Physically-Plausible Modelling of Biomolecular Systems: A Simplified, Energy-Based Model of the Mitochondrial Electron Transport Chain

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Abstract

Systems biology and whole-cell modelling are demanding increasingly comprehensive mathematical models of cellular biochemistry. These models require the development of simplified models of specific processes which capture essential biophysical features but without unnecessarily complexity. Recently there has been renewed interest in thermodynamically-based modelling of cellular processes. Here we present an approach to developing of simplified yet thermodynamically consistent (hence physically plausible) models which can readily be incorporated into large scale biochemical descriptions but which do not require full mechanistic detail of the underlying processes. We illustrate the approach through development of a simplified, physically plausible model of the mitochondrial electron transport chain and show that the simplified model behaves like the full system.

1. Introduction

In mathematical biology, and more widely, the relative merits of simple ‘toy’ models, which represent some key aspects of the system but not full mechanistic detail, and comprehensive mechanistically detailed representations have long been debated. Simple ‘toy’ models allow rigorous mathematical analysis and are generally easy to simulate, but are difficult to relate to the full system and measurements thereof. Full mechanistically detailed models on the other hand provide a straight-forward mapping to the real system, but are challenging to analyse and may require significant computational overhead to simulate.

Simple models of complex biochemical processes can elucidate basic behaviour and biologically significant trade-offs (Scott et al., 2014; Weiße et al., 2015) and as such can be used as an aid to synthetic biology (Darlington et al., 2018). Furthermore, models of individual processes may be used as part of a model of an overall system as, for example, in the Physiome Project (Hunter, 2016), or in whole-cell modelling Karr et al. (2012); Macklin et al. (2014); this require models to be modular and reusable (Neal et al., 2014; Nickerson et al., 2016).

Recently there has been renewed interest in thermodynamically-based mechanistic modelling of cellular processes (Mason and Covert, 2019; Pan et al., 2018b; Gawthrop et al., 2017; Klipp et al., 2016; Beard and Qian, 2010). A modular approach to energy-based modelling has been developed

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in the context of biomolecular systems (Gawthrop et al., 2015; Gawthrop and Crampin, 2016). This raises the question as to whether it is possible to develop energy-based models that are nevertheless simple.

Like engineering systems, living systems are subject to the laws of physics in general and the laws of thermodynamics in particular. This fact gives the opportunity of applying 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, 2017; Gawthrop et al., 2017; Gawthrop and Crampin, 2018a,b; Pan et al., 2018a,b).

When developing simplified models of biomolecular systems where energy transduction is important, it is essential that models be physically-plausible. A physically-plausible model of a physical system has two attributes: it is itself a model of a physical system (i.e. it does not contravene the laws of physics); and it shares key behaviours with the actual physical system (Gawthrop, 2003). Such an approach will, however, only be of use if there are complex physical systems which can indeed be represented by a simpler physical model. This paper shows that this is indeed the case. In particular we demonstrate that it is possible to develop a simplified model of the mitochondrial electron transport chain that is thermodynamically consistent, but which doesn’t represent full mechanistic detail, and show that it behaves like the full model.

Mitochondria make use of reduction-oxidation (redox) reactions in which the transfer of electrons is used to provide the power driving many living systems. As discovered by Mitchell (1961, 1976, 1993, 2011), 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 an electrochemical gradient known as the proton-motive force (PMF). The PMF is then used to power the synthesis of ATP – the universal fuel of living systems. Due to this central role in living systems, mathematical modelling of the key components of mitochondria is thus an important challenge to systems biology. Because mitochondria transduce energy, an energy-based modelling method is desirable, and Beard and colleagues have developed the most comprehensive such models to date (Beard, 2005; Wu et al., 2007; Beard and Qian, 2010; Beard, 2012; Bazil et al., 2016). A bond graph model of mitochondrial oxidative phosphorylation has been given by Gawthrop (2017). This model is based on modelling the redox reactions associated with complexes CI, CIII and CIV of the mitochondrial electron transport chain.

Below we briefly outline the bond graph approach to modelling energy flows in biochemical reactions, in particular describing the Faraday-equivalent potential approach to modelling electrochemical phenomena, and we describe a modified mass action kinetics approach which will be central to development of a simplified thermodynamic modelling approach. A set of Python based tools has been developed to assist the development and analysis of bond graph models and these tools are briefly outlined.

We then discuss the Mitochondrial Electron Transport Chain (ETC) as an example of a complex biomolecular system which can be successfully modelled by a simple physically-plausible model, and use data from Bazil et al. (2016) to derive parameters of the physically-plausible of the ETC to show that this simple model behaves the same as a fully mechanistic description of the ETC. Finally we conclude with suggestions for future research directions using simplified physically-plausible modelling as a strategy in systems and synthetic biology.
Figure 1: Bond Graph representation of A $\xrightarrow{r} 2$ B. The bond graph components Ce:A and Ce:B represent species A and B; the bond graph component Re:r represents the reaction the bonds $\rightarrow$ together with the zero 0 and one 1 junctions define the stoichiometry (Gawthrop and Crampin, 2014). The bonds carry the energy covariables chemical potential $\mu$ and and molar flow $v$.

