Flux-Concentration Duality in Dynamic Nonequilibrium Biological Networks

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ABSTRACT The structure of dynamic states in biological networks is of fundamental importance in understanding their function. Considering the elementary reaction structure of reconstructed metabolic networks, we show how appreciation of a gradient matrix, $\mathbf{G} = d\mathbf{v}/d\mathbf{x}$ (where $\mathbf{v}$ is the vector of fluxes and $\mathbf{x}$ is the vector of concentrations), enables the formulation of dual Jacobian matrices. One is for concentrations, $\mathbf{J}_x = \mathbf{S}\cdot\mathbf{G}$, and the other is for fluxes, $\mathbf{J}_v = \mathbf{G}\cdot\mathbf{S}$. The fundamental properties of these two Jacobians and the underlying duality that relates them are delineated. We describe a generalized approach to decomposing reaction networks in terms of the thermodynamic and kinetic components in the context of the network structure. The thermodynamic and kinetic influences can be viewed in terms of direction-driver relationships in the network.

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Fluxes and concentrations in reacting biochemical networks are related by kinetic and thermodynamic properties (1,2) and further elucidation of the nature of the relationship between these variables is necessary for the construction and analysis of dynamic networks. Here, we explore the general, underlying relationships between fluxes ($\mathbf{v}$) and concentrations ($\mathbf{x}$) through the use of net elementary reaction rates and the formulation of the gradient matrix $\mathbf{G}$ ($= d\mathbf{v}/d\mathbf{x}$). Subsequent analysis of the systems in terms of the gradient matrix results in characterization of the underlying relationship between fluxes and concentrations as dual variables, also enabling decomposition of the system into constituent direction-driver relationships resulting from the thermodynamic and kinetic influences in the network.

The mass balance equations describing the dynamic states of biochemical reaction networks are (1,2)

$$\frac{d\mathbf{x}(t)}{dt} = \mathbf{S}\cdot\mathbf{v}(\mathbf{x},k) \tag{1}$$

in which $\mathbf{x}$ is a metabolite or enzyme concentration vector in R^m, $\mathbf{v}$ is a reaction flux vector in R^n, and $\mathbf{S}$ is the $m \times n$ stoichiometric matrix, containing the coefficients of reactants and products for each reaction in the network (3), and k is a set of rate constants for the reaction rate expression.

A complete study of the system properties of Eq. 1 would result in the characterization of all four subspaces of $\mathbf{S}$ (4). The null and left null spaces of $\mathbf{S}$ have been thoroughly studied (3), containing steady-state pathways and time-invariant pools (5), respectively. Conversely, the focus of dynamic analysis is in the row and column spaces of $\mathbf{S}$, as the driving forces are in the row space and the thermodynamically determined direction of motion is in the column space. The drivers for change result from the inner product of a row in the stoichiometric matrix ($\mathbf{s}_i^v$) and the flux vector $\mathbf{v}$. If their inner product, $\langle \mathbf{s}_i^v, \mathbf{v}\rangle$, is nonzero, then a time derivative of concentration $\mathbf{x}$ changes. If the inner product, $\langle \mathbf{s}_i^v, \mathbf{v}\rangle$, is zero, then the flux vector is orthogonal to the row space vector and the time derivative of concentrations is equal to the null vector. The vector of the time derivatives of concentration variables is simply a linear combination of the columns of $\mathbf{S}$ (see Eq. 1) and hence is in the column space of $\mathbf{S}$.

