DFBA-LQR: An Optimal Control Approach to Flux Balance Analysis

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Systems biology is becoming an emerging field, as systems science is demonstrated as an essential tool in untangling biocomplexity. While various steady-state-based methods have been developed for study of metabolic networks, the study of the network dynamics is a less explored area, as it is inherently related to the regulatory network. The difficulty of regulatory network inference opens up the possibility of employing control techniques and methods for analysis of metabolic networks. In this work, the potential for application of optimal control theory to metabolic control problems is investigated. Considering that the regulatory network in essence is a cascade control structure, the proposed modeling approach relies on the postulate that evolution is a genetic optimization scheme leading to "optimal" regulatory networks. Thus the approach utilizes a modified linear quadratic regulator formulation to evaluate the optimal regulatory network for a given flux model. Utilizing a homeostasis-type objective, the proposed dynamic flux balance analysis with linear quadratic regulator (DFBA-LQR) approach enables constructing dynamic flux balance analysis models and produces analytical descriptions of the responses of the metabolic fluxes to disturbances. The analytical description can also be used to calculate the flux control coefficients and linearized reaction kinetics for the system. The application potential of the approach is demonstrated by a sample problem of modeling of lipid accumulation in rat hepatocytes cultures.

Introduction

Biological systems are extremely complicated. Each of the individual processes taking place in the metabolism is often regulated by multiple control loops in a heavily interconnected regulatory network that is currently far from being completely elucidated. While this complex structure gives the system its robustness and adaptability, it also presents a major difficulty for bioengineering purposes in two distinct ways. First, biocomplexity is difficult to understand and model, which is certainly a prime necessity for engineering a system.[1,2] Second, the regulatory network either reduces or negates the effects of straightforward ad hoc manipulations that are performed without a thorough understanding of how organisms react to forced changes.[3,4]

One intriguing idea for modeling biosystems is the principle of optimality. In addition to its uses in evolutionary biology, this approach is also utilized for cellular and metabolic modeling. Cybernetic modeling[5,6] assumes that the cell is an optimal resource allocator and has been successfully applied in various studies.[7−10] On the other hand, flux balance analysis (FBA) models, which are based on reaction stoichiometry of the metabolic reactions, have been particularly successful in modeling simpler organisms, such as *E. coli*, by assuming maximum biomass formation in a variety of conditions.[11−13] One advantage of flux models is that they are relatively easier to construct, as they are based solely on reaction stoichiometry, though they may require more information (thermodynamic, regulatory, and kinetic constraints) for obtaining acceptable accuracy.[14,15] A major advantage of such optimization-based approaches is that they are not strictly limited to interpolation, as is the case with most reductionist enzyme kinetics studies. The FBA models have even been demonstrated to be successful in prediction of adaptive evolution.[12] A thorough review of FBA studies has been presented by Kaufmann et al.[14]

Remarkably, these optimization-based studies have provided valuable insights into the regulatory mechanisms and strategies.[16] The fundamental idea in regulatory network inference with FBA is to identify multiple optimal solutions for a metabolic flux model[17] and elucidate the reasons and mechanisms that lead to the observed phenotype (i.e., the particular solution chosen by the cells[16]). It should be noted that this is a steady-state analysis of an inherently dynamic control system, and the reason for having multiple optimal solutions is that the actual problem is an initial value problem where the control system determines the final steady-state solution. The regulatory schemes identified with steady-state methods (either through FBA or other network inference techniques[18]), therefore, provide steady-state controller gains that are not unique. Also note that ignoring dynamics renders it impossible to identify or analyze the complete control system.[19] Proportional (P) control and proportional-integral (PI) control may give rise to very similar steady-state solutions, but the system dynamic behavior may be very different and sensitive to different factors. For a biological system, this would mean different optimal genetic manipulation targets, different causes for metabolic diseases, and corresponding therapeutic schemes.

The interest in using FBA methods to model unknown dynamic characteristics in metabolic flux models is a more recent trend.[13,20,21] Mahadevan et al.[13] extended FBA to the dynamics of metabolism as dynamic flux balance analysis (DFBA). This approach utilizes stoichiometric information about the metabolic reactions to calculate temporal flux profiles so that a metabolic objective is achieved. However, there are a number of limitations, including the possible necessity of kinetics of critical reactions for the models. In the study of Mahadevan et al.,[13] this is the kinetics of glucose uptake, which for *E. coli* in substrate-limited growth is essential in limiting and determining the growth and metabolic activity. Another issue is that the numerical solution approaches employed do not produce a closed form solution for the actual reaction kinetics, although the reaction dynamics are in fact predicted.

The FBA models present an interesting opportunity for development of methodologies based on process control techniques. The linear representation developed for metabolic flux modeling bears a strong similarity to state-space modeling, where the concentrations of biochemicals included in the model

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can be interpreted as the states and the reaction rates as the control variables, which are regulated by a complex regulatory network that can be considered as a state-feedback controller. Based on this premise, the applicability of optimal control to DFBA is investigated in this work. To achieve this, a metabolic flux model is combined with an optimal regulatory network that is based on a modified linear quadratic regulator (LQR) formulation to predict system transient responses. For demonstration purposes, the DFBA-LQR approach is applied to the modeling of fatty acid metabolism in rat hepatocytes cultures, with the aim of predicting the dynamics of lipid accumulation in these primary liver cell cultures when exposed to plasma.

Background

The underlying idea in metabolic flux analysis is to describe steady-state mass balance on the metabolites in the system in terms of the involved reaction rates (fluxes), i.e.,

$$\dot{\mathbf{z}} = \mathbf{M}\cdot\mathbf{v} = \mathbf{0} \tag{1}$$

where $\mathbf{z}$ is the vector of n metabolites, $\mathbf{v}$ is the vector of m fluxes (which can be considered as a group of individual reaction rates), and $\mathbf{M}$ is the $n \times m$ matrix of stoichiometric coefficients of the metabolites in reactions. Generally, there are more fluxes than metabolites, i.e., $m > n$. If some of the fluxes are measured, then the rest can be calculated by separating the flux vector to the measured and the unknown fluxes. Rearranging eq 1 gives

$$-\mathbf{M}_m\cdot\mathbf{v}_m = \mathbf{M}_u\cdot\mathbf{v}_u \tag{2}$$

where $\mathbf{v}_m$ and $\mathbf{v}_u$ are, respectively, the measured and the unknown fluxes. Sufficient measurements are necessary for solution. The measurements must be selected so as to make the right-hand side of eq 2 of full rank. With MFA, not only the number of measurements is reduced but also some of the difficult measurements (such as intracellular fluxes) can be avoided.[22]

Flux Balance Analysis. In the absence of actual data, flux distribution can be predicted through solving the following optimization problem, with the assumption that the cell maximizes a certain metabolic objective:

$$\max_{\mathbf{v}} I = f(\mathbf{v}) \tag{3}$$

s.t.

$$\mathbf{M}\cdot\mathbf{v} = \mathbf{0} \tag{4}$$

$$\mathbf{v}^{\min} \le \mathbf{v} \le \mathbf{v}^{\max} \tag{5}$$

A variety of alternative objectives have been suggested. Growth maximization is well accepted as a valid objective for *E. coli*, and it is the most common form.[14] This creates a linear objective function with fluxes corresponding to biomass formation in it. A linear objective function, which results in a linear programming (LP) problem, is the most common form of FBA, although other forms exist.[14] Also, particularly with a linear objective function, the constraints in eq 5 gain critical importance, and the accuracy of analysis critically depends on the accuracy of the information about flux limitations.[15]

However, FBA has not been very successful with mammalian cells. Two reasons can be proposed to explain this: (i) The metabolic objectives are not clear. For instance, the growth maximization is generally not a very reasonable assumption for mammalian cells which display a quite limited proliferation capacity. (ii) Linear FBA models critically rely on the constraints for accurate prediction. Such information is relatively easy to acquire for well studied organisms such as *E. coli* grown on simple culture mediums (e.g. glucose only medium, where substrate uptake is the only really critical data). However, acquiring such information is more difficult for mammalian cells given the very complex culture media used (typically containing amino acids, lipids, vitamins, serum, etc.). In these cultures, a large number of measurements may be necessary, to the point that direct application of MFA becomes more reasonable.

