Bond Graphs Unify Stoichiometric Analysis and Thermodynamics

Peter J. Gawthrop\*1,2

1 Systems Biology Laboratory, Department of Biomedical Engineering, Melbourne School of Engineering, University of Melbourne, Victoria 3010, Australia.
2 Systems Biology Laboratory, School of Mathematics and Statistics, University of Melbourne, University of Melbourne, Victoria 3010, Australia

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Abstract

Whole-cell modelling is constrained by the laws of nature in general and the laws of thermodynamics in particular. This paper shows how one prolific source of information, stoichiometric models of biomolecular systems, can be integrated with thermodynamic principles using the bond graph approach to network thermodynamics.

\*Corresponding author. peter.gawthrop@unimelb.edu.au
## Contents

1 Introduction .......................................................... 3

2 Bond Graphs Integrate Stoichiometry and Energy ................. 4

3 Chemostats, Flowstats and Pathways ................................ 7
   3.1  Illustrative example Noor (2018) ............................... 9
   3.2  Example: Glycolysis & Pentose Phosphate Pathways ............ 11
      3.2.1  Glycolysis .................................................... 12
      3.2.2  R\textsubscript{5}P & NADPH generation .................... 12
      3.2.3  R\textsubscript{5}P generation ..................................... 12
      3.2.4  NADPH generation ............................................ 13
      3.2.5  NADPH & ATP generation ..................................... 13

4 Modularity ..................................................................... 13
   4.1  Example: Metabolism ............................................... 14

5 FBA and EBA in a bond graph context ................................ 16
   5.1  Example: Parallel reactions ...................................... 17
   5.2  Example: three-reaction cycle ..................................... 18

6 Conclusion ..................................................................... 19

7 Acknowledgements .......................................................... 19

A Glycolysis & Pentose Phosphate Pathways: Reactions .......... 26

B Modular representation of Metabolism: Reactions .................. 27
   B.1  Glycolysis ............................................................ 27
   B.2  TCA cycle ............................................................. 27
   B.3  Electron Transport Chain .......................................... 28
   B.4  ATPase ................................................................. 28
1 Introduction

Whole-cell modelling has the potential to “predict phenotype from genotype” (Karr et al., 2012; Covert, 2015) and has the potential to “transform bioscience and medicine” (Szigeti et al., 2018). However, there are currently significant issues in achieving reproducibility (Medley et al., 2016) and integrating disparate sources of information (Goldberg et al., 2018). However, whatever the source of information, the whole-cell model is constrained by the laws of nature in general and the laws of thermodynamics in particular. Unfortunately, “The requirement for thermodynamic consistency, however, has not, in general, been adopted for whole-cell modelling” (Smith and Crampin, 2004). This paper shows how one prolific source of information, stoichiometric models of biomolecular systems, can be integrated with thermodynamic principles.

Stoichiometric analysis of biomolecular systems has been developed over the years (Heinrich and Schuster, 1996; Palsson, 2006, 2011, 2015) has had notable successes including modelling and analysis of the E.coli genome-scale reconstruction (Orth et al., 2011; Thiele et al., 2013; Swainston et al., 2016). The basic idea is to describe a biomolecular system as a (sparse) integer matrix – the \( n_X \times n_V \) stoichiometric matrix \( N \) connecting \( n_X \) species and \( n_V \) reactions. As discussed by Palsson (2015) the stoichiometric approach has a number of advantages:

1. The coefficients of \( N \) are integer; they can therefore be determined exactly.
2. Mass balance of species is ensured and, with the inclusion of the elemental matrix (Palsson, 2015, § 9.2.2), mass balance of elements is also ensured.
3. The sparse integer matrix representation is scaleable to include large systems; for example, the iJO1366 genome-scale reconstruction of the metabolic network of Escherichia coli has 2251 metabolic reactions, and 1136 unique metabolites (Orth et al., 2011).
4. Standard linear algebraic concepts such as the null spaces of a matrix can be invoked to provide precise and meaningful analysis of pathways and conserved moieties (Palsson, 2006, 2011, 2015; Klipp et al., 2016).
5. As discussed by Orth et al. (2011), the flux-balance analysis technique (Orth et al., 2010b) can be applied to predict metabolic flux distributions, growth rates, substrate uptake rates, and product secretion rates for large models.
6. Because the enzymes catalysing the reactions are related to the genome, the stoichiometric approach provides a bridge from genotype to phenotype (Palsson, 2015).
7. Comprehensive software tools are readily available (Ebrahim et al., 2013; Heirendt et al., 2019).

A number of works have discussed the fundamental significance of energy in the life sciences and evolution of living systems (Niven and Laughlin, 2008; Sousa et al., 2013; Martin

\footnote{The stoichiometric matrix has the symbol \( N \) is some works (Klipp et al., 2016) and \( S \) in others (Palsson, 2006, 2011, 2015).}
et al., 2014; Lane, 2014, 2018; Dai and Locasale, 2018; Niebel et al., 2019). In particularly, the efficiency (Smith et al., 2005; Lopaschuk and Dhalla, 2014; Niven, 2016; Park et al., 2016; Lark et al., 2016) of living systems is an evolutionary pressure. However, energy considerations are not explicitly included in the stoichiometric approach. This can lead to mass flows that are not thermodynamically possible; such non-physical flows can be detected and eliminated by adding additional thermodynamic constraints via Energy Balance Analysis (EBA) (Beard et al., 2002; Qian et al., 2003; Noor et al., 2014; Noor, 2018).

Like living systems, engineering systems are subject to the laws of physics in general and the laws of thermodynamics in particular. This fact gives the opportunity of applying energy-based engineering approaches to the modelling, analysis and understanding of living systems. The bond graph method of Paynter (1961) is one such well-established engineering approach (Cellier, 1991; Gawthrop and Smith, 1996; Gawthrop and Bevan, 2007; Borutzky, 2010; Karnopp et al., 2012) which has been extended to include biomolecular systems (Oster et al., 1971, 1973; Gawthrop and Crampin, 2014). The stoichiometric matrix of a biomolecular network can be derived from the corresponding bond graph (Gawthrop and Crampin, 2014; Gawthrop et al., 2015); this paper shows that the converse is true: the bond graph of a biomolecular system can be deduced from the stoichiometric representation. Thus the large repository of models of biomolecular systems available in stoichiometric form can be automatically converted to bond graph form.

