media, the correlation between enzyme turnover numbers and fluxes is higher when fluxes are predicted under aerobic conditions.
2.2 Utilizing Enzyme Kinetic Parameters within Genome-Scale Metabolic Modeling

The fact that enzyme turnover numbers and molecular weights are significantly correlated with metabolic flux rates suggests that the utilization of the latter data sources within metabolic modeling approaches may provide improved prediction accuracy of metabolic phenotypes. Towards this end, we developed a method called MetabOlic Modeling with ENzyme kineTics (MOMENT), which utilizes the kinetic parameters under the limitation of the total enzymatic pool available. Given a growth condition of interest, MOMENT predicts a flux distribution that satisfies stoichiometric mass-balance and enzyme directionality constraints, such that the total mass of enzymes required to catalyze the predicted flux is bounded by the total enzymatic mass. To achieve this, MOMENT is formulated as a linear programming optimization that jointly searches for a feasible flux distribution and for the required enzymatic mass. To formulate the dependencies between flux rate and the required enzymatic mass, MOMENT uses enzyme turnover numbers to compute an upper bound on enzyme concentrations required to catalyze the corresponding flux rates, and enzyme molecular weights to transform concentrations to units of mass. To account for isozymes, enzymatic complexes, and multi-functional enzymes, MOMENT makes use of detailed gene-to-reaction mapping that is commonly represented in CBM models via Boolean equations. For isozymes, the gene-to-reaction mapping denotes that the expression of one of several genes is required to catalyze a certain reaction, while for enzyme complexes, that the expression of several genes is jointly required. Notably, the entire set of gene-to-reaction mapping is formulated as part of the linear programming in a recursive manner, without requiring a more complex optimization such as mixed-integer linear programming that is
commonly used to model this mapping (Shlomi, Eisenberg et al. 2007; Kim and Reed 2010).
2.2.1 Implementation of MetabOlic Modeling with ENzyme kineTics (MOMENT)

Similarly to FBA, MOMENT searches for a feasible flux distribution vector \( v \) (mmol/g_{DW}/h), that satisfies mass-balance and reaction directionality constraints based on the following linear constraints:

\[
\begin{align*}
(i) & \quad S v = 0, \\
(ii) & \quad v_{lb} \leq v \leq v_{ub},
\end{align*}
\]

Where \( S \) denotes a stochiometric matrix \( S (N \times M) \) composed of \( N \) metabolites and \( M \) reactions \( S_{ij} \) corresponds to the stochiometric coefficient of metabolite \( i \) in reaction \( j \) and \( v_{lb} \) and \( v_{ub} \) represent known lower and upper bounds, respectively, on flux rates.

In addition to searching for a flux distribution, MOMENT searches for a vector of enzyme concentrations, denoted \( g \) (mmol/g_{DW}), such that each flux rate in \( v \) has a sufficiently high enzyme concentration to catalyze it. To associate flux rates with enzyme concentrations, we utilize the Boolean gene-to-reaction mapping that is included in the *E.coli* model of Feist et al (Feist, Henry et al. 2007), as follows:

1. For a reaction \( j \) catalyzed by single enzyme \( i \), we use the equation:

\[
(iii) \quad v_j \leq kcat_j \cdot g_i
\]

2. For a reaction \( j \) catalyzed by two isozymes \( a \) OR \( b \), we use the equation:

\[
(iv) \quad v_j \leq kcat_j \cdot (g_a + g_b)
\]

3. For a reaction \( j \) catalyzed by an enzyme complex consisting of gene products \( a \) AND \( b \), we use the equation:

\[
(v) \quad v_j \leq kcat_j \cdot \text{min}(g_a, g_b)
\]
This can be formulated in a linear equation by defining an auxiliary variable \( g_{a \& b} \) that is constrained to be smaller than both \( g_a \) and \( g_b \).

To account for more complex gene-to-reaction mappings, where multiple alternative enzyme complexes can catalyze a certain reaction, we applied the above rules recursively by adding auxiliary variables for AND and OR operators.

The enzymes solvent capacity constraint is formulated as:

\[
\sum g_i \cdot MW_i \leq C \left( \frac{\text{protein}}{g_{DW}} \right)
\]

where, \( MW_i \) denotes the molecular weight of protein coded by gene \( i \), and \( C \) denotes the total weight of proteins, which was assumed to be 56% out of the *E. coli* dry weight mass (Bremer 1996).

