Bond Graph Modelling of Chemiosmotic Biomolecular Energy Transduction

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Abstract

Engineering systems modelling and analysis based on the bond graph approach has been applied to biomolecular systems. In this context, the notion of a Faraday-equivalent chemical potential is introduced which allows chemical potential to be expressed in an analogous manner to electrical volts thus allowing engineering intuition to be applied to biomolecular systems. Redox reactions, and their representation by half-reactions, are key components of biological systems which involve both electrical and chemical domains. A bond graph interpretation of redox reactions is given which combines bond graphs with the Faraday-equivalent chemical potential. This approach is particularly relevant when the biomolecular system implements chemoelectrical transduction – for example chemiosmosis within the key metabolic pathway of mitochondria: oxidative phosphorylation.

An alternative way of implementing computational modularity using bond graphs is introduced and used to give a physically based model of the mitochondrial electron transport chain (ETC). To illustrate the overall approach, this model is analysed using the Faraday-equivalent chemical potential approach and engineering intuition is used to guide affinity equalisation: a energy based analysis of the mitochondrial electron transport chain.

1 Introduction

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 (Borutzky, 2010; Cellier, 1991; Gawthrop and Smith, 1996; Gawthrop and Bevan, 2007; Karnopp et al., 2012) which has been extended to include biomolecular systems (Oster et al., 1971, 1973). To quote from Paynter (1993):

Katchalsky’s breakthroughs in extending bond graphs to biochemistry are very much on my own mind. I remain convinced that BG models will play an increasingly important role in the upcoming century, applied to chemistry, electrochemistry and biochemistry, fields whose practical consequences will have a significance comparable to that of electronics in this century. This will occur both in device form, say as chemfets, biochips, etc, as well as in the basic sciences of biology, genetics, etc.

With this quotation in mind, this paper builds on the pioneering work of Katchalsky’s group (Oster et al., 1971, 1973), together with more recent investigations (Gawthrop and Crampin, 2016; Gawthrop, 2017; Gawthrop and Crampin, 2014; Gawthrop et al., 2015a) to give an engineering-inspired modelling approach to biomolecular systems which seamlessly combines biochemical reactions, electrons and protons using the concept of the Faraday-equivalent chemical potential.

In particular, this paper shows that combining electrical units for chemical potential with bond graph models of biomolecular systems not only provides a systematic methods for model development and analysis of biomolecular systems but also provides a bridge allowing application of electrical engineering methodology to biomolecular networks.

Redox reactions provide the energy required to sustain life (Atkins and de Paula, 2011; Sousa et al., 2013) and the notion of the redox potential is useful in describing energetic properties. This paper shows that both redox reactions and redox potential can be clearly and explicitly described using the bond graph approach and the use of the Faraday-equivalent chemical potential.

Mitochondria make use of redox reactions to provide the power driving many living systems. Mathematical modelling of the key components of mitochondria is thus an important challenge to systems biology. 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 the proton-motive force (PMF). This PMF is then used to power the synthesis of ATP – the universal fuel of living systems. Because mitochondria transduce energy, an energy-based modelling method (Beard and Qian, 2010; Hill, 1989; Qian and Beard, 2005; Wu et al., 2007) is desirable.

Modular bond graphs provide a way of decomposing complex biomolecular systems into manageable parts (Gawthrop and Crampin, 2016; Gawthrop et al., 2015a). This paper combines the modularity concepts of Neal et al. (2016) with the bond graph approach to give a more flexible approach to modularity. This paper suggests that such a modular bond graph approach, combined with electrical units, provides a flexible and powerful energy-inspired modelling method which brings engineering expertise to the analysis of biomolecular systems in general and chemiosmotic energy transduction in mitochondria in particular.

An alternative approach would use electrical networks to model chemical systems (Caravaca et al., 2014; Oster and Perelson, 1974; Županović and Juretić, 2004). Indeed, Oster and Perelson (1974) show the precise connection between the two approaches. However, the resultant circuit
diagrams can be unwieldy and the representation of stoichiometry is cumbersome. Therefore, in the author’s opinion, the more general bond graph approach is superior. Nevertheless, the equivalence discussed by Oster and Perelson (1974) should, in principle, allow circuit-theoretical approaches (Anderson and Vongpanitlerd, 2006) to be incorporated.

§ 2 introduces the Faraday-equivalent chemical potential and this is used in § 3 to provide bond graph models of redox reactions which seamlessly combine the chemical and electrical domains and provide a bond graph interpretation of redox potential. § 4 considers an approach to computational modularity in the context of bond graphs which is then used, together with the redox reaction models of § 3 in § 5 to give a modular bond graph model of the mitochondrial electron transport chain (ETC). § 6 uses this bond graph model to analyse how the intermediate electron transporters coenzyme Q and cytochrome c equalise the Faraday-equivalent potentials along the mitochondrial electron transport chain. § 7 describes how the bond graph representation of redox reactions can be generalised to include ATP hydrolysis and synthesis and how this can be combined with the ETC to give a modular bond graph representation of oxidative phosphorylation. § 8 concludes the paper and suggests directions for future research.

2 The Faraday-equivalent potential

The fundamental biophysical processes of life involve the transduction of chemical energy and electrical energy (Lane and Martin, 2010). For example, the chemiosmotic theory of Mitchell (1961, 1976, 1993, 2011) explains how a mixture of chemical and electrical energy is stored in a trans-membrane proton gradient and the theory of Hodgkin and Huxley (1952) shows how the mutual transduction of chemical and electrical energy gives rise to action potential in nerves.

Because the chemical and electrical domains are so intertwined, the analysis and understanding of such systems is enhanced by a common approach to the two domains. One example of this is the proton motive force PMF of chemiosmotic theory (Alberts et al., 2015; Berg et al., 2012; Mitchell, 1993; Nicholls and Ferguson, 2013) which reexpresses how a mixture of chemical and electrical energy is stored in a trans-membrane proton gradient and the theory of Hodgkin and Huxley (1952) shows how the mutual transduction of chemical and electrical energy gives rise to action potential in nerves.

A theme of this paper is that the notion of reexpressing chemical potential as electrical potential is not just confined to electrically-charged ions but can be generally applied to any chemical species – charged or not. Indeed, this can be regarded as one aspect of the concept of physical analogies introduced by Maxwell (1871) who pointed out that analogies are central to scientific thinking and allow mathematical results and intuition from one physical domain to be transferred to another. The central concept here is that conservation of energy holds across different physical domains.