Dynamic analysis of complex systems is normally carried out with the linearization of the right-hand side of Eq. 1. Noting that $\mathbf{S}$ is a matrix with constant coefficients, linearization of Eq. 1 follows from linearization of the reaction rates, $\mathbf{v}(\mathbf{x})$, following Taylor series expansion:

$$\mathbf{v}(\mathbf{x}) = \mathbf{v}(\mathbf{x}_0) + \frac{d\mathbf{v}}{d\mathbf{x}}\Big|_{\mathbf{x}_0}\cdot(\mathbf{x}-\mathbf{x}_0) + \frac{1}{2}\frac{d^2\mathbf{v}}{d\mathbf{x}^2}\Big|_{\mathbf{x}_0}\cdot(\mathbf{x}-\mathbf{x}_0)^2 + \ldots \tag{2}$$

$$\mathbf{v}(\mathbf{x}) = \mathbf{v}(\mathbf{x}_0) + \mathbf{G}\,|_{\mathbf{x}_0}\cdot(\mathbf{x}-\mathbf{x}_0) + \ldots. \tag{3}$$

The gradient matrix, $\mathbf{G}$, is a data matrix (6) which arises naturally from linearization of the flux vector. The interactions occurring in biological networks, including macromolecular interactions and enzymatic catalysis, are all fundamentally bilinear. Consequently, when reaction rates are written as net elementary reaction rates, it follows that $\mathbf{S}$ and $\mathbf{G}^T$ have a similar structure (6); the same elements in corresponding row and column entries have zero or nonzero entries (integers in $\mathbf{S}$, nonintegers in $\mathbf{G}$). Moreover, the elements in $\mathbf{G}$ reflect the response times of reactions to changes in concentrations. Different elements in networks typically have a wide range of response times; hence the gradient matrix gives rise to the characteristic hierarchical dynamics in biological systems as well as the wide range of concentrations. Note that since $\mathbf{G}$ is steady-state dependent, a single network may have many gradient matrices, reflecting different homeostatic states.

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The Jacobian Matrix for Concentrations

Specifying a steady state, $\mathbf{x}_0$, Eq. 1 becomes:

$$\frac{d\mathbf{x}'}{dt} = \mathbf{S}\cdot\mathbf{G}\cdot\mathbf{x}' \tag{4}$$

in which $\mathbf{x}'$ is the deviation variable, $(\mathbf{x} - \mathbf{x}_0)$. $\mathbf{J}_x = \mathbf{S}\cdot\mathbf{G}$, is the Jacobian matrix for the system of equations describing the concentration variables. The metabolite Jacobian can be analyzed, decomposed, and reconstructed in terms of topological, thermodynamic, and kinetic terms.

Factorization based on reaction physico-chemical properties

Note that this factorization separates the chemistry that specifies network topology (through **S**) and the kinetics and thermodynamics that give the driving forces and their timescale of action (residing in **G**). Kinetic and thermodynamic effects can be effectively separated by scaling the rows of **G** to unity as $\mathbf{G} = \kappa_v \cdot \mathbf{\Gamma}_v$ (6). The rows $(\boldsymbol{\gamma}_i{}^v)$ in $\mathbf{\Gamma}_v$ (a row-normalized gradient matrix with each row corresponding to a reaction) represent the direction of the driving forces (the thermodynamics) in the row space. The elements of the diagonal matrix κ_v represent the timescales on which the thermodynamic driver of a reaction acts.

Direction-driver decomposition

Equation 4 can be written as (10):

$$\frac{d\mathbf{x}'}{dt} = \sum_{i=1}^{m} \mathbf{s}_i^v \langle \mathbf{g}_i^v, \mathbf{x}' \rangle = \sum_{i=1}^{m} \kappa_i^v \, \mathbf{s}_i^v \langle \boldsymbol{\gamma}_i^v, \mathbf{x}' \rangle \tag{5}$$

in which the reaction vectors $\mathbf{s}_i{}^v$ are fixed and determine the direction of motion, whereas the inner product $\langle \boldsymbol{\gamma}_i{}^v, \mathbf{x}' \rangle$ is a time-dependent driving force along this direction, and $\kappa_i{}^v$ sets the motion's timescale. Thus, the rows of the gradient matrix represent drivers whereas the columns of the stoichiometric matrix give the directions of motion.