2. Modelling Bioenergetics of Biochemical Systems using Bond Graphs

Bond graphs provide a convenient modular framework for modelling energy flow within and across different physical domains: electrical, mechanical, chemical and so on; and as such are useful for representing biomolecular systems. In brief, bonds represent pairs of variables: potential and flow, whose product is power. In the biomolecular domain, the product of chemical potential $\mu$ (with units J mol$^{-1}$) and molar flow $v$ (with units mol s$^{-1}$) is power with units J s$^{-1}$ (Oster et al., 1971, 1973; Gawthrop and Crampin, 2014). Bonds connect components which represent either storage or dissipation of energy. In biomolecular systems, chemical potential is stored as concentration of chemical species, denoted Ce $^2$, whereas chemical reactions, denoted Re, in which chemical species are converted from one form to another are dissipative processes. The biochemical network stoichiometry is represented in the coupling of Ce components via the reactions Re using bonds which represent the flow of energy, connected using common potential 0 (‘zero’) and common flow 1 (‘one’) junctions.

To illustrate, Figure 1 is the bond graph representation of the chemical reaction:

$$A \xrightarrow{r} 2B \quad (2.1)$$

Ce components correspond to constitutive relations which relate the chemical potential to the amount of chemical species stored: the constitutive relations of Ce:A and Ce:B are:

$$\mu_A = RT \ln K_A x_A \quad (2.2)$$
$$\mu_B = RT \ln K_B x_B \quad (2.3)$$

where $x_A$ and $x_B$ are the concentrations of A and B, $K_A$ and $K_B$ are species thermodynamic constants (mol$^{-1}$) for A and B, specific to each chemical species, $R$ is the universal gas constant and $T$ the absolute temperature.

The constitutive relations for the reaction components Re provide the relationship between forward and backward chemical affinities $A$ (stoichiometric combinations of the chemical potentials) which provide the driving force for the reaction, and the molar flow (the reaction rate) $f$. The stoichiometry of reaction Re:r with formation of 2 molecules of species B for each molecule of A is represented by the two parallel bonds on the right hand side of Re:r. With mass-action kinetics, the

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$^2$In this paper, Ce components are used to represent chemical species and C components to represent electrical capacitors.
constitutive relation of $Re: r$ is

$$f = \kappa \left( \exp \frac{A^f}{RT} - \exp \frac{A^r}{RT} \right) \quad (2.4)$$

where $\kappa$ is a reaction rate constant (mol s$^{-1}$), specific to each reaction, and the forwards and backwards affinities are given by

$$A^f = \mu_A \quad (2.5)$$

and

$$A^r = 2\mu_B \quad (2.6)$$

Combining these expressions gives the familiar mass-action expression for the reaction flow $f$:

$$f = \kappa \left( K_A x_A - K_B^2 x_B^2 \right) \quad (2.7)$$

where the forward reaction rate constant $k^+ = \kappa K_A$ and the reverse reaction rate constant $k^- = \kappa K_B^2$.

The bond graph approach is naturally allied to stoichiometric concepts (Klipp et al., 2016; Palsson, 2006, 2011, 2015). In particular, the stoichiometric matrix $N$ can be automatically generated from the network represented in the system bond graph. $N$ can be used to give species flows $v_x$ in terms of reaction flows $v$ and, conversely, reaction affinity $A$ in terms of species potentials $\mu$:

$$v_x = Nv, \quad A = -N^T \mu \quad (2.8)$$

In the case of the system of Figure 1:

$$N = \begin{pmatrix} -1 & 2 \end{pmatrix}^T \quad (2.9)$$

2.1. Modified mass action kinetics

Simplified representation of biomolecular system requires a representation of the reaction network that approximates, but does not fully represent the full set of biochemical reactions in the network. Thus physically-plausible models of biomolecular systems will typically contain reactions which are an approximation to a sequence of elementary reactions. Thus even if elementary reaction steps have the mass action kinetics of Equation (2.4), this would not necessarily be the case for the overall reactions used to represent the system (see for example Atkins et al. (2018, chapter 17)).

In bond graph terms, one may represent the dissipative reaction component with any appropriate constitutive relation for the reaction flow $f$ in terms of the forward and reverse affinities: mass action, as given by (2.4) leading to (2.7) is one example. In particular, non-elementary reactions may be represented using rate equations where, unlike the mass-action formulation, the concentration exponents are not the stoichiometric coefficients. One particular case of this would be to divide all of the stoichiometric coefficients by an positive integer constant $\alpha$ in the rate equations. Thus, for example, if $\alpha = 2$, the reaction rate (2.7) corresponding to the reaction (2.1) would become:

$$f = \kappa \left( \sqrt{K_A x_A} - K_B x_B \right) \quad (2.10)$$

This corresponds to the non-integer stoichiometry

$$N_\alpha = \begin{pmatrix} -\frac{1}{2} & 1 \end{pmatrix}^T \quad (2.11)$$

Note that in the context of modelling the Mitochondrial Electron Transport Chain (ETC), the exponent $1/2$, corresponding to $\alpha = 2$, commonly appears in the flux expression for complex III, as given by
Beard (2005, (B72)) and Beard and Qian (2010, (7.38)) for example, and the exponent 1/4, corresponding to \( \alpha = 4 \), appears in the flux expression for complex IV given by Beard (2005, (B73)) and Beard and Qian (2010, (7.41)). This can be achieved by replacing the mass-action formula (2.4) by the \textit{modified mass-action} formula:

\[
\dot{f} = \kappa \left( \exp \frac{A_f}{aRT} - \exp \frac{A_r}{aRT} \right)
\]

(2.12)

which contains the additional parameter \( \alpha \), which is used below as an essential part of the model fitting process.