Once converted to bond graph form, the models are endowed with a number of additional features:

1. They are thermodynamically compliant and thus subsume the EBA approach.

2. As an energy based method, bond graphs can model multi-domain systems and thus readily incorporate charged species, electrons and protons in an integrated model (Gawthrop, 2017; Gawthrop et al., 2017; Pan et al., 2018b,a).

3. Bond graphs are modular (Gawthrop et al., 2015; Gawthrop and Crampin, 2016) a key requirement of any large-scale modelling endeavour.

4. Bond graph models can be simplified in an energetically coherent fashion (Gawthrop and Crampin, 2014; Pan et al., 2017; Gawthrop et al., 2019).

5. Bond graphs provide energy-based pathway analysis (Gawthrop and Crampin, 2017).

The *e.coli* Core Model (Orth et al., 2010a; Palsson, 2015) is a well-documented and readily-available stoichiometric model of a biomolecular system. This model is used in § 3.2 as an exemplar to illustrate how a bond graph can be automatically generated and to examine how it can be used for the energetic analysis of pathways.

## 2 Bond Graphs Integrate Stoichiometry and Energy

Bond graphs are, as the name implies, a graphical representation of a system. This has the advantage of clear visual representation when dealing with small systems, but such visualisation
becomes problematic for large systems. As meaningful biomolecular systems are large, this issue must be addressed. There are two approaches to overcoming this issue: modularity and a non-graphical representation. This paper uses both approaches: a recent concept of bond graph modularity (Gawthrop, 2017) is presented in § 4 and the recently developed BondGraphTools (Cudmore et al., 2019) ([https://pypi.org/project/BondGraphTools/]) is used throughout as a non-graphical representation.

The key concept is the energy bond represented by the $\rightarrow$ symbol. This bond carries energy in the form of an effort/flow pair: in the case of biomolecular systems this pair is chemical free energy $\phi$ J mol$^{-1}$ and molar flow $v$ mol s$^{-1}$. Bonds transmit, but do not store or dissipate energy. Within this context, the bonds connect four bond graph components:

**0 & 1 junctions** Provide a method of connecting a two or more bonds. The bonds impinging on a 0 junction share a common effort (chemical free energy); the bonds impinging on a 1 junction share a common flow. Both 0 & 1 junctions transmit, but do not store or dissipate energy. As discussed previously (Gawthrop and Crampin, 2014), the arrangement of bonds and junctions determines the stoichiometry of the corresponding biomolecular system and thus the relationship both between reaction and species flows and between species free energies and reaction forward and reverse free energies. As will be discussed, the reverse is also true: the stoichiometric matrix of a biomolecular system determines the bond graph.

**Ce** Represents species. Thus species A is represented by Ce:A with the equations:

$$x_A(t) = \int_0^t v_A(t')dt' + x_A(0) \quad (2.1)$$

$$\phi_A = \phi_A^\circ + RT \ln \frac{x_A}{x_A^\circ} \quad (2.2)$$

Equation (2.1) accumulates the flow $v_A$ of species A. Equation (2.2) generates chemical free energy $\phi_A$ in terms of the standard free energy $\phi_A^\circ$ at standard conditions $x_A^\circ$ where $R$ and $T$ are the universal gas constant and temperature respectively Atkins and de Paula (2011). Ce components store, but do not dissipate, energy.

**Re** Represents reactions. The flow associated with reaction 1 $v_1$ is given by the Marcelin – de Donder formula (Van Rysselberghe, 1958):

$$v_1 = \kappa_1 \left( \exp \frac{\Phi^f_1}{RT} - \exp \frac{\Phi^r_1}{RT} \right) \quad (2.3)$$

where $\Phi^f_1$ and $\Phi^r_1$ are the forward and reverse reaction free energies, or affinities. If $\kappa_1$ is constant, this represents the mass-action formula; in general, $\kappa_1$ is a function of $\Phi^f_1$, $\Phi^r_1$ and enzyme concentration (Gawthrop and Crampin, 2014). Re components dissipate, but

---

2 The symbol $\phi$ is used for chemical free energy in place of $\mu$.
do not store, energy. In general

\[ V = V(\Phi, \phi) \]  \hspace{1cm} (2.4)

where \( \Phi = \Phi^f - \Phi^r \)  \hspace{1cm} (2.5)

where \( V(\cdot) \) is dissipative in \( \Phi \) for all \( \phi \):

\[ V_i \Phi_i > 0 \]  \hspace{1cm} (2.6)

The key stoichiometric equations arising from bond graph analysis are (Gawthrop and Crampin, 2014):

\[ \dot{X} = NV \]  \hspace{1cm} (2.7)

\[ \Phi = -N^T \phi \]  \hspace{1cm} (2.8)

where \( X, \Phi \) and \( \phi \) are the species amounts, reaction free energies and species free energies respectively. \( N \) is the system stoichiometric matrix. The network of bonds and junctions transmits, but does not dissipate or store, energy. As discussed by Gawthrop and Crampin (2014), this fact can be used to derive Equation (2.8) from (2.7).

Moreover, the stoichiometric matrix \( N \) can be decomposed as (Gawthrop and Crampin, 2014):

\[ N = N^r - N^f \]  \hspace{1cm} (2.9)

where \( N^r \) corresponds to the positive entries of \( N \) and \( N^f \) to the negative entries. The forward and reverse reaction free energies \( \Phi^f \) and \( \Phi^r \) are given by:

\[ \Phi^f = N^f \phi \]  \hspace{1cm} (2.10)

\[ \Phi^r = N^r \phi \]  \hspace{1cm} (2.11)

Figure 1: Bond graphs of simple reactions.

