Supplementary material for "Energy-based Analysis of Biochemical Cycles using Bond Graphs"

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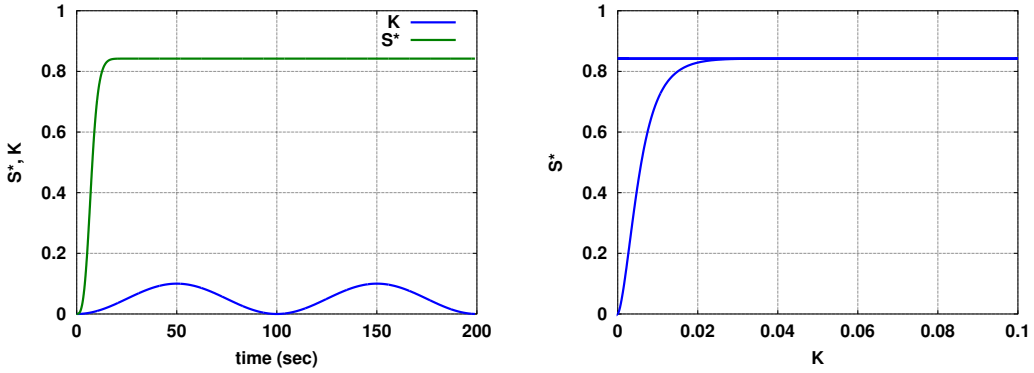
A A biochemical switch : further simulations



(a) $S^\star$ & K

(b) $S^\star$ v. K

(c) v_a – ATP flow

(d) ATP & S

Figure 1: A Biochemical Switch: simulation without ATP replenishment. The simulation is the same as that of Figure 7 of §5(b) of the paper except that ATP is not replenished and so the switch fails to function.

B Bimolecular reactions

Thermodynamic cycles typically involve bimolecular reactions where, in bond graph terms, the chain of reactions discussed in Section 4 is augmented by branches. Figure 2(a) shows a reaction of the form

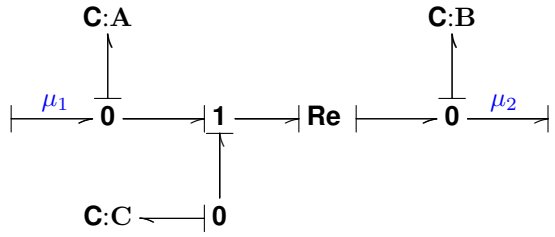
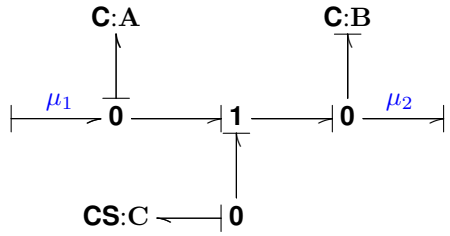
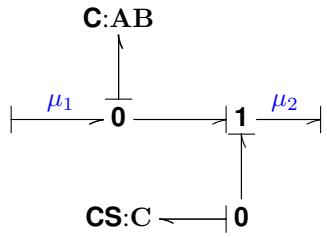
Name	Bond Graph
(a) Bimolecular reaction	
(b) Fast bimolecular reaction	
(c) Simplified bimolecular reaction	

Figure 2: Approximation of bimolecular reactions.

$A + C \rightleftharpoons B$ where the presence of C gives the branched bond graph structure. The bonds at the left and right of Figure 2(a) form connections to the rest of a reaction network.

There are two approximations made to simplify this bimolecular reaction. As in Section 4 it is assumed that the reaction is fast and thus the **Re** component can be removed as in Figure 2(b). It is further assumed that the concentration of C is approximately constant either due to replenishment or to a large pool; this is indicated in Figure 2(b) by replacing the component **C**:C by **CS**:C: a capacitive source. As in Section 4, the removal of the **Re** component changes the causality of **C**:B, but the causality of **CS**:C remains the same as that of **C**:C.

Again, the equilibrium induced by the removal of the **Re** component leads to the state x_b of **C**:B being determined by the states of the other two components:

$$\begin{aligned} x_b &= \frac{K_c x_c K_a x_a}{K_b} \\ &= \tilde{x}_c x_a \qquad (1) \\ \text{where } \tilde{x}_c &= \frac{K_c K_a}{K_b} x_c \qquad (2) \\ \text{thus } x_{ab} &= x_a + x_b = (1 + \tilde{x}_c) x_a \qquad (3) \end{aligned}$$

