

Optimality principles in nonequilibrium biochemical networks

R. M. T. Fleming

Department of Bioengineering, University of California, San Diego

C. M. Maes

Institute for Computational and Mathematical Engineering, Stanford University

M. A. Saunders and Y. Ye

Department of Management Science and Engineering, Stanford University

B. Ø. Palsson

Department of Bioengineering, University of California, San Diego

(Dated: August 12, 2009)

We derive a new optimization problem on a steady-state nonequilibrium network of biochemical reactions, with the property that Kirchhoff's loop law and the second law of thermodynamics both hold at the problem solution. This suggests a new optimality principle for biochemical networks and provides a computationally tractable method for enforcing **energy and thermodynamic constraints in addition to mass conservation**. The method may be used for predicting behavior in genome-scale biochemical networks such as those used by systems biologists in models of microorganisms. **The resulting solutions are biologically more realistic than for classical flux balance analysis alone.**

PACS numbers: ??

Genome sequences are now available that enable biologists to model the biochemical systems of many microorganisms including human and mammalian cells. Such systems are represented mathematically by a network of chemicals (nodes) and reactions (edges). To analyze these networks, systems biologists use a technique called flux balance analysis (FBA) [1]. Flux balance requires that the sum of fluxes into and out of each node in the network be zero. This is equivalent to Kirchhoff's current law in an electrical network. Recent work has sought to augment FBA with Kirchhoff's loop law for energy conservation as well as the second law of thermodynamics [2, 3]. **Incorporation of these constraints has produced results that are biologically more realistic and reveal greater insight into the control mechanisms operating in these complex biological systems [2].** However, the addition of these new constraints has been problematic because they are nonlinear and nonconvex. Previous attempts required computing the global solution of a non-convex optimization problem [2, 4] or solving an NP-hard problem [5].

The purpose of this work is to show that Kirchhoff's loop law and the second law of thermodynamics arise naturally from the optimality conditions of a convex optimization problem with flux balance constraints. Furthermore, every set of reaction fluxes that satisfies Kirchhoff's loop law and the second law of thermodynamics must be optimal for some instance of this problem. This suggests that there is an underlying optimality principle operating in biochemical networks, and leads to an efficient (scalable) method for computing fluxes that satisfy all three constraints.

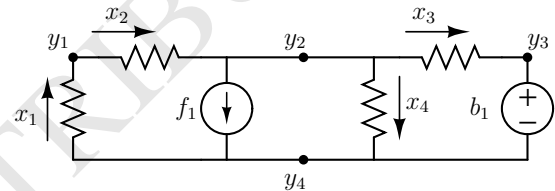


FIG. 1: A linear resistive network with currents x , potentials y , batteries b , and current sources f .

Consider a simple electrical circuit consisting of current sources, batteries, and resistors, as illustrated in Fig. 1. This is a linear resistive network with m nodes and n edges, where the node variables $y \in \mathbb{R}^m$ represent potentials and the edge variables $x \in \mathbb{R}^n$ represent flows (or currents) in the network. The circuit topology is defined by a node-edge incidence matrix $A \in \mathbb{R}^{m \times n}$, and properties of the network are encoded in a set of data vectors: $f \in \mathbb{R}^m$ is a vector of current sources, $b \in \mathbb{R}^n$ is a vector of batteries, and $r \in \mathbb{R}^n$ is a vector of resistances ($r > 0$).

To solve for the voltages and currents in the circuit we use three fundamental laws: Kirchhoff's current law (KCL) $Ax = f$, Kirchhoff's voltage (or loop) law (KVL) $w = b + A^T y$, and Ohm's law $w = Rx$ (where $w \in \mathbb{R}^n$ is a vector of voltages and $R = \text{diag}(r)$ is a positive-definite matrix).

An optimality principle underlies the circuit, which seeks a set of currents that minimize the heat (or power) dissipated subject to KCL. This is the convex optimization problem

$$\begin{aligned} & \text{minimize} && F(x) \equiv \frac{1}{2}x^T R x - b^T x \\ & \text{subject to} && Ax = f \quad : y \end{aligned} \quad (\text{QP})$$

where the node variables y are Lagrange multipliers for the equality constraints. The optimality conditions $\nabla F(x) = A^T y$ yield equations that enforce KVL and Ohm's Law, and the optimal variables x^* and y^* are a set of consistent potentials and currents for the circuit.