Reaction by reaction construction

$\mathbf{J}_x$ can be decomposed as (10):

$$\mathbf{J}_x = \sum_{i=1}^{m} \mathbf{s}_i^v \otimes \mathbf{g}_i^v = \sum_{i=1}^{m} \kappa_i^v \, \mathbf{s}_i^v \otimes \boldsymbol{\gamma}_i^v \tag{6}$$

in which $\otimes$ is the outer product. Equation 6 illustrates how each reaction contributes to the composition of $\mathbf{J}_x$.

The Jacobian Matrix for Fluxes

The gradient matrix enables the change of the system of equations from the concentration variables to a system of equations in terms of flux variables. Defining the flux deviation variable, $\mathbf{v}' = \mathbf{G}\cdot\mathbf{x}' = \mathbf{v} - \mathbf{v}^0$, and premultiplying Eq. 4 by the gradient matrix yields,

$$\frac{d\mathbf{v}'}{dt} = \mathbf{G}\cdot\mathbf{S}\cdot\mathbf{v}'. \tag{7}$$

Thus, the Jacobian matrix when treating the fluxes as the independent variables is: $\mathbf{J}_v = \mathbf{G}\cdot\mathbf{S}$. In a complementary manner, the flux Jacobian can also be investigated in terms of the topological, thermodynamic, and kinetic influences in this node view of networks.

Factorization based on node physico-chemical properties

G can be factored as $\mathbf{G} = \mathbf{\Gamma}_x \cdot \kappa_x$, where $\mathbf{\Gamma}_x$ has the columns of **G** (i.e., the columns, $\mathbf{g}_i{}^x$, corresponding to the compounds that influence the directions of the reactions) normalized and the diagonal matrix κ_x contains the length of these columns. κ_i represent the kinetic potential of compound or node, i, and $\boldsymbol{\gamma}_i{}^x$ represent the distribution of this potential among the links to the node and $\mathbf{J}_v = \mathbf{\Gamma}_x\cdot\kappa_x\cdot\mathbf{S}$.

Direction-driver decomposition

Equation 7 can be written as (10):

$$\frac{d\mathbf{v}'}{dt} = \sum_{i=1}^{n} \mathbf{g}_i^x \langle \mathbf{s}_i^x, \mathbf{v}' \rangle = \sum_{i=1}^{m} \kappa_i^x \boldsymbol{\gamma}_i^x \langle \mathbf{s}_i^x, \mathbf{v}' \rangle \tag{8}$$

The scaled kinetic connectivity vectors $\boldsymbol{\gamma}_i{}^x$ are fixed and determine the direction of motion of the flux vector, reflecting the kinetically-balanced outflow of a compound from a node if the concentration of the compound in that node is perturbed from steady state. The $\kappa_i{}^x$ sets the strength for this motion on the network flux state. The inner product $\langle \mathbf{s}_i{}^x, \mathbf{v}' \rangle$ is a time-dependent driving force along this direction. This driving force is simply a nonsteady-state mass balance on a node. Thus, the rows of the stoichiometric matrix represent drivers for flux whereas the columns of the gradient matrix give the directions of the flux distribution from a node.

Node by node construction

$\mathbf{J}_v$ can be decomposed as:

$$\mathbf{J}_v = \sum_{i=1}^{m} \mathbf{g}_i^x \otimes \mathbf{s}_i^x = \sum_{i=1}^{m} \kappa_i^x \boldsymbol{\gamma}_i^x \otimes \mathbf{s}_i^x \tag{9}$$

showing explicitly how each compound or node in the network contributes to its composition. $\mathbf{J}_v$ is thus reassembled compound by compound (or node by node), whereas $\mathbf{J}_x$ was assembled reaction by reaction (or link by link).