Unlike the unimolecular case, the chemical potentials μ_1 and μ_2 are different; in particular

$$\mu_2 = \mu_1 + RT \ln K_c x_c \qquad (4)$$

Comparing Figures 2(b) and 2(c):

$$\mu_1 = RT \ln K_{ab} x_{ab} = RT \ln K_a x_a \qquad (5)$$

It follows from Equation (3) that:

$$K_{ab} = K_a \frac{x_a}{x_{ab}} = \frac{K_a}{1 + \tilde{x}_c} \qquad (6)$$

Using Equations (4), (5) and (6) it follows that:

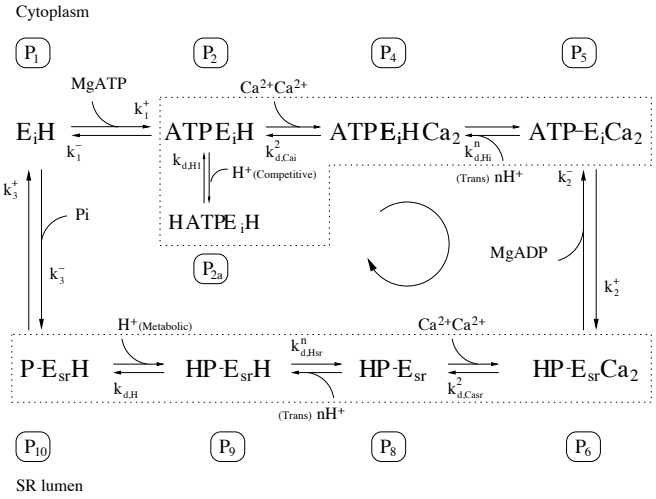
$$\begin{aligned} \mu_2 &= RT \ln K_{ab} x_{ab} + RT \ln K_c x_c \\ &= RT \ln K_{ab} x_{ab} K_c x_c \\ &= RT \ln K_{ab} x_{ab} \frac{K_b}{K_a} \tilde{x}_c \\ &= RT \ln K_{ba} x_{ab} \qquad (7) \\ \text{where} K_{ba} &= \frac{K_b \tilde{x}_c}{1 + \tilde{x}_c} \qquad (8) \end{aligned}$$

Equations (6) and (7) correspond to the equations for α_4^+ and α_3^- in the paper of Smith and Crampin (2004, Equations (30) & (31)).

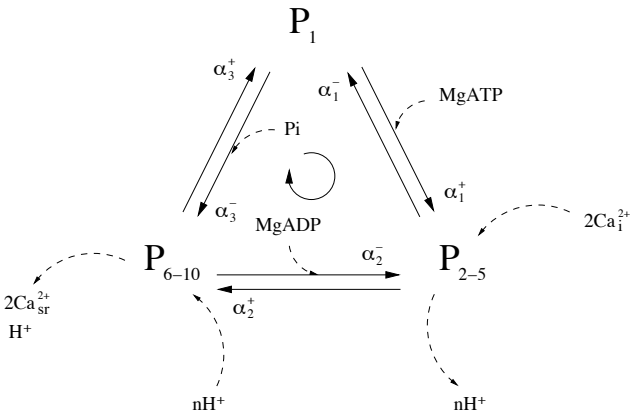
These simplification approaches for slow-fast reactions can naturally be applied to more complicated reaction schemes by application in a stepwise manner, each step of which preserves the underlying thermodynamic structure of the model. This can be automated, and can be used to generate different representations of an underlying model, as for example was done in our recent model of the cardiac sarcoplasmic/endoplasmic Ca^{2+} (SERCA) pump. Such models consider enzyme mechanisms to be thermodynamic cycles. These are discussed below.

C Example: model reduction of an enzymatic cycle model of the SERCA pump

Tran et al. (2009) present a thermodynamic enzyme cycle model of the cardiac sarcoplasmic/endoplasmic Ca^{2+} ATPase (SERCA) pump. A multi-state model is constructed which incorporates binding of different