2.1 Variables & Units

In the context of electrochemical systems, there are two ways of unifying the two domains: reexpress chemical potential as electrical potential (Bose et al., 2003) (as in the proton-motive force
concept (Mitchell, 1993, 2011) or reexpress electrical potential as chemical potential (Gawthrop et al., 2015b). Those with a physics or engineering background would be more familiar with electrical units and would therefore prefer the former choice. However there is a more general reason for choosing the electrical domain: it is better endowed with dedicated units.

Chemical potential is expressed as the compound unit of Joules per mole \( (J\,mol^{-1}) \) but does not have a dedicated unit\(^1\). In contrast, electrical potential has its own unit, the Volt \( (V) \). Although it would be possible to ignore this unit and use the equivalent compound unit of Joules per Coulomb \( (J\,C^{-1}) \), this would obscure the basic simplicity of electrical theory. Moreover, chemical flow can be expressed in compound units as moles per second \( (mol\,s^{-1}) \) but does not have a dedicated unit; in contrast, electrical flow has its own unit, the Amp \( (A) \). Again, it would be possible to be perverse and ignore this unit and use the equivalent compound unit of Coulombs per second \( (C\,s^{-1}) \).

The conversion factor relating the electrical and chemical domains is Faraday’s constant \( F \approx 96485 \, C \, mol^{-1} \). As discussed by Karnopp (1990) and Gawthrop et al. (2015b), this conversion can be represented by the bond graph \( TF \) component which enforces energy conservation. Like all physical quantities, Faraday’s constant \( F \) is composed of a real number (the measure) and a unit (Walton, 1996). In particular:

\[
F = F \times U \\
\text{where } F \approx 96485 \\
\text{and } U = 1 \, C \, mol^{-1}
\]

In bond graph terms, the single bond graph \( TF \) component representing \( F \) has been split into two \( TF \) components: one representing the the purely numerical conversion \( F \) and one representing the purely dimensional conversion \( U \).

Hence it is possible to define two new derived units, the Faraday-equivalent voltage \( \mathcal{V} = FJ\,mol^{-1} \) and the Faraday-equivalent current \( \mathcal{A} = \frac{1}{F}mol\,s^{-1} \). Using these units, the Faraday-equivalent potential \( \phi \), Faraday-equivalent affinity \( \Phi \) and the Faraday-equivalent flow \( f \) are defined in terms of chemical potential \( \mu \) and molar flow \( v \) as:

- Faraday-equivalent chemical potential
  \[
  \phi = \frac{\mu}{F} \, \mathcal{V}
  \]
- Faraday-equivalent reaction affinity
  \[
  \Phi = \frac{A}{F} \, \mathcal{V}
  \]
- Faraday-equivalent flow
  \[
  f = Fv \, \mathcal{A}
  \]

For example, consider NAD at standard conditions which has a chemical potential at standard conditions \( \mu_{\text{NAD}}^{\text{ST}} = 18100 \, J \, mol^{-1} \); the corresponding Faraday-equivalent potential is \( \phi_{\text{NAD}}^{\text{ST}} = 188 \, mV \). Similarly, a molar flow of \( v = 1 \mu mol\,s^{-1} \) has a Faraday-equivalent flow of about \( f = 97 \, mA \). Faraday-equivalent chemical potentials for some other species are given in Table 1.\(^4\)

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\(^1\) Job and Herrmann (2006) suggest the Gibbs \( (G) \) as a the unit of chemical potential, but this is not widely used.
2.2 The Bond Graph C component

The C component is the bond graph abstraction of an electrical capacitor. In the chemical context, it represents a chemical species with chemical potential replacing voltage and molar flow replacing current (Oster et al., 1971, 1973). In particular, following Gawthrop and Crampin (2014), the bond graph C component for biomolecular systems accumulates a chemical species A as the number of moles $x_A$ and generates the corresponding chemical potential $\mu_A$ in terms of the molar flow $x_A$:

$$x_A(t) = \int_0^t v_A(t')dt' + x_A(0) \quad (7)$$

$$\mu_A = \mu_A^\circ + RT \ln \frac{x_A}{x_A^\circ} \quad (8)$$

where $\mu_A^\circ$ is the chemical potential of $x_A$ when $x_A = x_A^\circ$. Equation (8) may be rewritten in two ways:

$$\mu_A = RT \ln K_A x_A \quad (9)$$

where $K_A = \frac{e^{\mu_A^\circ}}{x_A^\circ}$ (10)

and $\mu_A = \mu_A^\circ + RT \ln \left(1 + \frac{\tilde{x}_A}{x_A^\circ}\right)$ (11)

where $\tilde{x}_A = x_A - x_A^\circ$ (12)

Equation (10) is equivalent to that used previously (Gawthrop, 2017; Gawthrop and Crampin, 2014) and equation (12) is convenient when $\tilde{x}_A$ is small and so:

$$\mu_A \approx \mu_A^\circ + RT \frac{\tilde{x}_A}{x_A^\circ} \quad \text{when} \quad \frac{\tilde{x}_A}{x_A^\circ} \ll 1 \quad (13)$$

Using equations (4) and (5), equations (7) and (8) can be rewritten in Faraday-equivalent form as:

$$q_A(t) = \int_0^t q_A(t')dt' + q_A(0) \quad (14)$$

$$\phi_A = \phi_A^\circ + \tilde{\phi}_A \quad (15)$$

where $\phi_A^\circ = \frac{\mu_A^\circ}{F}$ (16)

$$\tilde{\phi}_A = V_N \ln \frac{q_A}{q_A^\circ} = V_N \ln \left(1 + \frac{\tilde{q}_A}{q_A^\circ}\right) \quad (17)$$

$$V_N = \frac{RT}{F} \approx 26 \text{ mV} \quad (18)$$

$$q_A = F x_A \quad (19)$$

$$q_A^\circ = F x_A^\circ \quad (20)$$

and $\tilde{q}_A = q_A - q_A^\circ \quad (21)$
### Table 1: Chemical and Faraday-equivalent Potentials.

| Species       | $\mu^\circ$kJ mol$^{-1}$ | $\phi^\circ$V |
|---------------|---------------------------|---------------|
| $O_2$         | 16.4                      | 0.169974      |
| $H^+$         | 0                         | 0             |
| $H_2O$        | -235.74                   | -2.44327      |
| NADH          | 39.31                     | 0.407419      |
| NAD$^+$       | 18.1                      | 0.187593      |
| $Q$           | 65.17                     | 0.675439      |
| QH$_2$        | -23.3                     | -0.241487     |
| Fe$^{3+}$     | -6.52                     | -0.067575     |
| Fe$^{2+}$     | -27.41                    | -0.284085     |
| ATP$^4^-$     | -2771                     | -28.7194      |
| ADP$^3^-$     | -1903.96                  | -19.7332      |
| HPO$_4^2^-$   | -1098.27                  | -11.3828      |
| $H^+_x$ (pH 7.78) | -44.408                  | -0.46026      |