Maximization of biomass production was used as an objective function for MOMENT and FBAwMC simulations, while the maximization of biomass yield was used as an objective function for FBA simulations unless otherwise mentioned. The commercial solver CPLEX running on 64-bit Linux machines was used for solving the LP and QP problems in this study.
2.3 Predicting *E. coli*’s Growth Rate Across Growth Media

To evaluate MOMENT’s ability to predict microbial growth rates, *E. coli*’s growth rates of 24 single substrate-limited media were experimentally measured (Table 1; details of the experiment are elaborated upon in the following section) in collaboration with Matthias Heinemann’s lab (Biomolecular Sciences and Biotechnology Institute, University of Groningen), and compared the predicted and measured rates. The predictions were obtained by applying MOMENT on the genome-scale metabolic network model of *E. coli* by (Feist, Henry et al. 2007). Reactions for which no turnover number was available were assigned with the median turnover number across all reactions. we found that growth rate predictions obtained by MOMENT are significantly correlated with the measured ones, with a Pearson correlation of 0.47 (*p*-value = 0.02; Figure 5 and 6a). To evaluate the importance of the utilized enzyme turnover numbers, we repeated the growth rate predictions with randomly shuffled turnover numbers, which were found to provide significantly lower prediction accuracy (*p*-value = 0.026).

Testing the prediction performance of the previously developed FBAwMC, showed that the growth rate prediction achieved are not significantly correlated with the measured ones (*p*-value = 0.14). A moderate correlation between measured and predicted growth rates in *E. coli* was previously reported by Vazaquez et al., for a smaller set of 10 media. Applying FBA to predict biomass yield under these 24 media showed that the predictions are not significantly correlated with the measured growth rates (*p*-value = 0.14; Figure 5 and 6b). For example, FBA falsely predicts a two-fold higher biomass production yield per unit of maltose uptake versus glucose uptake, as maltose contains twice the number of carbons. In contrast, MOMENT predicts a lower growth rate under maltose in agreement with experimental measurements.
Notably, normalizing the biomass production yields predicted by FBA by the molecular weight of the carbon nutrient (i.e. representing biomass yield as gr biomass/gr nutrient) also did not lead to a significant correlation between FBA’s predicted biomass yield and the measured growth rates ($p$-value = 0.74).

**Figure 5 - Growth rate prediction:** The prediction of *E.coli* growth rate under 24 different minimal media based on MOMENT, FBAwMC, FBA, and MOMENT with random enzyme turnover rates. As shown, only MOMENT (with the true turnover rates) achieves a statistically significant Pearson correlation between predicted and measured growth rates ($p$-values are shown on top of each bar).
2.3.1 Growth rate determination

Strains and medium

The wild-type Escherichia coli K-12 strain BW25113 was used for all experiments. Cells were cultivated in M9-minimal medium with different carbon source added. The carbon sources were added so that the amount of reducible carbon equaled the amount present in a concentration of 2 g/L glucose. M9 minimal medium was prepared in the following way: To 700 mL of autoclaved, purified water, 200 mL of 5x base salt solution (211 mM Na2HPO4, 110 mM KH2PO4, 42.8 mM NaCl, 56.7 mM (NH4)2SO4, autoclaved), 10 mL of trace elements (0.63 mM ZnSO4, 0.7 mM CuCl2, 0.71 mM MnSO4, 0.76 mM CoCl2, autoclaved), 1 mL 0.1 M CaCl2 solution (autoclaved), 1 mL 1 M MgSO4 solution (autoclaved), 2 mL of 500x thiamine solution (1.4 mM, filter sterilized) and 0.6 mL 0.1 M FeCl3 solution (filter sterilized) were added. The resulting solution was filled up to 1 liter with water. Carbon source were added to the medium from stock solutions adjusted to pH 7. All media were filtrated prior to use (Steritop-GP 500mL, Millipore). All chemicals used were obtained from Sigma-Aldrich if not indicated otherwise.