In other words, the stoichiometric matrix \( N \) can be derived from the system bond graph. This section shows that, conversely, the system bond graph can be derived from the stoichiometric matrix \( N \). The following constructive procedure is used:
1. For each species create a Ce component with appropriate name and a 0 junction; connect a bond from the 0 junction to the Ce component.

2. For each reaction create an Re component with appropriate name and two 1 junctions; connect a bond from one 1 junction to the forward port of the Re component and a bond from the reverse port of the Re component to the other 1 junction.

3. For each negative entry $N_{ij}$ in the stoichiometric matrix, connect $-N_{ij}$ bonds from the zero junction connected to the $i$th species to the one junction connected to the forward port of the $j$th reaction.

4. For each positive entry $N_{ij}$ in the stoichiometric matrix, connect $N_{ij}$ bonds from the one junction connected to the reverse port of the $j$th reaction to the zero junction connected to the $i$th species.

For example, the reaction $A \xrightleftharpoons{r_1} 2B$ has the stoichiometric matrix

$$N = \begin{pmatrix} -1 \\ 2 \end{pmatrix}$$  \hspace{1cm} (2.12)

and the bond graph of Figure 1(a). The reaction $B + C \xrightleftharpoons{r_2} D + E$ has the stoichiometric matrix

$$N = \begin{pmatrix} -1 \\ -1 \\ 1 \\ 1 \end{pmatrix}$$  \hspace{1cm} (2.13)

and has the bond graph of Figure 1(b).

### 3 Chemostats, Flowstats and Pathways

As discussed previously (Gawthrop and Crampin, 2016; Gawthrop, 2017), the notion of a chemostat (Polettini and Esposito, 2014) is useful in creating an open system from a closed system. As discussed by Gawthrop (2017), the chemostat has a number of interpretations:

1. one or more species are fixed to give a constant concentration (Gawthrop et al., 2015); this implies that an appropriate external flow is applied to balance the internal flow of the species.

2. as a Ce component with a fixed state.
3. as an external port of a module which allows connection to other modules.

In the context of stoichiometric analysis, the chemostat concept provides a flexible alternative to the primary and currency exchange reactions (Schilling et al., 2000; Palsson, 2006, 2015).

Gawthrop and Crampin (2016) discuss the dual concept of flowstats which again has a number of interpretations:

1. one or more reaction flows are fixed.
2. as an \( \mathbf{Re} \) component with a fixed flow.
3. as an external port of a module which allows connection to other modules.

In the context of stoichiometric analysis, the flowstat concept provides a way of isolating parts of a network by setting zero flow in the reactions connecting the parts. Such zero flow flowstats can also be interpreted as removing the corresponding enzyme via gene knockout.

In terms of stoichiometric analysis, the closed system equations (2.7) and (2.8) are replaced by:

\[
\dot{\mathbf{X}} = \mathbf{N}^{cd} \mathbf{V} \quad (3.1) \\
\Phi = -\mathbf{N}^T \phi \quad (3.2)
\]

where \( \mathbf{N}^{cd} \) is created from the stoichiometric matrix \( \mathbf{N} \) by setting rows corresponding to chemostats species and columns corresponding to flowstatted reactions to zero (Gawthrop and Crampin, 2016). As discussed by Gawthrop and Crampin (2016), system pathways corresponding to (3.1) are defined by the right-null space of \( \mathbf{N}^{cd} \) that is the columns of the matrix \( \mathbf{K}^{cd} \) where \( \mathbf{N}^{cd} \mathbf{K}^{cd} = 0 \). Further, then steady-state pathways are defined by:

\[
\mathbf{V} = \mathbf{K}^{cd} \mathbf{v} \quad (3.3)
\]

were \( \mathbf{v} \) is the pathway flow. It follows from Equation (3.1) that Equation (3.3) implies that \( \dot{\mathbf{X}} = 0 \). Gawthrop and Crampin (2017) define the pathway stoichiometric matrix \( \mathbf{N}_p \) as:

\[
\mathbf{N}_p = \mathbf{N} \mathbf{K}^{cd} \quad (3.4)
\]

In a similar fashion to equation (3.2), the pathway reaction free energies \( \Phi_p \) are given by

\[
\Phi_p = -\mathbf{N}_p^T \phi \quad (3.5)
\]

In the same way as the stoichiometric matrix \( \mathbf{N} \) relates reaction flows to species and thus represents a set of reactions, the pathway stoichiometric matrix \( \mathbf{N}_p \) also represents a set of reactions: these reactions will be called the pathway reactions.

Following Schilling et al. (2000), pathways can be divided into three categories according to the species corresponding to the non zero elements in the relevant column of the pathway stoichiometric matrix \( \mathbf{N}_p \):

I The species include primary metabolites; these pathways are of functional interest.
The species include currency metabolites only; these pathways dissipate energy without creating or consuming primary metabolites. Schilling et al. (2000) call these pathways *futile cycles*.

There are no species.

Pathway reactions for type I pathways contain both primary and currency metabolites; pathway reactions for type II pathways contain currency metabolites only; pathway reactions for type III pathways are empty.

Pathways have an equivalent bond graph obtained by applying the conversion method of § 2 to $N_p$ instead of $N$ Gawthrop and Crampin (2017); this fact can be utilised to give simple physically plausible models of complex systems Gawthrop et al. (2019).