Tables of chemical potentials at standard conditions are available (Atkins and de Paula, 2011). Table 1 lists some of these with their Faraday-equivalent potentials where the values for $\mu^\circ$ are taken from Wu et al. (2007). Given the chemical potential of substance $A$ at standard conditions $\mu^\circ_A$, and the corresponding Faraday-equivalent potential $\phi^\circ_A = F\mu^\circ_A$, the Faraday-equivalent potential $\phi^\circ_A$ at any other operating point can be computed from Equation (15) as

$$\phi^\circ_A = \phi^\circ_A + V_N \ln \rho_A$$

where

$$\rho_A = q^\circ_A \over q^\circ_A = c^\circ_A \over c^\circ_A$$

and $c^\circ_A$ and $c^\circ_A$ are the concentrations at the relevant conditions. For example, the following are required in §6

- **H$^+$ at pH 7**
  $$\phi^\circ = 0 + V_N \ln 10^{-7} = -414 \text{ mV}$$

- **H$_x^+$ at pH 6.88**
  $$\phi^\circ = 0 + V_N \ln 10^{-6.88} = -407 \text{ mV}$$

- **H$_x^+$ at pH 7.78**
  $$\phi^\circ = 0 + V_N \ln 10^{-7.78} = -460 \text{ mV}$$

- **O$_2$ at 200 $\mu$M**
  $$\phi^\circ = 170 + V_N \ln 2e-4 = -49 \text{ mV}$$

The pH values for $H^+_x$ and $H^+_i$ are taken from Porcelli et al. (2005).

### 2.3 Chemostats

As discussed by Gawthrop and Crampin (2016), the notion of a chemostat (Polettini and Esposito, 2014) is useful in creating an open system from a closed system; a similar approach is used by Qian and Beard (2005) who use the phrase “concentration clamping”. The chemostat has four interpretations:
1. one or more species is fixed to give a constant concentration (Gawthrop et al., 2015a); this implies that an appropriate external flow is applied to balance the internal flow of the species.

2. an ideal feedback controller is applied to species to be fixed with setpoint as the fixed concentration and control signal an external flow.

3. as a \( C \) component with a fixed state and

4. as an ideal source of Faraday-equivalent potential: \( \phi = \phi^\circ \).

In this paper, a further interpretation is added. In §4, a chemostat is interpreted as an external port of a module which allows connection to other modules.

### 2.4 The Bond Graph Re component

The \( R \) component is the bond graph abstraction of an electrical resistor. In the chemical context, a two-port \( R \) component represents a chemical reaction with chemical affinity (net chemical potential) replacing voltage and molar flow replacing current (Oster et al., 1971, 1973). As it is so fundamental, this two port \( R \) component is given a special symbol: \( \text{Re} \) (Gawthrop and Crampin, 2014). In particular, the \( \text{Re} \) component determines a reaction flow \( v_1 \) in terms of forward and reverse affinities \( A'_f \) and \( A'_r \) as the Marcelin – de Donder formula (Van Rysselberghe, 1958):

\[
v_1 = \kappa_1 \left( \exp \frac{A'_f}{RT} - \exp \frac{A'_r}{RT} \right)
\]  

(28)

in the special case of mass-action kinetics, \( \kappa \) is a constant. Otherwise \( \kappa \) is a function of the forward and reverse affinities \( A'_f \) and \( A'_r \). Using equations (5) and (6), equation (28) can be rewritten in Faraday-equivalent form as:

\[
f_1 = \frac{V_N}{r_1} \left( \exp \frac{\Phi'_f}{V_N} - \exp \frac{\Phi'_r}{V_N} \right)
\]  

(29)

where

\[
r_1 = \frac{V_N}{F\kappa_1}
\]  

(30)

\( V_N \) is given by Equation (18) and the resistance \( r_1 \) has units of ohms (\( \Omega \)). Alternatively:

\[
f_1 = \frac{V_N}{r_1} 2 \exp \frac{\Phi'_1}{V_N} \sinh \frac{\Phi'_1}{2}
\]  

(31)

where

\[
\bar{\Phi}_1 = \frac{\Phi'_f + \Phi'_r}{2}
\]  

(32)

and

\[
\Phi = \frac{\Phi'_f - \Phi'_r}{2}
\]  

(33)
When the normalised reaction affinity $\frac{\Phi_1}{V_N} \ll 1$:

$$f_1 \approx \left( \exp \frac{\Phi_1}{V_N} \right) \frac{\Phi_1}{r_1}$$ \hspace{1cm} (34)

3 Redox reactions

Figure 1: Redox Reactions. The redox reaction NADH + Q + H⁺ ⇌ NAD⁺ + QH₂ is divided into two half-reactions NADH $r_1^1$ NAD⁺ + H⁺ + 2e⁻ and Q + 2H⁺ + 2e⁻ $r_2^2$ QH₂ and the electron transfer is represented by e⁻ $r_1^1 \rightleftharpoons e_2^-$. The dashed box contains the electrical part of the system with linear components; the rest of the system is chemical and nonlinear.

Redox reactions ([Atkins and de Paula] 2011, Chapter 5) involve the transfer of electrons e⁻, and the corresponding free energy, from a donor species to an acceptor species. This can be explicitly represented using the concept of half-reactions ([Atkins and de Paula] 2011 §5.4). For example in the reaction

$$\text{NADH} + \text{Q} + \text{H}^+ \rightleftharpoons \text{NAD}^+ + \text{QH}_2$$ \hspace{1cm} (35)

NADH (reduced Nicotinamide Adenine Dinucleotide) donates two e⁻ (electrons) in forming NAD⁺ (oxidised Nicotinamide Adenine Dinucleotide) which are accepted by Q (oxidised Ubiquinone) to form QH₂ (reduced Ubiquinone) ([Alberts et al.] 2015, Panel 14.1).

Reaction (35) can be split into two half reactions as:

$$\text{NADH} \rightleftharpoons \text{NAD}^+ + \text{H}^+ + 2e^-$$ \hspace{1cm} (36)

$$\text{Q} + 2\text{H}^+ + 2e^- \rightleftharpoons \text{QH}_2$$ \hspace{1cm} (37)
where $e_1^-$ denotes electrons donated in half-reaction $a$ (36) and $e_2^-$ denotes electrons accepted in half-reaction $b$ (37).