Cultivation

The growth rates were determined using an automated cultivation device (Tecan Infinite 200 Pro plate reader). Cells were grown to a steady state in the respective growth medium in shake flasks, then washed twice in minimal medium. 4 μL of the washed cells were inoculated into a well on a 96-well plate (black, Greiner) with 196 μL of medium. The plate was covered with a transparent plastic cover and sealed with parafilm. Cultivation was done at the maximal linear shaking speed (160 min-1, 1 mm displacement) and the following settings for optical density (OD) measurement
(Interval time 5 min, shaking 4:42, reading (no shaking) 18 s; number of flashes 1; wavelength 600 nm, bandwidth 9 nm). The cells were grown to stationary phase or for at least 50 hours to ensure observation of steady state growth. The measured OD-values were corrected for the non-linearity of the device using an empirical function derived from samples with known OD-values (measured by spectrometry) from 10 to 0.001.

### Table 1 – Measured growth rates in 24 different single substrate-limited media:

Obtained from Matthias Heinemann’s lab (Biomolecular Sciences and Biotechnology Institute, University of Groningen).

| Carbon sources name      | Formula     | Molecular weight (g/mol) | Matthias max growth rate measured |
|--------------------------|-------------|--------------------------|------------------------------------|
| Acetate                  | 'C2H3O2'    | 59.0462                  | 0.29 ±0.02                         |
| N-acetylglucosamine      | 'C8H15NO6'  | 221.21024                | 0.61 ±0.03                         |
| Glycerol                 | 'C3H8O3'    | 92.09472                 | 0.47 ±0.03                         |
| Oxoglutarate             | 'C5H4O5'    | 144.08376                | 0.24 ±0.04                         |
| L-Alanine                | 'C3H7NO2'   | 89.09412                 | 0.24 ±0.03                         |
| Pyruvate                 | 'C3H3O3'    | 87.05502                 | 0.41 ±0.03                         |
| Fructose                 | 'C6H12O6'   | 180.15768                | 0.54 ±0.04                         |
| Guanosine                | 'C10H13N5O5' | 283.24392               | 0.37 ±0.03                         |
| Fumarate                 | 'C4H2O4'    | 114.05748                | 0.47 ±0.03                         |
| Ribose                   | 'C5H10O5'   | 150.1314                 | 0.41 ±0.01                         |
| Galactose                | 'C6H12O6'   | 180.15768                | 0.24 ±0.02                         |
| L-Lactate                | 'C3H5O3'    | 89.0709                  | 0.41 ±0.03                         |
| Gluconate                | 'C6H11O7'   | 195.14914                | 0.68 ±0.03                         |
| Sorbitol                 | 'C6H14O6'   | 182.17356                | 0.48 ±0.03                         |
| Sorbitol                 | 'C6H14NO5'  | 180.1809                 | 0.4 ±0.03                          |
| L-Malate                 | 'C4H4O5'    | 132.07276                | 0.55 ±0.03                         |
| Succinate                | 'C4H4O4'    | 116.07336                | 0.5 ±0.02                          |
| Glucose                  | 'C6H12O6'   | 180.15768                | 0.66 ±0.05                         |
| Maltose                  | 'C12H22O11' | 342.30008                | 0.52 ±0.02                         |
| Glucose 6-Phosp           | 'C6H11O9P'  | 258.121702               | 0.78 ±0.04                         |
| Mannitol                 | 'C6H14O6'   | 182.17356                | 0.61 ±0.03                         |
| Trehalose                | 'C12H22O11' | 342.30008                | 0.48 ±0.04                         |
| Mannose                  | 'C6H12O6'   | 180.15768                | 0.35 ±0.03                         |
| Xylose                   | 'C5H10O5'   | 150.1314                 | 0.51 ±0.03                         |
Figure 6 – MOMENT and FBA growth rate predictions capability: (a.) MOMENT predicted growth rates correlate with the measured growth rates. (b.) FBA predicted biomass yields correlate with the measured growth rates.
2.4 Predicting Metabolic Flux, Gene and Enzyme Expression Levels

To evaluate the performance of MOMENT in predicting intracellular fluxes, we obtained experimental flux measurements for 28 reactions in *E.coli* measured for unlimited batch growth on glucose under aerobic conditions (under exponential growth phase) by Schuetz et al (Schuetz, Kuepfer et al. 2007) and compared them with predicted fluxes. We found that flux predictions obtained by MOMENT achieve a Pearson correlation of 0.76 with the measured fluxes, significantly outperforming FBAwMC and FBA, which achieve correlations of 0.64 and 0.51, respectively. The utilization of the known enzyme turnover numbers, versus random sampling from the set of these parameters, was shown to significantly improve the prediction accuracy (Figure 7a).