### 3.1 Illustrative example Noor (2018)

![Bond graph](image)

![Pathway bond graph](image)

**Figure 2:** Bond graphs for illustrative example Noor (2018)

Noor (2018) gives a simple illustrative example of the three types of pathway; Figure 2(a) gives the corresponding bond graph. The reactions are:

\[
\begin{align*}
A & \underset{r_1}{\iff} B \quad (3.6) \\
\text{ATP} + B & \underset{r_2}{\iff} \text{ADP} + C \quad (3.7) \\
C & \underset{r_3}{\iff} D \quad (3.8) \\
D & \underset{r_4}{\iff} A \quad (3.9) \\
A & \underset{r_5}{\iff} C \quad (3.10) \\
C & \underset{r_6}{\iff} E \quad (3.11)
\end{align*}
\]
The there are seven species and six reactions giving states \( x \) and flows \( v \):

\[
\begin{align*}
\begin{bmatrix}
x_A \\
x_{ADP} \\
x_{ATP} \\
x_B \\
x_C \\
x_D \\
x_E
\end{bmatrix} = \\
\begin{bmatrix}
v_{r_1} \\
v_{r_2} \\
v_{r_3} \\
v_{r_4} \\
v_{r_5} \\
v_{r_6}
\end{bmatrix}
\end{align*}
\] (3.12)

The stoichiometric matrix is:

\[
N = 
\begin{pmatrix}
-1 & 0 & 0 & 1 & -1 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 \\
0 & -1 & 0 & 0 & 0 & 0 \\
1 & -1 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & 0 & 1 & -1 \\
0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1
\end{pmatrix}
\] (3.13)

Setting A, E, ATP and ADP as chemostats, \( N^{cd} \) is constructed by setting the corresponding rows of \( N \) to zero. The corresponding null space is three dimensional and corresponds to the three pathways:

1. \( r_1 + r_2 + r_3 + r_4 \)
2. \( r_3 + r_4 + r_5 \)
3. \( r_1 + r_2 + r_6 \)

Using (3.4), the pathway stoichiometric matrix \( N_p \) is:

\[
N_p = 
\begin{pmatrix}
0 & 0 & -1 \\
1 & 0 & 1 \\
-1 & 0 & -1 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 1
\end{pmatrix}
\] (3.14)

The three pathway reactions are:

\[
\text{ATP } \overset{P_1}{\longleftrightarrow} \text{ ADP}
\] (3.15)

\[
\text{ADP } \overset{P_2}{\longleftrightarrow} \text{ ATP}
\] (3.16)

\[
\text{A + ATP } \overset{P_3}{\longleftrightarrow} \text{ ADP + E}
\] (3.17)
Pathway reaction P1 corresponds to a type II pathway, pathway reaction P2 to a type III pathway and pathway reaction P3 to a type I pathway where A is converted to E driven by the conversion of ATP to ADP. The example is extended by assigning a set of nominal chemical free energies $\phi$ to the species: $\phi_A = 1$, $\phi_{ATP} = 0$, $\phi_{ADP} = 3$, $\phi_B = 1$, $\phi_C = 1$, $\phi_D = 1$, $\phi_E = 0$. The pathway reaction free energies are then computed using (3.5) as $\Phi_{P1} = -2$, $\Phi_{P2} = 0$, $\Phi_{P3} = -1$. As the free energy for each pathway only depends on the species appearing in the pathway reactions, the free energy of non-chemostatted species are irrelevant for this computation. In fact the free energies of the species will correspond to the steady-state values of concentrations of the non-chemostatted species arising from the flow patterns corresponding to the chemostat free energies (Gawthrop, 2018). The pathway bond graph appears in Figure 2(b).

### 3.2 Example: Glycolysis & Pentose Phosphate Pathways

The combination of the Glycolysis & Pentose Phosphate networks provides a number of different products from the metabolism of glucose. This flexibility is adopted by proliferating cells, such as those associated with cancer, to adapt to changing requirements of biomass and energy production (Vander Heiden et al., 2009).

The *e.coli* Core Model (Orth et al., 2010a; Palsson, 2015) is used as the basis for the examples in this section. In particular, the species, reactions and stoichiometric matrix were extracted from the spreadsheet *ecoli_core_model.xlsx* but with the biomass equations deleted and the reaction CYTBD (containing $\frac{1}{2}O_2$) multiplied by 2 to give integer stoichiometry. The submodel containing the reactions of the combined Glycolysis & Pentose Phosphate pathways was then extracted (see Appendix A for details) and converted to a bond graph in bond graph tools format using the algorithm of § 2. The following procedure was adopted to obtain physiologically-realistic values for the species free energies $\phi$.

1. The reaction free energies $\Phi$ were extracted from Table 4 provided by Park et al. (2016).

2. A set of consistent species free energies $\phi$ was obtained from equation (3.2) using

$$\phi = - \left(N^T\right)\dagger \Phi$$

(3.18)

where $\dagger$ denotes the pseudo inverse$^3$.

The reaction free energies for each reaction are given in Appendix A and, because of the above procedure, correspond to the reaction free energies listed by Park et al. (2016) Table 4.

As discussed by Garrett and Grisham (2017, § 22.6d), it illuminating to pick out individual paths through the network to see how these may be utilised to provide a variety of products. This is reproduced here by choosing appropriate chemostats and flowstats (§ 3) to give the results listed by Garrett and Grisham (2017, § 22.6d). In each case, the corresponding pathway reaction free energy is given. For consistency with Garrett and Grisham (2017, § 22.6d), each pathway starts with Glucose 6-phosphate (G$_6$P).

The following chemostat list is used (together with additional chemostats) in each of the following sections: \{ ADP, ATP, CO$_2$, G$_6$P, H, H$_2$O, NAD, NADH, NADP, NADPH, PI, PYR \}.

---

$^3$The pseudo inverse was implemented using the python linear algebra package function `linalg.pinv()`
3.2.1 Glycolysis

The glycolysis pathway is isolated from the pentose phosphate pathway by replacing the two connecting reactions (G6PDH2R and TKT2) by flowstats. This gives rise to the pathway:

- PGI + PFK + FBA + TPI + 2GAPD - 2PGK - 2PGM + 2ENO + 2PYK

The corresponding pathway reaction is:

\[ 3 \text{ADP} + \text{G}_6\text{P} + 2\text{NAD} + 2\text{PI} \xrightleftharpoons{P_1} 3\text{ATP} + \text{H} + 2\text{H}_2\text{O} + 2\text{NADH} + 2\text{PYR} \quad (-42.22 \text{kJ mol}^{-1}) \]

The pathway reaction \( P_1 \) is the overall glycolysis reaction Garrett and Grisham (2017, § 18.2). The negative reaction free energy indicates that the reaction proceeds in the forward direction.