For thermodynamic consistency, it is important that Equation (2.12) represents a dissipative system, that is any non zero flow dissipates energy (Willems, 1972; Polderman and Willems, 1997; Willems, 2007). With this in mind, it is now shown that the MMA equation can be rewritten in mass-action form but with the positive constant \( \kappa \) replaced by the positive function of concentration \( \kappa_\alpha \). As a simple example of this, it can be verified that Equation (2.10) can be rewritten as

\[
f = \kappa_\alpha \left( K_A x_A - K_B^2 x_B^2 \right)
\]

(2.13)

where \( \kappa_\alpha(x_A, x_B) = \frac{k}{\sqrt{K_A x_A + K_B x_B}} \) (2.14)

As \( x_A \) and \( x_B \) are positive, \( \kappa_\alpha \) is also positive. Thus (2.13) corresponds to the mass-action equation (2.4) with the positive constant \( \kappa \) replaced by the positive function of concentration \( \kappa_\alpha \). As shown in Appendix A, the general modified-mass action kinetics of Equation (2.12) can also be rewritten in mass-action form with the positive constant \( \kappa \) replaced by the positive \( \kappa_\alpha \):

\[
f = \kappa_\alpha(A^f, A^r) \left( \exp \frac{A_f}{RT} - \exp \frac{A_r}{RT} \right)
\]

(2.15)

\section*{2.2. Redox reactions}

Oxidative phosphorylation involves a series of electrochemical redox reactions. Nicholls and Ferguson write that ‘Whereas all redox reactions can quite properly be described in thermodynamic terms by their Gibbs energy changes, electrochemical parameters can be employed because the reactions involve the transfer of electrons.” (Nicholls and Ferguson, 2013, chapter 3.3). Rather than to deal directly with conversion between electrical and chemical potentials and associated variables, it is convenient to have a common system of units and convert the chemical energy covariables chemical potential and molar flow to equivalent electrical energy covariables voltage and current Gawthrop (2017). The relevant conversion factor is \textit{Faraday’s constant} \( F \approx 96,485 \text{ C mol}^{-1} \) (Nicholls and Ferguson, 2013; Gawthrop et al., 2017; Gawthrop, 2017). In particular, we define:

\[
\begin{align*}
\text{Faraday-equivalent potential} & \quad \phi = \frac{\mu}{F} V \\
\text{Faraday-equivalent flow} & \quad f = F v A
\end{align*}
\]

(2.16, 2.17)

Using these Faraday-equivalent variables, the \textbf{Ce} constitutive relations (2.2) and (2.3) become:

\[
\begin{align*}
\phi_A & = V_N \ln K_A x_A \\
\phi_B & = V_N \ln K_B x_B \\
\text{where} \quad V_N & = \frac{RT}{F} \approx 26 \text{ mV}
\end{align*}
\]

(2.18, 2.19, 2.20)
and the modified mass-action formula (2.12) becomes:

$$f = \kappa \left( \exp \frac{A_f}{aV_N} - \exp \frac{A_r}{aV_N} \right)$$  \hspace{1cm} \text{(2.21)}$$

As noted by Nicholls and Ferguson, an advantage of transforming the chemical potentials into equivalent electrical potentials in the treatment of redox reactions is: “the ability to dissect the overall electron transfer into two half-reactions involving the donation and acceptance of electrons, respectively.” (Nicholls and Ferguson, 2013, chapter 3.3). For example, the two half-reactions:

$$\text{A} \xrightleftharpoons{r_1} \text{C} + 2 e^- \quad \text{B} + e^- \xrightleftharpoons{r_2} \text{D} \quad \text{(2.22)}$$
electron donation in the first (oxidation of A), and acceptance in the second (reduction of B), correspond to the overall reaction:

$$\text{A} + 2 \text{B} \xrightleftharpoons{r} \text{C} + 2 \text{D} \quad \text{(2.23)}$$

Figure 2 shows a bond graph representation of these two half-reactions (2.22), explicitly representing the transfer of electrons \(e^-\) using the linear electrical capacitor represented by \(\text{C:E}\) with voltage \(V\); the two-electron stoichiometry of reaction \(r_1\) is represented by the two parallel bonds. If reaction \(r_1\) is in equilibrium, then the voltage \(V\) is exactly that required to stop reaction \(r_1\) from proceeding and thus \(V = -E_1\) where \(E_1\) is the redox potential of reaction \(r_1\). Conversely, if reaction \(r_2\) is in equilibrium, then the voltage \(V\) is exactly that required to stop reaction \(r_2\) from proceeding and thus \(V = -E_2\) where \(E_2\) is the redox potential of reaction \(r_2\).

2.3. BondGraphTools – a Python Toolkit

Computational tools necessary for model capture, parameterisation and simulation. As the name suggests, BondGraphTools is an application programming interface (API) for capturing, simplifying and simulating bond graph models, and is an important part of the bond graph approach.

BondGraphTools is written in Python and is built upon the Scientific Python (SciPy) libraries, all of which are open source and easily accessible. The core use-case of BondGraphTools is to turn bond graphs into a set of reduced equations which can be then passed into other SciPy libraries (parameter estimation routines, for example). As model reduction is performed symbolically, the simplification routines are free from numerical errors, which is important for systems involving parameters that are unknown.