Figure 1 includes the two chemical half-reactions (36) and (37) together with an electrical interconnection. Thus the bond graph component $\text{Re}_r1$, together with the components $C:\text{NADH}, C:\text{NAD}$ and $C:H$ and connecting bonds represents reaction $r1$ and the bond graph component $\text{Re}_r2$, together with the components $C:Q, C:QH2$ and $C:H$ and connecting bonds represents reaction $r2$. To obtain the appropriate bond graph, it is assumed that the electrons associated each reaction accumulate in electrical capacitors represented by $C:E_a$ and $C:E_b$ which generate voltages $V_1$ and $V_2$ respectively. The corresponding electrical currents are:

$$i_1 = 2f_1 - f$$
$$i_2 = f - 2f_1$$

It is further assumed that electrons can flow via the electrical resistor $\text{Re}_r$. This is represented by the reaction

$$\text{Re}_r: e_1^- \xrightleftharpoons{} e_2^-$$

and corresponds to the current:

$$f = \frac{V_1 - V_2}{r}$$

The chemoelectrical redox system of Figure 1 spans the two physical domains (chemical and electrical) discussed in §2. The standard approach to redox potential is to view the chemical part of the system from an electrical point of view; this is now shown to have bond graph interpretation.

In particular, consider the case where the electrical resistor is open-circuit so that the current $f = 0$. When the two separate parts of the system are in equilibrium, the two reaction flows are zero: $f_1 = 0, f_2 = 0$: this implies that the net affinity for reaction $\text{Re}_r1$ must be exactly balanced by the voltage on the electrical capacitor $C:E1$ and the net affinity for reaction $\text{Re}_r2$ must be exactly balanced by the voltage on the electrical capacitor $C:E2$:

$$2V_1 = \Phi_1$$
$$2V_2 = \Phi_2$$

Focusing on half-reaction 1, and using Table 1 and the potential for $H_2^+$ from Equation (26), the reaction affinity $\Phi_1$ is given by:

$$\Phi_1 = \Phi_1' - \Phi_1 = \phi_{\text{NADH}}^\circ - (\phi_{\text{NAD}}^\circ + \phi_{Hx}^\circ)$$
$$= 408 - (188 - 460) = 680 \text{ mV}$$

From Equation (41),

$$V_1 = \frac{1}{2} \Phi_1 = 340 \text{ mV}$$

$V_1$ is the redox potential of half-reaction 1. Similarly:

$$\Phi_2 = \phi_{\text{QH2}}^\circ - \phi_Q^\circ - 2\phi_{Hx}^\circ$$
$$= -241 - 675 + 920 = 4 \text{ mV}$$

$$V_2^\circ = \frac{1}{2} \Phi_2 = 2 \text{ mV}$$
Using the standard sign convention, $V_2^\circ$ is minus the redox potential of half-reaction 2. The overall redox potential is given by:

$$V^\circ = V_1^\circ - V_2^\circ = 338 \text{ mV}$$

(47)

If current is allowed to flow through the resistor, all the energy associated with the redox potential $V^\circ$ is wastefully dissipated. In contrast, the CI complex of the mitochondrial respiratory chain uses the flow of electrons to pump protons across the inner mitochondrial membrane against both a concentration and electrical gradient: thus much of the energy associated with $V^\circ$ is transduced and stored as the mitochondrial proton-motive force (Mitchell, 1993, 2011; Nicholls and Ferguson, 2013). This is examined in §§4 & 5.

4 Computational Modularity

![Diagrams](a) Proton pump  (b) Proton pump module

Figure 2: Modularity and Proton Pumps. (a) A model of an electron-driven proton pump; the two transformers $\text{TF:tf}$ and $\text{TF:tr}$ have the same modulus $n_p$; the number of protons pumped per electron. (b) A modular version of (a).

As discussed by Neal et al. (2014), models of biological systems should be modular and reusable. Modularity raises the issue of module interfaces and Neal et al. (2014) distinguish between black-box, code-level coupling using information-hiding interfaces at one extreme and white-box, biological-level coupling at the other.

Bond graphs naturally give rise to hierarchical modular modeling (Cellier, 1991, 1992). One approach uses explicit ports, represented by the bond graph SS (source-sensor) component, to define interfaces (Gawthrop and Bevan, 2007) and this has been used in the biomolecular context.
However, this does have the disadvantages of the black-box approach discussed by Neal et al. (2014). This paper uses an alternative approach to bond graph modularity inspired by the approach of Neal et al. (2016). The basic idea is simple: modules are self-contained and have no explicit ports; but any species, as represented by a C component has the potential to become a port. Thus if two modules share the same species, the corresponding C component in each module is replaced by an SS component with the same name, and the species is explicitly represented as a C component on a higher level. Moreover, each module can be individually tested by replacing the relevant C components by chemostats. Although not present in the current implementations, explicit connection to ontology databases such as the Ontology of Physics for Biology (Cook et al., 2011) or composite ontologies (Gennari et al., 2011) would be required for general use.

The mitochondrial proton pumps are complex molecules (Schultz and Chan, 2001). Nevertheless, their key energetic features can be modelled using simplified representations. Figure 3(a) shows a simple model of an electron-driven proton pump based on the generic biomolecular cycle of Hill (1989). The two electrical capacitors C:E1 and C:E2 correspond to those modelling redox potential in Figure 1; C:Hx and C:Hi correspond to the amount of protons in the mitochondrial matrix and intermembrane space respectively and C:P to the proton electrical potential across the membrane. Re:r determines the flow of protons through the membrane. In this particular case, all five C components are used as external connections in the modular form of the model shown in Figure 2(b). This model is reused three times (with differing values of $n_p$) in §5 as part of the models of the CI, CIII and CIV complexes of the mitochondrial electron transport chain (ETC).

As in any modelling endeavour, the complexity of the model should be appropriate to its use; the important issue is to enable the modelling approach to handle a range of levels of complexity whilst retaining a physically correct representation. Figure 3 shows one possible simplification of the module of Figure 2.
Figure 4: The Electron Transport Chain (ETC). The three complexes CI, CIII and CIV are represented by the modules mCI (Figure 5), mCIII (Figure 6) and mCIV (Figure 7) respectively. All three complexes pump protons $H^+_x$ accumulated in $C: \text{Hx}$ from the matrix across the inner membrane to protons $H^+_i$ accumulated in $C: \text{Hi}$ in the inter-membrane space; the corresponding electrical charge is accumulated in $C: \text{P}$. Ubiquone in reduced form $QH_2$ and oxidised form $Q$ is recycled around CI & CIII; cytochrome c in reduced form $Fe^{2+}$ and oxidised form $Fe^{3+}$ is recycled around CIII & CIV and the two cycles intersect.
5 The Mitochondrial Electron Transport Chain

The mitochondrial electron transport chain (ETC) is a key component of the bioenergetics of eukaryotic organisms (Nicholls and Ferguson, 2013; Rich and Maréchal, 2010). The electrons are transported by, and gain energy from, redox reactions such as that discussed in §3 and, as discussed in §4 partially transduce this energy by pumping protons across the inner membrane to create the proton-motive force (Mitchell, 1961, 1976, 2011).