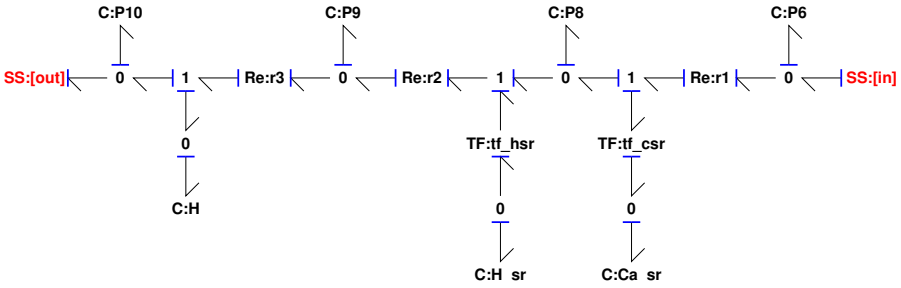


(a) 9-state (8-state cycle) model

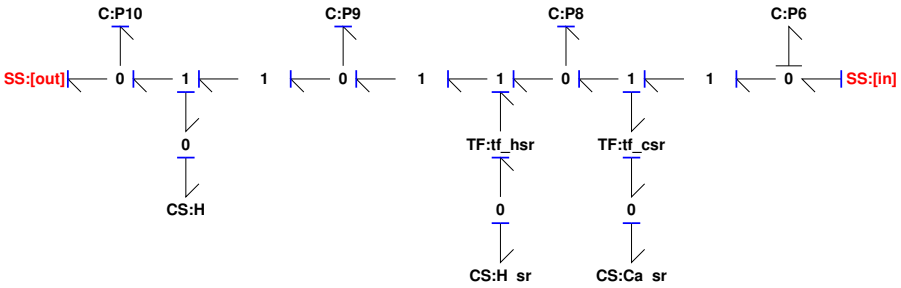


(b) 3-state reduced enzymatic cycle model

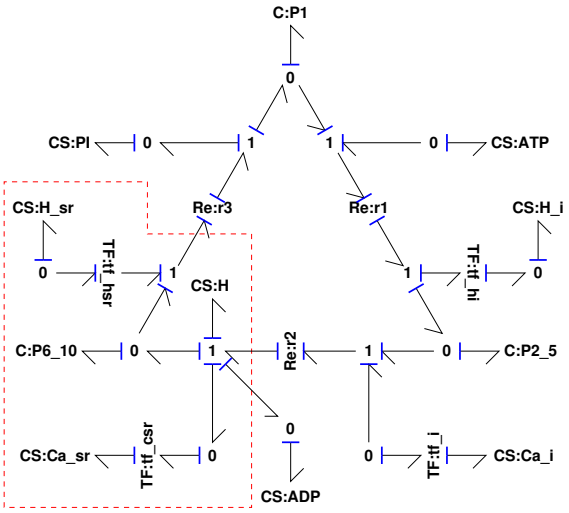
Figure 3: Schematic of SERCA pump model. (a) for the cardiac SERCA pump (where calcium binding mechanism is assumed to be fully cooperative); and (b) reduced 3-state model, modified from Tran et al. (2009). The dotted boxes in (a) show partial sub-systems of the model which are simplified to reduce the 9-state to the 3-state model.



(a) Bond graph of partial system



(b) Bond of simplified partial system



(c) Bond graph of simplified pump

Figure 4: Bond Graph of Partial SERCA pump model. (a) Shows the bond graph corresponding to the subsystem with states P_6–P_{10} of Figure 3(a) and the seven **C** components in integral causality correspond to the seven states. (b) The simplified subsystem has one **C** component in integral causality and thus only one state. (c) This subsystem is shown within the dashed box as part of the overall simplified model. **C:P6_10** represents the composite state of the subsystem and the **CS** components the corresponding constant concentrations.

molecular species to the SERCA protein, including transported calcium ions, co-transported and competitively binding hydrogen ions, ATP and its hydrolysis products ADP, Pi and hydrogen ion, and which represents conformational changes of the protein in the enzymatic cycle, and associated free energy transduction. This generates a thermodynamically constrained enzyme cycle model for SERCA, however the model has a large number of reaction steps and associated parameters, and, using methods akin to those in Section 4, the model is reduced from the nine-state, nine-reaction model of Figure 3(a) to the three-state, three-reaction model of Figure 3(b) by simplifying the reaction mechanism corresponding to states P_2–P_5 and to states P_6–P_{10} by assuming rapid equilibrium for calcium and hydrogen ion association-dissociation reactions.

To illustrate the bond graph equivalent of this procedure, Figure 4(a) gives the reaction mechanism corresponding to states P_6–P_{10}. Using the approach of Section B, this is reduced to the bond graph of Figure 4(b). The bond graph corresponding to Figure 3(b) is given in Figure 4(c) where the dotted line delineates the approximation to reaction mechanism corresponding to states P_6–P_{10}.