When it can be solved, the FBA problem forms an in silico model that can be used to predict the response of the cells to a variety of changes, either genetic or environmental, and identify certain regulatory motifs. FBA has been used for a variety of other biosystems engineering purposes.[11−18,23]

Another method worth mentioning is the energy balance analysis (EBA), which includes the analysis based on thermodynamic energy balance, in addition to the mass balance.[24,25] With EBA, the FBA model accuracy can be improved as thermodynamically infeasible solutions can be eliminated. More interestingly, since energy balances include metabolite concentrations as variables, it is possible to calculate corresponding metabolite concentrations, an evaluation not possible with pure MFA or FBA approaches.

Dynamic Flux Balance Analysis. DFBA is a problem similar to FBA, but the metabolite concentrations cannot be ignored as the steady-state assumption is revoked. The general DFBA problem formulation is delineated below.

$$\max_{\mathbf{v}(t),\mathbf{z}(t)} I = f(\mathbf{v},\mathbf{z}) \tag{6}$$

s.t.

$$\dot{\mathbf{z}} = \mathbf{M}\cdot\mathbf{v} \tag{7}$$

$$\mathbf{v}^{\min} \le \mathbf{v} \le \mathbf{v}^{\max} \tag{8}$$

$$\mathbf{z}(0) = \mathbf{z}_0, \mathbf{v}(0) = \mathbf{v}_0 \tag{9}$$

Note that in DFBA, an initial condition is necessary for the flux vector. As there is no metabolite term on the right-hand side of eq 7, initial metabolite values are not strictly necessary, and the initial metabolite concentrations can be arbitrarily set to zero. A nonzero final value would then indicate the net difference between the initial and the final metabolite concentrations. However, if the metabolic objective or flux constraint calculations require absolute metabolite concentrations, then this information may be necessary.

It is possible to have different objective functions in DFBA, including short-term (i.e., instantaneous) and long-term (i.e. time integrated) forms. Several alternative objective types (short-term and long-term growth maximization) were tested for *E. coli*.[13] It was observed that the instantaneous objective was clearly more successful. Long-term objectives were successfully applied by Dhurjati et al.[9] in cybernetic modeling, though were later abandoned in favor of the short-term optimal resource allocation formulation, due to computational ease and its ability to handle diauxic behavior.[7,8]

Metabolic Control Analysis. Metabolic control analysis (MCA) is a framework for the elucidation of the parameters in the control of flux so that the rational modification of metabolic fluxes becomes possible. The concept was developed from the ideas laid out by Kacser and Burns[26] and Heinrich and Rapoport.[27] In essence, MCA is a collection of methodologies

for calculating each of the flux control coefficients (FCCs) defined as

$$C_j^{J_k} = \frac{E_j}{J_k}\frac{\mathrm{d}J_k}{\mathrm{d}E_j} \tag{10}$$

where E_j is the activity of the enzyme governing reaction j and J_k is the steady-state flux of reaction k (the steady-state flux is identified separately as the reaction rate and the flux can mean different things when considering perturbations[22]). Essentially, an FCC is the change in the steady-state flux when a certain enzyme is perturbed. Thus, evaluation of these parameters is important in determination of the optimal engineering paths for the system studied. From a control perspective, FCCs are very similar to the steady-state controller gain. In fact, they are not constant as they change with time and system state. The FCCs can be further broken down to elasticity coefficients (ECs) and concentration control coefficients (CCCs) as follows

$$C_j^{J_k} = \pi_j^k + \sum_{i=1}^{n} \epsilon_{z_i}^k C_j^{z_i} \tag{11}$$

where

$$\epsilon_{z_i}^k = \frac{z_i}{J_k}\frac{\partial J_k}{\partial z_i} \tag{12}$$

$$C_j^{z_i} = \frac{E_j}{z_i}\frac{\partial z_i}{\partial E_j} \tag{13}$$

$$\pi_j^k = \frac{E_j}{J_k}\frac{\partial J_k}{\partial E_j} \tag{14}$$

where $\epsilon_{z_i}^k$ is the elasticity coefficient, which is the sensitivity of the kth flux with respect to the concentration of metabolite z_i; $C_j^{z_i}$ is the concentration control coefficient, which is the sensitivity of concentration of metabolite i with respect to enzyme activity j; and π_j^k is the parameter elasticity coefficient for flux k and enzyme j.

In general, π_j^k forms a diagonal matrix (1 if $j = k$; 0 otherwise). The elasticity coefficients are considered the critical variables, as they are directly related to the elusive enzyme kinetics. Further, once the ECs are determined, the CCCs and FCCs can be calculated through the connectivity and summation theorems of MCA. Evaluation of elasticity coefficients generally requires experimental data, either as perturbation experiments or time series data. A detailed introduction to MCA can be found in Stephanopoulos et al.,[22] and Rao et al.[28] present a good outline of connections between MCA and control theory.

DFBA − A Linear Quadratic Regulator Approach

The approach to be investigated here is the application of optimal control to DFBA problems. To apply optimal control methods, particularly the convenient linear quadratic regulator form, it is desirable to use a quadratic homeostasis type of objective function (i.e., preserving a nominal state by minimizing the deviations from this nominal state). There is sufficient empirical support for this type of objective. The responses of perturbed metabolic networks such as genetically engineered knock-out strains are found to be significantly better captured by the homeostasis objective.[29] Note that these modified strains have not been exposed to long-term evolutionary pressure; in the long term, *E. coli* was demonstrated to evolve to maximize its growth.[12,29] These results support the idea that homeostasis is a valid objective for the short-term responses. Considering that control of metabolism generally takes place within seconds, an assumption of homeostasis during short-term disturbance rejection periods appears reasonable. Optimal control of homeostasis has also been considered by Giuseppin and van Riel,[21] although control tuning is determined either heuristically or based on data fitting.

Another issue is the modeling of mammalian cells. Formulation of a traditional FBA objective for mammalian cells has proven difficult.[30] However, Uygun et al.[31] recently performed an objective identification study for cultured rat hepatocytes (primary liver cells), where the cellular objective identified suggests homeostasis is a possible goal. The homeostasis objective is particularly reasonable in the view of the results of Segre et al.,[29] since mammalian cells in general do not proliferate fast enough for evolution to be a significant factor in determining their culture behavior.

Assumptions. In modeling, the following four assumptions are made:

(1) The results are confined to the cases where homeostasis is a valid objective, which postulates that the cells strive to reach and/or maintain a nominal status that corresponds to an optimal level of its resources and activities.

(2) The analysis is for the control of metabolic reactions only; other signaling mechanisms or evolutionary changes are not considered. Essentially, it is assumed that the cellular regulatory network can be conceptually decoupled into multiple levels, with the quick metabolic regulation operating on a balancing mode, whereas the organism-wide regulation (such as the hormone mechanisms) or long-term changes (such as evolution) may have other objectives or effects. This decoupling enables representing the metabolic regulation as a feedback control scheme that aims to meet specified set points. The set points may be manipulated by a higher level of regulation (such as hormonal regulation) or altered by evolution.

