

Systems Biology and Metabolism: Kinetics, Flux and Energy Balance, and Thermodynamics

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September 22nd, 2004

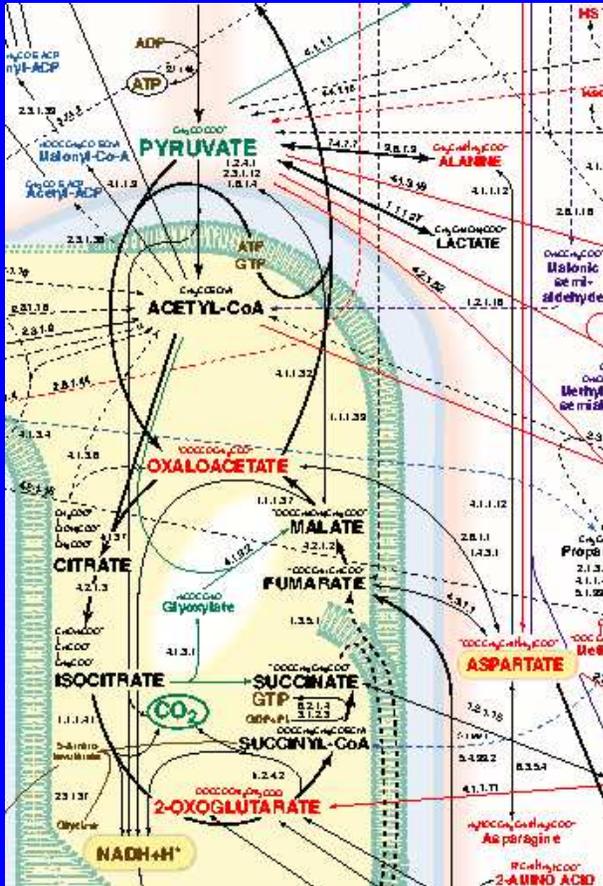
Systems Biology

Concerned with understanding how the parts of a system work together to give the behavior of that system as a whole. This approach includes many research areas in biology, from studying how components of cells interact to yield phenotypic functions to how populations of organisms form societies.

Abstract

Stoichiometric constraints-based models are an alternative to kinetic models when analyzing large-scale networks. Two analytic tools used in this type of modeling are Flux Balance Analysis (FBA) and Energy Balance Analysis (EBA), which use mass and energy balance constraints, respectively, to study the steady-state behavior of reaction networks. Such analysis has proven useful and successful, for example, when modeling cellular metabolism.

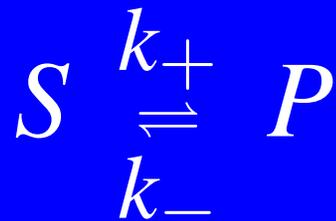
Large-Scale Complex Networks



Sigma

- A reaction network is typically very complex.
- Reaction rate constants are usually all unknown.
- ODE's describing the kinetics contain nonlinear terms.
- Typically unable to find analytic solutions.

