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## PERSPECTIVE

# Formulating genome-scale kinetic models in the post-genome era

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**The biological community is now awash in high-throughput data sets and is grappling with the challenge of integrating disparate data sets. Such integration has taken the form of statistical analysis of large data sets, or through the bottom-up reconstruction of reaction networks. While progress has been made with statistical and structural methods, large-scale systems have remained refractory to dynamic model building by traditional approaches. The availability of annotated genomes enabled the reconstruction of genome-scale networks, and now the availability of high-throughput metabolomic and fluxomic data along with thermodynamic information opens the possibility to build genome-scale kinetic models. We describe here a framework for building and analyzing such models. The mathematical analysis challenges are reflected in four foundational properties, (i) the decomposition of the Jacobian matrix into chemical, kinetic and thermodynamic information, (ii) the structural similarity between the stoichiometric matrix and the transpose of the gradient matrix, (iii) the duality transformations enabling either fluxes or concentrations to serve as the independent variables and (iv) the timescale hierarchy in biological networks. Recognition and appreciation of these properties highlight notable and challenging new *in silico* analysis issues.**

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**Subject Categories:** metabolic and regulatory networks; computational methods

**Keywords:** biochemical network; duality; gradient; hierarchical analysis; thermodynamics

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## Introduction

In the past decade, we have witnessed significant advances in the development of statistical analysis of genome-scale

networks (Slonim, 2002), which has been propelled by the availability of genome-scale high-throughput data sets and the successes of constraint-based modeling approaches (Pharkya *et al.*, 2004; Price *et al.*, 2004; Kummel *et al.*, 2006; Palsson, 2006; Reed *et al.*, 2006). The foundation of such genome-scale analysis is built on the stoichiometric matrix,  $\mathbf{S}$ , which describes all the biochemical transformations in a network in a self-consistent and chemically accurate matrix format. Much progress has been made with the genome-scale network reconstruction process and a growing number of genome-scale metabolic reconstructions are now available (Reed *et al.*, 2006; Feist *et al.*, 2007; Jamshidi and Palsson, 2007; Oh *et al.*, 2007; Resendis-Antonio *et al.*, 2007).

Reconstructions of genome-scale biochemical reaction networks (Reed *et al.*, 2006) have been analyzed by topological (Barabasi and Oltvai, 2004) and constraints-based (Price *et al.*, 2004) methods, but dynamic models, at this scale, still need development. It turns out that  $\mathbf{S}$  is not only a requisite for dynamic models but also a major determinant in their properties and thus it is important to have well-curated reconstructions available. The growing availability of metabolomic and fluxomic data sets (Goodacre *et al.*, 2004; Hollywood *et al.*, 2006; Sauer, 2006; Breitling *et al.*, 2008) and methods to estimate the thermodynamic properties (Mavrovouniotis, 1991; Henry *et al.*, 2006, 2007) of biochemical reactions has opened up the possibility to formulate large-scale kinetic models.

The structure of the workflow that leads to large-scale dynamic models is emerging and so are the associated data and mathematical challenges. Here, we will propose a framework for the data integration and mathematical challenges that come with the construction of genome-scale kinetic models. We proceed in four steps. First, we briefly describe the governing equations for dynamic analysis of genome-scale networks. Second, we will outline a proposed workflow for the formulation of large-scale kinetic models. Third, we describe the matrix format of the basic data and the information that it entails. Fourth, we outline the four newly identified key mathematical properties of the resulting models. These four properties are fundamental and the modeling community is now faced with the challenge of studying them in detail. We illustrate some of these properties with examples.

## The basic equations used for dynamic analysis

### The dynamic mass balances

The reconstruction of a genome-scale reaction network requires the identification of all its chemical components and the chemical transformations that they participate in. This process primarily relies on annotated genomes and detailed bibliomic assessment (Reed *et al.*, 2006). The result of this

process is the stoichiometric matrix,  $\mathbf{S}$ , that is used in the dynamic mass balances

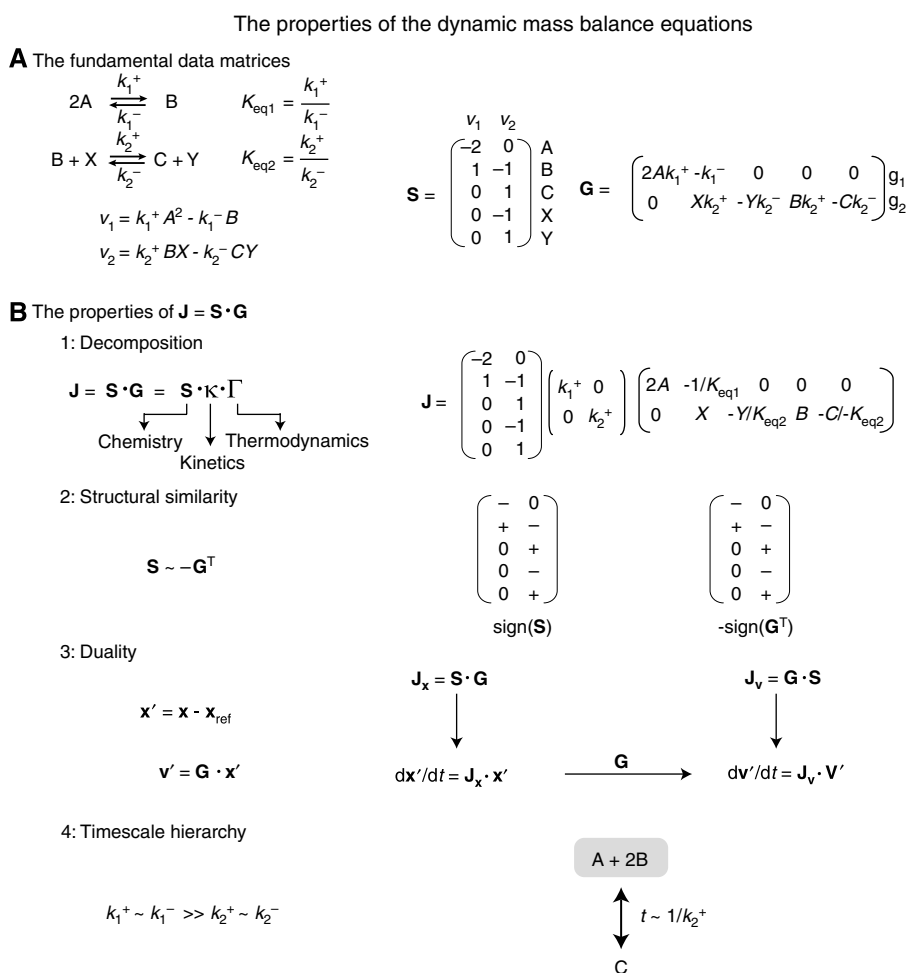
$$\frac{dx}{dt} = \mathbf{S} \cdot \mathbf{v}(\mathbf{x}) \quad \mathbf{x} = \mathbf{x}_0 \text{ at time } t = 0 \quad (1)$$