The three reaction components **Re**:$\mathbf{r1}$–**Re**:$\mathbf{r3}$ correspond to flows:

$$v_1 = \alpha_1^+ x_1 - \alpha_1^- x_2 \tag{9}$$

$$v_2 = \alpha_2^+ x_2 - \alpha_2^- x_3 \tag{10}$$

$$v_3 = \alpha_3^+ x_3 - \alpha_3^- x_1 \tag{11}$$

where x_1, x_2 and x_3 are the state occupancy probabilities of states **C**:$\mathbf{P_1}$, **C**:$\mathbf{P_{2-5}}$ and **C**:$\mathbf{P_{6-10}}$. Using methods akin to those of Section B, Tran et al. (2009) show that:

$$\alpha_1^+ = k_1^+ [\mathrm{MgATP}], \tag{12}$$

$$\alpha_2^+ = \frac{k_2^+ \widetilde{\mathrm{Ca}}_\mathrm{i}^{\,2}}{\widetilde{\mathrm{Ca}}_\mathrm{i}^{\,2}(1+\widetilde{\mathrm{H}}_\mathrm{i}^{\,\mathrm{n}}) + \widetilde{\mathrm{H}}_\mathrm{i}^{\,\mathrm{n}}(1+\widetilde{\mathrm{H}}_1)}, \tag{13}$$

$$\alpha_3^+ = \frac{k_3^+ \widetilde{\mathrm{H}}_\mathrm{sr}^{\,\mathrm{n}}}{\widetilde{\mathrm{H}}(1+\widetilde{\mathrm{Ca}}_\mathrm{sr}^{\,2}) + \widetilde{\mathrm{H}}_\mathrm{sr}^{\,\mathrm{n}}(1+\widetilde{\mathrm{H}})} \tag{14}$$

and the apparent backward rate constants are:

$$\alpha_1^- = \frac{k_1^- \widetilde{\mathrm{H}}_\mathrm{i}^{\,\mathrm{n}}}{\widetilde{\mathrm{Ca}}_\mathrm{i}^{\,2}(1+\widetilde{\mathrm{H}}_\mathrm{i}^{\,\mathrm{n}}) + \widetilde{\mathrm{H}}_\mathrm{i}^{\,\mathrm{n}}(1+\widetilde{\mathrm{H}}_1)}, \tag{15}$$

$$\alpha_2^- = \frac{k_2^- [\mathrm{MgADP}] \widetilde{\mathrm{Ca}}_\mathrm{sr}^{\,2} \widetilde{\mathrm{H}}_\mathrm{sr}^{\,\mathrm{n}}}{\widetilde{\mathrm{H}}(1+\widetilde{\mathrm{Ca}}_\mathrm{sr}^{\,2}) + \widetilde{\mathrm{H}}_\mathrm{sr}^{\,\mathrm{n}}(1+\widetilde{\mathrm{H}})}, \tag{16}$$

$$\alpha_3^- = k_3^- [\mathrm{Pi}] \tag{17}$$

where

$$\widetilde{\mathrm{Ca}}_\mathrm{i} = \frac{[\mathrm{Ca}^{2+}]_\mathrm{i}}{K_{d,Cai}}, \quad \widetilde{\mathrm{H}}_\mathrm{i} = \frac{[\mathrm{H}^+]}{K_{d,Hi}}, \quad \widetilde{\mathrm{H}}_1 = \frac{[\mathrm{H}^+]}{K_{d,H1}},$$

$$\widetilde{\mathrm{Ca}}_\mathrm{sr} = \frac{[\mathrm{Ca}^{2+}]_\mathrm{sr}}{K_{d,Casr}}, \quad \widetilde{\mathrm{H}}_\mathrm{sr} = \frac{[\mathrm{H}^+]}{K_{d,Hsr}}, \quad \widetilde{\mathrm{H}} = \frac{[\mathrm{H}^+]}{K_{d,H}}$$

As discussed further below, the purpose of this model reduction is not to reduce dynamical complexity but rather to reduce the number of unknown parameters to a value consistent with available experimental data. As demonstrated here, and discussed by Tran et al. (2009), this approach retains the thermodynamic properties of the full enzyme cycle model while reducing the number of unknown parameters.

The major advantage of this approach is that in constructing models such as this we usually do not know, a priori, the full set of parameters associated with the enzymatic cycle. A subset of the parameters, such as the free energy of hydrolysis of ATP, are known; and these values carry through to the reduced model. However, the majority of parameters (binding and unbinding rates, which are reduced to dissociation constants in the rapid equilibrium approximation) are typically not known and must be estimated

by fitting the resulting model to data (namely the steady state cycling rate of the model, as a function of concentrations of the different species, fitted to the data where rate of calcium transport is measured for different concentrations of calcium, pH and metabolites). This parameter estimation process is made significantly more tractable following reduction of the model to the simpler cycle, without compromising the thermodynamic properties and the prior knowledge incorporated in the full multi-state construction.

References

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Kenneth Tran, Nicolas P. Smith, Denis S. Loiselle, and Edmund J. Crampin. A thermodynamic model of the cardiac sarcoplasmic/endoplasmic Ca2+ (SERCA) pump. *Biophysical Journal*, 96(5):2029 – 2042, 2009. ISSN 0006-3495. doi: 10.1016/j.bpj.2008.11.045.