Appendix B briefly describes how BondGraphTools can be used to build a model of the chemical system shown in Figure 2.
3. A Simplified Physically-Plausible Model for the Mitochondrial Electron Transport Chain

Mitochondria make use of redox reactions to provide the power driving many living systems. The key process in the generation of ATP is chemiosmotic energy transduction, whereby a sequence of redox reactions pumps protons across the mitochondrial inner membrane to generate the proton-motive force (PMF), an electrochemical gradient which is then used to power the synthesis of ATP. Generation of the PMF is accomplished by the mitochondrial electron transport chain. Beard and colleagues have developed the most comprehensive thermodynamically consistent models of mitochondrial oxidative phosphorylation including the electron transport chain (Beard, 2005; Wu et al., 2007; Bazil et al., 2016). Recently Gawthrop (2017) provided a bond graph model of mitochondrial oxidative phosphorylation based on the redox reactions associated with complexes CI, CIII and CIV of the mitochondrial electron transport chain.

In contrast, here we develop a simple, but physically-plausible, model based on the overall chemical reaction of the Electron Transport Chain:

\[
2 \text{NADH} + \text{O}_2 + 18 \text{H}_x^+ \leftrightarrow 2 \text{NAD}^+ + 2 \text{H}_2\text{O} + 16 \text{H}_i^+ \quad (3.1)
\]

Reaction (3.1) can be rewritten as the weighted sum of three reactions:

\[
\text{NADH} \xrightarrow{r_1} \text{NAD}^+ + \text{H}_x^+ + 2e_1^- \quad (\times 2) \quad (3.2)
\]

\[
\text{O}_2 + 4\text{H}_i^+ + 4e_2^- \xrightarrow{r_2} 2\text{H}_2\text{O} \quad (\times 1) \quad (3.3)
\]

\[
5\text{H}_x^+ + e_1^- \xrightarrow{r_{\text{loss}}} 5\text{H}_i^+ + e_2^- \quad (\times 4) \quad (3.4)
\]

Reaction (3.2) converts NADH to NAD producing a proton \(\text{H}_x^+\) in the mitochondrial matrix and donating two electrons \(e_1^-\). Reaction (3.3) converts \(\text{O}_2\) and protons \(\text{H}_i^+\) in the mitochondrial inter-membrane space and consumes four electrons \(e_2^-\) to produce water \(\text{H}_2\text{O}\). Reaction (3.4) transfers electrons \(e_1^-\) to \(e_2^-\) and, in so doing, utilises the corresponding free energy to pump five protons from the mitochondrial matrix \(\text{H}_x^+\) to the mitochondrial inter-membrane space \(\text{H}_i^+\) against the \(\text{H}^+\) concentration gradient and the trans-membrane electrical potential \(\Delta\Psi\).

Figure 3 shows the bond graph of a physically-plausible model of the mitochondrial electron transport chain. The the components of the model are:
1. The electron donation reaction \( r_1 \) (3.2) is represented by \( \text{Re}:r_1 \) and the associated species by \( \text{Ce}:\text{NADH}, \text{Ce}:\text{NAD} \) and \( \text{Ce}:\text{Hx} \). The electrons \( e_1^- \) accumulate in the electrical capacitor \( C:E_1 \).

2. The electron consumption reaction \( r_2 \) (3.3) is represented by \( \text{Re}:r_2 \) and the associated species by \( \text{Ce}:\text{O}_2, \text{Ce}:\text{H}_2\text{O} \) and \( \text{Ce}:\text{Hi} \). The electrons \( e_2^- \) accumulate in the electrical capacitor \( C:E_2 \).

3. The electron transfer part of the electron transfer/proton pump (3.4) is modelled by the two electrical capacitors \( C:E_1 \) and \( C:E_2 \) and the (linear) electrical resistor (with resistance \( r_{loss} \)) \( R:\text{r loss} \). The voltage \( V_1 \) associated with \( C:E_1 \) is the redox potential of half reaction (3.2) and the voltage \( V_2 \) associated with \( C:E_2 \) is the redox potential of half reaction (3.3). The electrical capacitor \( C:dV \) with voltage \( \Delta V \) represents the net redox potential minus the potential drop associated with the resistor \( r_{loss} \):

\[
\Delta V = V_1 - V_2 - r_{loss}f
\]

4. Proton transfer is not explicitly modelled in Fig. 3. However, the corresponding membrane potential \( \Delta \Psi \) is given in terms of \( \Delta V \) as by:

\[
\Delta \Psi = \frac{\Delta V}{n_p} - \Phi_H
\]

where \( n_p = 5 \) is the number of protons pumped per electron and \( \Phi_H = \phi_{Hi} - \phi_{Hx} \), the chemical potential difference due to proton concentration difference across the membrane.

### 3.1. Physical Parameters

The \( \text{Ce} \) constitutive relation (2.18) can be rewritten in the alternative form:

\[
\phi_A = \phi_A^\varnothing + V_N \ln \frac{x_A}{x_A^\varnothing}
\]

where \( V_N \) is given by (2.20) and \( \phi_A^\varnothing \) is the potential of substance A at standard conditions where \( x_A = x_A^\varnothing \). Using tables of standard chemical potentials \( \mu^\varnothing \), equation (2.16) can be used to derive the corresponding potential \( \phi_A^\varnothing \). As discussed by Gawthrop (2017), the Faraday-equivalent chemical potential of substance A at nominal conditions \( \phi_A^\varnothing \) can be computed from Faraday-equivalent chemical potential at standard conditions \( \phi_A^\varnothing \) from the formula:

\[
\phi_A^\varnothing = \phi_A^\varnothing + V_N \ln \rho_A
\]

where \( \rho_A = \frac{x_A^\varnothing}{x_A} \)

where \( x_A^\varnothing \) and \( x_A \) are the concentrations of substance A at nominal conditions and standard conditions. Table 1 shows nominal values \( \phi_A^\varnothing \) for a number of different substances. These values will be used below to model the mitochondrial electron transport chain.