3.2.2 \( R_5P \) & NADPH generation

This pathway is isolated by setting PGI and TKT2 as flowstats and the product \( R_5P \) is added to the chemostat list. This gives rise to the pathway:

- G6PDH2R + PGL + GND + RPI

The corresponding pathway reaction is:

\[ \text{G}_6\text{P} + \text{H}_2\text{O} + 2\text{NADP} \xrightleftharpoons{P_1} \text{CO}_2 + 2\text{H} + 2\text{NADPH} + \text{R}_5\text{P} \quad (-17.01 \text{kJ mol}^{-1}) \]

The pathway reaction \( P_1 \) corresponds to the \( R_5P \) & NADPH synthesis discussed in comment 1 of Garrett and Grisham (2017, § 22.6d). The negative reaction free energy indicates that the reaction proceeds in the forward direction.

3.2.3 \( R_5P \) generation

This pathway is isolated by setting GAPD and G6PDH2R as flowstats and the product \( R_5P \) is added to the chemostat list. This gives rise to the pathway:

- - 5PGI - PFK - FBA - TPI - 4RPI + 2TKT2 + 2TALA + 2TKT1 + 4RPE

The corresponding pathway reaction is:

\[ \text{ADP} + \text{H} + 6\text{R}_5\text{P} \xrightleftharpoons{P_1} \text{ATP} + 5\text{G}_6\text{P} \quad (20.30 \text{kJ mol}^{-1}) \]

The pathway reaction \( P_1 \) corresponds to the \( R_5P \) synthesis discussed in comment 2 of Garrett and Grisham (2017, § 22.6d). The positive reaction free energy indicates that the reaction proceeds in the reverse direction.
3.2.4 NADPH generation

This pathway is isolated by setting GAPD as a flowstat. This gives rise to the pathway:

- $\text{5PGI} - \text{PFK} - \text{FBA} - \text{TPI} + 6\text{G6PDH2R} + 6\text{PGL} + 6\text{GND} + 2\text{RPI} + 2\text{TKT2} + 2\text{TALA} + 2\text{TKT1} + 4\text{RPE}$

The corresponding pathway reaction is:

$$\text{ADP} + G_6P + 6H_2O + 12\text{NADP} \xrightarrow{\text{P1}} \text{ATP} + 6CO_2 + 11H + 12\text{NADPH} \ (-81.79 \text{ kJ mol}^{-1})$$

The pathway reaction P₁ corresponds to the NADPH synthesis discussed in comment 3 of Garrett and Grisham (2017, § 22.6d). The negative reaction free energy indicates that the reaction proceeds in the forward direction.

3.2.5 NADPH & ATP generation

This pathway is isolated by setting PGI as flowstat. This gives rise to the pathway:

- $2\text{PFK} + 2\text{FBA} + 2\text{TPI} + 5\text{GAPD} - 5\text{PGK} - 5\text{PGM} + 5\text{ENO} + 5\text{PYK} + 3\text{G6PDH2R} + 3\text{PGL}$
  - $3\text{GND} + \text{RPI} + \text{TKT2} + \text{TALA} + \text{TKT1} + 2\text{RPE}$

The corresponding pathway reaction is:

$$8\text{ADP} + 3G_6P + 5\text{NAD} + 6\text{NADP} + 5\text{PI} \xrightarrow{\text{P1}} 8\text{ATP} + 3\text{CO}_2 + 8\text{H} + 2\text{H}_2\text{O} + 5\text{NADH} + 6\text{NADPH} + 5\text{PYR} \ (-146.44 \text{ kJ mol}^{-1})$$

The pathway reaction P₁ corresponds to the NADPH and ATP synthesis discussed in comment 4 of Garrett and Grisham (2017, § 22.6d). The negative reaction free energy indicates that the reaction proceeds in the forward direction.

4 Modularity

As discussed by Gawthrop and Crampin (2016), there are two related but distinct concepts of modularity: computational modularity where physical correctness is retained and behavioural modularity where module behaviour (such as ultra-sensitivity) is retained. It is the former that is discussed in this section. As discussed by Gawthrop (2017), modular bond graphs provide a way of decomposing complex biomolecular systems into manageable parts (Gawthrop et al., 2015; Gawthrop and Crampin, 2016). In particular, this paper combines the modularity concepts of Neal et al. (2016) with the bond graph approach to give a more flexible approach to modularity. The basic idea (Gawthrop, 2017) is simple: modules are self-contained and have no explicit ports; but any species, as represented by a Ce component has the potential to become a port. Thus if two modules share the same species, the corresponding Ce component in each module is replaced by a port with the same name, and the species is explicitly represented as a Ce component on a higher level. Moreover, each module can be individually tested by replacing the relevant Ce components by chemostats.

The algorithm is:
1. Within each module, each Ce component corresponding to a common species is exposed – replaced by a port component. Note that the algorithm of § 2 ensures that each Ce is attached to a 0 junction.

2. For each common species, create a Ce component connected to a 0 component.

3. Connect all module ports associate with each species to the 0 junction associated with the species; all instances of Ce components corresponding to each species are thus unified.

Figure 3: Modularity. Modules M1 and M2 correspond to Figures 1(a) & 1(b) respectively. The common species B is exposed as a port in each module and connected to the new Ce:B component via a 0 junction.

