Computing Biomolecular System Steady-states

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Abstract

A new approach to computing the equilibria and steady-states of biomolecular systems modelled by bond graphs is presented. The approach is illustrated using a model of a biomolecular cycle representing a membrane transporter and a model of the mitochondrial electron transport chain.

1 Introduction

There are many ways of deriving the dynamical equations describing the time course of flows and concentrations in biomolecular systems [1, 2]. As physical systems, biomolecular systems are subject to the laws of thermodynamics [3, 4] and number of such energy-based approaches have been developed including the bond graph of Oster et al. [5, 6] further developed by Greifeneder and Cellier [7] and Gawthrop and Crampin [8, 9, 10]. The bond graph approach is particularly appropriate for systems which combine different physical domains and thus are particularly appropriate for electrogenic biomolecular systems Gawthrop et al. [11], Gawthrop [12]. In common with other approaches, the bond graph approach can be used to derive ordinary differential equations (ode) describing the time evolution of the chemical species, electrical charges and chemical and electrical flows.

In some cases, it is of interest to find values corresponding to a situation where the amount of each chemical species and electrical charge is constant; this is termed a steady-state of the system. System steady-states correspond to constant chemical and electrical flows; in the special case the the flows are zero, the system is said to be in equilibrium. A number of approaches to finding the steady-state of biomolecular networks have been suggested including simulation, flux balance analysis [13] and the diagram methods of King and Altman [14] and Hill [15].

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The simulation approach is exemplified by Bazil et al. [16] who state that “For the steady state analysis, the model was simulated until the absolute values of the state variable derivatives were less than $10^{-10}$ ...”. A similar approach is to simulate starting from equilibrium with slowly-varying chemostats followed by an algebraic solver for each simulation time point starting with the simulated state Gawthrop and Crampin [10]. Such approaches can be numerically challenging and time consuming.

Flux balance analysis (FBA) [13] is based on the system stoichiometric matrix but ignores reaction kinetics. Essentially, the system stoichiometric matrix constrains all steady-state flows $V$ to lie within the right null subspace of the chemodynamic stoichiometric matrix $N^{cd}$. These constraints are not enough to deduce the steady-state flows $V$. Specific values for $V$ are found by adding extra constraints together with a quadratic (in the flows) cost function and applying linear programming. Energy considerations are not directly addressed, but can be added on [17].

The diagram method of King and Altman [14] and Hill [15] can be used to generate explicit rate equations for reaction networks using, for example, the software described by Qi et al. [18].

The approach to steady-state analysis proposed here is based on two ideas: using the Faraday equivalent potential [12] to unify chemical and electrical potential and using the positive-pathway approach [10] to represent the steady-state flow pathways.

The approach suggested here is like the FBA approach insofar as it uses pathway analysis of steady-state flows, and like the simulation approach in that it uses reaction kinetics. When based on bond graph models, the approach ensures thermodynamic compliance.

§ 2 gives the equations required in the rest of the paper. § 3 shows how conventional mass-action kinetics, expressed in terms of forward $\Phi_f$ and reverse $\Phi_r$ reaction affinities, can be re-expressed in terms of reaction affinity $\Phi = \Phi_f - \Phi_r$ and summed reaction affinity $\Phi^* = \Phi_f + \Phi_r$. This involves the hyperbolic functions sinh and tanh. § 4 shows how the equilibrium values of the free potentials can be deduced from the fixed potentials. § 5 contains the main results of the paper: how the steady-state values of the free potentials can be deduced from the fixed potentials for a given pathway flow. § 6 uses the biomolecular cycle of Hill [15] to illustrate the approach. § 7 uses the Mitochondrial Electron Transport Chain as a more detailed illustrative example. § 8 concludes the paper and suggest future research directions.

## 2 Background

This section contains the background material required in the rest of the paper. More information is to found in a companion paper [12].