The stoichiometric equations (2.8) can be rewritten in Faraday-equivalent form as

\[
f_x = Nf \quad \Phi = -N^T \phi
\]

In the case of the half-reaction (3.2)

\[
\text{NADH} \leftrightarrow \text{NAD}^+ + \text{H}_x^+ + 2e_1^-
\]
Table 1: Physical Parameters of the Physically-plausible model. $x^\phi$ is the concentration at nominal conditions relative to standard conditions, $\phi^\phi$ and $\phi^\phi$ are the Faraday-equivalent potentials at standard and nominal conditions related by Equation (3.8). $H_i^+$ and $H_e^+$ are protons in the inter-membrane space and matrix respectively.

| Substance | $\rho$ | $\phi^\phi$ V | $\phi^\phi$ V |
|-----------|--------|---------------|---------------|
| $H_2O$    | 1.000  | $-2.443$      | $-2.443$      |
| $H_i^+$   | $1.318 \times 10^{-7}$ | 0.000         | $-3.729 \times 10^{-1}$ |
| $H_e^+$   | $1.660 \times 10^{-8}$ | 0.000         | $-4.217 \times 10^{-1}$ |
| $NAD^+$   | $1.500 \times 10^{-3}$ | 1.876 $\times 10^{-1}$ | 3.454 $\times 10^{-2}$ |
| NADH      | $1.500 \times 10^{-3}$ | 4.074 $\times 10^{-1}$ | 2.544 $\times 10^{-1}$ |
| $O_2$     | $2.500 \times 10^{-5}$ | 1.700 $\times 10^{-1}$ | $-7.945 \times 10^{-2}$ |

The stoichiometric matrix is:

$$N^T = \begin{pmatrix} -1 & 1 & 1 & 2 \end{pmatrix}$$ (3.12)

It follows that the reaction potential $\Phi$ is:

$$\Phi = N^T \phi = \phi^\phi_{\text{NADH}} - \phi^\phi_{\text{NAD}} - \phi^\phi_{\text{Hx}} - 2\phi^\phi_E$$ (3.13)

At equilibrium, $\Phi = 0$ and so:

$$V = \phi^\phi_E = \frac{1}{2} \left( \phi^\phi_{\text{NADH}} - \phi^\phi_{\text{NAD}} - \phi^\phi_{\text{Hx}} \right)$$

$$= \frac{1}{2} \left( 254.4 - 34.54 - (-421.7) \right) \approx 320 \text{ mV}$$ (3.14)

and so this corresponds to a redox potential of $E = -V = -320 \text{ mV}$ for this half-reaction.

In the case of the half-reaction (3.3)

$$O_2 + 4H_i^+ + 4e^- \rightleftharpoons 2H_2O$$ (3.15)

the stoichiometric matrix is:

$$N^T = \begin{pmatrix} -1 & -4 & -4 & 2 \end{pmatrix}$$ (3.16)

It follows that the reaction potential $\Phi$ is:

$$\Phi = -\phi^\phi_{O2} - 4\phi^\phi_{Hi} - 4\phi^\phi_E + 2\phi^\phi_{H2O}$$ (3.17)

At equilibrium, $\Phi = 0$ and so:

$$\phi^\phi_E = \frac{1}{4} \left( 2\phi^\phi_{H2O} - \phi^\phi_{O2} - 4\phi^\phi_{Hi} \right)$$

$$= \frac{1}{4} \left( 2 \times -2443 - (-79.5) - 4 \times -372.9 \right) \approx -829 \text{ mV}$$ (3.18)

and thus this corresponds to a redox potential of $E = -V = 829 \text{ mV}$.

3.2. An explicit formula

From a systems point of view, the model of the ETC can be characterised by the voltage/current relationship of the bond graph component $C \cdot dV$. This represents the steady state relationship between the flow (rate of electron transport along the ETC, or equivalently the rate of oxygen consumption)
and the mitochondrial membrane potential which is established. Letting \( n_1 \) and \( n_2 \) be the number of bonds connecting reactions \( r_1 \) and \( r_2 \) to the electrical subsystem, the steady-state flows are related by:

\[
f = n_1 f_1 = n_2 f_2
\]

and the steady-state potentials by Equation (3.5) with:

\[
V_1 = \frac{1}{n_1} \Phi_1
\]

and

\[
V_2 = \frac{1}{n_2} \Phi_2
\]

Using the modified mass action formula (2.21), the reaction flows are given by

\[
f_1 = \kappa_1 \left( \exp \frac{\Phi f_1}{aV_N} \right) (3.22)
\]

\[
f_2 = \kappa_2 \left( \exp \frac{\Phi f_2 + n_2 V_2}{aV_N} \right) - \exp \frac{\Phi f_2}{aV_N} \right) (3.23)
\]

where the parameter \( \alpha \) remains to be determined (by fitting to data). At equilibrium, the flows are zero and thus:

\[
V_1 = V_{eq}^1 = \frac{1}{n_1} \left( \Phi f_1 - \Phi r_1 \right) = \frac{1}{n_1} \Phi_1 (3.24)
\]

\[
V_2 = V_{eq}^2 = \frac{1}{n_2} \left( \Phi r_2 - \Phi f_2 \right) = -\frac{1}{n_2} \Phi_2 (3.25)
\]