Elementary Chemical Reaction

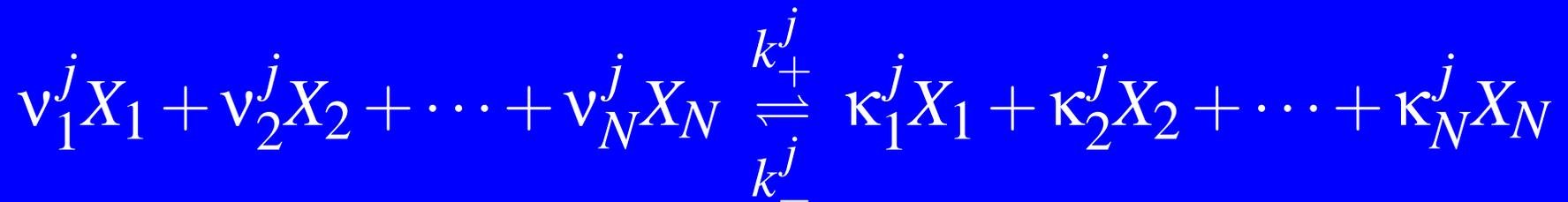


$$\frac{ds}{dt} = k_- p - k_+ s$$

$$\frac{dp}{dt} = k_+ s - k_- p$$

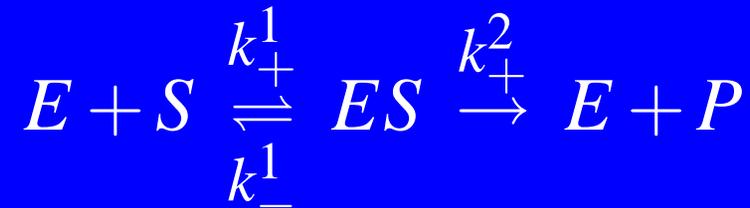
General Chemical Reaction

If we have M reactions involving N species:



$$\frac{dx_i}{dt} = \sum_{j=1}^M (\kappa_i^j - v_i^j) (k_+^j x_1^{v_1^j} x_2^{v_2^j} \cdots x_N^{v_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \cdots x_N^{\kappa_N^j})$$

Michaelis-Menten Enzyme Kinetics



First step is rapid. Enzyme forms an intermediate complex which changes slowly relative to the rate of change of s and p . So we have coupled differential equations:

$$\frac{d(s)}{dt} = k_{-}^1(es) - k_{+}^1(e)(s)$$

$$\frac{d(es)}{dt} = k_{+}^1(e)(s) - k_{-}^1(es) - k_{+}^2(es)$$

$$\frac{d(p)}{dt} = k_{+}^2(es)$$

$$\frac{d(es)}{dt} \approx 0$$

$$\frac{d(es)}{dt} = k_{+}^1(e)(s) - k_{-}^1(es) - k_{+}^2(es) = 0$$

$$(es) = \frac{k_{+}^1(e)(s)}{k_{-}^1 + k_{+}^2}$$

$$K_M = \frac{k_{-}^1 + k_{+}^2}{k_{+}^1}$$

$$(es) = \frac{(e)(s)}{K_M} = \frac{(e_0)(s)}{K_M + (s)}$$

If we assume S is continuously supplied to the system and P is continuously taken out of the system, each at some rate J , then we arrive at steady-state when:

$$J = \frac{k_+^2 (e_o)(s)}{K_M + (s)} = \frac{V_{max}(s)}{K_M + (s)}$$

where $(e_o) = (e) + (es)$ is the total concentration of enzyme (free and bound).

Reversible Michaelis-Menten Kinetics

$$J = \frac{(e_o)}{1 + \frac{(s)}{K_{M,S}} + \frac{(p)}{K_{M,P}}} \left((s) \frac{k_+^2}{K_{M,S}} - (p) \frac{k_-^1}{K_{M,S}} \right)$$

$$K_{M,S} = \frac{k_-^1 + k_+^2}{k_+^1} \quad K_{M,P} = \frac{k_-^1 + k_+^2}{k_-^2}$$

Note that if $J = 0$ this method predicts chemical equilibrium

when:

$$\frac{(p)}{(s)} = \frac{k_+^1 k_+^2}{k_-^1 k_-^2} = K_{eq} = \frac{V_f K_{M,P}}{V_r K_{M,S}}$$

which is known as the Haldane relationship.

The Stoichiometry-Based Model

Instead of representing the ODE's as:

$$\frac{dx_i}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j})$$

We can simplify our model to:

$$\frac{dx_i}{dt} = \sum_{j=1}^M S_i^j J^j$$

where \mathbf{S} is the $N \times M$ stoichiometry matrix and $\mathbf{J} = \mathbf{J}_+ - \mathbf{J}_-$ is the M -dimensional flux vector with

$$J_+^j = k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} \quad \text{and} \quad J_-^j = k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}$$

Including External Fluxes

In the case of an open system which has external injection fluxes of species, for example:



we have:

$$\frac{dx_i}{dt} = \sum_{j=1}^M S_i^j J^j + J_i^e$$

which can be rearranged to give:

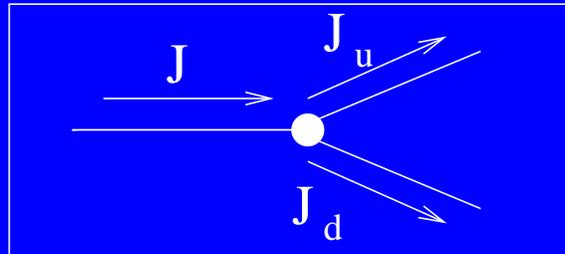
$$\frac{dx_i}{dt} = \sum_{j=1}^{\hat{M}} \hat{S}_i^j \hat{J}^j$$

Equilibrium vs. Non-equilibrium Steady-State

If there is no external flux, then the system of reactions is at equilibrium when $\mathbf{S}\mathbf{J} = \mathbf{0}$. However, if there are external fluxes acting on the system, then the system is at non-equilibrium steady-state (NESS) when $\mathbf{S}\mathbf{J} = -\mathbf{J}^e$.

Kirchoff's Current Law and Flux Balance Analysis

Current Law: At any junction, the sum of the currents into that junction is zero.

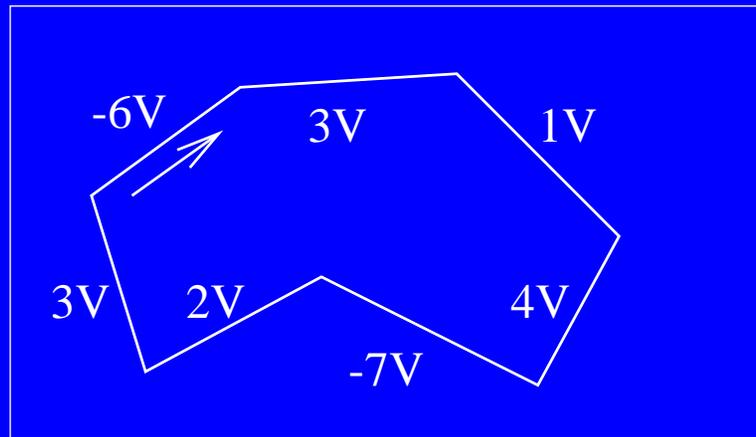


$$J = J_u + J_d$$

Any flux, J , which satisfies the equilibrium or NESS conditions satisfies the flux balance condition.

Kirchoff's Voltage Law

Voltage Law: The change in potential, when summed around any loop within a circuit, is zero.



$$V_1 + V_2 + \cdots + V_N = 0$$

Thermodynamics

If the chemical potential difference for each internal reaction is defined as ΔG then from thermodynamics we know, for the sample reaction:



$$\Delta G = \Delta G^o + k_B T \ln \frac{c^{\alpha_3} d^{\alpha_4}}{a^{\alpha_1} b^{\alpha_2}}$$

and

$$\Delta G^o = -k_B T \ln \frac{k_+}{k_-}$$

Therefore,

$$\Delta G = k_B T \ln \frac{J_-}{J_+}$$

Singular Value Decomposition

Consider now, only the internal reactions without the external fluxes. By using Singular Value Decomposition (SVD), we can decompose \mathbf{S} such that $\mathbf{S} = \mathbf{D}\Sigma\mathbf{B}^T$. Σ is the diagonal matrix containing the singular values of \mathbf{S} and has the form:

$$\Sigma = \begin{pmatrix} \sigma_1 & \dots & 0 & 0 & \dots & 0 \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0 & \dots & \sigma_N & 0 & \dots & 0 \end{pmatrix},$$

the columns \mathbf{D} contain the left singular vectors of \mathbf{S} , and \mathbf{B} is the inverse of the matrix which has columns containing the right singular vectors of \mathbf{S} .