Biochemical networks are significantly more complicated than linear resistive networks. However, some of the same underlying network concepts apply. In this work we construct an optimization problem in a form similar to Problem (QP), where the potentials are Lagrange multipliers for an equality constraint on the flow variables, and the optimality conditions of the problem yield equations that enforce Kirchhoff's loop law and the second law of thermodynamics. Previous work noted a connection between Lagrange multipliers and chemical potentials in traditional FBA [6]. Here, we establish a quantitative relation between the Lagrange multipliers and chemical potential for this new optimization problem.

The mathematical representation of a biochemical network is the *stoichiometric matrix* S . Like A above, S is a sparse $m \times n_{\text{tot}}$ incidence matrix that encodes the network topology. However, biochemical networks, unlike linear resistive networks, are nonlinear networks or hypergraphs. That is, a single edge may link many nodes to many nodes, and the entries in S , which are integer stoichiometric coefficients, are not confined to the set $\{-1, 0, 1\}$. Each row of S corresponds to an individual chemical compound, and each column of S corresponds to an individual elementary reaction. In practice, $m < n_{\text{tot}}$ and S does not have full row-rank. A model of a system is called genome-scale if a large proportion of the system's genes are represented. In current genome-scale models of the metabolic system of *E. coli*, m and n_{tot} are several thousand.

Flux balance analysis (FBA) computes a set of fluxes that satisfy mass-conservation constraints and are optimal for a biological objective function. A *flux* is a reaction rate; it represents flow through the network and is analogous to current in an electrical circuit. We denote the flux of the j th reaction by the variable $v_j \in \mathbb{R}$. The concentration of the i th chemical in the network is denoted $x_i \in \mathbb{R}$. The fundamental equation of FBA is the dynamic mass-balance equation—a differential equation relating the change in chemical concentration to reaction fluxes via the stoichiometric matrix:

$$\frac{dx}{dt} = Sv.$$

Here $x \in \mathbb{R}^m$ is a vector of chemical concentrations and $v \in \mathbb{R}^{n_{\text{tot}}}$ is a vector of reaction fluxes. Each row of this vector equation states that the rate of change in concentration for a chemical is the sum of fluxes that synthesize or degrade that chemical. We assume the biochemical system is operating in steady state and therefore the chemical concentrations are constant. Thus, we

have $dx/dt = 0$ and therefore $Sv = 0$. This equation is directly analogous to Kirchhoff's current law in an electrical network.

So far we have considered a whole stoichiometric matrix S and flux vector v . However, it is useful to separate S and v into three components: $S = [S_f \ S_r \ S_e]$, where $S_f \in \mathbb{R}^{m \times n}$, $S_r \in \mathbb{R}^{m \times n}$, and $S_e \in \mathbb{R}^{m \times n_e}$, with corresponding vectors v_f , v_r , v_e . To understand this splitting, consider a reversible reaction $A + 2B \rightleftharpoons C$, which we split into two one-way reactions: forward ($A + 2B \rightarrow C$) and reverse ($A + 2B \leftarrow C$). The fluxes for these one-way reactions form the vectors v_f and v_r . The *net flux* is then $v^{\text{net}} = v_f - v_r$.

The matrix S_f contains all the columns corresponding to forward reactions, and S_r contains all columns corresponding to the reverse reactions. Because of the way reactions are represented, the column for a reverse reaction is the negative of the column of the forward reaction, and thus $S_r = -S_f$. These columns correspond to *internal reactions* (those occurring inside the system).

A living biochemical system interacts with its surroundings and operates in *nonequilibrium steady state* (NESS) [7]. The concentrations of chemicals within the system remain constant, but *exchange fluxes* transport chemicals into and out of the system. These fluxes are represented by the vector v_e associated with S_e . Exchange fluxes are infinite sources and sinks of chemicals and are analogous to current sources in an electrical network. Fig. 2 illustrates a biochemical network.

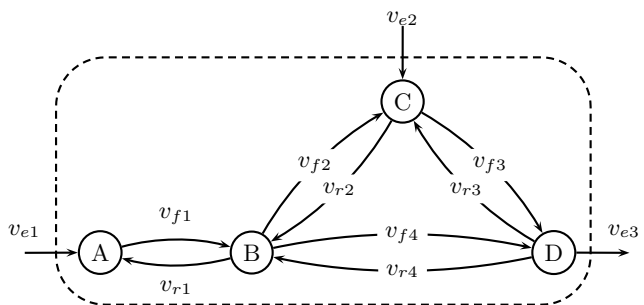


FIG. 2: An example of a simple biochemical network. Chemicals are represented as nodes, reactions as edges. The boundary of the network is represented by a dashed line. Reaction fluxes are labeled as exchange, forward, or reverse.