Writing \( \Delta V_1 = V_1 - V_{eq}^1 \) and \( \Delta V_2 = V_2 - V_{eq}^2 \) it follows that the flows can be rewritten as:

\[
f_1 = (1 - \lambda_1) K_1 \]

\[
f_2 = (\lambda_2 - 1) K_2 \]

where

\[
\lambda_1 = \exp \frac{n_1 \Delta V_1}{aV_N} \]

\[
\lambda_2 = \exp \frac{n_2 \Delta V_2}{aV_N} \]

\[
K_1 = \kappa \exp \frac{\Phi f_1}{aV_N} \]

\[
K_2 = \kappa \exp \frac{\Phi f_2}{aV_N} \]

Hence using (3.26)

\[
\lambda_1 = 1 - \frac{f}{n_1 K_1} \]

\[
\lambda_2 = 1 + \frac{f}{n_2 K_2} \]

Using (3.27) and (3.5)

\[
\Delta V = \Delta V^{eq} + \frac{aV_N}{n_1} \ln \left( 1 - \frac{f}{n_1 K_1} \right) - \frac{aV_N}{n_2} \ln \left( 1 + \frac{f}{n_2 K_2} \right) - r_{loss} f \]

(3.30)

where \( \Delta V^{eq} = V_{eq}^1 - V_{eq}^2 \). Using the results of § 3.1

\[
\Delta V^{eq} = 320 + 829 = 1149 \text{ mV}
\]

This formula, derived from the simplified model using modified mass action, thus provides a voltage/current steady state relationship that describes the operation of the ETC. Using equation (3.6) and a value of \( \Phi_H = 25 \text{ mV} \) this corresponds to an equilibrium mitochondrial membrane potential \( \Delta \Psi_{eq} \) of: \( \Delta \Psi_{eq} = \frac{1149}{5} - 25 = 205 \text{ mV} \).
3.3. Model Fitting

The simplified, physically plausible model of the electron transport chain derived above contains physical parameters which are known \textit{a-priori}, as well as parameters which are model-dependent and must be obtained by fitting to relevant data. In particular, the explicit formula (3.30) relating flow $f$ to potential difference $\Delta V$ has four known physical parameters: $\Delta V_{eq}$, $V_N$, $n_1$ and $n_2$ and four unknown parameters: $\alpha$, $K_1$, $K_2$ and $r_{loss}$. Bazil et al. (2016) develop a detailed physically based mathematical model of mitochondrial oxidative phosphorylation and ROS generation and compare the results with \textit{in-vitro} experimental data. These results are used below to fit the parameters of the physically-plausible model developed above.

Figure 2B in Bazil et al. shows four datasets: two sets of simulation results corresponding to two concentrations of inorganic phosphate Pi: $[\text{Pi}] = 1 \text{ mM}$ and $[\text{Pi}] = 5 \text{ mM}$, and two sets of experimental data corresponding to these two conditions. In each case, the mitochondrial membrane potential $\Delta \Psi_{\text{mV}}$ is plotted against the rate of oxygen consumption $V_{O_2} \text{ nmol/min}$. For the purposes of this paper, the flow is normalised by $f_{\text{norm}} = 150 \text{ nmol/min}$ such that

$$f = \frac{1}{f_{\text{norm}}} V_{O_2}$$

(3.32)

Inorganic phosphate Pi does not appear in the physically plausible model developed above and therefore it is not possible to take account of the variation of Pi. However, the experimental data shown in Figure 2B of Bazil et al. does not show a strong dependence of this part of the system on Pi and so the lack of dependence of our simplified model on Pi is reasonable. For this reason, experimental data for both values of Pi are considered for the purposes of model fitting to the raw data. We note that other components of mitochondrial metabolism not modelled here, such as ATPase, do depend strongly on Pi.

Known physical parameters $\Phi^{\ominus}$ of Table 1 are drawn from the supplementary material of Wu et al. (2007), the concentrations of NAD and NADH from Bazil et al. (2016), the pH values from Porcelli et al. (2005) and the $O_2$ concentration from Murphy (2009). Parameter fitting was implemented using the Python function within the SciPy.optimize module opt.minimize with method = "L-BFGS-B" and parameter bounds of $10^{-12}$ and $\infty$ as the parameters are all positive. The cost function $\epsilon$ is the root-mean-square (RMS) difference between the value of $\Delta \Psi$ predicted by equations (3.6) and (3.30) and the data for each value of (normalised) flow.

The form of the cost function was examined by fixing one of the four parameters and minimising with the other three and plotting the cost against the fixed parameter. Figure 4(a) shows the dependence of the cost function on parameters $K_1$ and $\alpha$, and indicates that the parameter $K_1$ has little effect as long as it is greater than about $K_1 = 5$. For the rest of this paper, $K_1$ was fixed at a large positive value and not included further in the optimisation. Thus there are three significant parameters: $\alpha$, $K_2$ and $r_{loss}$. Figure 4(b) shows that the parameter $\alpha$ has a significant effect. For the two simulation data sets, there is a clear minimum at $\alpha \approx 5$. The rows 1–3 of Table 2 show the optimal parameters $\alpha$, $K_2$ and $r_{loss}$ together with the minimal cost $\epsilon$ for the two simulations and the experimental data. Rows 4–6 correspond to fixing $\alpha = 5$ and estimating the remaining two parameters $K_2$ and $r_{loss}$.