For example, let modules M1 and M2 correspond to Figures 1(a) & 1(b) respectively. In Figure 3, the common species B is exposed as a port in each module and connected to the new Ce:B component via a 0 junction. The composite system contains the two reactions:

\[
\begin{align*}
A & \xrightarrow{r_1} 2B \\
C + B & \xrightarrow{r_1} D + E
\end{align*}
\]  (4.1)  (4.2)

Choosing the set of chemostats to be \{A, C, D, E\} the corresponding pathway stoichiometric matrix \(N_p\) is

\[
N_p = \begin{pmatrix}
-1 \\
-2 \\
2 \\
2 \\
0
\end{pmatrix}
\]  (4.3)

where the species are \{A, C, D, E, B\} and the reactions \{r_1, r_2\}. The pathway reaction \(P_1\) is then:

\[
A + 2C \xrightleftharpoons{P_1} 2D + 2E
\]  (4.4)

4.1 Example: Metabolism

As in § 3.2, the e.coli Core Model (Orth et al., 2010a; Palsson, 2015) is used. In particular, reactions corresponding to four modules (Glycolysis, TCA cycle, Electron Transport Chain and
ATPase) were extracted as detailed in Appendix B. For simplicity, reaction PDH (converting PYR to ACCOA) and reaction NADTRHD (converting NADP/NADPH to NAD/NADH) were included in the TCA cycle module.

These modules can be analysed individually. For example the TCA cycle module can be analysed using the set of chemostats:

\{PYR, CO_2, ADP, ATP, H_2O, NAD, NADH, PI, H, Q_8, Q_8H_2\}

The two pathways are

1. FRD7 + SUCDI

2. PDH + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + NADTRHD

These two pathways correspond to the two pathway reactions:

\(
P_1 \quad ADP + 2H_2O + 4NAD + PI + PYR + Q_8 \xrightarrow{P_2} ATP + 3CO_2 + 2H + 4NADH + Q_8H_{2p}\)

The first is a type III reaction and the second a type I reaction which utilises the free energy of PYR to generate two NADH, one NADHP, one ATP and one Q_8H_2 whilst releasing two CO_2 and two H.

The overall metabolic system comprises the four modules (Glycolysis, TCA cycle, Electron Transport Chain and ATPase) connected together. Using the approach of § 4, the modules are interconnected by declaring the set of species that the modules have in common:

\{PYR, ATP, ADP, PI, H, H_E, NAD, NADH, H_2O, Q_8, Q_8H_2\}

These species are unified as described in § 4. To analyse the composite system, the set of chemostats was chosen as:

\{GLCD_E, CO_2, O_2, ADP, ATP, H_2O, PI, H\}.

The three pathways are

1. PFK + FBP

2. FRD7 + SUCDI

3. 2 GLCPTS + 2 PGI + 2 PFK + 2 FBA + 2 TPI + 4 GAPD - 4 PKG - 4 PGM + 4 ENO + 2 PYK + 4 PDH + 4 CS + 4 ACONTA + 4 ACONTB + 4 ICDHYR + 4 AKGDH - 4 SUCOAS - 4 FRD7 + 4 FUM + 4 MDH + 4 NADTRHD + 20 NADH16 + 12 CYTBD + 27 ATPS4R

15
These three pathways correspond to the three pathway reactions:

\[
\begin{align*}
ATP + H_2O & \overset{P_1}{\Rightarrow} ADP + PI + H \\
2 GLCD_E + 12 O_2 + 35 ADP + 35 PI + 35 H & \overset{P_2}{\Rightarrow} 12 CO_2 + 35 ATP + 47 H_2O \\
\end{align*}
\]

As in § 3.1, pathway reaction P1 corresponds to a type II pathway, pathway reaction P2 to a type III pathway and pathway reaction P3 to a type I pathway. Pathway 3 corresponds to the metabolic generation of ATP using the free energy of \( GLCD_E \). The ratio of ATP to \( GLCD_E \) is 17.5; this is the value quoted by Palsson (2015, § 19.2).

### 5 FBA and EBA in a bond graph context

The standard FBA approach is to create open systems from closed systems by adding “exchange reactions” to species which connect to the outside world – for example: \( ATP \iff \varnothing \). In contrast, the bond graph approach would declare ATP to be a chemostat. Chemostats provide a more flexible approach as they can be created without changing system structure and are used in the sequel.

FBA (Orth et al., 2010b) uses the linear equation (3.3) within a constrained linear optimisation to compute pathway flows. EBA adds two sorts of nonlinear constraint arising from thermodynamics. This section shows that the bond graph approach automatically includes the EBA constraint equations by considering Inequality (2.6) and Equation (3.2). In particular:

1. Inequality (2.6) corresponds to Equation 8 of Beard et al. (2002). This inequality can be re-expressed as:

\[
\Phi_i = r_i(\phi)V_i
\]

where \( r_i(\phi) > 0 \).

2. If \( K \) is the right null matrix of \( N \), it follows from Equation (3.2) that

\[
K^T\Phi = 0
\]

This corresponds to Equation 7 of Beard et al. (2002). Note that \( K \) defines the pathways of the closed system system (with no chemostats).

Moreover, the pathways of the open system as defined by \( K^{cd} \) can be considered by defining \( R = \text{diag } r_i \) and using Equation (3.3):

\[
K^T R K^{cd} v = 0
\]

Equation (5.4) and inequality (5.2) constrain the pathway flows \( v \); this is illustrated in the following examples drawn from Beard et al. (2002).
5.1 Example: Parallel reactions

Figure 4: Bond graphs corresponding to examples from Beard et al. (2002) (1 junctions are not shown for clarity). (a) (Beard et al., 2002, Fig. 2), (b) (Beard et al., 2002, Fig. 3)

Beard et al. (2002, Fig. 2) motivate EBA using the example of two resistors in parallel. Figure 4(a) shows the bond graph of the analogous reaction system: the species A and B are joined by two reactions:

\[
\begin{align*}
A & \xrightleftharpoons{r_1} B \\
A & \xrightleftharpoons{r_2} B
\end{align*}
\]  

(5.5)  

(5.6)

The stoichiometric matrix is:

\[
N = \begin{pmatrix}
-1 & -1 \\
1 & 1
\end{pmatrix}
\]  

(5.7)

and the null space matrix \( K \) is

\[
K = \begin{pmatrix}
-1 \\
1
\end{pmatrix}
\]  

(5.8)

corresponding to the pathway: \(-r_1 + r_2\).