Energy Balance Analysis

If \mathbf{S} has rank r then columns $r + 1$ through M of \mathbf{B} span the null-space of \mathbf{S} . So we can define \mathbf{K} such that

$$\mathbf{K} = (\mathbf{B}(:, r + 1) \dots \mathbf{B}(:, M))$$

Then each row of \mathbf{K} provides the exact weights needed to balance the internal chemical reactions of the network.

Energy Balance Analysis

Relating back to the thermodynamics, let us define the chemical potential of each reactant i as G_i . Then the chemical potential of each reaction is given by:

$$\sum_{i=1}^N G_i S_i^j = \Delta G^j$$

and multiplying by \mathbf{K} on the right we have:

$$G^T S K = \Delta G^T K = 0$$

which is a statement of global free energy balance for the network and is equivalent to Kirchoff's voltage law.

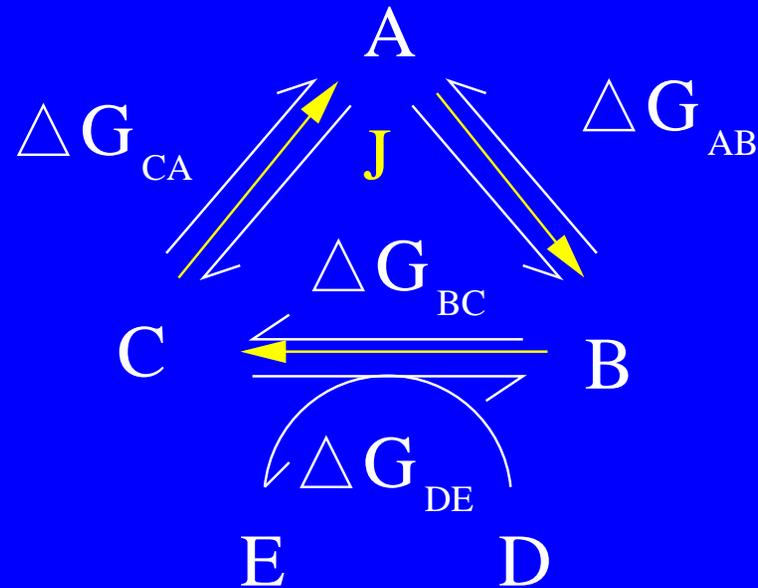
Second Law of Thermodynamics

The second law of thermodynamics requires that each reaction must dissipate energy. Therefore, in terms of J^j , which is the turnover per unit time, and ΔG^j , which is the chemical potential change of turnover, we have:

$$-J^j \Delta G^j = -k_B T (J_+^j - J_-^j) \ln \frac{J_-^j}{J_+^j} \geq 0.$$

By summing over all reactions, this formula can be used to compute the total heat dissipation rate, which is necessarily equal to the entropy production rate of the isothermal biochemical network in a steady-state.

Energy Balance for Simple Loops



$$\Delta G_{AB} + \Delta G_{BC} + \Delta G_{CA} = \Delta G_{DE}$$

$$J\Delta G_{AB} + J\Delta G_{BC} + J\Delta G_{CA} = J\Delta G_{DE}$$

Rate of heat dissipation = Rate of energy pumped in

Easy Example

$$A + 2B \rightleftharpoons C$$

$$C + D \rightleftharpoons 2A + B$$

$$A + B \rightleftharpoons 2D$$

$$A + C \rightleftharpoons B + 2D$$

$$B \rightleftharpoons D$$

\Rightarrow

$$\mathbf{S} = \begin{pmatrix} -1 & 2 & -1 & -1 & 0 \\ -2 & 1 & -1 & 1 & -1 \\ 1 & -1 & 0 & -1 & 0 \\ 0 & -1 & 2 & 2 & 1 \end{pmatrix}$$

$$\frac{d}{dt} \begin{pmatrix} a \\ b \\ c \\ d \end{pmatrix} = \begin{pmatrix} -1 & 2 & -1 & -1 & 0 \\ -2 & 1 & -1 & 1 & -1 \\ 1 & -1 & 0 & -1 & 0 \\ 0 & -1 & 2 & 2 & 1 \end{pmatrix} \begin{pmatrix} J^1 \\ J^2 \\ J^3 \\ J^4 \\ J^5 \end{pmatrix}$$