that are the basis of all kinetic models. Here  $d(\cdot)/dt$  denotes the time derivative,  $\mathbf{x}$  is the vector of the concentrations of the compounds in the network and  $\mathbf{v}(\mathbf{x})$  is the vector of the reaction rates. All biochemical transformations are fundamentally uni- or bi-molecular. Such reactions can be represented by mass action kinetics, or generalizations thereof (Segel, 1975). The net reaction rate for every elementary reaction in a network can be represented by the difference between a forward and reverse flux (e.g. see Figure 1).

This commonly used formulation is based on several well-known assumptions, such as constant temperature, volume and homogeneity of the medium. If  $\mathbf{S}$ ,  $\mathbf{v}(\mathbf{x})$  and the initial conditions ( $\mathbf{x}_0$ ) are known, then these ordinary differential equations can be numerically solved for a set of conditions of interest.

### Linear form

The characterization of the dynamic states of networks can be studied through numerical simulation or through using mathematical analysis. A simulation is context dependent and represents a case study. Mathematical methods for the analysis of model characteristics typically rely on studying the



**Figure 1** (A) The fundamental matrices describing the dynamic states of biological networks: the stoichiometric matrix  $\mathbf{S}$  and the gradient matrix  $\mathbf{G}$ . The corresponding stoichiometric and gradient matrices (for mass action kinetics) are shown. (B) The decomposition of the Jacobian matrix into the stoichiometric and gradient matrices looks similar to other mathematical factorizations; however, this approach is biologically meaningful and driven by the underlying chemistry, kinetics and thermodynamics. There are different ways to factor  $\mathbf{G}$  into  $\mathbf{\Gamma}$  and the diagonal  $\mathbf{K}$  matrix. In this example, the forward rate constant is factored out of  $\mathbf{G}$ . The structural similarity between  $\mathbf{S}$  and  $\mathbf{G}^T$  is highlighted by the fact that their row-reduced forms are equivalent and illustrated for a simple example involving two reactions. A duality exists between the concentrations and fluxes. The practical significance of this duality is that in the linear regimen, the relationship between fluxes and concentrations can be determined independently of the specific rate law formulation, if one can approximate the gradient matrix. When certain reactions occur much faster than others, the related metabolites pool together into aggregate variables. For the example in (A), when the forward and reverse rate constants of the first reaction ( $2A \leftrightarrow B$ ; dimerization reaction) are much faster than the rate constants for the second reactions ( $B + X \leftrightarrow C + Y$ ; cofactor exchange reaction), then the substrate and products of the first reaction form an aggregate pool. Since B is a 'dimer' of A, the pool will consist of A and B in a 1:2 ratio. In this example, the ratios between the metabolites can also be found in the left null space. Furthermore, since the first reaction occurs much more quickly than the second, the interaction between the  $A + 2B$  pool and the C (X and Y are considered cofactors in this example) is determined by the rate constant(s) for the second reaction.

properties of the transformation between the concentrations and fluxes. The analysis of such fundamental properties normally relies on the linearization of the governing equations at a defined condition. The linearization of the dynamic mass balance equations comes down to the linearization of the reaction rate vector to form the gradient matrix

$$\mathbf{G} = [g_{ij}] = \left[ \frac{dv_i}{dx_j} \right] \quad (2)$$

and then forming the Jacobian matrix at a reference state  $\mathbf{x}_{\text{ref}}$ :

$$\frac{d\mathbf{x}'}{dt} = \mathbf{S} \cdot \mathbf{G} \cdot \mathbf{x} = \mathbf{J} \cdot \mathbf{x}' \quad (3)$$

where  $\mathbf{x}' = \mathbf{x} - \mathbf{x}_{\text{ref}}$  and  $\mathbf{J}$  is the well-known Jacobian matrix. Analysis of the characteristics of Jacobian matrix is standard procedure in mathematical analysis of system dynamics (e.g. Strogatz, 1994). The application of these methods to biochemical networks has been carried out for decades (Heinrich *et al.*, 1977, 1991; Heinrich and Sonntag, 1982; Palsson *et al.*, 1987) and in recent years there has been renewed interest and recently further developments in the dynamic analysis of the properties of  $\mathbf{J}$  have appeared (Teusink *et al.*, 2000; Kauffman *et al.*, 2002; Famili *et al.*, 2005; Bruggeman *et al.*, 2006; Steuer *et al.*, 2006; Grimbs *et al.*, 2007).