Because the electron transport chain is primarily concerned with chemiosmotic energy transduction, it is natural to use the bond graph approach for modelling this system. Because the system is complex, a modular approach enhances understanding. With this in mind, Figure 4 gives a top-level bond graph representation of the electron transport chain with the following features:

1. The external substrates are NADH and O₂ and the external products are NAD⁺ and H₂O and are represented by C:NADH, C:O₂, C:NAD and C:H₂O respectively. As discussed in §2.3 these components are regarded as chemostats.

2. The reactions within the three complexes may consume or produce protons H⁺ and thus are dependent on the proton concentration often expressed as pH. Protons exist in two separate volumes with different pH: the mitochondrial matrix, where they are denoted by H⁺x and the inter-membrane space where they are denoted by H⁺i.

3. The three complexes (CI, CIII & CIV) can be represented as redox reactions with an explicit flow of electrons e⁻ combined, as in §4 with a proton pump to transduce the electron energy into proton energy by pumping protons across the mitochondrial inner membrane. The matrix protons accumulate in C:Hx, the inter-membrane protons accumulate in C:Hi and C:P holds the corresponding electrical charge. Because the mitochondrial inner membrane separating the matrix from the intermembrane space has an electrical voltage across it, the C:P stores electrical energy. For convenience, the net proton charge represented by C:P is denoted P within reactions to clarify stoichiometry.

4. Ubiquone in reduced form QH₂ and oxidised form Q (sometimes denoted coenzyme Q or CoQ) is recycled around CI & CIII; cytochrome c in reduced form Fe²⁺ and oxidised form Fe³⁺ is recycled around CIII & CIV and the two cycles intersect. As discussed in §6 this structure allows the Faraday-equivalent potentials to be equalised across the three complexes CI, CIII and CIV.

5. Although not included in this paper, the explicit representation of electron e⁻ flow allows electron leakage, and the concomitant generation of reactive oxygen species (ROS) such as superoxide O₂⁻ and hydrogen peroxide H₂O₂ (via Superoxide Dismutase) (Murphy, 2009) to be explicitly modelled.

Following Atkins and de Paula (2011), Fe²⁺ is used to represent reduced cytochrome c (otherwise known as C(red)) and Fe³⁺ is used to represent oxidised cytochrome c (otherwise known as C(ox)).
5.1 Complex CI

The bond graph of complex CI given in Figure 5 is based on the redox reaction of Figure 1 but instead of the electron-motive force $V_1 - V_2$ being dissipated in the resistor $r$, it is used to drive the proton pump described in §4.

The proton pump is represented by the bond graph component $\text{mppn:pp}$ where $\text{mppn}$ is the modular version of the proton pump described in Figure 3(b) and $\text{pp}$ provides a label indicating a particular instance (in this case with $n_p = 2$). Following previous notation (Gawthrop 1998; Gawthrop and Bevan 2007; Gawthrop et al. 2015a), the five ports are labelled $[\text{E1}]$, $[\text{E2}]$, $[\text{Hx}]$, $[\text{Hi}]$ and $[\text{P}]$ corresponding to the five ports designated by the SS:components of Figure 3(b).

In a similar fashion to §3, the redox reaction

$$\text{NADH} + \text{Q} + \text{H}_x^+ \rightleftharpoons \text{NAD}^+ + \text{QH}_2$$  \hspace{1cm} (48)

is split into the two half-reactions:

$$\text{NADH} \overset{r_1}{\rightleftharpoons} \text{NAD}^+ + \text{H}_x^+ + 2e^- \hspace{1cm} (49)$$

$$\text{Q} + 2\text{H}_x^+ + 2e^- \overset{r_2}{\rightleftharpoons} \text{QH}_2 \hspace{1cm} (50)$$

and these two half reactions are represented by bond graph components in the same way. There are two differences from the reactions of §3.
1. The energy from the redox reaction is no longer dissipated in the component \( \text{Re}:r \) but are used to drive the proton pump represented by \( \text{mppn}:pp \). The pump removes protons \( \text{H}_x^+ \) from the matrix and deposits them as \( \text{H}_i^+ \) in the intermembrane space; as discussed above, the matrix protons accumulate in \( \text{C}:\text{Hx} \), the inter-membrane protons accumulate in \( \text{C}:\text{Hi} \) and \( \text{C}:\text{P} \) holds the corresponding electrical charge.

2. The hydrogen ions \( \text{H}_x^+ \) are explicitly associated with the mitochondrial matrix.

In this case, \( n_p = 2 \) and thus two protons are pumped from the matrix to the intermembrane space for each electron associated with the redox reaction. As two electrons are associated with each molecule of NADH, four protons are pumped for each molecule of NADH consumed in the reaction. In addition, reaction \( \text{Re}:r1 \) produces one, and reaction \( \text{Re}:r2 \) consumes two, protons in the matrix. Thus the overall reaction represented by Figure 5 is:

\[
\text{NADH} + \text{Q} + 5\text{H}_x^+ \overset{\text{CI}}{\rightleftharpoons} \text{NAD}^+ + \text{QH}_2 + 4\text{H}_i^+ + 4\text{P} \quad (51)
\]

In Figure 16 of his Nobel Lecture, Mitchell (1993) draws insightful comparisons between fuel cells and mitochondria. In particular, he notes that the two half-reactions are coupled by electrons (electricity) and protons (proticity). The difference is that fuel cells are designed to generate electricity whereas the electron transport chain of mitochondria generates proticity. This is also the situation in Figures 5, 6 and 7 where electrons flow in the upper part of the diagram and protons in the lower and the two half reactions are to the left and the right. Thus the bond graph representation of complexes CI, CIII and CIV reflects the situation depicted by Mitchell (1993, Figure 16).