Add External Fluxes

Add $\rightarrow A$

Remove $\leftarrow D$

$$\mathbf{S} = \begin{pmatrix} -1 & 2 & -1 & -1 & 0 & 1 & 0 \\ -2 & 1 & -1 & 1 & -1 & 0 & 0 \\ 1 & -1 & 0 & -1 & 0 & 0 & 0 \\ 0 & -1 & 2 & 2 & 1 & 0 & -1 \end{pmatrix}$$

$$\frac{d}{dt} \begin{pmatrix} a \\ b \\ c \\ d \end{pmatrix} = \begin{pmatrix} -1 & 2 & -1 & -1 & 0 & 1 & 0 \\ -2 & 1 & -1 & 1 & -1 & 0 & 0 \\ 1 & -1 & 0 & -1 & 0 & 0 & 0 \\ 0 & -1 & 2 & 2 & 1 & 0 & -1 \end{pmatrix} \begin{pmatrix} J^1 \\ J^2 \\ J^3 \\ J^4 \\ J^5 \\ J^I \\ J^O \end{pmatrix}$$

Optimization using FBA

Biological networks are assumed to optimize a certain biologically meaningful objective function.

$$\begin{aligned} & \max_{\mathbf{J}} \quad \mathbf{c}^T \mathbf{J} \\ \text{s.t.} \quad & \mathbf{S} \mathbf{J} = \mathbf{0}, \quad \alpha^j \leq \mathbf{J}^j \leq \beta^j \end{aligned}$$

Sample Problem With D Output

$$\begin{aligned} & \max_{\mathbf{J}} \mathbf{J}^O \\ & \text{s.t. } \mathbf{S}\mathbf{J} = \mathbf{0} \\ & J^I = J^O = 1 \end{aligned}$$

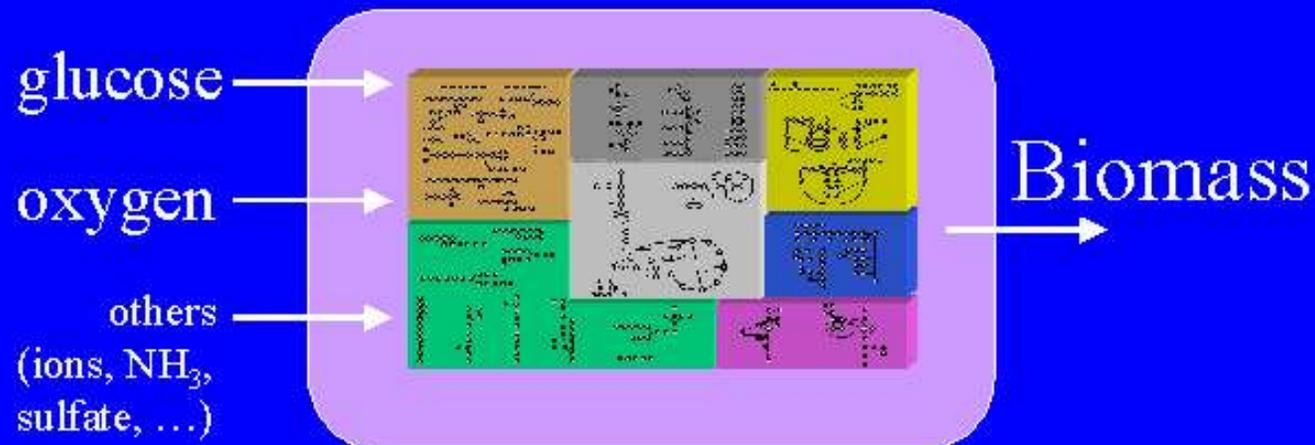
$$\text{A input} \Rightarrow \mathbf{J} = \begin{pmatrix} 0 \\ -0.3 \\ 0.1 \\ 0.3 \\ -0.1 \end{pmatrix}$$

$$\text{B input} \Rightarrow \mathbf{J} = \begin{pmatrix} -1 \\ -0.5 \\ 0.5 \\ -0.5 \\ 0.5 \end{pmatrix}$$

E. Coli Growth Objective Function

Edwards and Palsson *PNAS. USA* 97: 5528-5533, (2000)

- ⊛ Precursors to cell growth.
 - ★ Production of Biomass (growth is maximized).
- ⊛ Components: amino acids, cell wall, DNA, RNA, energy storage cofactors, polyamines.
- ⊛ Stoichiometry of growth factors determined experimentally.