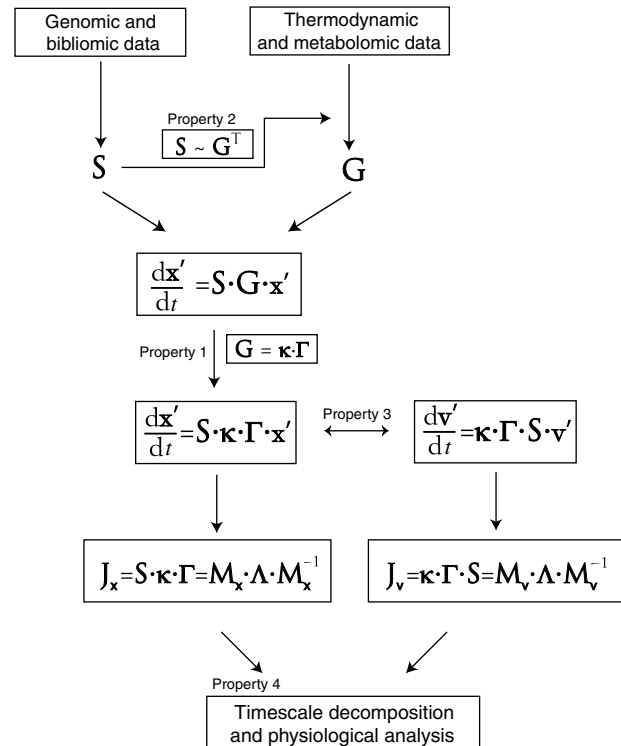
The Jacobian matrix for biochemical reaction networks is the product of two data matrices. Prior to looking at the fundamental properties of  $\mathbf{J}$ , we consider the workflows and data properties that relate to  $\mathbf{S}$  and  $\mathbf{G}$ .

## The workflow associated with constructing large-scale dynamic models

The equations used to describe the dynamic states of networks and outlined above are fairly well known, with the exception of the explicit representation of the gradient matrix. This factorization of the Jacobian matrix turns out to be important in formulating the workflows and in the analysis of the properties of these basic equations.

The integration of genomic, bibliomic, fluxomic and metabolomic data with thermodynamic information into dynamic models of metabolism is illustrated in Figure 2. The process of reconstructing  $\mathbf{S}$  is now well developed (Palsson, 2006). The formulation of  $\mathbf{G}$  can now be performed based on metabolomic data and methods to estimate thermodynamic properties. As discussed below the chemical equations that make up  $\mathbf{S}$  determine the location of the non-zero elements in  $\mathbf{G}$  and hence its structure.

This workflow brings up two important issues. First, the representation and the properties of the data that the two key matrices are made up of. Second, the mathematical challenges associated with analyzing the resulting equations. The matrices represent data, and the equations represent physical laws. Thus, these two issues basically represent data analysis challenges under the constraints of the governing physical laws. We now discuss both of these issues.



**Figure 2** The workflow for constructing approximations to genome-scale kinetic networks and how the four properties discussed in this article and highlighted in Figure 1 enable such reconstructions.

## Key data and fundamental scientific considerations are found in a matrix format

Factoring  $\mathbf{J}$  into the  $\mathbf{S}$  and  $\mathbf{G}$  matrices is not simply a mathematical exercise, but represents a decomposition of  $\mathbf{J}$  into two fundamental factors each with its own relevance (Table I). Comparing the properties of  $\mathbf{S}$  and  $\mathbf{G}$  further highlights the contributions of each matrix to characteristics of biological networks. For example, the formation of pools and reaction co-sets is determined by  $\mathbf{S}$  (Heinrich and Schuster, 1996; Papin *et al.*, 2004; Jamshidi and Palsson, 2006), whereas timescale separation is in the realm of  $\mathbf{G}$ . This decomposition factors the various underlying data needed for the formulation of genome-scale kinetic models. Furthermore, it illustrates the various underlying disciplinary interests that need to be considered and integrated to successfully achieve the analysis of dynamic states of genome-scale networks.

## Bioinformatic considerations

$\mathbf{S}$  is primarily derived from an annotated genomic sequence fortified with any direct bibliomic data on the organisms' gene products. The construction of  $\mathbf{G}$  will rely on fluxomic and metabolomic data, in addition to direct kinetic characterization of individual reactions and assessment of thermodynamic properties.

**Table 1** A comparison of the properties of the stoichiometric and gradient matrices

Properties	Stoichiometric matrix	Gradient matrix
Biological	Species differences Distal causation	Individual differences Proximal causation
Genetic	Genomic characteristics represents a species	Genetic characteristics represents an individual
Informatic	Annotated genome Bibliomic Comparative genomics	Kinetic data Metabolomics Fluxomics
Physico-chemical	Chemistry Conservation laws	Kinetics Thermodynamics
Mathematical	Integer entries Knowable matrix	Real numbers Entries have errors
Numerical	Sparse Well conditioned Non-stiff	Sparse Ill conditioned Leads to stiffness
Systemic	Pool formation Network structure	Timescale separation Dynamic function

### Physico-chemical considerations

**S** represents chemistry (i.e. stoichiometry of reactions), while **G** represents kinetics and thermodynamics. The chemical information is relatively easy to get, the thermodynamics information harder but possible (Henry *et al*, 2007), and the kinetic information is the hardest to acquire. The former two represent hard physico-chemical constraints, while the third represents biologically manipulable numbers; i.e. reaction rates are accelerated by enzymes.

### Biological and genetic considerations

The matrix **S** is reconstructed based on the content of a genome and is a property of a species. It has thus been used productively for the analysis of distal causation (Ibarra *et al*, 2002; Fong and Palsson, 2004; Pal *et al*, 2006; Harrison *et al*, 2007). Distal (or ultimate) causation results from (genomic) changes that occur from generation to generation, in contrast to proximal (or proximate) causation that occurs against a fixed genetic background (i.e. an individual) (Mayr, 1961). **G** is genetically derived and can represent the variations among individuals in a biopopulation. It is important in studying proximal causation and the differences in phenotypes of individuals in a biopopulation. For example, many disease states in higher order organisms result from disruptions or deficiencies in enzyme kinetics (Jamshidi *et al*, 2002). These changes are reflected in **G** since this contains the information about kinetics, consequently the analysis of disease states, inter-individual differences and transitions from a healthy to disease state in a particular individual will in general focus on **G**.