The Faraday-equivalent potential [12] of a substance A is given in terms of the potential $\phi_A$ when the amount of the substance is $x_A$mol as:

$$\phi_A = \phi_A^\circ + \phi_N \ln \frac{x_A}{x_A^\circ}$$

where $\phi_N = \frac{RT}{F}$

(1)

$R = 8.314 \text{ JK}^{-1}\text{mol}^{-1}$ is the universal gas constant, $T \text{ K}$ is the absolute temperature and $F \approx 96.5 \times 10^3 \text{C mol}^{-1}$ is Faraday’s constant.
1. For a given temperature, $\phi_N$ is a constant given by (1) and, at $T = 300 \, \text{C}$ has a value of about 26 mV.

2. Equation (1) has two parameters:
   (a) $\phi_A^\circ$, the potential of the substance in the relevant compartment at nominal conditions and
   (b) $x_A^\circ$, the amount of the substance in the relevant compartment at the nominal conditions defining the nominal potential $\phi_A^\circ$.

3. Given the two parameters $\phi_A^\circ$ and $x_A^\circ$, and the constant $\phi_N$, the amount $x_A$ may be deduced from $\phi_A$ by inverting Equation (1):

   $$x_A = x_A^\circ \exp \frac{\phi_A - \phi_A^\circ}{\phi_N}$$

4. The potential $\phi_A^\circ$ of substance A at nominal conditions can be derived from the potential $\phi_A^\circ$ at standard conditions using the formula [4]:

   $$\phi_A^\circ = \phi_A^\circ + \phi_N \ln \frac{x_A^\circ}{x_A^\circ}$$

   Such standard potentials are listed for common substances by Atkins and de Paula [4].

5. Equation (1) can be rewritten as:

   $$\phi_A = \phi_N \ln K_A x_A$$

   where $K_A = \frac{\exp \frac{\phi_A^\circ}{\phi_N}}{x_A^\circ}$

   The forward $\Phi^f$ and reverse $\Phi^r$ reaction affinities can be deduced from the Faraday-equivalent potentials $\phi$ of the substances using the forward $N^f$ and reverse $N^r$ stoichiometric matrices as:

   $$\Phi^f = N^f T \phi$$

   $$\Phi^r = N^r T \phi$$

   Furthermore, the net reaction affinity $\Phi$ is defined as

   $$\Phi = \Phi^f - \Phi^r$$

   and so $\Phi = -N^T \phi$ where $N = N^r - N^f$

   and $N$ is the system stoichiometric matrix.

   Mass-action reaction kinetics can be written as:

   $$v = \kappa \left( v_0^+ - v_0^- \right)$$

   where $v_0^+ = \exp \frac{\Phi^f}{\phi_N}$

   and $v_0^- = \exp \frac{\Phi^r}{\phi_N}$
where \( \kappa \) is a constant and \( \Phi^f \) and \( \Phi^r \) are the forward and reverse reaction affinities.

From equations (8) – (10), zero reaction flow \( v = 0 \) implies that

\[
v_0^+ = v_0^-
\]

and \( \Phi = \Phi^f - \Phi^r = 0 \) \( (11) \)

As discussed in the literature, the system ode can be written as:

\[
\dot{X} = NV
\]

where \( \dot{X} \) is the rate of change of the vector of chemical species and electrical charge, \( N \) is the system stoichiometric matrix and \( V \) is the vector of chemical and electrical flows.

The notion of a chemostat \([19, 9]\), the chemical analogue of a voltage source, is useful when considering the steady-states of open biomolecular systems. In particular, as discussed by Gawthrop and Crampin \([9]\), in the presence of chemostats:

\[
\dot{X} = N^{cd}V
\]

where \( N^{cd} \) is \( N \) with the rows corresponding to the chemostats set to zero. In either case, the reaction potentials \( \Phi \) are given by Equation (7).

The left null space matrix \( G \) of \( N^{cd} \) has the property that:

\[
GN^{cd} = 0
\]

hence \( G\dot{X} = GN^{cd}V = 0 \) \( (15) \)

The \( n_{cm} \) rows of \( G \) thus define the \( n_{cm} \) conserved moieties given by:

\[
GX = GX_0
\]

where \( X_0 \) is the initial state.