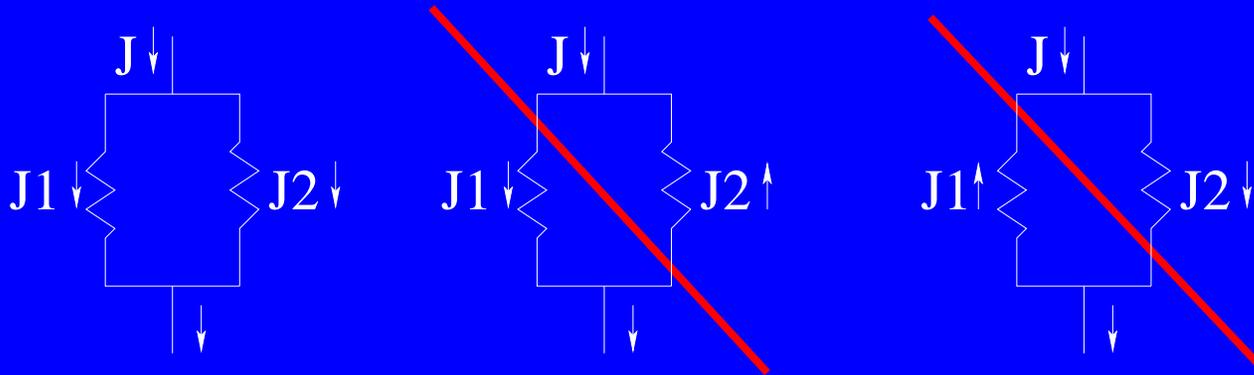
In Silico Predictions of *E. Coli* Growth

Edwards and Palsson *PNAS. USA* 97: 5528-5533, (2000)

7 -/+ of 79					<i>In silico</i> /Experimental				
Gene	Glucose	Glycerol	Succinate	Acetate	Gene	Glucose	Glycerol	Succinate	Acetate
<i>aceEF</i>	+/+				<i>pgl</i>	+/+			
<i>aceA</i>				-	<i>prtAB</i>	+/+	+/+	+/+	+/+
<i>aceB</i>				-	<i>gik</i>	+/+			
<i>ackA</i>				+/+	<i>ppc</i>	+/+	+/+	+/+	+/+
<i>acs</i>				+/+	<i>pta</i>				+/+
<i>acn</i>	-	-	-	-	<i>pts</i>	+/+			
<i>cyd</i>	+/+				<i>pyk</i>	+/+			
<i>cyo</i>	+/+				<i>rpi</i>	-	-	+	+
<i>eno</i>	+/+	+/+	-	-	<i>sdhABCD</i>	+/+			
<i>fxa</i>	+/+				<i>tpi</i>	+/+	-	+	+
<i>fbp</i>	+/+	-	-	-	<i>unc</i>	+/+		+/+	-
<i>gap</i>	-	-	-	-	<i>zwf</i>	+/+			
<i>glfA</i>	-	-	-	-	<i>sucAD</i>	+/+			
<i>gnd</i>	+/+				<i>zwf, prt</i>	+/+			
<i>idh</i>	-	-	-	-	<i>pck, mez</i>			+	+
<i>rdh</i>	+/+	+/+			<i>pck, pps</i>			+	+
<i>nuo</i>	+/+	+/+			<i>pgi, zwf</i>	-			
<i>pfk</i>	+/+				<i>pgi, gnd</i>	-			
<i>pgi</i>	+/+	+/+			<i>pta, acs</i>				+
<i>pgk</i>	-	-	-	-	<i>tklA, tklB</i>	-			

-/+ Essential gene not predicted by model.