(3) The external pool of metabolites does not affect the pathway kinetics; they are controlled at a steady-state level. This is a common assumption in MCA.[22]

(4) Metabolites are homogeneously distributed in the system, or the metabolite transport dynamics are much faster than the regulation dynamics; hence, they are negligible. This is another common assumption in MCA.[22]

DFBA Model Formulation. With a homeostasis objective, small deviations from the nominal, in the face of external disturbances, would be tolerable, especially on a short-term basis. As the magnitude and time interval of the deviation increases, the system would assign more and more importance to the deviation, exerting an increased effort to correct the deviation. A proper mathematical way of representing such behavior is to penalize these deviations as augmented penalty terms in the metabolic objective, which leads to a very convenient linear quadratic regulator formulation. It can be, therefore, postulated that the objective of metabolic regulation is appropriately captured by the following DFBA model formulation.

$$\min_{\mathbf{v}(t)} I = \frac{1}{2}\int_0^{\infty} [(\mathbf{z}-\mathbf{z}^n)^T\cdot\mathbf{Q}\cdot(\mathbf{z}-\mathbf{z}^n) + (\mathbf{v}-\mathbf{v}^n)^T\cdot\mathbf{R}_\alpha\cdot (\mathbf{v}-\mathbf{v}^n) + \dot{\mathbf{v}}^T\cdot\mathbf{R}_\beta\cdot\dot{\mathbf{v}}]\mathrm{d}t \tag{15}$$

s.t.

$$\dot{\mathbf{z}} = \mathbf{M}\cdot\mathbf{v} \tag{16}$$

$$\mathbf{z}(0) = \mathbf{z}_0 \tag{17}$$

$$\mathbf{v}(0) = \mathbf{v}_0 \tag{18}$$

where $\mathbf{z}^n$ and $\mathbf{v}^n$ are, respectively, the metabolite concentrations and flux values at the nominal steady state; $\mathbf{Q}$ and $\mathbf{R}_\alpha$ are

positive semidefinite symmetric weight matrices; and $\mathbf{R}_\beta$ is a positive definite, symmetric weight matrix. The definiteness limitations on the matrices are required for solution existence. Also note that $\mathbf{R}_\alpha$ can be semidefinite, so that it is possible to set $\mathbf{R}_\alpha$ to 0. However $\mathbf{R}_\beta$ has to be positive definite, i.e., must have nonzero elements and must be of full rank. The coefficient, 1/2, is for convenience in the analytical solution. It is possible that nondiagonal elements may be employed to penalize opposing reactions (e.g., glycolysis vs gluconeogenesis) or factor in the isozymes, though at this point only diagonal weight matrices will be used.

The first term of the quadratic metabolic objective function in eq 15 reflects a goal of maintaining homeostasis of intracellular metabolite concentrations around a nominal metabolite concentration vector, and the deviations from this nominal state are penalized using the quadratic error form. The second term can be considered basically as homeostasis for enzyme concentrations (to be technically correct, enzyme activities, as allosteric control can directly modify catalytic capacity of the enzymes). Accordingly, this term extends the homeostasis assumption to the fluxes, hence stating that the cell will also have an optimum metabolic activity rate, $\mathbf{v}^n$. Note that the metabolic steady state is a dynamic equilibrium; without this term, the model would accept a solution where all metabolites reach their target concentrations, and then all metabolic activity entirely shuts down, which is clearly not the actual case.

If only these two terms are used in the objective, the solution will be a classical state-feedback proportional controller. One problem with this approach is that the control responses to a perturbation are discrete; to achieve this, the biological network would have to respond *instantaneously* to adjust enzyme activities and reaction rates.[32] In reality, there is always some delay, as the regulation mechanisms involve some intermediate reactions that take some time, even if very short. Depending on the number and nature of the reactions involved, this delay ranges from the near-instantaneous to hours or days. Genetic regulation, for instance, involves rather tedious transcription, translation, and post-translation processes, which is in the order of hours. As it is not practical to try to include all these individual processes in one model (considering most are still not fully elucidated), a more feasible alternative is to include a term to account for these delays. Rather than modifying the flux model itself with delay terms, an alternative way is to augment a penalty term to the flux changes. This last term, which does not appear in traditional LQR formulations, therefore requires that the manipulations of the fluxes are kept relatively small (in fact minimized, as long as it does not prevent reaching the nominal state). Hence, a flux with high penalty term $\mathbf{R}_\beta$ would be the one that is difficult to adjust and would have a slower response time.

Matrices $\mathbf{Q}$ and $\mathbf{R}_\alpha$ state the importance of each biochemical species to the system. Also, each weight in the objective function can be interpreted as the difficulty of change for the related variables. A smaller weight would enable the corresponding variable to change quickly and move away from the nominal values when necessary, with a certain amount of freedom in the short term. A large weight would force the system to keep the variable close to the nominal values, at the expense of other variables if necessary. Obviously, the protein conformation dynamics are the fastest. This will in turn affect the reaction rates that should hence be relatively slower to change. This in turn determines the changes in metabolite concentrations, which have the slowest dynamics relatively. Accordingly, it can be posed that the weights should be roughly inversely proportional to the average time scales (i.e. the characteristic times of the reactions) of the corresponding dynamics.

Solution. The dynamic optimization problem defined in eqs 15−18 can be solved using calculus of variations to obtain an analytical solution[33] (see the Appendix for detailed derivation). This solution, in terms of the original variables, yields the optimal control law as

$$\dot{\mathbf{v}} = -[\mathbf{K}_1 \;\; \mathbf{K}_2]\cdot\begin{bmatrix}\mathbf{z}-\mathbf{z}^n\\ \mathbf{v}-\mathbf{v}^n\end{bmatrix} \tag{19}$$

Equivalently, it can be stated

$$\mathbf{v}(t) = \mathbf{K}_\mathrm{P}\cdot(\mathbf{z}(t)-\mathbf{z}^n) + \mathbf{K}_\mathrm{I}\cdot\int_0^t(\mathbf{z}(t)-\mathbf{z}^n)\mathrm{d}t \tag{20}$$

where

$$\mathbf{K}_\mathrm{P} = -(\mathbf{K}_2\cdot\mathbf{M}^{-1}) \tag{21}$$

$$\mathbf{K}_\mathrm{I} = -\mathbf{K}_1 \tag{22}$$

The solution in eq 20 is a state-feedback proportional-integral control law, which smoothes the control movements and ensures zero steady-state bias for the metabolites (see ref 34 for a discussion of the importance of integral control for signaling pathways). It is important to note that the calculated gain matrices are only a function of the system and weight matrices in the objective function, and they remain constant even if different targets are set. In other words, it is a unique property for a system, independent of the disturbances/stimuli applied. As such, the identified control gain should be valid for all possible experiments. However, the identified controller (eq 20) *is* a function of the nominal state. Further, as it will be discussed in the following sections, it can be shown that eq 20 corresponds to a linearized form of a nonlinear kinetic equation. Therefore, it can be stated that the identified control description is a linearization around the nominal state; the linearization is not apparent explicitly but is inherent in the postulate of the homeostasis objective. Accordingly, eq 20 should be valid only within the range where homeostasis around the identified nominal state is a valid assumption.

Data Requirement. Two types of information are necessary for application of the method. The first type of information is the metabolic objective weights, which is necessary for evaluation of the optimal gain matrices. The second type is steady-state data about the initial and nominal conditions, i.e., the flux distribution and metabolite concentrations before and after the experiment, which are necessary for performing dynamic simulations. The initial values are necessary since the model in eqs 15−18 is an initial value problem. The final conditions are necessary since they should be specified as the nominal values for the system in the control description in eq 20. There are two ways to gather this information. With the data-based approach, the initial and nominal values can be obtained with experiments and MFA studies. The objective weights can be evaluated by a dynamic data fit problem when there is some additional time-series data. However, if a full EBA model is available, then this would enable calculation of both initial and nominal steady states in silico, without experimental data. The metabolic objectives in a FBA/EBA model may be used to obtain the objective weights, although steady-state information may not be sufficient for evaluating matrix $\mathbf{R}_\beta$.

DFBA and MCA. An intriguing connection exists between DFBA and MCA. In DFBA, the problem is finding a flux profile

$\mathbf{v} = \mathbf{v}(\mathbf{z}, t)$ so as to predict the system dynamics. In general, the solution is numerical,[11,13] and a closed form for the fluxes is not acquired, although this correlation is effectively evaluated. It seems there is a lack of effort on bridging this particular gap. However, in DFBA-LQR, eqs 19 and 20 provide a closed form for the flux profiles. Accordingly, if we take the derivative of $\mathbf{v}(t)$ in eq 20 with respect to $\mathbf{z}$, the elasticity coefficients can be calculated as follows (note that the integral error will be zero at a steady state):

$$\epsilon_{z_i}^k = \frac{z_i}{J_k} K_{Pk,i} \tag{23}$$

Hence, the ECs are correlated to the proportional gain calculated in DFBA-LQR. Once the optimal gain is calculated, and assuming the intracellular metabolite concentrations are known, the ECs can be calculated from eq 23. The FCCs and CCCs can then be determined through the connectivity and summation theorems as necessary.