In this paper, the order of the \( i \)th conserved moiety is defined as the the number of non zero entries in the \( i \)th row of \( G \) minus 1. Thus zero-order moieties correspond to chemostats and first order moieties to two substances whose net amount is constant.

### 3 Reaction Kinetics

The mass-action kinetics of eqns. (8) – (10) are expressed in terms of the forward and reverse reaction affinities \( \Phi^f \) and \( \Phi^r \). One property of eqns. (8) – (10) is that flow \( v \) is zero if \( \Phi^f = \Phi^r \) or \( \Phi = \Phi^f - \Phi^r = 0 \) where \( \Phi \) is the (net) reaction affinity.

To obtain further insight into mass-action reaction kinetics it is therefore convenient to rewrite eqn. (8) explicitly in terms of the reaction affinity \( \Phi \) and sumed reaction affinity \( \Phi^* \) where:

\[
\Phi = \Phi^f - \Phi^r \quad \text{and} \quad \Phi^* = \Phi^f + \Phi^r
\]

(17)

These definitions imply that

\[
\Phi^f = \frac{\Phi^* + \Phi}{2} \quad \text{and} \quad \Phi^r = \frac{\Phi^* - \Phi}{2}
\]

(18)
and hence that

\[ v_0^+ = \kappa^* \exp \frac{\Phi_f}{2\phi_N} \quad \text{and} \quad v_0^- = \kappa^* \exp \frac{-\Phi_f}{2\phi_N} \]  \hspace{1cm} (19)

where \( \kappa^* = \exp \frac{\Phi^*}{2\phi_N} \) \hspace{1cm} (20)

Introducing the difference \( v_0 \) and sum \( v_0^* \) of \( v_0^+ \) (9) and \( v_0^- \) (10):

\[ v_0 = v_0^+ - v_0^- = 2\kappa^* \sinh \frac{\Phi}{2\phi_N} \] \hspace{1cm} (21)

\[ v_0^* = v_0^+ + v_0^- = 2\kappa^* \cosh \frac{\Phi}{2\phi_N} \] \hspace{1cm} (22)

where \( \sinh \) and \( \cosh \) are the hyperbolic \( \sin \) and \( \cos \) functions respectively.

Using these equations, the mass-action kinetics of eqn. (8) can be rewritten in two alternative forms:

\[ v = 2\kappa\kappa^* \sinh \frac{\Phi}{2\phi_N} \] \hspace{1cm} (23)

\[ v = \kappa v_0^* \tanh \frac{\Phi}{2\phi_N} \] \hspace{1cm} (24)

where \( \kappa^* = \exp \frac{\Phi_f + \Phi_r}{2\phi_N} \) \hspace{1cm} (25)

and \( v_0^* = \exp \frac{\Phi_f}{\phi_N} + \exp \frac{\Phi_r}{\phi_N} \) \hspace{1cm} (26)

\( \tanh \) is the hyperbolic \( \tan \) function, (25) follows from (17)&(20) and (26) follows from Equations (9)&(10). Equation (23) is explicitly in terms of the reaction affinity \( \Phi \) (7) and, via \( \kappa^* \) (20), \( \Phi^* \) (17) and via (5), the potentials \( \phi \). Equation (24) is also explicitly in terms of the reaction affinity \( \Phi \) (7) but, unlike Equation (23), depends on \( v_0^* \) rather than \( \kappa^* \).