Ruling Out Unrealistic Scenarios



By only using FBA constraints to analyze complex networks, one allows some nonphysical solutions to be included in the feasible set of solutions. EBA and thermodynamic constraints need to be used in combination with FBA to rule out these unrealistic scenarios.

Easy Example

$$A + 2B \rightleftharpoons C$$

$$C + D \rightleftharpoons 2A + B$$

$$A + B \rightleftharpoons 2D$$

$$A + C \rightleftharpoons B + 2D$$

$$B \rightleftharpoons D$$

 \Rightarrow

$$\mathbf{S} = \begin{pmatrix} -1 & 2 & -1 & -1 & 0 \\ -2 & 1 & -1 & 1 & -1 \\ 1 & -1 & 0 & -1 & 0 \\ 0 & -1 & 2 & 2 & 1 \end{pmatrix}$$

$$\mathbf{SB} = \mathbf{U} \Sigma$$

 \Rightarrow

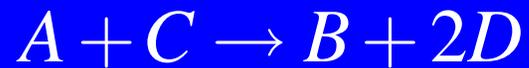
$$\mathbf{K} =$$

$$\begin{pmatrix} 0 \\ -0.2236 \\ -0.6708 \\ 0.2236 \\ 0.6708 \end{pmatrix}$$

 \Rightarrow

$$\mathbf{SK} = \mathbf{0}$$

K Balances Internal Reactions



E. Coli Growth with EBA and Thermodynamics

Beard, Liang, and Qian *Biophysical Journal*, 83, 79-86 (2002)

Enzyme	Gene(s)	Wild Type			pyk knock out			pgi knock out			eno knock out			pyk knock out		
		flux	ΔG_j	c	flux	ΔG_j	c	flux	ΔG_j	c	flux	ΔG_j	c	flux	ΔG_j	c
Glucose phosphotransferase	ptsG	10	-4	2.5	10	-4	2.5	10	-4	2.5	0	-5.6448	0	1.3418	-0.5367	2.5
Glucokinase	glk	0	-7.0082	0	0	-4.3242	0	0	-4.1363	0	10	-4	2.5	5.1759	-2.8512	1.8154
PGI (G-6-P / F-6-P)	pgi	2.3309	-1.2083	1.9291	1.2058	-1.8777	0.6422	0	-7.3982	0	0.2327	0.0307	0.9175	0.2815	0.1029	0.8180
PGI (bDDG-6-P / G-6-P)	pgi	-1.5104	0.6041	2.5	-2.3471	0.9388	2.5	0	3.6991	0	0.6734	0.2694	2.5	7.9806	-3.0780	2.5
PGI (bDDG-6-P / F-6-P)	pgi	1.5104	-0.6041	2.5	2.3471	-0.9388	2.5	0	-3.6991	0	0.6734	0.2694	2.5	7.9806	-3.0780	2.5
Glucose-1-phosphatase	agg	0	-1.2708	0	0	-0.951	0	0	-0.9552	0	0	-6.6044	0	0.0006	-1.8055	0.0004
Phosphofructokinase	pfkFB	7.1478	-2.8591	2.5	7.0635	-2.8254	2.5	5.8813	-4.8729	1.2069	5.7591	-13.364	0.4309	0	-6.4622	0
Fructose 1,6-bisphosphatase	fbp	0	-6.6906	0	0	-3.4008	0	0	-1.1738	0	0	-3.8448	0	0	0	Eq.
Fructose 1,6-bp aldolase	fba	7.1478	-2.8591	2.5	7.0635	-2.8254	2.5	5.8813	-3.6991	1.5899	5.7591	-9.5194	0.605	0	-19.267	0
Triphosphate isomerase	tpis	-7.0564	2.8226	2.5	-6.9735	2.7894	2.5	-5.7915	3.6991	1.5656	-5.6936	9.5194	0.5981	0.5695	-1.0127	2.5
GAP-dehydrogenase	gapAC1C2	15.774	-6.3097	2.5	15.71	-6.2841	2.5	14.532	-5.8127	2.5	14.776	-5.9103	2.5	5.6279	-19.267	0.2921
Phosphoglycerate kinase	pgk	15.774	-6.3097	2.5	15.71	-6.2841	2.5	14.532	-5.8127	2.5	14.776	-5.9103	2.5	5.6279	-19.267	0.2921
Phosphoglycerate mutase	gpmAB	14.322	-5.7286	2.5	14.279	-5.7116	2.5	13.191	-5.2766	2.5	0	0	Eq.	5.2105	-9.3348	0.5689
Enolase	eno	14.322	-5.7286	2.5	14.279	-5.7116	2.5	13.191	-5.2766	2.5	0	9.9992	0	5.2105	-9.3348	0.5689
Phosphoenolpyr. synthetase	ppsA	0	-4.3797	0	0	-2.828	0	0	-2.0828	0	2.1528	-0.8611	2.5	0.0008	-0.0003	2.5
Pyruvate kinase	pykAF	1.3145	-0.8041	1.6348	0	-2.5288	0	0.2368	-2.7292	0.0868	0	-14.854	0	0.0023	-1.7574	0.0013
Pyruvate dehydrogenase	pdhA, aceVF	9.2368	-3.6947	2.5	9.2577	-3.7031	2.5	5.0462	-2.0185	2.5	0	0	Eq.	0	-11.287	0