This connection between DFBA-LQR and MCA is very useful, as MCA has been an accepted tool and ECs are considered as very important information for metabolic engineering studies.[22] Further, it shows a potential venue for direct calculation of the weights in the metabolic objective from MCA results.

Since there is a known connection between elasticity coefficients and kinetic coefficients, it is also possible to evaluate the apparent reaction kinetics based on the DFBA-LQR approach. Using power-law kinetics also used in the biological systems theory[35,36] yields

$$v_j = \alpha_j \prod_i z_i^{a_{ji}} \prod_k v_k^{b_{jk}} \tag{24}$$

where α_j is the apparent rate constant for reaction j, and a_{ji} and b_{ji} are the apparent kinetic orders for metabolites and enzymes, respectively. The MCA representation is equivalent to the linearized form of eq 20, and the apparent kinetic orders are equivalent to the respective ECs and CCCs. Consequently, the optimal gains calculated in the DFBA-LQR can be used to construct the apparent reaction kinetic descriptions. However, note that the power-law representation does not include a term correlated to the integral error part of the flux distribution in eq 23; hence, the equivalence of DFBA-LQR with BST and MCA in this regard only exists at steady state. Also note that similar to MCA, the identified kinetics is a linearized form of eq 24 around the nominal state; hence it has limited predictive ability.

Case Study—Fatty Acid Metabolism in Rat Hepatocytes

A particularly interesting target for metabolic modeling is the fatty acid metabolism in liver and its association with intracellular triglyceride accumulation. Triglyceride accumulation is a key characteristic of several liver diseases including hepatic steatosis, pre-eclampsia, and acute liver toxicity. Lipid accumulation (steatosis) in liver is observed to be a precursor to more serious pathological conditions,[37] and the risk of more serious pathologies correlates with the severity of steatosis. In addition, evidence shows that some individuals may be predisposed to develop fatty liver, suggesting that a specific metabolic defect may be involved in some cases. Furthermore, steatosis is a problem in bioartificial liver (BAL) studies, as the cultured hepatocytes are prone to reduced hepatic functionality and intracellular lipid accumulation,[38,39] and a direct correlation between the two has been observed.[38] Enhanced understanding of this problem may aid both in analysis of liver diseases and improvement of the efficiency of BAL devices.

To demonstrate its efficacy, the DFBA-LQR method is applied to study the fatty acid metabolism in hepatocytes. The metabolic pathway model developed by Lee et al.[40] and Chan et al.[41−43] is used as the base metabolic flux model. As lipid accumulation is of specific interest, a minor addition to the model is made. Triglyceride (TG) is separated to two metabolites: free TG and stored TG, where the free TG is assumed to be at steady state, and the observed lipid accumulation is compartmentalized as the stored TG flux. An extra flux is added for direct TG uptake/secretion that can be calculated now that free TG is assumed to be at steady state. As depicted in Figure 1 and listed in Tables 1 and 2, the model includes 46 metabolites and 77 fluxes in nine pathways, and it spans most of the fluxes related to lipid metabolism.

The objective weight matrices are selected based on the approximate relative relaxation times of the metabolite and enzyme dynamics, with metabolite changes considered roughly an order of magnitude slower than the reaction rates, which are in turn considered roughly an order of magnitude slower than the quick protein conformational changes which dictate the changes in the reaction rates,[18,22] such that $\mathbf{Q} = 10\mathbf{I}_{n\times n}$, $\mathbf{R}_\alpha = 1\mathbf{I}_{m\times m}$, and $\mathbf{R}_\beta = 0.1\mathbf{I}_{m\times m}$.

Figure 2 depicts the correlations in the evaluated optimal gain matrix. The entries are not surprising; each reaction is predicted to have a self-controlling mechanism (as can be observed in the diagonal negative entries in the right side in Figure 2). The other entries are such that products reduce reaction rates, and reactants have positive effects. Secondary correlations (i.e., smaller gain entries) are also observed: a reaction that produces the reactants has a small positive effect on the reaction, and a reaction that produces the same product has a negative effect. As discussed before, these entries correspond to the elasticity coefficients, and the trends observed in the optimal gain are as expected.

To test the findings of the DFBA-LQR method, a simulation of the dynamics of plasma exposure is performed, which corresponds to a standard hepatocyte culture experiment where the cells postisolation are preconditioned in culture medium and then exposed to plasma. This is also the typical configuration for a BAL operation. For the initial and nominal flux distributions, the experimental data by Chan et al.[41,42] are used (Specifically, the unsupplemented, high insulin preconditioning data are used. The contents of the DMEM and plasma mediums can be found elsewhere.[41]). In the absence of data, it is assumed that the initial and final intracellular metabolite concentrations are roughly equal, which is a reinforcement of the homeostasis assumption. For validation, the time response data of Matthew et al.[38] and Uygun et al.[44] are used. These two works share the same experimental setup with the work of Chan et al.[41−43] with the exception of plasma sources (rat and bovine vs human, respectively).

Figures 3−5 depict the model predictions versus data. The predictions demonstrate an excellent fit. All predictions are satisfactory when the data sources are consistent. A significant difference is observed in the initial FFA uptake values (Figure 5), which may have been caused by the difference in plasma sources. Note that the initial values are based on the data. Hence these differences are not caused by the DFBA-LQR dynamic predictions but are due to the differences in the source data. Perhaps more important is the match of trends between predictions and data in the short-term dynamics (first 24 h),

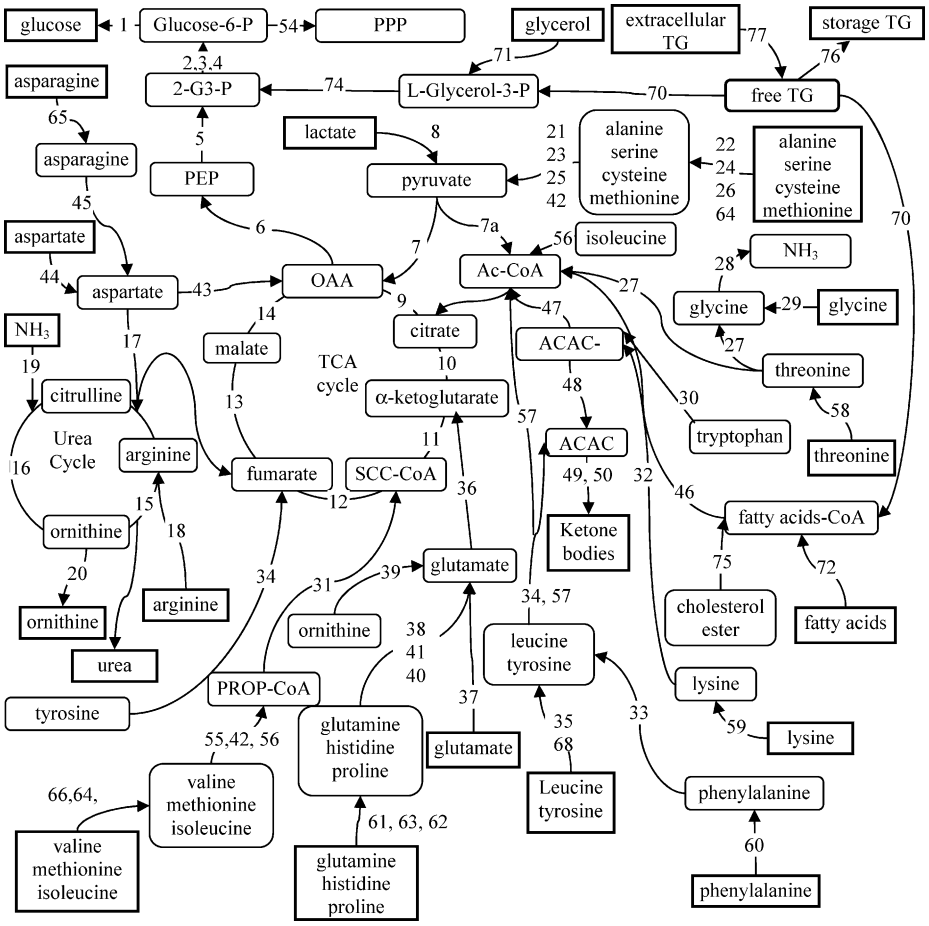

Figure 1. Metabolic network model for hepatocyte plasma cultures (Chan et al.[41]).

which is where the predictions of DFBA-LQR model are most relevant. The quick and well-dampened settling of β-hydroxybutyrate (Figure 3) and urea (Figure 4) to its final (plasma) values predicted coincide particularly well with the data, and the FFA uptake dynamics follow the same s-shaped second-order-like trend. It is also interesting to note that this nonoscillatory behavior is observed with the heuristic selection of the weight matrix, hence supporting the proposed correlation between relative relaxation times and the DFBA-LQR objective weights.