### Mathematical and numerical considerations

Whereas **S** is a ‘perfect’ matrix comprised of integers (i.e. digital), **G** is an analog matrix whose entries are real numbers and we may only know within an order of magnitude. From a numerical and mathematical standpoint, **S** is a well-conditioned matrix comprised of integers (−2, −1, 0, 1, 2), whereas **G** is an ill-conditioned matrix of real numbers that can differ up to 10 orders of magnitude in their numerical values. This property leads to timescale separation. Both matrices are sparse, that is, most of their elements are zero.

### The four fundamental properties

Study of the result from the linearization of the dynamic mass balance equations yields four properties that are of fundamental importance (Figure 1). These properties are illustrated using the familiar glycolytic pathway (Figure 3A).

#### Property 1. Fundamental structure of the Jacobian

The wide differences in the numerical values of the entries of **G** lead to its factorization ( $\mathbf{G}=\boldsymbol{\kappa}\cdot\boldsymbol{\Gamma}$ ) by scaling it by the length of its rows to yield a factorization of **J** into three matrices:

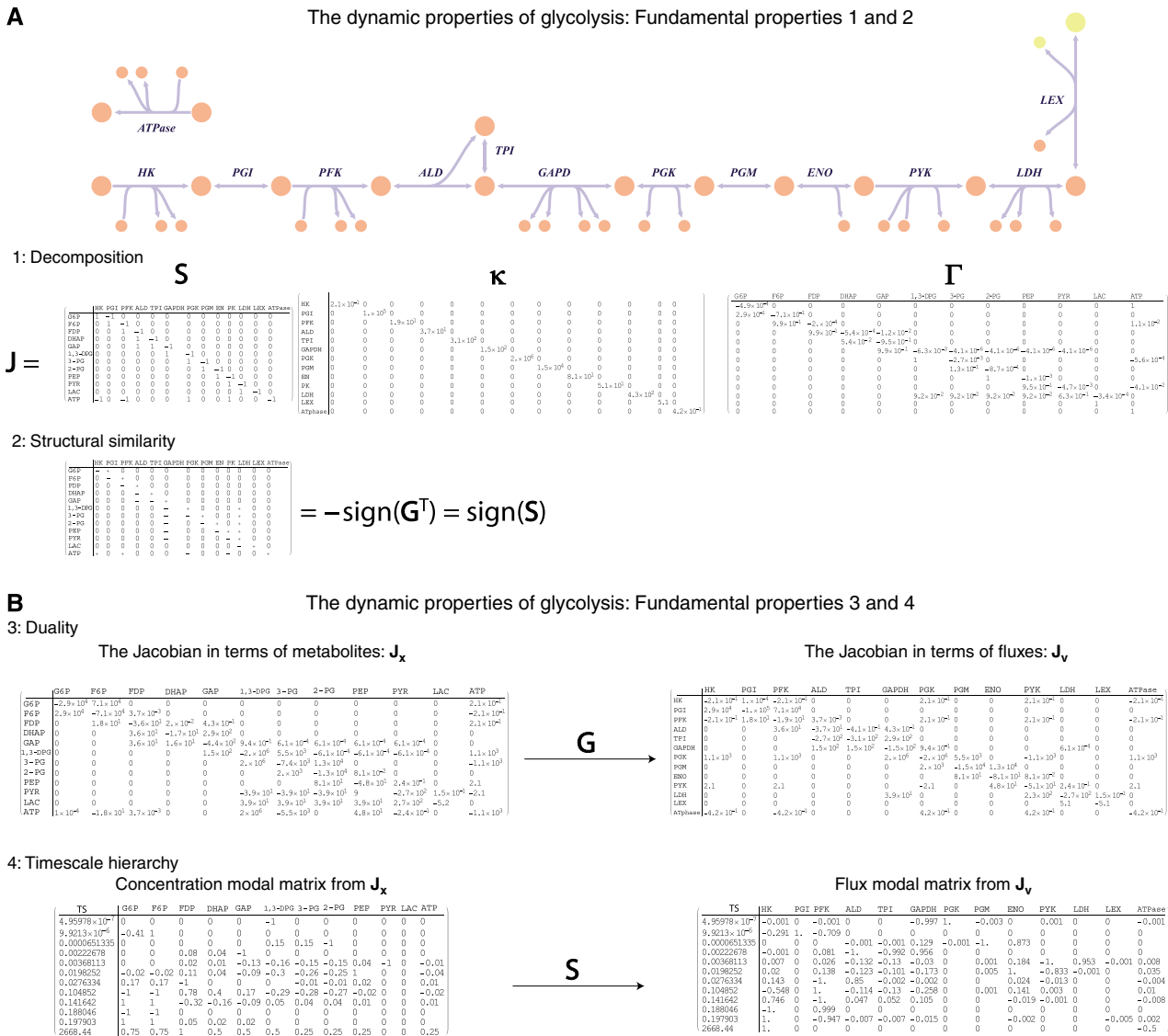
$$\mathbf{J} = \mathbf{S} \cdot \boldsymbol{\kappa} \cdot \boldsymbol{\Gamma} \quad (4)$$

where  $\boldsymbol{\kappa}$  is a diagonal matrix of the norms of the rows in **G** (Figure 1B1). We emphasize that *i*th column of **S** contains the stoichiometric coefficients of the *i*th reaction in the network and the *i*th row in **G** contains the forward and reverse rate constants of that same reaction. Thus, the reciprocal of the diagonal entries,  $1/\kappa_{ii}$ , corresponds to characteristic time constants of the corresponding reactions. Their numerical values will differ significantly.