As a further control, we tested an alternative objective function of maximizing ATP yield per sum of flux square, previously shown by Schuetz et al to improve flux prediction accuracy (Schuetz, Kuepfer et al. 2007). To maximize ATP yield per flux unit, \( \frac{v_{ATP}}{\sum v_i^2} \), as performed by Schuetz et al., we utilized the following quadratic optimization criteria:

\[
(i) \quad \frac{v_{ATP}}{v_{glucose}} - \epsilon \sum v_i^2
\]

where \( \epsilon \) represents a trade-off between ATP maximization and minimization of flux norm. To find an optimal solution, the analysis performed for a wide range of \( \epsilon \), and the solution maximizing ATP yield per flux unit had been chosen. We found that predictions obtained by the latter approach achieve a Pearson correlation of 0.68 with the measured fluxes, which is still markedly lower than MOMENT’s prediction accuracy (Figure 7a). Similar results were obtained when quantifying the performance
of the various prediction methods using the fidelity score presented in Schuetz et al. Specifically, the fidelity score for MOMENT was 0.014, while for an optimization of ATP yield per sum of flux square it was significantly worse, with a value of 0.16.

To further evaluate the performance of MOMENT in predicting enzymatic activity on a larger-scale, we extracted data on gene expression changes in *E.coli*, in batch cultivations under glucose minimal media, between low and high growth rates (in which over-flow metabolism occurs) (Veit, Polen et al. 2007), and compared it to predicted changes in enzyme concentrations. We found that changes in enzyme concentrations predicted by MOMENT (Figure 7b) between the low and high growth rates achieve a Pearson correlation of 0.32 ($p$-value = 3e-7) with the measured changes in gene expression. The performance of FBAwMC is significantly lower in this case (Figure 7b), with a Pearson correlation of 0.18 ($p$-value = 1e-3). FBA was not evaluated here as it predicts only biomass production yield, and hence cannot be applied to predict differential metabolism across growth rates.

As a further validation of the enzymes’ concentration prediction ability a quantitative measurement of over 50 proteins conducted by Ishii et al. (in glucose-limited chemostat cultures, at several different dilution rates) (Ishii, Nakahigashi et al. 2007) in multiple genetic and environmental perturbations was compared to MOMENT predictions. The different environmental conditions were simulated using MOMENT which resulted in a strong correlation (mean Pearson correlation of $R=0.62$) between the predicted and the measured enzymes concentrations that had a known $k_{cat}$ value (Figure 8), with a significant $p$ value for all the different conditions. Since the experiments were conducted with relatively low growth rates, FBAwMC and FBA predicted the same flux distribution for all the different conditions and therefore corresponded to the same gene product concentrations.
Figure 7 - Metabolic state prediction: (a.) The prediction of flux rates in *E.coli* under exponential growth phase at aerobic conditions based on MOMENT in comparison to FBAwMC, FBA, and MOMENT with random enzyme turnover rates. (b.) The prediction of differential gene expression in *E.coli* across different growth rates. *p* values are shown above each bar.

Figure 8 - Proteomics prediction: Mean Pearson correlation between proteomic measurements and the predicted one simulated under several growth conditions and gene knockouts for several dozen proteins (Ishii, Nakahigashi et al. 2007).
3 Discussion

The development of genome-scale metabolic modeling approaches that account for enzyme kinetic considerations is a major open challenge in metabolic modeling. Here, we provide further motivation for utilizing enzyme turnover numbers and molecular weights for metabolic modeling, showing that these characteristics of enzymes are significantly correlated with measured metabolic flux. Incorporating these parameters within MOMENT is shown to improve the prediction accuracy of several metabolic phenotypes including growth rates, intracellular fluxes, and gene expression of enzyme-coding genes.