Define \( \kappa_{eq} \) and \( v_{eq}^0 \) as the equilibrium values of \( \kappa^* \) and \( v_0^* \) respectively. At equilibrium, \( \Phi_f = \Phi_r \) and thus, from Equations (25) and (26) it follows that

\[ v_{eq}^0 = 2\kappa_{eq} \] \hspace{1cm} (27)

Equations (23) and (24) can be rewritten as:

\[ v = 2\kappa \rho_s \sinh \frac{\Phi}{2\phi_N} \quad \text{where} \quad \rho_s = \frac{\kappa^*}{\kappa_{eq}} \] \hspace{1cm} (28)

\[ v = 2\kappa \rho_t \tanh \frac{\Phi}{2\phi_N} \quad \text{where} \quad \rho_t = \frac{v_0^*}{v_{eq}^0} \] \hspace{1cm} (29)

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and $\bar{\kappa} = \kappa \kappa^{eq} = \kappa^{eq}_{\frac{\kappa}{2}}$. Near equilibrium, $\Phi \approx 0$ and these two equations can each be approximated by functions of $\Phi$ only as:

$$v \approx 2\bar{\kappa} \sinh \frac{\Phi}{2\phi_N}$$  \hspace{1cm} (30)

$$v \approx 2\bar{\kappa} \tanh \frac{\Phi}{2\phi_N}$$  \hspace{1cm} (31)

They may be further approximated by the linear equation:

$$v \approx \bar{\kappa} \frac{\Phi}{\phi_N}$$  \hspace{1cm} (32)

### 3.1 Example: A Basic Electrogenic Network

![Bond graph of a basic electrogenic network](image)

Figure 1: A Basic Electrogenic Network. The system bond graph has two $C$ components: $C:A$ and $C:B$ representing the chemical species A and B respectively and a third $C$ component $C:E$ representing electrical trans-membrane charge. The $Re$ component represents the reaction and the 0 and 1 junction connect components appropriately.

Figure 3.1 gives the bond graph of a basic electrogenic network where the trans-membrane reaction:

$$A \overset{r}{\Rightarrow} B$$  \hspace{1cm} (33)

accumulates charge on the capacitor $C:E$. For illustration, it is assumed that:

$$\phi^\circ_A = \phi^\circ_B = 0$$  \hspace{1cm} (34)

The state vector is chosen as

$$X = (x_A \ x_B \ x_E)^T$$  \hspace{1cm} (35)
where $x_A$, $x_B$ and $x_E$ are the amounts of $A$, $B$ and charge respectively. The stoichiometric matrices $N$, $N^f$ and $N^r$ are given by:

\[
N = \begin{pmatrix} -1 \\ 1 \\ 2 \end{pmatrix}, \quad N^f = \begin{pmatrix} 1 \\ 0 \\ -1 \end{pmatrix}, \quad N^r = \begin{pmatrix} 0 \\ 1 \end{pmatrix}
\]  

Hence the corresponding reaction affinities are given by Equations (5) – (7) as

\[
\Phi^f = \phi_A - \phi_E \quad \Phi^r = \phi_B + \phi_E
\]

and the corresponding difference and summed reaction affinities are:

\[
\Phi = \phi_A - \phi_B - 2\phi_E \quad \Phi^* = \phi_A + \phi_B
\]

Consider first the non-electrogenic case where $\phi_E = 0$. Then using the formula (1) and eqn. (22) for chemical potential:

\[
v^*_0 = \exp \frac{\Phi^f}{\phi_N} + \exp \frac{\Phi^r}{\phi_N} = \exp \frac{\phi_A}{\phi_N} + \exp \frac{\phi_B}{\phi_N} = K_A x_A + K_B x_B
\]

For the purposes of illustration, further assume that $K_A = K_B = 1$ thus

\[
v^*_0 = x_A + x_B
\]

As $A$ and $B$ form a conserved moiety, $v^*_0$ is constant and thus the tanh approximation (31) is exact. Figure 2(a) shows the exact flow $v$ together with the approximations (30) and (31) for this case.

In contrast, Figure 2(b) shows the case where $x_b = 0.5$ and $x_a$ varies. There is no conserved moiety: $x_A + x_B \neq constant$. In this case neither approximation is exact, though the tanh approximation is better than the sinh approximation.