To further demonstrate the capabilities of the proposed approach, an in silico experiment is performed to predict the effects of elevated oxygen levels (19−60%, as described by Matthew et al.[38]) in the culture environment. This is achieved by including a disturbance term in the model such that an additional oxygen uptake is forced on the cells due to the supraphysiological oxygen concentrations. Since the oxygen concentration is increased by ∼200%, a disturbance term equivalent to twice the amount at the steady-state value reported[41,42] is added as a forced oxygen source to the model. That is

$$\dot{\mathbf{z}}_{\text{oxygen}} = \left(\sum_{j}^{m} \mathbf{M}_{\text{oxygen},j} \cdot \mathbf{v}_j\right) + 2\mathbf{v}^{n}_{\text{oxygen}} \qquad (25)$$

The nominal state values are kept the same as the previous values, as oxygen at the level of 19% appears to be close to the optimum for this culture environment.[38] Note that this analysis does not require a recalculation of the optimal gains.

Figures 6 and 7 describe the predictions of the model as compared to the data at 60% oxygen. The predictions demonstrate an excellent match both in terms of response times and the new steady-state values, providing empirical support that the MCA coefficients calculated by the DFBA-LQR would be indeed close to their actual values. Also note that, at this new steady state, the oxygen uptake fluxes have changed but are not tripled; the original and new (predicted) values are 15.9 and 23.0 μmol/day/million cells, respectively (see Table 3). The metabolic regulation compensates for the remainder, in particular by increasing the β-oxidation as observed in Figure 6, but is not capable of completely absorbing the disturbance. The increase in β-oxidation evidently reduces the carbon flux input to the urea cycle, causing significantly reduced urea synthesis (and hence reduced hepatic functionality), as can be observed in both the model predictions and the data in Figure 7. Note that while the flux control coefficients in the system have not been explicitly evaluated (in fact this is not possible since metabolite concentrations are unknown), this in silico experiment is equivalent to a dynamic MCA study. In addition to evaluating the postdisturbance steady state based on the DFBA-LQR gains that relay information equivalent to the flux control coefficients, the full dynamic transition period can also be simulated.

A Note on the Selection of Objective Weights. It should be noted that the heuristic selection of the objective weights is a current bottleneck for the application of the method. It is desirable to create correlations of the individual weights to other information such as relaxation times or failing that to identify appropriate weights from available dynamic data. To test the selection of the weights, an optimization study (i.e. a dynamic parameter fit problem where the weights in the objective are selected so as to minimize the difference between the time data in Figures 3−5 and the model predictions) is performed to

Table 1. Balanced Metabolites

1	glucose-6-phosphate
2	fructose-6-phosphate
3	fructose-1,6-bisphosphate
4	glyceraldehyde-3-phosphate
5	phosphoenolpyruvate (PEP)
6	pyruvate
7	oxaloacetate
8	nicotinamide adenine dinucleotide, reduced form (NADH)
9	acetyl-coenzyme A (CoA)
10	citrate
11	α-ketoglutarate
12	Succinyl-CoA
13	fumarate
14	flavin adenine dinucleotide, reduced form ($FADH_2$)
15	malate
16	ornithine
17	arginine
18	citrulline
19	aspartate
20	NH_4^+
21	alanine
22	serine
23	cysteine
24	glutamate
25	glycine
26	O_2
27	propionyl-CoA
28	acetoacetate
29	tyrosine
30	acetoacetyl-CoA
31	nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)
32	threonine
33	lysine
34	phenylalanine
35	glutamine
36	proline
37	histidine
38	methionine
39	asparagine
40	tryptophan
41	valine
42	isoleucine
44	glycerol
45	palmitate
46	triglycerides (TG)

determine the relative weights (only three free parameters used such that $\mathbf{Q} = p_1\mathbf{I}_{n\times n}$, $\mathbf{R}_\alpha = p_2\mathbf{I}_{m\times m}$, and $\mathbf{R}_\beta = p_3\mathbf{I}_{m\times m}$) that minimize prediction errors. It is observed that the error-minimizing weights are similar (6.1:1:0.18) to the heuristic selection, and the average prediction error does not decrease significantly if these weights are used instead of the heuristic selection. This indicates that the selected weights are reasonable, although this is a temporary solution and development of more rigorous objective identification methods for DFBA is necessary. Since the amount of time data is not sufficient to statistically analyze the data fit problem (only three out of 123 variables are observed), and in order to avoid complicating the study with the issues of the dynamic data fit problem, the heuristic selection of the weights (10:1:0.1) was used throughout the rest of the study.

The parameter fit described above led to two significant observations: (i) It may in fact be possible that the objective weights can actually be guessed/calculated based on relaxation times of the processes involved or similar general information, which would reduce or waive experimental data necessity for the model development. (ii) The sensitivity of the model predictions to the free parameters (objective weights) was fairly low. It was observed that as long as the correlation of $p_1 > p_2 > p_3$ was preserved, the same general trends did hold in simulated variables. This low sensitivity indicates that the model (i.e. optimal control of homeostasis with a state-feedback proportional-integral controller) is a good estimator of the situation. Further, it indicates that properties such as model sensitivity to measurement errors, propagation of errors in the model, and model accuracy will be mathematically "well behaved" and are unlikely to significantly complicate the usability of the method in the future.

Concluding Remarks

In this paper, a new DFBA-LQR framework is introduced for metabolic modeling of transitional responses in homeostasis problems. Based on the LQR formulation and flux models, the developed DFBA framework enables deducing analytic descriptions for the short-term metabolic regulation, and the calculated optimal gain is shown to correlate with the flux control coefficients in MCA. Further, as the developed model is fully defined, it can be used to predict the transitory responses of the system to certain changes (including environmental changes and potentially metabolic diseases), provided that the initial and nominal state conditions are available. The combination of computational ease in obtaining solutions and the scalability to large problems renders the DFBA-LQR method a promising technique for dynamic flux balance analysis and metabolic control analysis. The application of the proposed method to the hepatic lipid metabolism has demonstrated the potential of the approach.

From the methodology perspective, the first challenge in DFBA and other FBA studies are determination of the weights in the objective function. The heuristically selected weights are observed to be quite successful for the case study. A rigorous objective determination method, possibly similar to the objective identification procedure of Burgard and Maranas,[45] is necessary in the future.

It should be noted that the DFBA-LQR method has several limitations. The applicability is limited to cases where the homeostasis objective holds true or at least is a reasonable approximation. Similarly, the dynamic predictions based on the method require that the nominal state is accurate, which necessitates either a MFA or a FBA/EBA study. Also, the introduced method shares some limitations with MCA, as while the calculated optimal gain matrix can be used to evaluate the apparent reaction kinetics, these kinetics are linear approximations, hence having limited predictive capability beyond the point of normalization.

Another issue is the utilization of a long-term (i.e. involving time integral) type objective function. It can be easily argued that a cell intelligently choosing a long term goal (as opposed to an instantaneous objective maximization) is not plausible. However, given a number of possible alternative control mechanisms, evolution may choose a mechanism that is optimal in the long-term, time averaged control performance, over a mechanism that maximizes "instantaneous gratification"; and in fact, this is the primary postulate of this work. It should be noted that this is strictly an assumption.