Up Regulated
 Down Regulated
 Reverse Direction
 Knock Out

$$c^j = \frac{-J^j}{\Delta G^j} = \text{measure of the activation level of the pathway}$$

Conclusions

- ⊗ By using a Stoichiometric Constraints-Based Model, we are able to avoid the nasty details of the complex reaction kinetics. By doing so, we create models which depend only on invariant properties of the system.
- ⊗ It is important to combine the Flux Balance, Energy Balance, and thermodynamic constraints in order to get physically realistic results. Such results have proven to be quite accurate in predicting the behavior of a network under various conditions.
- ⊗ These models are also easily adaptable in studying systems biology. To understand the behavior of a system, the different parts of the system can be studied individually or collectively.

Stability of the Network

“The key idea upon which the theory of stoichiometric dynamical systems is built is that in general the complete set of steady states can easily be calculated. It can always be represented parametrically in a simple form. Once this is done, any static or dynamical property that can be related to the steady states is ripe for investigation.” - Bruce L. Clark

Extensions to Ecology and Economics

The idea of stoichiometric modeling can be extended to many ecological and economic models as well, as long as the stoichiometries are real numbers and the reaction rates are proportional to continuous functions which are positive in the interior of the domain.

For the General Model

For reactions in chemistry and interactions, birth processes, death processes, and immigrations in ecology:

$$\frac{dx_i}{dt} = \sum_{j=1}^M b_{ij} J_+^j - d_{ij} J_-^j$$

where the \mathbf{J}_+ 's can be birth processes and \mathbf{J}_- 's can be death process, expressed as

$$J_+^j = k_+^j \prod_{i=1}^N x_i^{g_{ij}}$$

$$J_-^j = k_-^j \prod_{i=1}^N x_i^{h_{ij}}$$

For Chemical Reaction Networks

We have certain relationships between the matrices:

$$\mathbf{B} = \mathbf{D} = \mathbf{H} - \mathbf{G}$$

Equilibrium always exists with $\mathbf{J}_+ = \mathbf{J}_- \geq \mathbf{0}$. The linear stability is determined by the Jacobian matrix which can be shown to have all real, non-positive eigenvalues.

Duality

A sufficient and necessary condition that the system:

$$\sum_{i=1}^N G_i S_i^j J^j \leq 0$$

admits a nontrivial solution (i.e. at least one $G_i \neq 0$) is that
it's dual problem:

$$\sum_{j=1}^M S_i^j J^j y^j \leq 0 \quad y^j \geq 0$$

admits only a trivial solution.