FBA has been implemented using linear programming [8]. We take the FBA problem to be

$$\begin{aligned} & \underset{v_f, v_r, v_e}{\text{maximize}} && d^T v_e \\ & \text{subject to} && S_f v_f - S_f v_r + S_e v_e = 0 \\ & && \ell \leq v_e \leq h, \quad v_f, v_r \geq 0. \end{aligned} \quad (\text{FBA})$$

Often, the lower bounds ℓ and upper bounds h on the exchange fluxes come from laboratory measurements (e.g. the uptake of glucose in a particular culture of *E. coli*).

The vector d is chosen to optimize a biological objective (e.g. maximizing replication rate in unicellular organisms). Note that classical FBA does not explicitly solve for v_f and v_r but rather $v^{\text{net}} = v_f - v_r$.

We now state a lemma about alternative solutions to Problem (FBA).

Lemma 1. *If there is an optimal solution to Problem (FBA), there is an optimal solution with strictly positive internal fluxes v_f and v_r .*

FBA predicts fluxes that satisfy mass conservation but not energy conservation or the second law of thermodynamics. Recent work has tried to add constraints based on Kirchhoff's loop law and the second law of thermodynamics to Problem (FBA) [2–4]. However, these constraints are problematic because they are nonlinear and nonconvex. We now describe these constraints.

The loop law for chemical potentials in a biochemical network is directly analogous to Kirchhoff's voltage law for electrical circuits. It states that the stoichiometrically weighted sum of chemical potentials around any closed loop of chemical reactions is zero. The *chemical potential* is a measure of a chemical's tendency to change (e.g. changing its composition by undergoing a reaction, or changing its location or concentration) [9]. We explicitly model a chemical potential $u_i \in \mathbb{R}$ for each of the chemicals in the network, and we define the *change in chemical potential* for all internal reactions in the network as the vector

$$\Delta u \equiv S_f^T u \quad (\in \mathbb{R}^n), \quad (1)$$

where $u \in \mathbb{R}^m$ is the vector of chemical potentials. This ensures that Kirchhoff's loop law is satisfied [10].

Assuming mass-action kinetics, constant temperature and pressure, and uniform spatial concentrations (i.e. a well mixed system), it is known (e.g. [11]) that the change in chemical potential may be expressed in terms of elementary one-way reaction rates as

$$\Delta u = \rho \log(v_r ./ v_f), \quad (2)$$

where $./$ denotes component-wise division of vectors, and $\rho = RT > 0$ is the gas constant multiplied by temperature. Equation (2) leads directly to a macroscopic (long-term) application of the second law of thermodynamics:

$$-\Delta u_j v_j^{\text{net}} \geq 0,$$

which says that the net flux for each elementary reaction j must be down the gradient of potential, that the system must dissipate heat, and that entropy must increase as a result of work being done on the system through the exchange fluxes [7]. The total heat dissipation rate of the biochemical system in NESS is given by $-\Delta u^T v^{\text{net}} \geq 0$.

Under the specified assumptions, we now define a thermodynamically feasible flux.

Definition 1. For a network described by a stoichiometric matrix $S = [S_f \ -S_f \ S_e]$ with a given set of exchange fluxes v_e , a set of *thermodynamically feasible fluxes* is a pair of internal flux vectors $(v_f, v_r) > 0$ that satisfy steady-state mass-balance,

$$S_f v_f - S_f v_r = -S_e v_e, \quad (3)$$

and for which there exists an underlying vector of chemical potentials $u \in \mathbb{R}^m$ that satisfies (1) and (2):

$$\Delta u \equiv S_f^T u = \rho \log(v_r ./ v_f) = \rho \log v_r - \rho \log v_f. \quad (4)$$

We now present the main theorem of this Letter. The theorem introduces a new convex optimization problem with the same flux balance constraints as Problem (FBA) but with a negative entropy objective function. It states that the thermodynamic constraints (3) and (4) hold at its unique solution.