The method introduced here essentially ignores the noise in the system, despite the fact that it is the inherent reality of the stochastic nature of the genetic regulation, and omitting this source of uncertainty may lead to significant errors in modeling.[46] It can be argued that in the face of measurement noise, the cell behavior may be modeled as a linear-quadratic gaussian regulator (LQG), where a Kalman filter is used to filter the noise in data from the signal.[47] Provided measurement noise covariance data can be acquired/estimated, extension of the LQR controller proposed here to a LQG type controller may be

Table 2. Reactions Included in the Model − a. Gluconeogenesis/Glycolysis, b. Lactate Metabolism, TCA, and Urea Cycles, c. Amino Acid Uptake and Catabolism, and d. Lipid Metabolism and Other Pathways

a. Gluconeogenesis/Glycolysis

flux no.	gluconeogenesis pathway (for plasma cultures)	flux no.	glycolysis pathway (for medium cultures during preconditioning)
1	glucose-6-P + H_2O → glucose + P_i	1a	glucose + P_i → glucose-6-P + H_2O
2	fructose-6-P ↔ glucose-6-P	2a	glucose-6-P ↔ fructose-6-P
3	fructose-1,6-P_2 + H_2O → fructose-6-P + P_i	3a	fructose-6-P + P_i → fructose-1,6-P_2 + H_2O
4	2-glyceraldehyde-3-P ↔ fructose-1,6-P_2	4a	fructose-1,6-P_2 ↔ 2 glyceraldehyde-3-P
5	phosphoenolpyruvate + NADH + H^+ + ATP + H_2O ↔ glyceraldehyde-3-P + P_i + NAD + ADP	5a	glyceraldehyde-3-P + P_i + NAD^+ + ADP ↔ phosphoenol-pyruvate + NADH + H^+ + ATP + H_2O
6	oxalacetate + GTP → phosphoenolpyruvate + GDP + CO_2	6a	phosphoenolpyruvate + ADP → pyruvate + ATP
7	pyruvate + CO_2 + ATP + H_2O → oxaloacetate + ADP + P_i + $2H^+$	7a	pyruvate + CoA + NAD+ → acetyl-CoA + CO_2 + NADH

b. Lactate Metabolism, TCA, and Urea Cycles

flux no.	lactate metabolism and TCA cycle	flux no.	urea cycle, ammonia, arginine, and ornithine uptake
8	lactate + NAD^+ ↔ pyruvate + NADH + H^+	15	arginine + H_2O → urea + ornithine
9	oxaloacetate + acetyl-CoA + H_2O → citrate + CoA + H^+	16	ornithine + (CO_2 + NH_4 + + 2ATP) + H_2O citrulline + 2ADP + 2P_i + $3H^+$
10	citrate + NAD^+ ↔ α-ketoglutarate + CO_2 + NADH		
11	α-ketoglutarate + NAD^+ + CoA → succinyl-CoA + CO_2 + NADH + H^+	17	citrulline + aspartate + ATP → arginine + fumarate + AMP + PP_i
12	succinyl-CoA + P_i + GDH + FAD ↔ fumarate + GTP + $FADH_2$ + CoA	18	arginine uptake
		19	NH_4^+ uptake
13	fumarate + H_2O ↔ malate	20	ornithine secretion
14	malate + NAD^+ ↔ oxaloacetate + NADH + H^+		

c. Amino Acid Uptake and Catabolism

flux no.	amino acid uptake and catabolism	flux no.	amino acid uptake and catabolism
21	alanine + 0.5NAD^+ + 0.05$NADP^+$ + H_2O ↔ pyruvate + NH_4^+ + 0.5NADH + 0.5NADPH + H^+	40	proline + 0.5O_2 + 0.5NAD^+ + 0.5$NADP^+$ → glutamate + 0.5NADH + 0.5NADPH + H^+
22	alanine uptake	41	histidine + H_4folate + 2H_2O → NH_4^+ + N_5-formiminoH$_4$folate + glutamate
23	serine → NH_4^+ + pyruvate		
24	serine uptake	42	methionine + ATP + serine + NAD^+ + CoA → PPi + Pi + adenosine + cysteine + NADH + CO_2 + NH_4^+ + propionyl-CoA
25	cysteine + 0.5NAD^+ + 0.5$NADP^+$ + H_2O + SO_3^{2-} ↔ pyruvate + thiosulfate + NH_4^+ + 0.5NADH + 0.5NADPH + H^+		
26	cysteine uptake	43	aspartate + 0.5NAD^+ + 0.5$NADP^+$ + H_2O ↔ oxaloacetate + NH_4^+ + 0.5NADH + 0.5NADPH + H^+
27	threonine + NAD^+ → glycine + acetyl-CoA + NADH		
28	glycine + NAD^+ + H_4folate ↔ N^5,N^{10}-CH_2−H_4folate + NADH + CO_2 + NH_4^+ + H^+	44	aspartate uptake
		45	asparagine + H_2O → aspartate + NH_4^+
29	glycine uptake	55	valine + 0.5$NADP^+$ + CoA + 2H_2O + 3.5NAD^+ + FAD → NH_4^+ + 0.5NADPH + 3H^+ + 3.5NADH + $FADH_2$ + 2CO_2 + propionyl-CoA
30	tryptophan + 3H_2O + 3O_2 + CoA + 3NAD^+ + FAD → 3CO_2 + $FADH_2$ + 3NADH + 4H^+ + NH_4^+ + acetoacetyl-CoA		
		56	isoleucine + 0.5$NADP^+$ + 2H_2O + 2.5NAD^+ + FAD + 2CoA → NH_4^+ + 0.5NADPH + 3H^+ + 2.5NADH + $FADH_2$ + CO_2 propionyl-CoA + acetyl-CoA
31	propionyl-CoA + ATP + CO_2 → succinyl-CoA + AMP + PPi		
32	lysine + 3H_2O + 5NAD^+ + FAD + CoA → 2NH_4^+ + 5H^+ + 5NADH + 2CO_2 + $FADH_2$ + acetoacetyl- CoA	57	leucine + 0.5$NADP^+$ + H_2O + 1.5NAD^+ + FAD + ATP + CoA → NH_4^+ + 1.5NADH + 0.5NADPH + 2H^+ + $FADH_2$ + ADP + P_i + acetoacetate + acetyl-CoA
33	phenylalanine + H_4biopterin + O_2 → H_2biopterin + tyrosine + H_2O		
34	tyrosine + 0.5NAD^+ + 0.5$NADP^+$ + H_2O + 2O_2 → NH_4^+ + 0.5NADH + 0.5NADPH + H^+ + CO_2 + fumarate + acetoacetate	58	threonine uptake
		59	lysine uptake
		60	phenylalanine uptake
35	tyrosine uptake	61	glutamine uptake
36	glutamate + 0.5NAD^+ + 0.5$NADP^+$ + H_2O ↔ NH_4^+ + α-ketoglutarate + 0.5NADPH + 0.5NADPH + H^+	62	proline uptake
		63	histidine uptake
		64	methionine uptake
37	glutamate uptake	65	asparagine uptake
38	glutamine + H_2O → glutamate + NH_4^+	66	valine uptake
39	ornithine + NAD^+ + $NADP^+$ + H_2O → glutamate + NH_4^+ + NADH + NADPH + H^+	67	isoleucine uptake
		68	leucine uptake

d. Lipid Metabolism and Other Pathways

flux no.	fatty acid metabolism	flux no.	fatty acid metabolism
46	palmitate + ATP + 7FAD + 7NAD^+ → 8acetyl-CoA + 7$FADH_2$ + 7NADH + AMP + PP_i	47	2acetyl-CoA ↔ acetoacetyl-CoA + CoA
		48	acetoacetyl-CoA + H2O → acetoacetate + CoA
46a	8acetyl-CoA + 7ATP + 14NADPH + 14H^+ → palmitate + 8CoA + 6H_2O + 7ADP + 7P_i + 14$NADP^+$	49	acetoacetate production
		50	acetoacetate + NADH + H^+ ↔ β-OH-butyrate + NAD^+