The factorization of the Jacobian in equation (4) shows that the study of the dynamic properties of biochemical networks can be formally decomposed into chemistry (represented by **S**), kinetics (represented by  $\boldsymbol{\kappa}$ ) and thermodynamic driving forces (represented by **Γ**). The effects of each can thus be formally determined. Chemistry and thermodynamics are physico-chemical properties, whereas the kinetic constants are biologically set through a natural selection process. The particular numerical values (chosen through the selection process) lead to the formation of biologically meaningful dynamic properties of the network. These biological features of the network can be assessed through timescale decomposition, see property 4 below.

### Glycolysis

We use the familiar glycolytic pathway (Figure 3A) to illustrate this and the properties below (See Supplementary Information for the data matrices). A full kinetic model of red cell metabolism (Jamshidi *et al*, 2001) is available and the stoichiometric and gradient matrices are readily obtained for its glycolytic pathway. **G** can be factored into  $\boldsymbol{\kappa}$  and (Figure 3A). We see that the elements in  $\boldsymbol{\kappa}$  are spread over approximately 10 ( $\log[\kappa_{\max}/\kappa_{\min}]=9.7$ ) orders of magnitude. The matrix **S** is universal and so are the elements of **G** for a given set of physico-chemical conditions, such as temperature.



**Figure 3** (A) An example of the first two properties for the glycolytic pathway. A reaction map of the glycolytic pathway is shown. The decomposition of the Jacobian ( $\mathbf{J}_x$ ) into the stoichiometric,  $\mathbf{\kappa}$ , and  $\mathbf{\Gamma}$  matrices follows below (1—norm used for the factorization of  $\mathbf{\Gamma}$ ). The negative transpose of the gradient matrix is shown directly below the stoichiometric matrix, demonstrating the structural similarity. (B) Explicit illustration of the third and fourth properties via the resulting data matrices. The Jacobian duals are shown; they are related by the gradient matrix. Hierarchical analysis can be carried out of the network in terms of metabolites or fluxes. The resultant modal matrices can be related to one another via the stoichiometric matrix. As illustrated in Figure 4, sometimes it is convenient to think about the hierarchical structure in terms of metabolites and sometimes it is more intuitive to think in terms of the fluxes. The network was constructed using equilibrium constants and concentrations from a kinetic red cell model, which has been validated in the literature. Network dynamics were then described using mass action kinetics for a particular steady state. Abbreviations: ALD, fructose-bisphosphate aldolase; ATPase, ATP hydrolysis (demand/utilization); ENO, enolase; GAPD, glyceraldehyde phosphate dehydrogenase; HK, hexokinase; LDH, lactate dehydrogenase; LEX, lactate transporter; PFK, phosphofructokinase; PGI, phosphoglucoisomerase; PGK, phosphoglucokinase; PGM, phosphoglucomutase; PYK, pyruvate kinase; TPI, triose-phosphate isomerase; TS, timescales in hours.

**Property 2. The structural relationship between the stoichiometric and gradient matrices**

It can be readily shown from equation (2) that if  $s_{ij}=0$  then  $g_{ji}$  is also zero, that is, if a compound does not participate in a reaction it has no kinetic effect on it. Conversely, if  $s_{ij} \neq 0$  then  $g_{ji}$  is also not zero. When elementary rate law formulations are used, this relationship holds for allosteric regulation as well, for net reactions. Further inspection reveals the property that  $\mathbf{S}$  is structurally similar to  $-\mathbf{G}^T$  as illustrated in Figure 1B2. Thus, the non-zero entries in  $\mathbf{S}$  have corresponding non-zero

elements in  $-\mathbf{G}^T$ , but with a different numerical value. This fundamental feature shows that the topology of the network as reflected in  $\mathbf{S}$  has a dominant effect on its dynamic features, providing another example of the biological principle that structure has a dominant effect on function.

**Glycolysis**

The structural similarity between the stoichiometric matrix and negative of the transpose of the gradient matrix for glycolysis is immediately apparent (Figure 3A1).



### Property 3. Duality—either fluxes or concentrations can be used as the independent variables

A flux deviation variable,  $\mathbf{v}'$  can be defined such that  $\mathbf{v}' = \mathbf{G} \cdot \mathbf{x}'$ , from which it follows that

$$\frac{d\mathbf{v}'}{dt} = \mathbf{G} \cdot \mathbf{S} \cdot \mathbf{v}' = \mathbf{J}_v \cdot \mathbf{v}' \quad (5)$$

This transformation illustrates the switch from concentrations to fluxes as the independent variables. While concentrations have historically been used as the independent variables, the use of fluxes has grown in recent years as they tie together the multiple parts of a network to form its overall functions. Furthermore, the ability to relate the fluxes and concentrations independently of a specific rate law formulation, if the elements of  $\mathbf{G}$  can be approximated, has significant implications for the construction and analysis of kinetic networks.

The two Jacobian matrices,  $\mathbf{G} \cdot \mathbf{S}$  and  $\mathbf{S} \cdot \mathbf{G}$ , are similar and share eigenvalues. Equation (5) is the 'dynamic' flux balance equation since the variables in it are the fluxes,  $\mathbf{v}'$ . One can thus analyze network properties either in terms of concentrations or fluxes as illustrated within Figure 1B3. The fluxes are 'network' variables, as they tie all the components together, while the concentrations are 'component' variables. Note that since  $\mathbf{J}_v$  has not been fully recognized and studied in this field, when not otherwise specified,  $\mathbf{J}$  is the metabolite Jacobian, or  $\mathbf{J}_x$ .

### Glycolysis

The duality between fluxes and concentrations highlights a deep relationship in network dynamics. The gradient matrix can be used to convert a set of differential equations in terms of metabolites into a set of differential equations in terms of fluxes. Consequently, the Jacobian can be defined in terms of metabolites or fluxes (Figure 3B3).