Now consider the electrogenic, but constant concentration, case where $\phi_E$ varies but $\phi_A$ and $\phi_B$ are constant. It follows from Equation (38) that $\kappa^*$ is constant and thus the sinh approximation (30) is exact. Combining equations (30) and (38), it follows that:

\[
\phi_E = \frac{\phi_A - \phi_B}{2} - \phi_N \sinh \frac{v}{2\kappa}
\]

This is plotted in Figure 3.1 for a particular choice of parameters. This result is qualitatively similar to the experimentally obtained results of Bazil et al. [16, Figure 2B] for the Mitochondrial Electron Transport Chain. This is explored further in § 7.
Figure 2: Non-electrogenic case. Flow $v$ computed exactly and using the sinh and tanh approximations. (a) $x_A + x_B = 10.2$. The tanh approximation is exact in this case. (b) $x_B = 5$. Neither approximation is exact in this case.
Figure 3: Electrogenic case with fixed concentrations. The capacitor potential $\phi_E$ plotted against reaction flow when $\phi_A = 500$ mV and $\phi_B = 100$ mV. The reaction constant (28) is chosen as $\kappa_s = 0.01$. At $v = 0$, $\phi_E = \frac{\phi_A - \phi_B}{2} = 200$ mV; as $v$ increases, $\phi_E$ decreases as the inverse hyperbolic sin of (41).

4 Equilibria

In typical biomolecular systems, the reaction kinetics of § 3 are embedded in large networks. This section considers the equilibria of such networks. In this paper, a system is said to be in equilibrium if efforts (e.g., chemical potentials and voltages) are such that all flows are zero,

$$V = 0 \quad (42)$$

which implies from the arguments leading to equation (11) that the reaction affinities $\Phi = 0$.

As discussed in § 2, the affinities $\Phi$ are given by equation (7) ($\Phi = -N^T \phi$). Equation (7) is linear and therefore finding the potentials $\phi$ to give zero affinities $\Phi = 0$ is a linear problem. However, the $n_V \times n_X$ matrix $N^T$ is usually not full rank as usually $n_V < n_X$ and therefore (7) cannot be used directly to deduce the potentials $\phi$ from reaction affinities $\Phi$.

However, the conserved moieties of Equation (16) give a further set of constraints on the potentials $\phi$. In particular, using Equation (2), Equation (16) becomes:

$$G \left( X^\diamond \cdot \exp \left( \frac{\phi - \phi^0}{\phi_N} \right) \right) = GX_0 \quad (43)$$

where $\cdot$ denotes elementwise multiplication. Equation (43) is nonlinear in $\phi$. 

9
4.1 Conserved Moieties with order zero

In the special case that all conserved moieties are order 0, Equation (43) can be rewritten as the linear equation.

\[ G\phi = \phi^c_m \]  \hspace{1cm} (44)

where \( \phi^c_m = G\phi_0 \) and \( \phi_0 = \phi^c + \phi_N \ln \frac{X_0}{X^c} \)  \hspace{1cm} (45)

In this case Equations (7) and (44) can be combined as:

\[ \begin{pmatrix} -N^T \\ G \end{pmatrix} \phi = \begin{pmatrix} \Phi \\ \phi^c_m \end{pmatrix} \]  \hspace{1cm} (46)

Once again, equilibria are defined by setting \( \Phi = 0 \) and the solution of Equation (46) is a linear problem.

4.2 Conserved Moieties with order greater than zero

The order-zero case gives the linear set of equations (46). This section shows how the non-linear equations arising when the conserved moiety is not zero-order may be solved.

Define \( G_{cm} \) as the matrix containing the non-zero-order rows of \( G \).

1. The non-zero-order moieties are converted to order-zero by deleting all but one element of the rows of \( G_{cm} \).

2. A \( \phi_{cm} \) for the new chemostats is chosen and the linear problem (46) solved.

3. Compute \( G_{cm}X \) and evaluate discrepancy with respect to \( X_{cm} \).

4. Choose new \( \phi_{cm} \) to reduce discrepancy and repeat from step 2.

This is conveniently automated using \texttt{fsolve} in Octave [20].