Theorem 1. *Let v_e^* be any set of optimal exchange fluxes from Problem (FBA), and define $b = -S_e v_e^*$. The convex equality-constrained problem*

$$\begin{aligned} & \underset{v_f, v_r > 0}{\text{minimize}} && v_f^T \log(v_f) + v_r^T \log(v_r) + c^T(v_f + v_r) \\ & \text{subject to} && S_f v_f - S_f v_r = b \quad : y \end{aligned} \quad (\text{EP})$$

is then feasible, and for any $c \in \mathbb{R}^n$ its solution (v_f^, v_r^*) is a set of thermodynamically feasible internal fluxes. The chemical potentials u may be obtained from the Lagrange multiplier $y \in \mathbb{R}^m$ for the equality constraints. The combined vector (v_f^*, v_r^*, v_e^*) is thermodynamically feasible and optimal for Problem (FBA).*

Proof. First note that the constraints $v_f, v_r > 0$ are implied by the domain of the logarithm; they have no associated Lagrange multipliers. From Lemma 1, we know that if v_e^* is optimal for Problem (FBA) and $b = -S_e v_e^*$, there must be corresponding positive internal fluxes v_f and v_r that satisfy $S_f v_f - S_f v_r = b$. Therefore, Problem (EP) with this choice of b is always feasible.

Define the objective function as $\phi(v_f, v_r)$ and note that it is bounded below and strictly convex because $\nabla^2 \phi(v_f, v_r)$ is positive-definite for all $v_f, v_r > 0$. Problem (EP) is thus a convex linear equality-constrained problem with a unique optimal solution that satisfies the following optimality conditions (where e is a vector of ones):

$$S_f^T y^* = \nabla_{v_f} \phi = \log(v_f^*) + e + c \quad (5)$$

$$-S_f^T y^* = \nabla_{v_r} \phi = \log(v_r^*) + e + c \quad (6)$$

$$S_f v_f^* - S_f v_r^* = b. \quad (7)$$

Subtracting (5) from (6) gives $S_f^T(-2y^*) = \log(v_r^* ./ v_f^*)$. Taking $u = -2y^*/\rho$ we see that (v_f^*, v_r^*) is a pair of thermodynamically feasible fluxes with underlying chemical potentials u for the exchange fluxes v_e^* . **The combined vector (v_r^*, v_f^*, v_e^*) is feasible for Problem (FBA), and is optimal because the objective $d^T v_e^*$ is unchanged.** \square

In summary, to compute an optimal solution (v_f^*, v_r^*, v_e^*) to Problem (FBA) that is thermodynamically feasible, perform the following steps: solve Problem (FBA) to find an optimal exchange flux vector v_e^* , form $b = -S_e v_e^*$, choose a vector c , and solve Problem (EP) to find $v_f^*, v_r^* > 0$.

The next theorem proves that if there is a set of thermodynamically feasible fluxes in a biochemical network, it *must* be the solution to an optimization problem in the form of Problem (EP).

Theorem 2. *Every set of thermodynamically feasible fluxes v_f, v_r (and chemical potentials u) is the solution (and corresponding Lagrange multiplier) of a convex optimization problem in the form of Problem (EP).*

Proof. We show how to choose the vector $c \in \mathbb{R}^n$ so that the given v_f, v_r , and u are optimal. Since v_f and v_r satisfy (3), they are feasible. From (4) we have $S_f^T u = \rho \log(v_r ./ v_f)$. Letting $u = -2\rho y$ we have

$$-2S_f^T y = \log(v_r) - \log(v_f).$$

If we take $2c = -2e - \log(v_r) - \log(v_f)$ then from the above equation we have

$$\begin{aligned} 2S_f^T y &= 2\log(v_f) + 2e + 2c \\ -2S_f^T y &= 2\log(v_r) + 2e + 2c, \end{aligned}$$

which with (3) are the optimality conditions for Problem (EP) as given by (5)–(7). Therefore, with $c = -e - \frac{1}{2}\log(v_r) - \frac{1}{2}\log(v_f)$, the given v_f and v_r are the optimal solution, with Lagrange multiplier vector $y = -\frac{1}{2\rho}u$. \square

Note that Problem (EP) has a unique solution for every value of c and Theorem 2 states that every thermodynamically feasible flux has an associated vector c . The value of c influences the relationship between v_f and v_r through the relation $v_f .* v_r = \exp(-2e - 2c)$.

This vector c is a set of free parameters. Varying c may change v_f and v_r but not the fact that they are thermodynamically feasible and optimal for (FBA). We see this property of the model as an advantage rather than a disadvantage. There is much biological variation and many acceptable biological states. It would be surprising, and biologically unrealistic, if the model produced a unique solution without additional data or constraints. Further research is needed to explore and refine the potential set of fluxes. For instance, systems biologists might require that fluxes also satisfy mass-action kinetics, or that the computed chemical potentials be consistent with the directions of reactions. Alternatively, changing $c^T(v_f + v_r)$ to $c_f^T v_f + c_r^T v_r$ in Problem (EP)'s objective provides another mechanism for satisfying (4). The authors have had some success in using $c_f = -c_r$ to bias the directionality of reactions by incorporating standard transformed chemical potentials [12].