### Property 4. Multi-timescale analysis of network dynamics

The properties of the Jacobian matrix that determine the characteristics of the network dynamics are its eigen properties. The eigenvalues are network-based time constants (in contrast to the reaction-based time constants in  $\kappa$ ). Formally, the standard eigen analysis is performed by the diagonalization of the Jacobian matrix as:

$$\mathbf{J} = \mathbf{M} \cdot \Lambda \cdot \mathbf{M}^{-1} \quad (7)$$

where  $\mathbf{M}$  is the matrix of eigenvectors,  $\Lambda$  is a diagonal matrix of the eigenvalues and  $\mathbf{M}^{-1}$  is the matrix of eigenrows.

If the decomposition of equation (7) is introduced into equation (3), we obtain differential equations for the modes ( $\mathbf{m} = \mathbf{M}^{-1} \cdot \mathbf{x}'$ )

$$\frac{d\mathbf{m}}{dt} = \Lambda \cdot \mathbf{m}, \text{ or } \frac{dm_i}{dt} = \lambda_i \cdot m_i, \text{ or} \quad (8)$$

$$m_i(t) = m_{i,0} \exp(\lambda_i t)$$

or a set of completely decoupled dynamic variables, that is, each  $m_i$  moves on its own timescale defined by  $\lambda_i$  independent of all the other  $m_j$ . The eigenrows give lumped or aggregate variables that move independently on each timescale since  $\mathbf{m}$

is a set of dynamically independent variables. The eigenvectors form a natural coordinate system to describe the dynamic motion of the modes. We note that this decomposition can be applied to  $\mathbf{J}_x$  or  $\mathbf{J}_v$  (Figure 3B4). The eigenvalues will be the same whereas the eigenvectors and eigenrows will differ since the variable sets (concentrations versus fluxes) will not be the same.

The distribution of the numerical values of the eigenvalues is the basis for timescale separation. Timescale separation forms the basis for decomposition of biochemical reaction networks in time and the interpretation of the biochemical events that take place on the various time constants. Timescale separation has been analyzed in the context of biological networks and shown to lend insight and enable the simplification of these networks (Reich and Selkov, 1981; Heinrich and Sonntag, 1982; Palsson *et al*, 1987). Glycolysis provides an example.

### Glycolysis

Pooling of variables (metabolites or fluxes) refers to the formation of aggregate groups of variables, in which the group of variables move together in a concerted manner. Pools that form on very fast timescales reflect the formation of chemical equilibrium pools, whereas pooling that occurs on the slower timescales reflects physiologically relevant interactions. Moving from the very fast timescales to the slower ones (Figure 4), one observes the well-known examples of pool formation between hexose phosphates (HPs) and phosphoglycerates (PGs). With successive removal of modes on the slower timescales, more and more of the metabolites begin to form aggregate variables and move together in a concerted fashion at fixed ratios. For glycolysis alone, the successive aggregation of chemical moieties (i.e. HP, PG) culminates in, on the slowest timescale, the formation of a physiologically meaningful pool that represents the sum of high-energy phosphate bonds found in the glycolytic intermediates (i.e. their ATP equivalents) (Figures 3.4 and 4). The last row of  $\mathbf{M}^{-1}$  for  $\mathbf{J}_v$  shows that this pool is moved by hexokinase as the input and ATPase as the output (Figure 3B4).

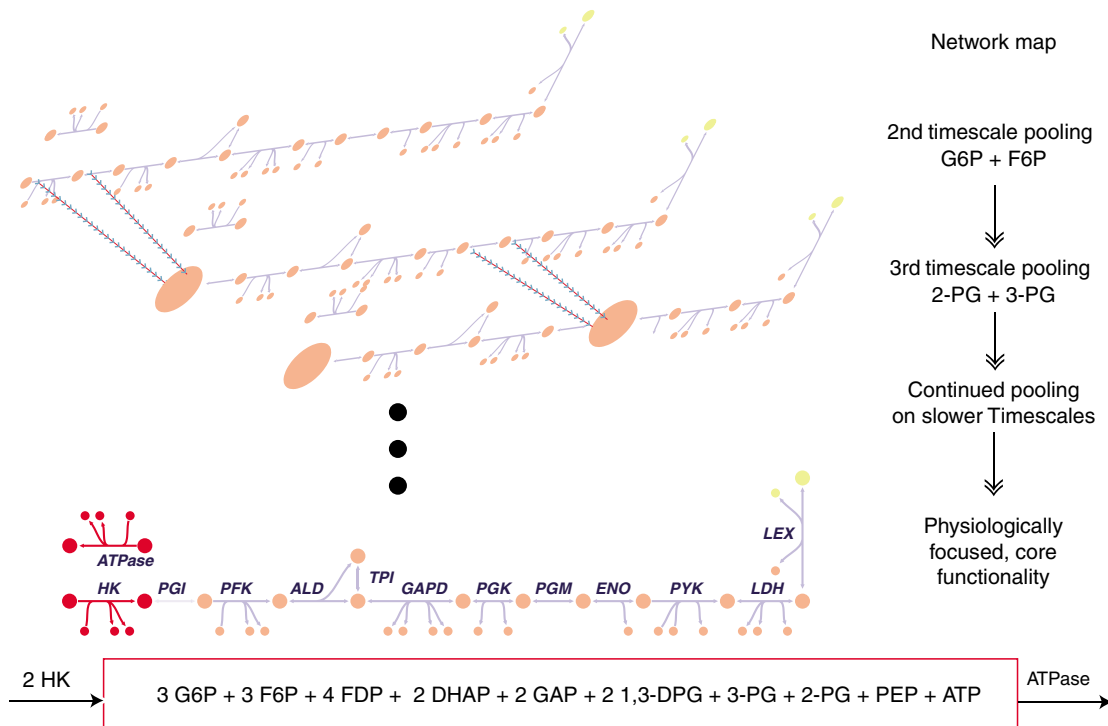
### Recapitulation

Once recognized, these four properties will require further study.