4.3 Example: A Basic Electrogenic Network (continued)

Consider first the case where both A and E are chemostats. Then \( N^{cd} \) (13) is given by \( N \) with the first and third rows deleted:

\[ N^{cd} = \begin{pmatrix} 0 & 1 & 0 \end{pmatrix}^T \]  \hspace{1cm} (47)

One choice of \( G \) is

\[ G = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix}^T \]  \hspace{1cm} (48)
Figure 4: Electrogenic system: Equilibria, order-zero moieties. A and E are chemostats; B is free to vary. (a) The potentials $\phi_A$ and $\phi_B$ when B is a chemostat with $\phi_B = 0$ and the electrical potential $\phi_E$ varies. (b) The corresponding states $x_A$ and $x_B$. 
As there is only one non-zero entry in each row of $G$, the linear equation (46) can be used with:

$$
\begin{pmatrix}
-1 & 1 & 2 \\
1 & 0 & 0 \\
0 & 0 & 1
\end{pmatrix}
\times
\begin{pmatrix}
N^T \\
G
\end{pmatrix}
= 
\begin{pmatrix}
0 \\
\phi_A \\
\phi_E
\end{pmatrix}
$$

Figure 4 shows the solution of equation (46) for $\phi_A, \phi_B$ plotted against $\phi_E$ when the potential chemostat A is fixed at $\phi_A = 0$ and $\phi_E$ varies from 0 to 2. In this case the solution is simply that $\phi_B = -2\phi_E$ as can be readily obtained from Equation (38) by setting $\Phi = \phi_A = 0$.

Figure 5: Electrogenic system: Equilibria, order-one moieties. E is a chemostat, A & B form a conserved moiety. (a) The potentials $\phi_A$ and $\phi_B$ when A and B form a conserved moiety with $x_A + x_B = 2$ and the electrical potential $\phi_E$ varies. (b) The corresponding states $x_A$ and $x_B$. Note that $x_A + x_B = 2$ for all flows.

Consider secondly the case where only E is a chemostat. Then $N^{cd}$ (13) is given by $N$ with
the third row deleted:

\[ N^{cd} = \begin{pmatrix} -1 & 1 & 0 \end{pmatrix}^T \] (50)

One choice of \( G \) is

\[ G = \begin{pmatrix} 1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}^T \] (51)

As there are two non-zero entries in the first row of \( G \), there is a order-one conserved moiety and so the method of § 4.2 must be used. In this case

\[ G_{cm} = \begin{pmatrix} 1 & 1 & 0 \end{pmatrix} \] (52)

Deleting the second element of the first row of \( G \) gives the same linear equation as for the two-chemostat case; the difference is that \( \phi_A \) must be chosen to satisfy the conserved moiety \( x_A + x_B = x_{AB} \). In this example, choose \( x_{AB} = 2 \) and thus \( \phi_{cm} = \phi_N \ln 2 \). Figure 5 shown the results of the procedure of § 4.2 where \( \phi_A, \phi_B \) plotted against \( \phi_E \) when the potential chemostat A is fixed at \( \phi_A = 0 \) and \( \phi_E \) varies from 0 to 2.

5 Steady-states

In this paper, a system is said to be in steady-state if efforts are such that all system states are constant. Thus in the context of stoichiometric models:

\[ \dot{X} = N^{cd}V = 0 \] (53)

where \( N^{cd} \) is the chemodynamic stoichiometric matrix [9]. In the special case of equilibrium (42), \( V = 0 \) and Equation (53) is automatically satisfied.

Pathway analysis determines the set of non-zero flows such that Equation (53) is satisfied. In particular such analysis determines the pathway matrix \( K_p \) such that flows \( V \) given by

\[ V = K_p v_p \] (54)

satisfy Equation (53) for any choice of \( v_p \).