Note the structural similarity of Problems (QP) and (EP). In both cases the primal variables are the flows in the networks, the constraints impose Kirchhoff's current law, and the dual variables for these constraints are the potentials in the network. There is a clear physical interpretation of the objective function in Problem (QP) and an optimality principle in operation. We believe there must be an optimality principle in operation in the biochemical networks as well. However, it is not clear to the authors what this is, or why it appears in the form of the negative entropy objective. Perhaps, by maximizing the entropy of the internal fluxes, the optimization problem produces the most unbiased prediction [13] of internal elementary fluxes, subject to mass-conservation constraints and boundary conditions imposed by exchange fluxes.

The theorems of this Letter have practical import. They provide the theoretical basis for efficiently computing thermodynamically feasible fluxes for current genome-scale, and even larger, biological networks. The convexity of Problem (EP) is a crucial property. Efficient polynomial-time algorithms exist for solving convex optimization problems of this form, based on interior methods [14]. These algorithms are guaranteed to return a solution, optimal to within roundoff error, or a certificate that the original problem is infeasible. In practice, computing thermodynamically feasible fluxes using interior methods should take no longer than performing standard flux balance analysis.

The authors believe that Problem (EP) is flexible enough to incorporate additional biological data (via the vector c) and make quantitative predictions for a range of biochemical systems, including metabolic, regulatory, and signaling networks. Furthermore, the theorems in this Letter extend to any potential network for which flux balance holds and the change in potential can be written as a difference in a monotone function of fluxes: $\Delta u = g(v_r) - g(v_f)$, where $g(\cdot) : \mathbb{R}^n \rightarrow \mathbb{R}^m$ is monotone. These potential networks admit a convex optimization model whose solution is unique and whose potentials are Lagrange multipliers for the flux balance constraint. Problems (QP) and (EP) are simply two examples of such network models [15].

Although the ultimate value of this work lies in what is accomplished with it in future biological studies, we believe this Letter makes an important step forward by providing a computationally tractable method for implementing energy and thermodynamic constraints, and thus for reducing the set of biologically feasible states for complex biochemical systems.

We would like to acknowledge valuable assistance from Ines Thiele and some helpful discussions with Hong Qian and John Ross. The reviewer's comments were also invaluable.

-
- [1] A. Varma and B. Ø. Palsson, *J. Theoretical Biology* **165**, 477 (1993).
- [2] D. Beard, S. Liang, and H. Qian, *Biophysical J.* **83**, 79 (2002).
- [3] N. Price, I. Thiele, and B. Ø. Palsson, *Biophysical J.* **90**, 3919 (2006).
- [4] D. Nagrath, M. Avila-Elchiver, F. Berthiaume, A. W. Tilles, A. Messac, and M. L. Yarmush, *Ann. Biomedical Eng.* **35**, 863 (2007).
- [5] F. Yang, H. Qian, and D. A. Beard, *Metabolic Engineering* **7**, 251 (2005).
- [6] P. B. Warren and J. L. Jones, *Physical Review Letters* **99**, 108101 (2007).
- [7] H. Qian, *Annual Review of Physical Chemistry* **58**, 113 (2007).
- [8] B. Ø. Palsson, *Systems Biology: Properties of Reconstructed Networks* (Cambridge University Press, New York, 2006).
- [9] R. Baierlein, *American J. Physics* **69**, 423 (2001).
- [10] M. Planck, *Treatise on Thermodynamics* (Courier Dover Publications, New York, 1945).
- [11] J. Ross, *Thermodynamics and fluctuations far from equilibrium*, vol. 80 of *Springer Series in Chemical Physics* (Springer, New York, 2008).
- [12] R. A. Alberty, *Thermodynamics of Biochemical Reactions* (Wiley-Interscience, Hoboken, NJ, 2003).
- [13] E. T. Jaynes, *Probability Theory – The Logic of Science* (Cambridge University Press, Cambridge, 2003).
- [14] Y. Ye, *Interior Point Algorithms: Theory and Analysis*, Interscience Series in Discrete Mathematics and Optimization (Wiley, 1997).
- [15] R. T. Rockafellar, *Network Flows and Monotropic Optimization* (Wiley, New York, 1984).