### 5.2 Complex CIII

The bond graph of complex CIII given in Figure 6 is similar to that of complex CI given in Figure 5. The difference is that it now represents the redox reaction:

\[
\text{QH}_2 + 2\text{Fe}^{3+} \rightleftharpoons \text{Q} + 2\text{Fe}^{2+} + 2\text{H}_x^+ \quad (52)
\]

with the half-reactions

\[
\text{QH}_2 \overset{r_1}{\rightleftharpoons} \text{Q} + 2\text{H}_x^+ + 2\text{e}^- \quad (53)
\]

\[
\text{Fe}^{3+} + \text{e}^- \overset{r_2}{\rightleftharpoons} \text{Fe}^{2+} \quad (54)
\]

Note that the first half reaction of CIII (53) is the reverse of the second half reaction of CI (50).

As with complex CI, the energy associated with the redox reaction is used to pump protons across the inner mitochondrial membrane. In this case \( n_p = 1 \) and thus two protons are pumped from the matrix to the intermembrane space for each \( \text{QH}_2 \) consumed. The first half-reaction donates two protons \( \text{H}_x^+ \) to the matrix for each \( \text{QH}_2 \) consumed. The first half-reaction produces

\[^3\text{QH}_2 \text{ and Q exist within the inner membrane. The assumption made here is that the the corresponding protons are part of the matrix pool. Other assumptions could easily be accommodated by modifying Figures 5 and 6.}\]
two electrons for each QH$_2$ consumed and the second half-reaction consumes one electron for each Fe$^{3+}$ consumed. Thus the overall reaction represented by Figure 6 is:

$$\text{QH}_2 + 2\text{Fe}^{3+} \xleftrightarrow{\text{CIII}} \text{Q} + 2\text{Fe}^{2+} + 2\text{H}^+ + 2\text{P}$$  \hspace{1cm} (55)$$

5.3 Complex CIV

The bond graph of complex CIV given in Figure 7 is similar to that of complex CI given in Figure 5. The difference is that it now represents the redox reaction:

$$4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+_i \xleftrightarrow{\text{CIV}} 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$$  \hspace{1cm} (56)$$

with the half-reactions

$$\text{Fe}^{2+} \xrightarrow{r_1} \text{Fe}^{3+} + e^-_1$$  \hspace{1cm} (57)$$

$$\text{O}_2 + 4\text{H}^+_i + 4e^-_2 \xrightarrow{r_2} 2\text{H}_2\text{O}$$  \hspace{1cm} (58)$$

Note that the first half reaction of CIV (57) is the reverse of the second half reaction of CIII (54). As with complex CI, the energy associated with the redox reaction is used to pump protons across the inner mitochondrial membrane. In this case $n_p = 2$ and thus eight protons are pumped
Figure 7: Complex CIV

from the matrix to the intermembrane space for each oxygen molecule O$_2$ consumed. The first half-reaction consumes two protons H$^+$ from the intermembrane space. Thus the overall reaction represented by Figure 7 is:

$$4\text{Fe}^{2+} + \text{O}_2 + 8\text{H}^+_x \rightleftharpoons 4\text{Fe}^{3+} + 2\text{H}_2\text{O} + 4\text{H}^+_i + 8\text{P} \quad (59)$$

Using the methods of Gawthrop and Crampin (2016) applied to the bond graphs of Figures 5, 6 and 7 gives the following overall chemical equation for the electron transport chain:

$$2\text{NADH} + \frac{1}{2}\text{O}_2 + 9\text{H}^+_x \rightleftharpoons 2\text{NAD}^+ + 2\text{H}_2\text{O} + 16\text{H}^+_i + 20\text{P} \quad (60)$$

An alternative approach is to note that CI (51) corresponds to 2e$^-$ pumping 4 protons, CIII (55) corresponds to 2e$^-$ pumping 2 protons and CIV (59) corresponds to 4e$^-$ pumping 8 protons. Thus equation (60) arises from multiplying the stoichiometry of CI and CIII by 2 and adding the resultant equations to that for CIV; the total number of protons pumped by 4e$^-$ passing down the ETC is thus $2 \times 4 + 2 \times 2 + 1 \times 8 = 20$.

Equation (60) is sometimes rewritten with non-integer stoichiometry as:

$$\text{NADH} + \frac{1}{2}\text{O}_2 + 9\text{H}^+_x \rightleftharpoons \text{NAD}^+ + \text{H}_2\text{O} + 8\text{H}^+_i + 10\text{P} \quad (61)$$

The integer stoichiometry version of Equation (60) is used in the following section.
6 Energy transduction and affinity equalisation

As an illustration of the potential of the bond graph approach, this section uses the electron transport chain model of §5 to show how the electron-transporting complexes Q/\(\text{QH}_2\) and Fe\(^{3+}/\text{Fe}^{2+}\) equalise the Faraday-equivalent potentials along the mitochondrial electron transport chain. There are three redox reactions (corresponding to the three complexes), each of which has two half reactions. However, the first half-reaction of CIII is a reversed version of the second half-reaction of CI and the first half-reaction of CIV is a reversed version of the second half-reaction of CIII. Hence there are just four half reactions to be considered: those involving NADH, Q, Fe and O\(_2\).

Using Table I, the four reaction affinities are:

\[
\Phi_{\text{NADH}}^\circ = \phi_{\text{NADH}}^\circ - \phi_{\text{NAD}^+}^\circ - \phi_{\text{H}_x}^\circ = 407 - 187 + 460 = 680 \text{ mV}
\]

\[
\Phi_Q^\circ = \phi_Q^\circ - \phi_{\text{QH}_2}^\circ + 2\phi_{\text{H}_x}^\circ = 675 + 241 - 922 = -4 \text{ mV}
\]

\[
\Phi_{\text{Fe}}^\circ = \phi_{\text{Fe}^{3+}}^\circ - \phi_{\text{Fe}^{2+}}^\circ = -67 + 284 = 217 \text{ mV}
\]

\[
\Phi_{\text{O}_2}^\circ = \phi_{\text{O}_2}^\circ - 2\phi_{\text{H}_2\text{O}}^\circ + 4\phi_{\text{H}_x}^\circ = -49 + 4887 - 1628 = 3210 \text{ mV}
\]
The corresponding complex affinities are

\[
\Phi_{\text{CI}}^{C} = \Phi_{\text{NADH}}^{C} + \Phi_{Q}^{C} = 680 - 4 = 676 \text{ mV}
\]

\[
\Phi_{\text{CIII}}^{C} = -\Phi_{Q}^{C} + 2\Phi_{\text{Fe}}^{C} = 4 + 434 = 438 \text{ mV}
\]

\[
\Phi_{\text{CIV}}^{C} = -4\Phi_{\text{Fe}}^{C} + \Phi_{O_{2}}^{C} = -868 + 3210 = 2344 \text{ mV}
\]

\[
\Phi_{\text{ETC}}^{C} = 2\Phi_{\text{CI}}^{C} + 2\Phi_{\text{CIII}}^{C} + \Phi_{\text{CIII}}^{C} = 1352 + 876 + 2344 = 4572 \text{ mV}
\]

where \(\Phi_{\text{ETC}}^{C}\) is the overall affinity of the electron transport chain summarised by Equation (60).