Gawthrop and Crampin [10] present an energy-based approach to pathway analysis. This forms the basis of the method discussed in this section. In particular, the starting point for the method proposed here is to apply the positive-pathway analysis of Gawthrop and Crampin [10] to the system where the chemostats have been chosen to give exactly one pathway described by \( K_p \).

The method is as follows:

1. Choose a vector of pathway flows \( v_p \) and deduce all the relevant steady-state flows from:

\[ V = K_p v_p \] (55)
2. Choose a starting value for the potentials $\phi$ – for example that corresponding to equilibrium – for all flows.

3. Compute the $\kappa^*$ – based on $\phi$ – for all flows.

4. Deduce the reaction affinities by inverting the approximation (30)

$$\Phi = 2\phi_N \sinh \frac{V}{2\kappa}$$

(56)

5. Using the approach of § 4, deduce the free-to-vary potentials from Equation (46):

$$N_v\phi_v = \Phi - N_{cs}\phi_{cs}$$

(57)

6. If $\phi$ has not converged, then return to step 4.

Assuming convergence, this method yields the steady-state potentials $\phi$ as well as the corresponding $\kappa^*$.

An alternative approach would use the tanh formulation (24) and $v_0^*$ in place of the sinh formulation (23) and $\kappa^*$.

6 A Biomolecular Cycle

This example is based on that of Gawthrop and Crampin [10, § 5] which in turn corresponds to the seminal monograph, “Free energy transduction and biomolecular cycle kinetics” of Hill [15]. As discussed by Gawthrop and Crampin [10, § 5], the biomolecular cycle of Figure 1.1 of Hill [15] may be represented as the bond graph of Figure 6(b). For the purposes of illustration the thermodynamic constants of the ten species are $\phi^\bigcirc = 0$ and $X^\bigcirc = 1$ and the rate constants of the six reactions are $\kappa = 10$. This system has a conserved moiety of order 5 involving the the total amount of the six species $E$, $EM$, $LEM$, $E^\ast$, $E^\ast M$ and $LE^\ast M$ and therefor the method of § 4.2 is used. For the purposes of illustration, the total amount is taken as $E_{tot} = 10$. Following [10], $M_o$, $L_i$, $L_o$ and $M_i$ are chemostats and the amount of $M_o$ and $L_i$ is taken as unity, the amount of $L_o$ as two and $M_i$ is variable. With these parameters, as derived in the Supplementary Material of [10], the maximum flow is given by $v_{max} = \frac{100}{21} \approx 4.76$.

Figure 7(a) shows flow $v_P$ is plotted against the variable potential $\phi_Mi\mathrm{mV}$. The maximum flow $v_{max}$ is marked as a horizontal line. Figure 7(b) shows the amounts of the six species involved in the conserved moiety; the sum remains 10 but one of the amounts reaches zero at the maximum flow: this is why there is a maximum flow. Figure 7(c) shows the values of $\rho_s$ (23) for the six reactions. By definition, $\rho_s = 1$ at zero flow (equilibrium), but is not unity for non-zero flow $v_p$. Again, one of the $\rho_s$ reaches zero at the maximum flow.
7 Mitochondrial Electron Transport Chain

As discovered by Mitchell [21], the key feature of mitochondria is the chemiosmotic energy transduction whereby a chain of redox reactions pumps protons across the mitochondrial inner membrane to generate the proton-motive force (PMF). This PMF is then used to power the synthesis of ATP – the universal fuel of living systems. The bond graph model of chemiosmosis of Figure 8 is described in detail by Gawthrop [12] and its equilibrium properties analysed. This paper uses this model to illustrate the steady state analysis of this biologically significant system and compares the theoretical results to experimental results of Bazil et al. [16].

Bazil et al. [16] compare experimental results with a detailed ode model of mitochondria which includes not only the ETC but also the TCA cycle and ATPase. The model is simulated to steady-state for a variety of conditions and a number of model outputs are compared with experimental data. One of these outputs is the membrane potential $\psi$ which is compared with experimental data in [16, Figure 2B] (Two sets of data are given: for low Pi and high Pi – the low Pi data is used here). The values of intermembrane and matrix pH were used to compute the corresponding PMF.