Duality of Fluxes and Concentrations

Thus, there are two Jacobian matrices describing the same network, $\mathbf{J}_x = \mathbf{S}\cdot\mathbf{G}$ and $\mathbf{J}_v = \mathbf{G}\cdot\mathbf{S}$, depending on which variables, concentrations or fluxes, are used as state variables. The former gives a link (reaction)-centric view of the dynamics, whereas the latter gives a node (compound)-centric view. These are complementary views of the same system. The $\mathbf{J}_v$ and $\mathbf{J}_x$ are structurally similar to the reaction adjacency matrix ($\mathbf{A}_v = \widehat{\mathbf{S}}^T\widehat{\mathbf{S}}$) (11) and the compound adjacency matrix ($\mathbf{A}_x = \widehat{\mathbf{S}}\widehat{\mathbf{S}}^T$), respectively (see Chapter 7 of Palsson et al. 2006 (3)), demonstrating that network topology has an overarching effect on the dynamics. One can view $\mathbf{J}_x$ as a weighted version of $\mathbf{A}_x$ and $\mathbf{J}_v$ as a weighted version of $\mathbf{A}_v$.

Modal decomposition of the Jacobian has previously been applied in biological network analysis (7). We note that the two Jacobian matrices share the same eigenvalues. The eigenvectors/rows of $\mathbf{J}_x$, relate to pool formation on various timescales (7–9), whereas the eigenvectors/rows of $\mathbf{J}_v$, relate the formation of groups of fluxes that move these pools.

Example: series of linear reactions

The above principles and concepts will be considered for the simple reaction scheme:

$$\overset{b_1}{\rightarrow} x_1 \overset{v_1}{\rightleftharpoons} x_2 \overset{v_2}{\rightleftharpoons} x_3 \overset{v_3}{\rightarrow}. \qquad (10)$$

Treating the input, (b_1), as a constant leaves three dynamic dimensions. The stoichiometric matrix, gradient matrix, and rate expressions for elementary kinetics are depicted in Fig. 1. Note that sign($\mathbf{S}$) = sign($-\mathbf{G}^T$).

Driving forces and motions

As noted, the driving forces of motion are in the row space and the directions of motions in the column space. The explicit direction-driver relationships for the fluxes as well as concentrations are shown in the top panel of Fig. 2.

The driving forces for the flux motions are nonflux balance variables ($\mathbf{S}\cdot\mathbf{v} \neq \mathbf{0}$) on the nodes. The direction of motion for the flux vector is the columns, $\mathbf{g}^x$, viewed as kinetic connectivity of the metabolites. The driving forces on the concentration are nonequilibrium-type thermodynamic variables. The direction of motion for the concentrations is given by the reaction vectors ($\mathbf{s}^v$).

Forming the dual Jacobian matrices

The dual Jacobian matrices can be decomposed into a summation of rank one matrices (Fig. 2). The reaction based decomposition of $\mathbf{J}_x = \mathbf{S}\cdot\mathbf{G}$ has the logical scaling factors $\kappa_i{}^v = -\langle \mathbf{g}_i{}^v, \mathbf{s}_i{}^v \rangle$, whose inner products are the absolute lengths of the reaction row ($\mathbf{g}^v$), corresponding to the timescale of the corresponding reaction. When the scaling factors differ significantly, each rank one component matrix in $\mathbf{J}_x$ will contribute very differently to the Jacobian matrix, determined by the response time of a reaction and its equilibrium constant.

Analogously, decomposition of $\mathbf{J}_v = \mathbf{G}\cdot\mathbf{S}$ in terms of the compounds (nodes) with logical scaling factors $\kappa_i{}^x = -\langle \mathbf{s}_i{}^x, \mathbf{g}_i{}^x \rangle$, whose inner products are the absolute lengths of the connectivity column ($\mathbf{g}^x$), correspond to the kinetic potential of the corresponding compound (node). The relative contribution of each node to $\mathbf{J}_v$ is determined by the response time of the fastest reaction connected to the node.