Each complex drives protons across the inner membrane against the PMF \(\Delta p\); but each complex drives a different number of protons: \(n_{\text{CI}} = 4\), \(n_{\text{CIII}} = 2\) and \(n_{\text{CIV}} = 8\). The overall number of protons transferred by the electron transport chain is

\[
n_{\text{ETC}} = 2n_{\text{CI}} + 2n_{\text{CIII}} + n_{\text{CIV}} = 20
\]

\[
\bar{\Phi}_{\text{CI}}^{C} = \frac{\Phi_{\text{CI}}^{C}}{n_{\text{CI}}} = \frac{676}{4} = 169 \text{ mV}
\]

\[
\bar{\Phi}_{\text{CIII}}^{C} = \frac{\Phi_{\text{CIII}}^{C}}{n_{\text{CIII}}} = \frac{438}{2} = 219 \text{ mV}
\]

\[
\bar{\Phi}_{\text{CIV}}^{C} = \frac{\Phi_{\text{CIV}}^{C}}{n_{\text{CIV}}} = \frac{2344}{8} = 293 \text{ mV}
\]

\[
\bar{\Phi} = \frac{\Phi_{\text{ETC}}^{C}}{n_{\text{ETC}}} = \frac{4572}{20} = 228 \text{ mV}
\]

Thus with these values of working concentrations, the maximum PMF \(\Delta p\) is determined by the smallest of these values, namely 169 mV for complex CI and the other two complexes have wasted affinity.

Complex CIII acts as an electronic bridge between the the Q/QH\(_2\) and the Fe\(^{3+}/Fe^{2+}\) pools and therefore can regulate the entire electronic transport chain (Sarewicz and Osyczka 2014). In particular, allowing the concentrations relating to the Q and Fe half reactions to vary gives two degrees of freedom to equalise the complex affinities per proton. That is:

\[
\Phi_{\text{CI}} = \Phi_{\text{CI}}^{C} + \bar{\Phi}_{\text{CI}} = \Phi_{\text{CI}}^{C} + \bar{\Phi}_{Q}
\]

\[
\Phi_{\text{CIII}} = \Phi_{\text{CIII}}^{C} + \bar{\Phi}_{\text{CIII}} = \Phi_{\text{CIII}}^{C} - \bar{\Phi}_{Q} + 2\bar{\Phi}_{\text{Fe}}
\]

\[
\Phi_{\text{CIV}} = \Phi_{\text{CIV}}^{C} + \bar{\Phi}_{\text{CIV}} = \Phi_{\text{CIV}}^{C} - 4\bar{\Phi}_{\text{Fe}}
\]

\(^4\) Equation (60) corresponds to \(4e^{-}\) passing down the ETC. The non-integer stoichiometry version (61) corresponds to \(2e^{-}\) passing down the ETC and thus the corresponding affinity is \(\frac{1}{2}\Phi_{\text{ETC}} = 2286 \text{ mV} = 220 \text{ kJmol}^{-1}\). This is the figure quoted in the literature (Nath 2016; Nicholls and Ferguson 2013).
As $\Phi_{ETC}$ is not affected by $\tilde{\Phi}_Q$ or $\tilde{\Phi}_{Fe}$, the three complex affinities per proton can all be set to

$$\Delta p = \Phi = \frac{\Phi_{ETC}^\circ}{n_{ETC}}$$

(78)

Hence equations (75) and (77) can rewritten as:

$$\tilde{\Phi}_Q = \Phi_{CI} - \Phi_{CI}^\circ = n_{CI}\Phi - \Phi_{CI}^\circ$$

$$= 912 - 676 = 236 \text{ mV}$$

(79)

$$\tilde{\Phi}_{Fe} = -\frac{1}{4} (\Phi_{CIV} - \Phi_{CIV}^\circ) = -\frac{1}{4} (n_{CIV}\Phi - \Phi_{CIV}^\circ)$$

$$= -\frac{1}{4} (1824 - 2344) = 130 \text{ mV}$$

(80)

It can be verified that this choice of $\tilde{\Phi}_Q$ and $\tilde{\Phi}_{Fe}$ equalises the three complex affinities per proton:

$\Phi_{CI} = \Phi_{CIII} = \Phi_{CIV} = \Phi$. The corresponding concentration ratios are:

$$\rho_Q = \exp \frac{\tilde{\Phi}_Q}{V_N} = 1.0328 \times 10^4$$

(81)

$$\rho_{Fe} = \exp \frac{\tilde{\Phi}_{Fe}}{V_N} = 151.0$$

(82)

The formulae (78), (79) & (80) can be used to evaluate the PMF $\Delta p$, and the incremental changes in the poll affinities $\tilde{\Phi}_Q$ & $\tilde{\Phi}_{Fe}$ as the concentration of $O_2$, as reflected in the potential $\phi_{O_2}$ changes.

Figure 8 shows the results of changing the concentration of $O_2$ so that the potential is varied by $\phi_{O_2}$. Figure 8(a) shows how the PMF $\Delta p$ and voltage $\Delta \psi$ vary and Figure 8(b) shows how the potentials $\phi_Q$ & $\phi_{Fe}$ of the two pools vary. This corresponds to the fact that hypoxia effects mitochondrial oxidative metabolism (Solaini et al., 2010).