Setting A and B as chemostats:

\[
N^{cd} = \begin{pmatrix}
0 & 0 \\
0 & 0
\end{pmatrix}
\]  

(5.9)

\[
K^{cd} = \begin{pmatrix}
1 & 0 \\
0 & 1
\end{pmatrix}
\]  

(5.10)

Equation (5.4) then becomes:

\[-r_1 v_1 + r_2 v_2 = 0\]

(5.11)

As \( r_i > 0 \), it follows that \( v_1 \) and \( v_2 \) must either be zero or have the same sign.
5.2 Example: three-reaction cycle

Beard et al. (2002, Fig. 3) give the example of a three-reaction cycle. Figure 4(b) shows the corresponding bond graph. The species A, B and C are joined by three reactions:

\[
\begin{align*}
A & \xleftarrow{r_1} B \quad (5.12) \\
B & \xleftarrow{r_2} C \quad (5.13) \\
C & \xleftarrow{r_3} A \quad (5.14)
\end{align*}
\]

The stoichiometric matrix is:

\[
N = \begin{pmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix} \quad (5.15)
\]

and the null space matrix \( K \) is

\[
K = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \quad (5.16)
\]

corresponding to the pathway: \( r_1 + r_2 + r_3 \).

Setting A and B as chemostats:

\[
N_{cd} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 1 & -1 \end{pmatrix} \quad (5.17)
\]

\[
K_{cd} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} \quad (5.18)
\]

Equation (5.4) then becomes:

\[
r_1 v_1 + r_2 v_2 + r_3 v_2 = r_1 v_1 + (r_2 + r_3) v_2 = 0 \quad (5.19)
\]

As \( r_i > 0 \), it follows that \( v_1 \) and \( v_2 \) must either be zero or have the opposite sign.

Alternatively, setting A, B and C as chemostats:

\[
N_{cd} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad (5.20)
\]

\[
K_{cd} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 0 \end{pmatrix} \quad (5.21)
\]
Equation (5.4) then becomes:

\[ r_1 v_1 + r_2 v_2 + r_3 v_3 = 0 \]  

(5.22)

As \( r_i > 0 \), there are three possibilities: all flows are zero; one of the three pathway flows must have one sign and the other two flows the opposite sign; or one flow is zero and the other two have opposite signs.

6 Conclusion

1. It has been shown that the bond graph of a biomolecular system can be derived from the stoichiometric matrix. Thus the plethora of existing stoichiometric models can be automatically endowed with a number of features including

   (a) thermodynamic compliance
   (b) modularity
   (c) explicit energy flows allowing exploration of, for example, efficiency (Gawthrop and Crampin, 2018)
   (d) generation of reduced-order models using pathway analysis (Gawthrop and Crampin, 2017; Gawthrop et al., 2019).
   (e) energy compliant connections to other physical domains including models of chemoelectric transduction (Gawthrop et al., 2017; Gawthrop, 2017), membrane transporters (Pan et al., 2019), cardiac action potential (Pan et al., 2018a), chemomechanical transduction and photosynthesis.

2. The key equations of the EBA approach of Beard et al. (2002) have been shown to be implicit in the system bond graph.

3. Via the modular approach of § 4, the Re components of § 2, representing mass-action kinetics, can be replaced by thermodynamically compliant models of more complex kinetics Cornish-Bowden (2013) driven by enzymes and inhibitors including feedback inhibition, allosteric modulation and cooperativity.

4. This approach provides a basis for thermodynamically compliant whole-cell models.

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A Glycolysis & Pentose Phosphate Pathways: Reactions

\[
\begin{align*}
\text{GLCD} + \text{PEP} & \xrightarrow{\text{GLCPTS}} \text{G}_6\text{P} + \text{PYR} \quad (-26.81 \text{ kJ mol}^{-1}) \\
\text{G}_6\text{P} & \xrightarrow{\text{PGI}} \text{F}_6\text{P} \quad (-1.60 \text{ kJ mol}^{-1}) \\
\text{ATP} + \text{F}_6\text{P} & \xrightarrow{\text{PFK}} \text{ADP} + \text{FDP} + \text{H} \quad (-24.71 \text{ kJ mol}^{-1}) \\
\text{FDP} & \xrightarrow{\text{FBA}} \text{DHAP} + \text{G}_3\text{P} \quad (-1.98 \text{ kJ mol}^{-1}) \\
\text{DHAP} & \xrightarrow{\text{TPI}} \text{G}_3\text{P} \quad (-0.79 \text{ kJ mol}^{-1}) \\
\text{G}_3\text{P} + \text{NAD} + \text{PI} & \xrightarrow{\text{GAPD}} \text{G}_6\text{PGL} + \text{H} + \text{NADH} \quad (-1.32 \text{ kJ mol}^{-1}) \\
\text{3PG} + \text{ATP} & \xrightarrow{\text{PGK}} \text{13DPG} + \text{ADP} \quad (-1.42 \text{ kJ mol}^{-1}) \\
\text{2PG} & \xrightarrow{\text{PGM}} \text{3PG} \quad (-3.17 \text{ kJ mol}^{-1}) \\
\text{2PG} & \xrightarrow{\text{ENO}} \text{H}_2\text{O} + \text{PEP} \quad (-2.75 \text{ kJ mol}^{-1}) \\
\text{ADP} + \text{H} + \text{PEP} & \xrightarrow{\text{PYK}} \text{ATP} + \text{PYR} \quad (-7.09 \text{ kJ mol}^{-1}) \\
\text{G}_6\text{P} + \text{NADP} & \xrightarrow{\text{GPDHR}} \text{6PG} + \text{H} + \text{NADPH} \quad (-0.96 \text{ kJ mol}^{-1}) \\
\text{6PG} + \text{H}_2\text{O} & \xrightarrow{\text{PGI}} \text{6PGC} + \text{H} \quad (-0.96 \text{ kJ mol}^{-1}) \\
\text{6PGC} + \text{NADP} & \xrightarrow{\text{GND}} \text{CO}_2 + \text{NADPH} + \text{RU}5\text{PD} \quad (-15.08 \text{ kJ mol}^{-1}) \\
\text{RU}3\text{PD} & \xrightarrow{\text{RPI}} \text{R}3\text{P} \quad (-0.00 \text{ kJ mol}^{-1}) \\
\text{E}4\text{P} + \text{XU}3\text{PD} & \xrightarrow{\text{TKT}2} \text{F}6\text{P} + \text{G}3\text{P} \quad (-1.61 \text{ kJ mol}^{-1}) \\
\text{G}3\text{P} + \text{S}3\text{P} & \xrightarrow{\text{TALA}} \text{E}4\text{P} + \text{F}6\text{P} \quad (-5.43 \text{ kJ mol}^{-1}) \\
\text{R}3\text{P} + \text{XU}3\text{PD} & \xrightarrow{\text{TKT}1} \text{G}3\text{P} + \text{S}3\text{P} \quad (-0.40 \text{ kJ mol}^{-1}) \\
\text{RU}3\text{PD} & \xrightarrow{\text{RPE}} \text{XU}3\text{PD} \quad (-0.08 \text{ kJ mol}^{-1})
\end{align*}
\]
B Modular representation of Metabolism: Reactions