Recapitulation

Herein we showed how the gradient matrix underlies the relationship between the fluxes and concentrations. The gradient matrix enables dynamic descriptions of biochemical reaction networks via dual Jacobian matrices, $\mathbf{S}\cdot\mathbf{G}$ and $\mathbf{G}\cdot\mathbf{S}$. The ability to convert from one set of variables into another

$$S = \begin{pmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix} \qquad v = \begin{pmatrix} k_1 x_1 - k_{-1} x_2 \\ k_2 x_2 - k_{-2} x_3 \\ k_3 x_3 \end{pmatrix}$$

$$G = \begin{pmatrix} k_1 & -k_{-1} & 0 \\ 0 & k_2 & -k_{-2} \\ 0 & 0 & k_3 \end{pmatrix} \qquad \text{sign}(-G^T) = \begin{pmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix}$$

FIGURE 1 Set of data matrices (S and G) and flux vector (v) for the example reaction scheme.

Driver Direction Relationships

$$\frac{dx}{dt} = \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix}(k_1 x_1 - k_{-1} x_2) + \begin{pmatrix} 0 \\ -1 \\ 1 \end{pmatrix}(k_2 x_2 - k_{-2} x_3) + \begin{pmatrix} 0 \\ 0 \\ -1 \end{pmatrix}(k_3 x_3)$$

$$\frac{dv}{dt} = \begin{pmatrix} k_1 \\ 0 \\ 0 \end{pmatrix}(b_1 - v_1) + \begin{pmatrix} -k_{-1} \\ k_2 \\ 0 \end{pmatrix}(v_1 - v_2) + \begin{pmatrix} 0 \\ -k_{-2} \\ k3 \end{pmatrix}(v_2 - v_3)$$

Dual Jacobian: Rank One Matrix Decomposition

$$J_x = \begin{pmatrix} -k_1 & k_{-1} & 0 \\ k_1 & -k_{-1} & 0 \\ 0 & 0 & 0 \end{pmatrix} + \begin{pmatrix} 0 & 0 & 0 \\ 0 & -k_2 & k_{-2} \\ 0 & k_2 & -k_{-2} \end{pmatrix} + \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -k_3 \end{pmatrix}$$

$$J_v = \begin{pmatrix} -k_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} + \begin{pmatrix} -k_{-1} & k_{-1} & 0 \\ k_2 & -k_2 & 0 \\ 0 & 0 & 0 \end{pmatrix} + \begin{pmatrix} 0 & 0 & 0 \\ 0 & -k_{-2} & k_{-2} \\ 0 & k_3 & -k_3 \end{pmatrix}$$

FIGURE 2 Direction-driver relationships and the dual Jacobian matrices expressed as summations of rank one matrices for a simple linear reaction scheme.

highlights the underlying root of the physical relationship between fluxes and concentrations and how they determine dynamic states via thermodynamic and kinetic influences.

The thermodynamic and kinetic influences can be decomposed into direction-driver relationships that illustrate the link or node viewpoint of the dynamic states of a network. These relationships can be used to formulate timescale hierarchy through scaling of $\mathbf{G}$ that enables a decomposition into reaction (link based) physico-chemical properties on one hand and, in terms of network level (node based), physico-chemical properties on the other. This dual representation can be used for any biochemical reaction network that is represented in terms of net elementary reactions. The recent availability of metabolomic and fluxomic data makes the construction of the gradient matrix feasible in homeostatic states where measurements are made, thus making the reduction to practice of the analysis methods described here possible.

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10. A column in $\mathbf{S}$ corresponds to a reaction, designated by $\mathbf{s}_i{}^v$. Similarly, a row in $\mathbf{S}$ corresponds to a compound, and we designate a row as $\mathbf{s}_i{}^x$. For the matrix $\mathbf{G}$ the opposite is the case, thus $\mathbf{g}_i{}^v$ designates a row in $\mathbf{G}$ and $\mathbf{g}_i{}^x$ designates a column.
11. $\hat{\mathbf{S}}$ refers to the binary form of the stoichiometric matrix.