Finally, the current-voltage relationship derived above and given in (3.30) is plotted in Figure 5 for different sets of fitted parameters given in Table 2, along with simulation results from the full model of Bazil et al. (2016), and the corresponding experimental data, showing that the explicit formula for the steady state current/voltage behaviour of the ETC is well captured by the physically plausible model. Figures 5(a)–5(c) correspond to rows 4–6 of Table 2; Figure 5(d) corresponds to row 3 of Table 2.
Figure 4: Fitting $K_1$ and $\alpha$. (a) The RMS error $\epsilon$ as parameter $K_1$ is varies; all other parameters are optimised. The value of $K_1$ is not important if greater than about 2; the reaction r1 is in equilibrium. (b) The RMS error $\epsilon$ as parameter $\alpha$ of the modified mass-action kinetics (2.21) is varies; all other parameters are optimised. For data sets sim-0 and sim-1 (data from the experimentally fitted model of Bazil et al. (2016)) there is a clear minimum at about $\alpha = 5$.

| Source | $\alpha$ | $K_2$ | $r_{loss}$ (mΩ) | $\epsilon$ (mV) |
|--------|---------|-------|-----------------|-----------------|
| sim_0  | 5.4     | $3.0 \times 10^{-4}$ | $3.6 \times 10^{-1}$ | $2.5 \times 10^{-2}$ |
| sim_1  | 5.0     | $5.7 \times 10^{-4}$ | $5.3 \times 10^{-1}$ | $3.1 \times 10^{-2}$ |
| exp    | 8.8     | $2.9 \times 10^{-3}$ | $9.9 \times 10^{-2}$ | 1.3              |
| sim_0  | 5.0     | $2.2 \times 10^{-4}$ | $4.5 \times 10^{-1}$ | $1.2 \times 10^{-1}$ |
| sim_1  | 5.0     | $5.5 \times 10^{-4}$ | $5.4 \times 10^{-1}$ | $3.5 \times 10^{-2}$ |
| exp    | 5.0     | $5.2 \times 10^{-4}$ | $6.7 \times 10^{-1}$ | 1.7              |

Table 2: Optimal parameters. The table summarises the fitting results using the two sets of simulation data (sim_0 & sim_1) and the experimental data (exp) from Bazil et al. (2016). Rows 1–3 correspond to free $\alpha$ and the rows 4–6 to fixed $\alpha = 5$. 
Figure 5: Model fitting. All four plots show four data sets: sim-0 and sim-1 are simulation data from the experimentally fitted simulation of Bazil et al. (2016) for two values of [Pi]; exp is the corresponding experimental data; pp-model is the simulation of the physically-plausible model using the formula of § 3.2. (a) The physically-plausible model is fitted to the experimentally fitted simulation of Bazil et al. (2016) with [Pi] = 1 mM using fixed $\alpha = 5$ and estimating $K_2$ and $r_{loss}$. See row 4 of Table 2. (b) As (a) but with [Pi] = 5 mM. See row 5 of Table 2. (c) The physically-plausible model is fitted to the experimental points combining both values of Pi using fixed $\alpha = 5$ and estimating $K_2$ and $r_{loss}$, see row 6 of Table 2. (d) As (c) except that $\alpha$ is also estimated, see row 3 of Table 2.
4. Discussion

Simplified models of biochemical and biophysical processes have a central role to play in the development of physiome-scale and whole-cell models, and the use of such models in biomedical and synthetic biology applications. Here we have argued that such simplified models need to be physically plausible, in the sense that they are consistent with the laws of physics (for example, that they obey mass conservation, are consistent with thermodynamic principles, and so on) as well as providing a suitable fit to available data sets. Here we have demonstrated that bond graphs provide a useful framework for the development of such models, and have demonstrated that by using a modified form of mass action we can generate a simple physically-plausible model of the mitochondrial electron transport chain that is able to reproduce experimentally measured properties of the system.

A particular advantage of a simple model over a fully mechanistic model is the possibility of deriving explicit formulae for key properties and behaviours of the system. Here we have shown that using the physically plausible modelling approach we can derive an explicit formula for the flux through the electron transport chain to the PMF that is generated across the mitochondrial membrane, given in equation (3.30).

The physically plausible model of the electron transport chain derived above contains physical parameters which are known a-priori, as well as parameters which are model-dependent and therefore obtained by fitting to relevant data. In particular, the explicit formula (3.30) relating flow $f$ to potential difference $\Delta V$ has four known physical parameters: $\Delta V^{eq}$, $V_N$, $n_1$ and $n_2$ and four unknown parameters: $\alpha$, $K_1$, $K_2$ and $r_{loss}$. Using an optimization approach to model fitting, we have shown that the value of the parameter $K_1$ appears unimportant, as long as $K_1 > 10$; this corresponds to the rate constant $\kappa_1$ of reaction $r_1$ being large enough so that there is negligible potential drop across the reaction; in other words, this requires that the reaction (3.2) is operating essentially at equilibrium under the experimental conditions considered.

In contrast, the $\alpha$ parameter of the modified mass-action equation (2.21) is found to be important. Choosing $\alpha = 5$ gives a good fit for both the experimentally-fitted simulations and experimental data. This dependence is to be expected as the physically plausible model subsumes a number of individual reactions and this is known to lead to non-stoichiometric exponents (Atkins et al., 2018, chapter 17).

We have shown that despite its simplicity, the physically-plausible model fits the (complex) simulation data from Bazil et al. (2016) closely (RMS error $\epsilon \ll 1$ mV) for both values of $P_i$ and for free $\alpha$ and fixed $\alpha = 5$. Furthermore the experimental data can be fitted with the model with an error $\epsilon$ of about 2 mV.