The first property is a result of the explicit reconstruction process and the incorporation of different data types. The properties, completeness and accuracy of the data can be explicitly traced to dynamic properties. This decomposition will not only tie the models directly back to the data but also explicitly gives us the parts of the model that are under biological control and subject to change with adaptation or evolution. Measurement uncertainties are primarily in  $\kappa$  and are subject to evolutionary changes. These 'biological' design parameters will likely need to be dealt with through the use of methods that bracket the range of values within uncertainty limitations.

The second property is a result of the full delineation of the chemical equations that make up a network and ideally their representation as net combinations of elementary reactions

## Hierarchical reduction of glycolysis



**Figure 4** Illustration of pooling for glycolysis. The pools that form on the fastest timescales reflect achievement of chemical equilibria between particular metabolites, such as the hexose phosphates (HPs) on the second timescale and the partial phosphoglycerate (PG) pool on the third timescale. Pools that form on the slower timescales reflect more biologically relevant interactions. For this example of glycolysis, the slowest timescale reflects the sum total of the high-energy phosphate bonds, which can be viewed in terms of the metabolites or fluxes (see Figure 3B3 and 3B4). From the flux point of view, the net balance can be described by the hexokinase flux and the ATPase (in a ratio of 2:1). On this very slow timescale, all of the other glycolytic metabolites have pooled together and the physiological function of the pathway emerges; namely, the sum of the high-energy phosphate bonds found in the glycolytic intermediates. The bottom illustration and corresponding equation detail the metabolites and fluxes comprising the final mode, in the fixed ratios. Metabolite abbreviations: ATP, adenosine triphosphate; DHAP, dihydroxyacetone phosphate; 1,3-DPG, 1,3-bis-phosphoglycerate; F6P, fructose-6-phosphate; FDP, fructose-2,6-phosphate; G6P, glucose-6-phosphate; GAP, glyceraldehyde-3-phosphate; LAC, lactate; PEP, phosphoenolpyruvate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; PYR, pyruvate.

(i.e.  $v_{\text{net}} = v^+ - v^-$ ). In this format, we not only determine the structure of the gradient matrix but also make integration of multiple networks possible and enable the explicit analysis of the effects of regulatory molecules. Furthermore, it explicitly recognizes the underlying bilinear kinetic nature of network dynamics, as all chemical reactions are combinations of bilinear interactions, including regulatory processes (Segel, 1975).

The third property is helpful now that we have systems biological thinking developing fast in the community. The systems biology paradigm of ‘components → networks → computational models → physiological states’ naturally leads to the use of fluxes as variables to characterize the functional states of a network. Fluxomic data tie components in a network together and are interpreted through network models, whereas concentration data are component data. Fluxes have been widely used for steady-state analysis and can now be used to study dynamic states as well.

The fourth property has been known and studied for several decades (Heinrich *et al.*, 1977; Reich and Selkov, 1981; Heinrich and Sonntag, 1982; Palsson *et al.*, 1987). Such studies have primarily been performed for small-scale models today, but their conceptual foundation has been established. At larger

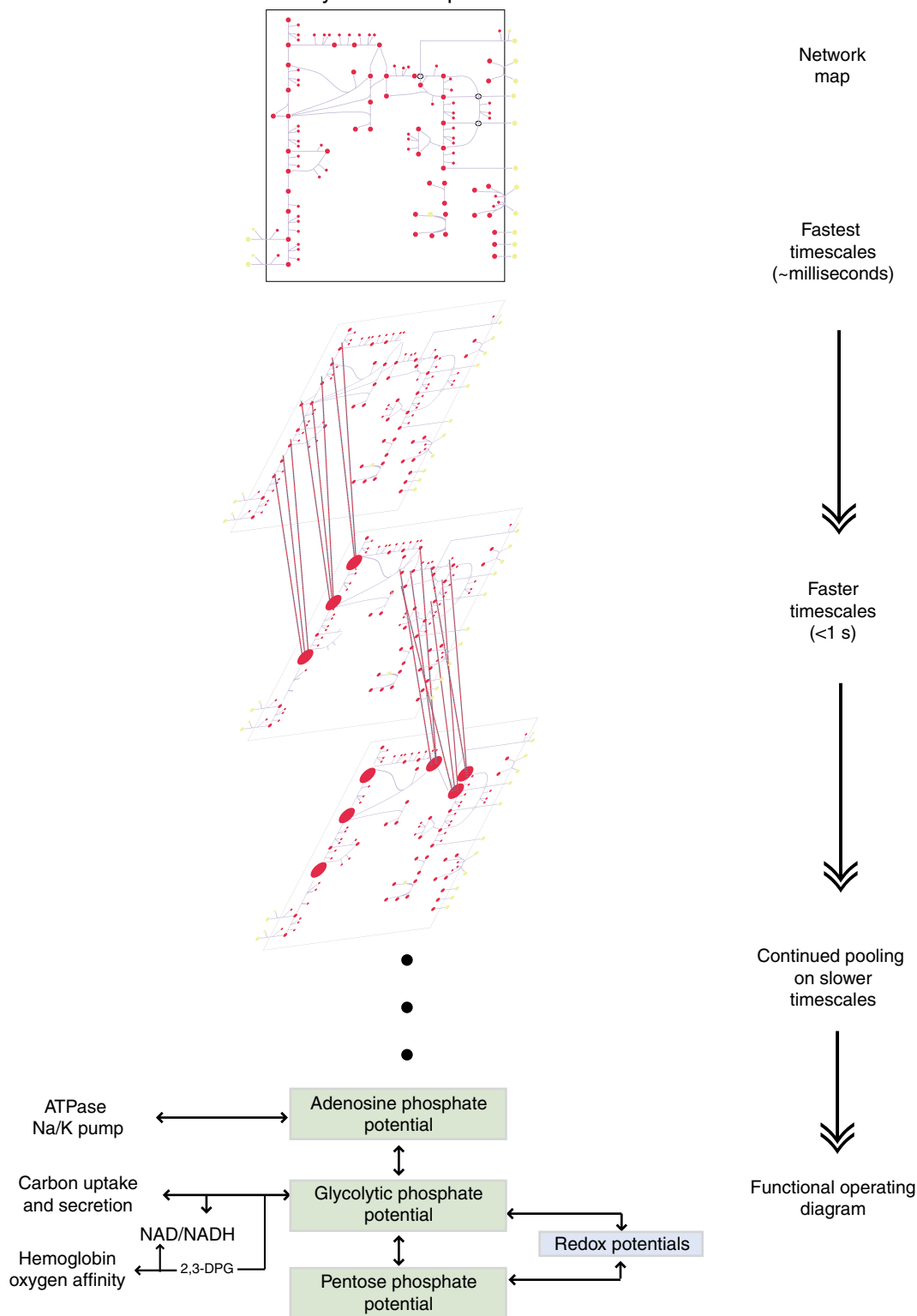
scales new issues will arise. These are likely to include, data sensitivity, course graining and modularization of physiological functions in time. New methods to study the bases vectors in  $\mathbf{M}$  and  $\mathbf{M}^{-1}$  that directly relate them to biochemistry and physiological functions need to be established. The promise of the elucidation of (dynamic) structure–(physiological) function relationships (Palsson *et al.*, 1987) may now grow to large-scale networks.