flux no.	oxygen uptake and electron transport system	flux no.	oxygen uptake and electron transport system
51	NADH + H^+ + 0.5O_2 + 3ADP → NAD^+ + H_2O + 3ATP	53	oxygen uptake
52	$FADH_2$ + 0.5O_2 + 2ADP → FAD + H_2O + 2ATP		

flux no.	pentose phosphate pathway	flux no.	albumin synthesis
54	glucose-6-P + 12$NADP^+$ + 7H_2O → 6CO_2 +12NADPH + 12H^+ + P_i	69	24ARG + 32ASP + 61ALA + 24SER + 35CYS + 57GLU + 17GLY + 21TYR + 33THR + 53LYS + 26PHE + 25GLN + 30PRO + 15HIS + 6MET + 20ASN + TRP + 35VAL + 13ISO + 56LEU + 2332 ATP → albumin + 2332ADP + 2332P_i

flux no.	lipid (TG,CE) and glycerol metabolism, glycogen storage and fatty acid uptake	flux no.	lipid (TG,CE) and glycerol metabolism, glycogen storage and fatty acid uptake
70	TG + 3H_2O → glycerol + 3palmitate + 3H^+	74	glycerol + NAD^+ ↔ glyceraldehyde-3-P + NADH + H^+
71	glycerol uptake	75	cholesterol ester + H_2O → cholesterol + palmitate
72	palmitate uptake	76	TG stored
73	glucose-6-P + UTP + H_2O → glycogen + 2P_i + UDP	77	TG uptake

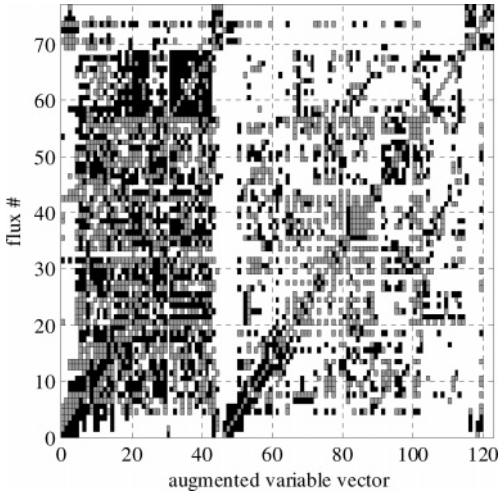

Figure 2. The gain matrix, **-K**, map representation (black indicates positive, gray indicates negative). The *x*-axis is the augmented vector **w**: the left part corresponds to the metabolites (1−46), followed by the fluxes (47−123).

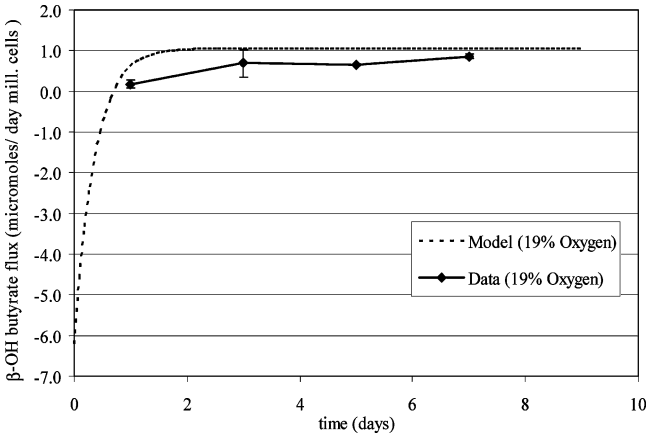

Figure 3. β-Hydroxybutytrate predictions (19% oxygen, nominal state). Data obtained from ref 38.

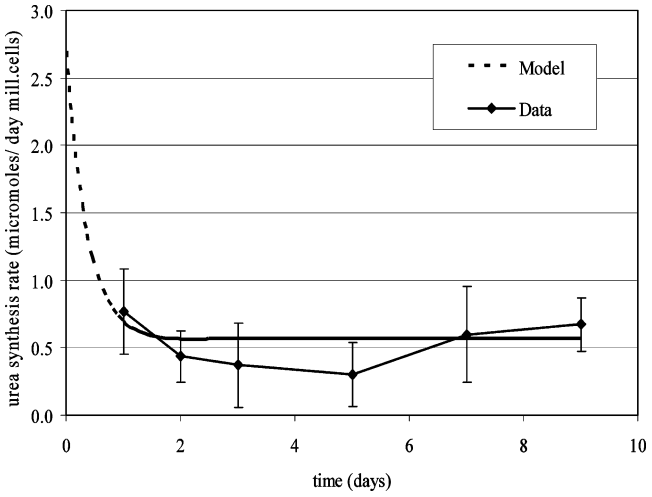

Figure 4. Urea synthesis predictions. Data obtained from ref 38.

straightforward, assuming the noise is random. It would also be interesting to determine what portion of the variations in data could be attributed to the inherent cellular noise, and whether this information can be used to identify metabolic maladjustments in patients before they fully progress to a disease state. For example, identifying lipid accumulation leading to

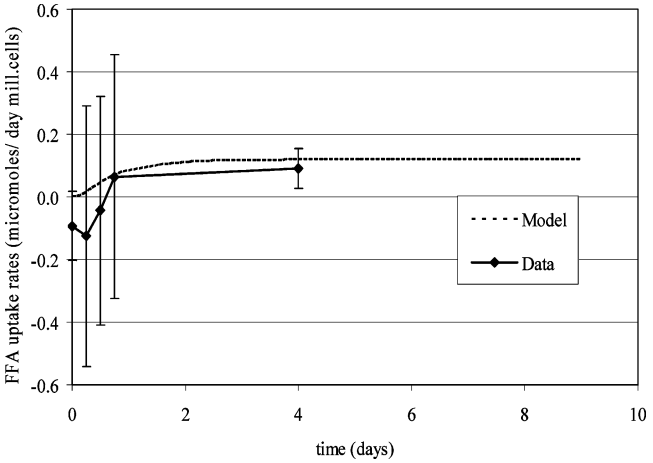

Figure 5. FFA uptake predictions. Data obtained from ref 44.

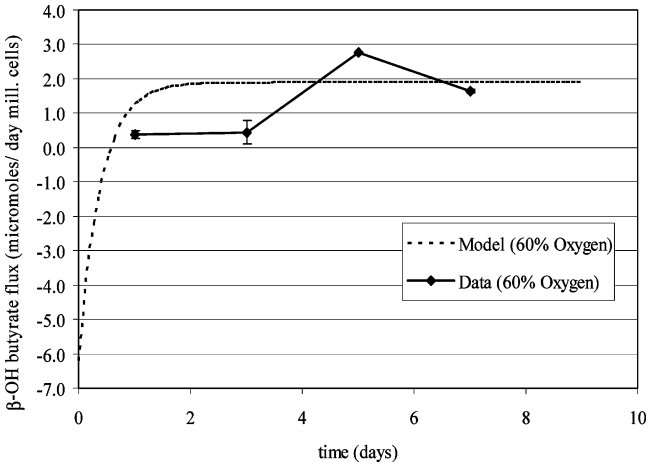

Figure 6. β-Hydroxybutytrate predictions (simulations at 60% oxygen). Data obtained from ref 38.

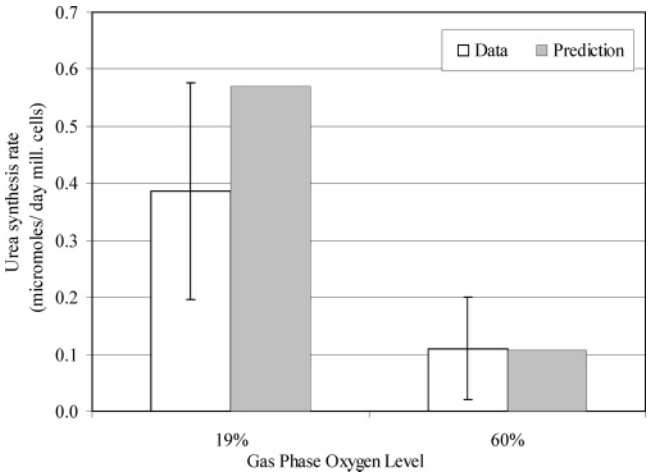

Figure 7. Urea synthesis comparison at different oxygen levels. Data obtained from ref 38.

acute liver toxicity by evaluating the limits of normal variation within each patients' personal metabolic norms.