B.1 Glycolysis

\[ \text{GLCD}_E + \text{PEP} \xrightleftharpoons{\text{GLCPTS}} \text{G}_6\text{P} + \text{PYR} \quad (B.1) \]
\[ \text{G}_6\text{P} \xrightarrow{\text{PGI}} \text{F}_6\text{P} \quad (B.2) \]
\[ \text{ATP} + \text{F}_6\text{P} \xrightarrow{\text{PFK}} \text{ADP} + \text{FDP} + \text{H} \quad (B.3) \]
\[ \text{FDP} + \text{H}_2\text{O} \xrightarrow{\text{FBP}} \text{F}_6\text{P} + \text{PI} \quad (B.4) \]
\[ \text{FDP} \xrightarrow{\text{FBA}} \text{DHAP} + \text{G}_3\text{P} \quad (B.5) \]
\[ \text{DHAP} \xrightarrow{\text{TPI}} \text{G}_3\text{P} \quad (B.6) \]
\[ \text{G}_3\text{P} + \text{NAD} + \text{PI} \xrightarrow{\text{GAPD}} 1_3\text{DPG} + \text{H} + \text{NADH} \quad (B.7) \]
\[ 3\text{PG} + \text{ATP} \xrightarrow{\text{PGK}} 1_3\text{DPG} + \text{ADP} \quad (B.8) \]
\[ 2\text{PG} \xrightarrow{\text{PGM}} 3\text{PG} \quad (B.9) \]
\[ 2\text{PG} \xrightarrow{\text{ENO}} \text{H}_2\text{O} + \text{PEP} \quad (B.10) \]

\[ \text{ADP} + \text{H} + \text{PEP} \xrightleftharpoons{\text{PYK}} \text{ATP} + \text{PYR} \quad (B.11) \]

B.2 TCA cycle

\[ \text{COA} + \text{NAD} + \text{PYR} \xrightarrow{\text{PDH}} \text{ACCOA} + \text{CO}_2 + \text{NADH} \quad (B.12) \]
\[ \text{ACCOA} + \text{H}_2\text{O} + \text{OAA} \xrightarrow{\text{CS}} \text{CIT} + \text{COA} + \text{H} \quad (B.13) \]
\[ \text{CIT} \xrightarrow{\text{ACONTA}} \text{ACONC} + \text{H}_2\text{O} \quad (B.14) \]
\[ \text{ACONC} + \text{H}_2\text{O} \xrightarrow{\text{ACONTB}} \text{ICIT} \quad (B.15) \]
\[ \text{ICIT} + \text{NADP} \xrightarrow{\text{ICDHYR}} \text{AKG} + \text{CO}_2 + \text{NADPH} \quad (B.16) \]
\[ \text{AKG} + \text{COA} + \text{NAD} \xrightarrow{\text{AKGDH}} \text{CO}_2 + \text{NADH} + \text{SUCCOA} \quad (B.17) \]
\[ \text{ATP} + \text{COA} + \text{SUCC} \xrightarrow{\text{SUCCOA}} \text{ADP} + \text{PI} + \text{SUCCOA} \quad (B.18) \]
\[ \text{FUM} + \text{Q}_8\text{H}_2 \xrightarrow{\text{FRD}_7} \text{Q}_8 + \text{SUCC} \quad (B.19) \]
\[ \text{Q}_8 + \text{SUCC} \xrightarrow{\text{SUCCI}} \text{FUM} + \text{Q}_8\text{H}_2 \quad (B.20) \]
\[ \text{FUM} + \text{H}_2\text{O} \xrightarrow{\text{FUM}} \text{MALL} \quad (B.21) \]
\[
\begin{align*}
\text{MALL} + \text{NAD} & \xrightarrow{\text{MDH}} \text{H} + \text{NADH} + \text{OAA} \quad \text{(B.22)} \\
\text{NAD} + \text{NADPH} & \xrightarrow{\text{NADTRHD}} \text{NADH} + \text{NADP} \quad \text{(B.23)}
\end{align*}
\]

**B.3 Electron Transport Chain**

\[
\begin{align*}
4 \text{H} + \text{NADH} + \text{Q}_8 & \xrightarrow{\text{NADH}_{16}} 3 \text{H}_E + \text{NAD} + \text{Q}_8 \text{H}_2 \quad \text{(B.24)} \\
4 \text{H} + \text{O}_2 + 2 \text{Q}_8 \text{H}_2 & \xrightarrow{\text{CYTBD}} 2 \text{H}_2 \text{O} + 4 \text{H}_E + 2 \text{Q}_8 \quad \text{(B.25)}
\end{align*}
\]

**B.4 ATPase**

\[
\begin{align*}
\text{ADP} + 4 \text{H}_E + \text{PI} & \xrightarrow{\text{ATPS}_{4R}} \text{ATP} + 3 \text{H} + \text{H}_2 \text{O} \quad \text{(B.26)}
\end{align*}
\]