## Example of a cell-scale kinetic model

With fluxomic, metabolomic and thermodynamic data, we can anticipate the ability to generate large-scale kinetic models where more complicated structures of chemical and physiological pool formation will be found. Currently, human red cell is the only cell-scale kinetic model available, whose formulation followed a 30-year history of iterative model building (summarized in Joshi and Palsson, 1989, 1990). Analysis of the dynamic structure of this model resulted in the simplification of network dynamics and the description of the cellular functions in terms of physiologically meaningful pools of metabolites.



### Hierarchical analysis and simplification of red cell metabolism



**Figure 5** Hierarchical simplification of metabolic dynamics in the human red cell. Illustration of the reduction of biological networks through the formation of aggregate pools on progressively slower timescales, for a full kinetic model of the human red cell. Pool formation, as observed for the simple glycolytic pathway in Figure 4, occurs in a more complicated form in the red cell metabolism. A map for the complete kinetic model of human red cell metabolism is illustrated as pools form on progressive timescales. Ultimately, the function of the red cell can be reduced to redox and adenosine ‘potentials’ in a similar spirit to Atkinson’s energy charge (Atkinson, 1968). The adenosine phosphate potential drives the sodium potassium ATPase pump, the redox potential reflects redox state of the cell and determines the oxidative loads that it can withstand. The glycolytic phosphate potential interacts with the redox and adenosine and pentose phosphate potentials. It also affects the redox state of hemoglobin and the oxygen-binding affinity via NADH and 2,3-DPG. This ‘functional block diagram’ describes the principle functionalities of red cell metabolism.

Analysis of the hierarchical dynamics of the human red cell model resulted in a richer and more complex physiological pool formation (Kauffman *et al.*, 2002) that is detailed above for glycolysis alone. An overview of the hierarchical reduction of the network into a functional diagram is schematized in Figure 4. For example, the adenosine phosphate potential is defined analogously to Atkinson's energy charge (Atkinson, 1968). As originally elaborated by Reich and Selkov (1981), this 'potential' is the ratio of the number of energy-rich phosphate bonds and the ability or capacity to carry such bonds. The different pools of metabolites in the red cell contribute to phosphate potentials and/or oxidation/reduction potentials. The result of the pool formation is an 'operating diagram' (bottom of Figure 5) that describes the function of the metabolic network in the red cell slower timescales (minutes to hours).

This example shows how physiologically meaningful dynamic structures form as a result of the particular numerical values in **G** and how they overlay on the network structure given by **S**. Not all sets of numerical values give this dynamic structure. It has been shown that genetic variation, as represented by sequence polymorphism in particular enzymes (pyruvate kinase and glucose-6-phosphate dehydrogenase), can disrupt this dynamic structure and lead to pathological states (Jamshidi *et al.*, 2002).

## Concluding remarks

Large-scale kinetic models have not been successfully constructed to date, with the human red blood cell being an exception (Heinrich *et al.*, 1977; Heinrich, 1985; Joshi and Palsson, 1989, 1990; Mulquiney and Kuchel, 1999; Jamshidi *et al.*, 2001). The chief reason for this lack of success is the large number of kinetic parameters required to define the system that is confounded by the fact that *in vitro* measurements of kinetic constants may not be representative of their numerical values *in vivo* (e.g. for a recent example, see Teusink *et al.*, 2000). Thus, the probability of achieving other cell-scale models using these approaches appears to be very low. Recently, there have been efforts by investigators to develop methods to fill the gap between constraint-based models and kinetic models (Famili *et al.*, 2005; Smallbone *et al.*, 2007; Ishii *et al.*, 2008).

As metabolomic data become available and drives toward genome-scale coverage (Brauer *et al.*, 2006; Wishart *et al.*, 2007) and approaches for approximating thermodynamic quantities using computational approaches (Mavrovouniotis, 1991) are being realized on the genome-scale (Henry *et al.*, 2007), the data needed to build large-scale kinetic models will become available. In anticipation of the completion of these developments, we present here a workflow for the formulation of large-scale dynamic models and identification of four fundamental properties of the governing equations that genome-scale dynamic analysis will be based on. By focusing on the key structural and dynamic properties of networks and the inherent relationships between fluxes and concentrations, it will become possible to achieve dynamic descriptions of genome scale models, as illustrated in Figure 2.

## Supplementary information

Supplementary information is available at the Molecular Systems Biology website ([www.nature.com/msb](http://www.nature.com/msb)).

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