MOMENT is implemented via standard linear programming and thus can be easily solved similarly to the commonly used FBA approach. The improved prediction performance of metabolic flux and growth rates suggests that the incorporation of MOMENT within computational methods for microbial metabolic engineering (e.g. OptKnock, RobustKnock, OptStrain(Burgard, Pharkya et al. 2003; Pharkya, Burgard et al. 2004; Tepper and Shlomi 2010), etc) may significantly contribute to optimal strain design.
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וני קלטר

ודיר גידול במיקרואורגניזמים

רשת מתכונתית בשילוב פרמטרים קינטיים

אנדימיטים

רוני אדרי
חיזוי קצבי גידול במיקרורגניזמים بواسطة
רשת מתבנית בשילוב פרמטרים קינטיים
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הנה<[הנהי]ף לпотенци המובנים – מכון מטלורא לתאני
2011
The author expresses his gratitude to Dr. Tomer Shulov for his guidance and assistance throughout his studies. Without his support, Tomer Shulov, in my opinion, would not have been able to complete his research.

I would like to thank Leonid Yatzek for his financial support during my studies. I also wish to acknowledge the assistance of Dr. Shulov, who provided me with valuable insights and encouragement throughout my work.

I hereby declare that the thesis is my own work and that it does not contain any material that has been published or submitted elsewhere. I also confirm that the research conducted in this thesis is my original work.

Tomer Shulov
תקציר

 førarez

mitevulit, איה המפריעים הביסוס ביצורי ביבילון. איה התהליכים המתרחשים ביצורים חיים, אותם המז_transaksiים ביצורים חיים, בהם ביצורים חיים, בהם בהתקはずים של חייתיות. מרכיבים נוספים של תהליך מטבוליזם, הם מהסביבה ויצורים ליצירת אנרגיה ובניית תאים. עיבוד חומרים ליצירת אנרגיה והבנה של Processes מובולם. מתוכם כל תהליך מתבצע על אלפים של אנזימים נוספים ויצורים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים ליצירת אנרגיה והבנה של תאים. עיבוד חומרים L

Technion - Computer Science Department - M.Sc. Thesis MSC-2011-14 - 2011
Over-flow metabolism

This work focuses on the development of kinetic models that are built upon metabolic and regulatory networks and are capable of predicting high and unrealistic growth rates.

The network of a given organism can be modeled using CBM, and the kinetic model of the network can be used to predict the growth rate of the organism. However, this model is only as good as the kinetic parameters that are used to build it. Therefore, efforts have been made to incorporate kinetic information into the model, which can improve the accuracy of the predictions.

The model is then used to predict the growth rate of the organism under different conditions, and the results are compared with experimental data. The model can also be used to predict the effect of different nutrients on the growth rate of the organism.

Over-flow metabolism is a phenomenon that is observed in many organisms, and it is characterized by the production of high amounts of metabolites that are not needed by the organism. This can lead to an increase in the growth rate, but it can also lead to the production of toxic compounds that can harm the organism.

In conclusion, the development of kinetic models is an important tool for understanding the metabolism of organisms and for predicting their growth rates. These models can be used to predict the effects of different nutrients and to optimize the growth of organisms in different environments.
Growth and productivity of E.coli through turnover numbers of metabolic pathways. The turnover numbers of enzymes in E.coli were found to be related to their molecular weight, based on this result. Turnover numbers and the weight of the enzymes contribute to the turnover numbers of the reactions, forming a high correlation in different environmental conditions.

The metabolic modeling with ENzyme kineTics (MOMENT) which was carried out under different conditions, found that the total weight of all enzymes is limited by the turnover numbers, minimizing the catalytic activity of enzymes. The reaction rates are calculated accurately based on the kinetic constants and multi-functional enzymes. The reaction rate of different metabolic pathways is calculated using the MOMENT method, which is not limited to the growth rate, but is calculated based on the input rate of the components of the growth media. The MOMENT method is also used to calculate the growth rate of E.coli, which is improved significantly by the MOMENT method. Moreover, the method allows the calculation of multi-environmental cases, in which the input rate of the components of the growth media is not known. The method also improves the calculation of phenotypes, such as turnover numbers and productivity of E.coli.

The MOMENT method is presented as a basis for existing metabolic engineering methods such as OptKnock, OptStrain, OptKnock, and RobustKnock. The method is presented as a powerful tool for metabolic engineering.