Finally, because the physically-plausible model is energy based, any or all of the Ce components can provide connections with energy-based models of other parts of the mitochondria system such as the TCA cycle, ATPase and ROS generation. In bond graph terminology, the Ce components become ports (Gawthrop and Crampin, 2018b) with which to connect to other bond graph components for simulation and analysis of larger-scale models of mitochondrial and cellular bioenergetics.

It remains to be determined how different possible modifications to mass action, or indeed other constitutive relations that may be used to relate chemical potential and reaction rate, affect the ability of simple physically plausible models to represent complex biochemical processes. In our future work we intend to further explore this issue, as well as to use the simplified model to investigate in larger models of mitochondrial function the basis of ROS generation and damage.

Furthermore, the physically-plausible model of the mitochondrial electron transport chain developed here provides the basis for simplified but thermodynamically realistic models of mitochondrial energy production to be used in simulation of spatially-distributed networks of mitochondria (Jarosz et al., 2017; Ghosh et al., 2018), for which detailed mechanistic models of mitochondrial bioenergetics.
have too high a computational overhead.

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Appendix A. Modified mass-action kinetics

Theorem. If the reaction flow $f$ is given by the modified mass-action formula

$$f = \kappa \left( \exp \frac{A^f}{\alpha RT} - \exp \frac{A^r}{\alpha RT} \right)$$  (A.1)

where $\kappa > 0$ and $\alpha$ is a positive integer, then $f$ is also given by the alternative formula

$$f = \kappa_\alpha(A^f, A^r) \left( \exp \frac{A^f}{RT} - \exp \frac{A^r}{RT} \right)$$  (A.2)

where $\kappa_\alpha(A^f, A^r) = \kappa_\sigma > 0$ and $\sigma = \sum_{i=0}^{\alpha-1} \exp \frac{(\alpha-1-i)A^f}{\alpha RT} \exp \frac{iA^r}{\alpha RT} > 0$.

Proof. Let $f_f = \exp \frac{A^f}{RT}$ and $f_r = \exp \frac{A^r}{RT}$ and define $p = f_f^{\frac{1}{\alpha}}$ and $q = f_f^{\frac{1}{\alpha}}$, then, from Equation (A.1), $f = \kappa (p - q)$. It is a fact that $p^{\alpha} - q^{\alpha} = (p - q)\sigma$ where $\sigma = \sum_{i=0}^{\alpha-1} p^{\alpha-1-i}q^i$. Hence Equation (A.1) may be rewritten as Equation (A.2) where $\kappa_\alpha = \frac{\kappa}{\sigma}$, $p$ and $q$ are the exponentials of real numbers and therefore positive, it follows that $\sigma$ and $\kappa_\alpha$ are positive.

Example. Consider the system of § 2.1 where $\alpha = 2$, $A^f = RT \ln K_{A^f}$ and $A^r = 2RT \ln K_{B^f}$. Then

$$\sigma = \exp \frac{A^f}{2RT} \cdot \exp 0 + \exp 0 \cdot \exp \frac{A^r}{2RT}$$
$$= \exp \frac{\ln K_{A^f}}{2} + \exp \ln K_{B^f}$$
$$= \sqrt{K_{A^f}} + K_{B^f}$$

This corresponds to Equation (2.14).

Appendix B. Using BondGraphTools

Building a model using BondGraphTools involves instantiating new components from a library (and setting global parameters), adding them to a blank bond graph, then defining the power bonds which connect the components. Listing B.6 shows how BondGraphTools can be used to build the simple example in Figure 1. Once built, model equations can be generated by accessing the constitutive relations and passed into parameter estimators or nonlinear solvers.

BondGraphTools are available via the Python Package Index (PyPI) and the source code is made available at https://github.com/BondGraphTools/ under the Apache 2.0 open source licence.
import BondGraphTools as bgt

# Create a new blank model
model = bgt.new()

# Define the Constants
constants = {'R': 1, 'T': 1}

# Define the symbolic parameters
import sympy as sp
K_a, K_b = sp.symbols('K_a, K_b')
kappa = sp.Symbol(r'\kappa')

# Instantiate the components
Ce_A = bgt.new('Ce', library='BioChem',
               value={'k': K_a, 'R': 1, 'T': 1})
Ce_B = bgt.new('Ce', library='BioChem',
               value={'k': K_b, 'R': 1, 'T': 1})
one_A = bgt.new('1')
one_B = bgt.new('1')
zero = bgt.new('0')
Re = bgt.new('Re', library='BioChem',
            value={'r': kappa, 'R': 1, 'T': 1})

# Add them to the model
model.add(Ce_A, Ce_B, one_A, one_B, zero, Re)

# Define the tail and head pairs of each bond.
bonds = [(Ce_A, one_A),
         (one_A, Re),
         (Re, one_B),
         (one_B, zero),
         (one_B, zero),
         (zero, Ce_B)]

# Connect everything up by connecting each pair
for tail, head in bonds:
    bgt.connect(tail, head)

# Print out the equations of motion in implicit form.
model.constitutive_relations
# >>> [ K_a * kappa * x_0 - K_b * 2 * kappa * x_1 * 2 + dx_0,
#       -2 * K_a * kappa * x_0 + 2 * K_b * 2 * kappa * x_1 * 2 + dx_1 ]

Figure B.6: The model reaction \( A \leftrightarrow 2B \) in Figure 1 can be programmatically built in BondGraphTools by adding components to a blank model, then connecting all the power bonds. Once the model is build, the equations of motion can be accessed via the constitutive relations.