## 7 Synthesis of ATP

ATP hydrolysis is a key energy-generating reaction in biochemistry. Following Berg et al. (2012), it may be written as:

$$\text{ATP}^4^- + H_2O \rightleftharpoons \text{ADP}^3^- + \text{HPO}_4^{2-} + H^+$$

(83)

where $\text{HPO}_4^{2-}$ is the orthophosphate ion commonly referred to as inorganic phosphate or Pi. Using the proton-motive force generated by the electron transport chain of §5, this reaction can be driven in reverse to synthesise $\text{ATP}^4^-$ from $\text{ADP}^3^-$ and $\text{HPO}_4^{2-}$. As discussed in §3, the standard decomposition of redox reactions into half reactions has a neat bond graph representation which allows the coupling of a proton pump in a natural way. The essence of the half-reaction approach is that the two half reactions are coupled by one or more electrons $e^-$. Here, it is suggested that this idea can be generalised by allowing the coupling to be some arbitrary chemical entity. In
particular, although the ATP hydrolysis reaction of Equation (83) is not a redox reaction, it can be decomposed into two half reactions as:

\[
\begin{align*}
\text{ATP}^{4-} & \rightleftharpoons \text{ADP}^3^- + \text{PO}_3^- \quad (84) \\
\text{H}_2\text{O} + \text{PO}_3^- & \rightleftharpoons \text{HPO}_4^{2-} + \text{H}^+ \quad (85)
\end{align*}
\]

coupled by the entity \( \text{PO}_3^- \). As discussed in §5.1, these two half-reactions can be coupled to a proton pump. In the ATPase complex of vertebrates, three \( \text{ATP}^{4-} \), and thus three \( \text{PO}_3^- \) pump 8 protons (Nicholls and Ferguson, 2013, §3.6.2). In a similar fashion to the redox reaction representation of §3, reactions (84) and (85) can be represented in bond graph form as in Figure 9. The overall reaction represented by Figure 9 is:

\[
3\text{ATP}^{4-} + 3\text{H}_2\text{O} + 5\text{H}_x^+ \rightleftharpoons 3\text{ADP}^3^- + 3\text{HPO}_4^{2-} + 8\text{H}_i^+ + 8\text{P}
\]

Using the approach of §4 to modularise the electron transport chain of Figure 4 and the phosphorylation reaction of Figure 9, oxidative phosphorylation can be represented in modular bond graph terms as Figure 10. The coupling of the two modules via the proton motive force represented by \( \text{C:H}_i \), \( \text{C:P} \) and \( \text{C:H}_x \) is clearly visible and the hydrolysis reaction \( \text{mPhos} \) is driven in reverse by the ETC \( \text{mOx} \).
Figure 10: Oxidative phosphorylation. mOx and mPhos are the modular versions of the ETC of Figure 4 and the ATP hydrolysis reaction of Figure 9.
8 Conclusion

It has been shown that combining previous work on the bond graph modelling of biomolecular systems with the Faraday-equivalent chemical potential and an alternative concept of bond graph modularity gives a seamless approach to modelling complex chemiosmotic biological systems involving biochemical reactions, electrons and protons. Using a new bond graph representation of redox reactions, the approach has been applied to give a model of the mitochondrial electron transport chain. As an illustration, this model is then used to show how the electron-transporting complexes \( \text{Q/QH}_2 \) and \( \text{Fe}^{3+}/\text{Fe}^{2+} \) equalise the Faraday-equivalent potentials along the mitochondrial electron transport chain. More generally, the approach of this paper provides an approach to analysing and understanding energy flows in complex biomolecular systems – for example, those within the Physiome Project (Hunter, 2016).

The appropriate level of complexity of a given model depends on the use to which the model is put. For example, it would be helpful to extend the mitochondrial electron transport chain to include the Q-cycle (Hunte et al., 2003) in complex CIII, the production of reactive oxygen species (ROS) (Bazil et al., 2016; Murphy, 2009; Vinogradov and Grivennikova, 2016) and the corresponding cellular control systems (Cosentino and Bates, 2012; Dunn et al., 2015; Vinnakota et al., 2016). On the other hand, for some purposes the model of this paper may be too detailed; in this case the energy-based pathway analysis of Gawthrop and Crampin (2016) can be used to give a reduced model retaining the key thermodynamic features. Versions of a model of a particular biomolecular subsystem (for example, CIII) can be encapsulated as modules and used and reused within larger systems to give the appropriate complexity.

It has been argued by Nath and Villadsen (2015) that Mitchell’s chemiosmotic theory is deficient in that “the energy transducing complexes involved in oxidative phosphorylation and photosynthesis are proton-dicarboxylic acid anion cotransporters” rather than just proton transporters. It would be interesting to create bond graph models corresponding to this hypothesis and compare the models with those of this paper.

The energy balance of biomolecular systems has been discussed in the literature (Ghafuri et al., 2014; Gibbs and Chapman, 1985; Harris et al., 2012; Sengupta and Stemmler, 2014) and summarised by Nath (2016) in the context of oxidative phosphorylation. The energy-based approach used here forms the basis of an alternative efficiency analysis of biomolecular systems and this is the subject of current research (Gawthrop et al., 2015b).

Although not discussed here, the bond graph approach leads to dynamic models which can be used to generate time-course data via simulation. Moreover, stability issues can be considered in this context (Gawthrop and Crampin, 2016). This is the subject of current research. Although not discussed here, spatial variation issues are of interest. Externally, mitochondria change their shape, size and clustering configuration (Jarosz et al., 2016) and, according to the mechano-chemiosmotic model, they change their shape internally (Kasumov et al., 2015). It would be interesting to include spatial effects within the bond graph formulation of this paper.

In addition to the oxidative phosphorylation model of Figure 10, a model of mitochondrial metabolism would include glycolysis, the conversion of pyruvate to acetyl coenzyme A, and the citric acid cycle (Alberts et al., 2015; Berg et al., 2012). The modular energy-based approach of this paper will be extended to more complete model making use of the pre-existing modular
bond graph model of glycolysis (Gawthrop et al., 2015a).

Because mitochondria are critical to life, mitochondrial dysfunction is hypothesised to be the source of ageing (Alberts et al., 2015; Wellstead, 2012), neuro-degenerative diseases (Cloutier et al., 2012; Drion et al., 2012; Francis et al., 2012; Le Masson et al., 2014; Poliquin et al., 2013; Wellstead, 2012; Wellstead and Cloutier, 2012), cancer (Gogvadze et al., 2008; Marin-Hernandez et al., 2014; Solaini et al., 2011) and other diseases (Nunnari and Suomalainen, 2012; Wallace, 2005). Although mathematical models of mitochondria exist already (Bazil et al., 2016; Cortassa and Aon, 2014; Vinnakota et al., 2016; Wu et al., 2007), it is hoped that the engineering-inspired bond graph approach of this paper will shed further light on the function and dysfunction of mitochondria. This is the subject of current research.

The equations describing the examples are worked out in some detail in the paper; however, the results can also be automatically generated from the system bond graphs. To illustrate this, a Virtual Reference Environment (Hurley et al., 2014) is available for this paper at doi:10.5281/zenodo.166046. This contains a ISO image of the software, bootable by a virtual machine, which not only generates all figures in the paper but also automatically generates information about the systems and modules discussed in the paper.

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