The main goal of this work is to demonstrate that there are control techniques that can be applied to biosystems with relative ease, provided that the problems are identified correctly and appropriately. Biological systems feature some of the most sophisticated control systems, and their analysis can be hardly performed without the knowledge of control methodologies. Optimality has been a demonstrated quality in biological systems. Others include robustness, resiliency, and redundancy.

Table 3. Flux Values[a,b]

flux no.	initial (in DMEM)	nominal (plasma, 19% oxygen)	predicted (60% oxygen)	flux no.	initial (in DMEM)	nominal (plasma, 19% oxygen)	predicted (60% oxygen)
1	−2.7000	−0.1705	−0.1124	40	−0.0489	0.1172	0.6897
2	0.7343	0.4386	0.5163	41	0.0541	−0.0169	−0.2795
3	0.7343	0.4386	0.5163	42	0.1580	0.0154	−0.2255
4	0.7343	0.4386	0.5163	43	−6.1548	−0.5154	−0.6365
5	−7.4314	0.3472	0.4667	44	−3.7215	0.0060	−0.4730
6	0.0000	0.3472	0.4667	45	−0.0048	0.0058	−0.2432
7	0.0000	0.3059	−0.0883	46	−0.1400	0.5986	0.8428
8	0.0840	0.2502	0.2598	47	−0.0436	−0.2505	0.0452
9	7.0524	5.2749	5.6220	48	0.1800	0.1100	0.3941
10	7.0524	5.2749	5.6220	49	6.7971	−0.9163	0.0989
11	10.1930	5.2782	5.1415	50	−6.2041	1.0491	1.9044
12	10.4680	5.2759	4.8235	51	62.1620	21.7339	23.0511
13	13.2070	5.8317	6.8136	52	10.9000	9.8000	10.4857
14	13.2070	5.8317	6.8136	53	37.4000	15.9000	23.0017
15	2.7000	0.5700	0.1067	54	0.2441	0.0093	−0.0291
16	2.4179	0.5243	−0.1131	55	0.0435	−0.0163	0.1805
17	2.4179	0.5243	−0.1131	56	0.0737	−0.0015	−0.2730
18	0.2900	0.0480	0.2448	57	0.0915	−0.0089	−0.4939
19	0.2200	0.0440	−0.0875	58	0.1600	−0.0015	−0.2287
20	0.1000	0.0640	0.3305	59	0.2414	0.3656	0.4053
21	0.0109	0.2343	0.1023	60	0.2600	0.0140	1.7125
22	0.0310	0.2400	0.1661	61	3.1000	0.0990	−0.1446
23	0.0241	−0.1477	−0.1795	62	−0.0390	0.1200	0.7211
24	0.1900	−0.1300	−0.3800	63	0.0590	−0.0155	−0.2639
25	0.2205	−0.0309	−0.2709	64	0.1600	0.0160	−0.2193
26	0.0740	−0.0430	−0.0088	65	0.0018	0.0077	−0.2223
27	0.1491	−0.0046	−0.2632	66	0.0550	−0.0130	0.2171
28	0.4935	0.1338	0.0107	67	0.0780	−0.0003	−0.2594
29	0.3500	0.1400	0.2917	68	0.1100	−0.0036	−0.4354
30	−0.0003	−0.0001	−0.0010	69	0.0003	0.0001	0.0010
31	0.2752	−0.0024	−0.3180	70	0.0000	0.0300	0.0875
32	0.2239	0.3606	0.3499	71	8.9000	0.5000	0.4785
33	0.2514	0.0116	1.6853	72	0.0025	0.1200	0.0841
34	0.3215	0.0316	2.1032	73	3.1902	0.5998	0.6579
35	0.0770	0.0220	0.4399	74	8.9000	0.5300	0.5659
36	3.1402	0.0033	−0.4805	75	−0.1375	0.6286	0.6645
37	−0.1200	−0.1700	−0.5495	76	0.1300	0.8300	0.8013
38	3.0918	0.0966	−0.1707	77	0.1300	0.8600	0.8887
39	0.1821	−0.0183	−0.1108				

[a] All fluxes in μmol/day million cells. [b] Data from ref 41.

The utilization of such control criteria for analysis of regulatory networks are profitable lines of research for control researchers interested in expanding their examples to problems of biology.

Acknowledgment

This work was in part supported by the Charles H. Gershenson Distinguished Faculty Fellowship of Wayne State University to the third author.

Appendix

Derivation of eq 19, based on the model in eqs 15−18.

First, let us define the following new variables for this problem:

$$\mathbf{u} = \dot{\mathbf{v}} \tag{A-1}$$

$$\mathbf{w}^T = [(\mathbf{z} - \mathbf{z}^n)^T \ \ (\mathbf{v} - \mathbf{v}^n)^T] \tag{A-2}$$

Also,

$$\hat{\mathbf{Q}} = \begin{bmatrix} \mathbf{Q} & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_\alpha \end{bmatrix} \tag{A-3}$$

$$\hat{\mathbf{R}} = \mathbf{R}_\beta \tag{A-4}$$

$$\hat{\mathbf{A}} = \begin{bmatrix} \mathbf{0} & \mathbf{M} \\ \mathbf{0} & \mathbf{0} \end{bmatrix} \tag{A-5}$$

$$\hat{\mathbf{B}} = \begin{bmatrix} \mathbf{0} \\ \mathbf{I} \end{bmatrix} \tag{A-6}$$

Rewriting eq 15 in a traditional LQR form yields

$$\min_{\text{w.r.t.}\mathbf{u}(t)} I = \frac{1}{2}\int_0^\infty (\mathbf{w}^T \cdot \hat{\mathbf{Q}} \cdot \mathbf{w} + \mathbf{u}^T \cdot \hat{\mathbf{R}} \cdot \mathbf{u}) \mathrm{d}t \tag{A-7}$$

s.t.

$$\dot{\mathbf{w}} = \hat{\mathbf{A}} \cdot \mathbf{w} + \hat{\mathbf{B}} \cdot \mathbf{u} \tag{A-8}$$

The solution to this problem can be derived through solving the algebraic Riccati equation:[33] where $\mathbf{S}$ is the steady-state

$$\hat{\mathbf{A}}^T \cdot \hat{\mathbf{S}} + \hat{\mathbf{S}} \cdot \hat{\mathbf{A}} - \hat{\mathbf{S}} \cdot \hat{\mathbf{B}} \cdot \hat{\mathbf{R}}^{-1} \cdot \hat{\mathbf{B}}^T \cdot \hat{\mathbf{S}} + \hat{\mathbf{Q}} = 0 \tag{A-9}$$

$$\hat{\mathbf{K}} = \hat{\mathbf{R}}^{-1} \cdot \hat{\mathbf{B}}^T \cdot \hat{\mathbf{S}} \tag{A-10}$$

$$\mathbf{u} = -\hat{\mathbf{K}} \cdot \mathbf{w} \tag{A-11}$$

Riccati matrix, and $\mathbf{K}$ is the optimal control gain. In terms of the original variables, this yields the optimal control law as

$$\dot{\mathbf{v}} = -[\mathbf{K}_1 \ \ \mathbf{K}_2] \cdot \begin{bmatrix} \mathbf{z} - \mathbf{z}^n \\ \mathbf{v} - \mathbf{v}^n \end{bmatrix} \tag{19}$$

Note that in the last equation the gain matrix, $\hat{\mathbf{K}}$, is split into two submatrices, $\mathbf{K}_1$ and $\mathbf{K}_2$, with appropriate dimensions.

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