The bond graph [12] involves 10 chemical species: NADH, NAD, O$_2$, H$_2$O, Hx, Hi, Q, QH$_2$, Fe$_3$, Fe$_2$, the transmembrane potential $\psi$ and the electrical charges used in modelling the redox reactions. Q and QH$_2$ correspond to oxidised and reduced ubiquinone respectively and Fe$_3$ and Fe$_2$ correspond to oxidised and reduced cytochrome c respectively. There are 9 reaction components[12]: the left & right half redox reactions and the proton pump corresponding to each
Figure 7: Example: A Biomolecular Cycle. (a) The flow $v_P$ is plotted against the variable potential $\phi_{Mi}$ mV. The maximum flow $v_{max}$ is marked as a horizontal line. (b) The six states corresponding to the conserved moiety are plotted against the pathway flow $v_P$. The maximum flow $v_{max}$ is marked by a vertical line and corresponds to the flow where a state ($x_E$ in this case) becomes zero. (c) The values of $\rho_s$ (23) for the six reaction are, by definition, unity at zero flow (equilibrium), but are not unity for non-zero flow $v_P$. 

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(a) Pathway flow $v_P$ and $\phi_{Mi}$ mV

(b) Conserved moiety

(c) $\rho_s$
Figure 8: Mitochondrial Electron Transport Chain Following [12], the three complexes CI, CIII and CIV are bond graph modules interconnected by energy bonds.

Figure 9: Electron Transport Chain: PMF as flow varies. The firm line shows the results of steady-state analysis using the parameter values discussed in the text. The three points show the (normalised) experimental data of Bazil et al. [16, Figure 2B (low Pi data)] and the dashed line the simulation results that they present. As discussed in the text, a single parameter was adjusted to make the firm line fit the data.
of the three complexes CI, CIII and CIV.

Following the analysis of Gawthrop and Crampin [10], the positive pathway matrix is:

$$K_p = \begin{pmatrix} 2 & 2 & 4 & 2 & 4 & 4 & 1 & 4 \end{pmatrix}^T$$ (58)

The corresponding reaction rates $\kappa_i$ of Equation (28) are denoted $\kappa_{iL}$, $\kappa_{iR}$ and $\kappa_{iP}$ corresponding to the left & right half reactions and the proton pump of the three complexes [12]. For the steady-state analysis of Figure 9, $\bar{\kappa}_{iL} = \kappa_{iR} = \infty$, $i = 1 \ldots 3$ and $\bar{\kappa}_{2P} = \bar{\kappa}_a$, $\bar{\kappa}_{1P} = \bar{\kappa}_{3P} = \infty$ where $\bar{\kappa}_a$ is the sole adjustable parameter.

As indicated in Figure 9, when the sole adjustable parameter $\bar{\kappa}_a = 0.025$, this simple choice of adjustable parameters, together with the Faraday-equivalent potentials listed in [12, Table 1], gives a steady-state solution consistent with the experimental data of Bazil et al. [16].

8 Conclusion

The mass-action reaction kinetics equation – expressed in terms of forward and reverse reaction affinity – is rewritten in terms of reaction affinity and summed reaction affinity. Combining this with stoichiometric pathway analysis [10] leads to an algorithm for computing steady state potentials corresponding to non-zero steady-state reaction flows. This is illustrated using introductory examples, a bond graph model of a biomolecular cycle, and a bond graph model of the mitochondrial electron transport chain.

Future work will extend the method to include non mass-action kinetics such as Michaelis-Menten and to handle more than one pathway. It would also be of interest to examine the relative merits of the sinh and tanh and to analyse and enhance numerical properties of the approach. The illustrative example of § 7 just matches the PMF to the data [16]. It would be interesting to use the other free parameters to match the other types of data analysed by Bazil et al. [16].

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