Energy-based Feedback Control of Biomolecular Systems with Cyclic Flow Modulation

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Abstract

Energy-based modelling brings engineering insight to the understanding of biomolecular systems. It is shown how well-established control engineering concepts, such as loop-gain, arise from energy feedback loops and are therefore amenable to control engineering insight. The approach is illustrated using a class of metabolic cycles with activation and inhibition leading the concept of Cyclic Flow Modulation.

1 Introduction

The bond graph implementation of Network Thermodynamics was introduced some 50 years ago as an energy-based approach to modelling biomolecular systems [1]. “Graphical representations similar to engineering circuit diagrams can be constructed for thermodynamic systems. ... such diagrams do increase one’s intuition about system behaviour.”[2].

The design of linear feedback circuits also has a long history and the correspondingly well-established theory of control systems [3] has been applied to biomolecular systems [4–6] and has led to a number of control concepts such as feedback and integral action being used in the biomolecular context [5].

Classical linear control theory is based on transfer function models of dynamical systems. In contrast, the energy-based approach of this paper uses the bond graph paradigm for modelling

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biomolecular systems. There has been limited work on the bond graph approach to control [7–12]. For this reason, a novel method is introduced to allow the transfer function based approach of classical linear control to be utilised in the analysis of feedback systems modelled by bond graphs and thus combine energy-based modelling with control systems analysis.

As discussed by Gawthrop and Crampin [13], the bond graph approach gives the set of non-linear ordinary differential equations describing the biomolecular system being modelled. Linearisation of non-linear systems is a standard technique in control engineering. Linearisation in the context of bond graph models of biomolecular systems was introduced by Gawthrop and Crampin [13] and is used here.

The role of metabolic cycles in the regulation of metabolic flux is well established [14–17]. Such cycles are involved in a number of substrate conversions including those between fructose-6-phosphate and fructose-1,6-biphosphate, fructose-6-phosphate and fructose-2,6-biphosphate, triglyceride/fatty acid, glucose and glucose-6-phosphate, and glycogen and glucose 1-phosphate [14, 16, 17]. To illustrate the fusion of network thermodynamics and control theory, this paper will focus on the first two inter-conversions involving fructose-6-phosphate (F$_6$P). Because of the cyclic nature of these two reactions, and the fact that flow is modulated, the term Cyclic Flow Modulation (CFM) is used to describe such reaction systems.

The use of CFM requires energy and there is a trade-off between quality of control and energy consumed [15]. It is therefore important to account for energy flows when modelling biomolecular systems and this is done here using the fusion of the network thermodynamics paradigm, as implemented using bond graphs, with control theory.

Criteria for robust biochemical reaction networks have been established which ensure zero steady-state error [18–20]; but these papers make no mention of energy and therefore entirely ignore thermodynamic constraints and the consequent performance-energy trade-off.

Building complex systems is simplified using modularity; but it is essential to distinguish two different concepts of modularity: computational modularity where physical correctness is retained and behavioural modularity where module behaviour (such as ultra-sensitivity) is retained [13]. As well as providing computational modularity, bond graphs provide a natural formulation of behavioural modularity and reveal the sources of retroactivity [13]. Chemostats [13, 21] are used to create an open system from a closed system and also provide a convenient way of providing ports to connect bond graph modules.

§ 2 introduces the bond graph based approach to the analysis of feedback control systems using an enzyme catalysed reaction with competitive inhibition as an illustrative example. § 3 shows how cyclic flow modulation (CFM) can be used to build effective feedback controllers with approximate integral action. § 4 concludes the paper and gives directions for future work.

## 2 Bond graph based control analysis

Figure 1(a) depicts a conventional feedback control system in transfer-function form. The four transfer functions $G_{con}(s)$, $G_{sys}(s)$, $G_{w}(s)$ and $G_{g}(s)$ represent the controller, the system uni-

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1 The pejorative term “futile cycle” is often used to describe such cycles; this will be avoided in this paper.
Figure 1: Feedback control. (a) A classical feedback loop block diagram representing the linearisation of a non-linear system. The blocks represent transfer functions which are connected by signals. $y$ is the controlled output, $d$ a disturbance and $w$ the setpoint, or desired value of $y$. (b) A bond graph feedback loop. CON and SYS are bond graph modules with ports denoted by []. Ce:P, Ce:D and Ce:P0 are bond graph components representing species corresponding to product, disturbance and reference species respectively. The $\rightarrow$ symbol indicates an energetic connection between two subsystems; the half-arrow indicates the direction corresponding to positive energy flow.
under control, the setpoint and disturbance transfer functions respectively where $s$ is the Laplace variable. The four signals $y$, $u$, $w$ and $d$ represent the system output, system input, setpoint and disturbance respectively.

The closed-loop transfer function is:

$$y = \frac{L(s)}{1 + L(s)} G_w(s)w + \frac{1}{1 + L(s)} G_d(s)d$$

(1)

where $L(s) = G_{con}(s)G_{sys}(s)$

(2)

$L(s)$ is referred to as the feedback loop gain. In the engineering context, $G_{con}(s)$ and $G_{sys}(s)$ would arise from separate physical entities; nevertheless, the loop gain $L(s)$ (2) appearing in equation (1) only requires the product of $G_{con}(s)$ and $G_{sys}(s)$. This is important for biomolecular systems where there is no clear physical distinction between controller and system: it is the feedback loop itself that is of fundamental importance.

Typically, such control systems are analysed in the frequency domain by setting $s = j\omega$ where $j = \sqrt{-1}$ and $\omega$ is frequency in rad/sec. At those frequencies where $L(j\omega)$ is large, equation (1) can be approximated by $y \approx G_w(s)w$. In other words, a large loop gain $L(j\omega)$ is desirable insofar as the system output $y$ is a close match to the desired value $G_w(s)w$ despite disturbances represented by $d$. However, incorrect choice of the the loop gain $L(s)$ can lead to instability and $L(s)$ is, moreover, subject to fundamental constraints [3].

To summarise, there are two potentially conflicting issues in controller design: good disturbance rejection and stability; these are both captured in the loop gain $L(s)$.

Figure 1(a) implicitly assumes that the connection between subsystems, such as those represented by $G_{con}(s)$ and $G_{sys}(s)$ is one-way as indicated by the arrows. However, the physical controller needs to be designed to make sure this one-way interaction is correct; this requires the use of energy. It has been argued that this approach is misguided, even in the context of engineering systems. This has lead to the concept of physical-model based control [7–12].

In the context of biomolecular systems, the concept of retroactivity [6] has been introduced to explain why interaction is not one-way and thus design based on simplistic application of the approach of Figure 1(a) often fails.

There are two reasons why the bond graph approach is superior to the transfer function approach of Figure 1(a) in the context of feedback control:

1. It explicitly accounts for the two-way interaction found in physical systems in general and biomolecular systems in particular.

2. It explicitly accounts for energy flows and thus can directly expose performance/energy consumption trade-offs.

For this reason, the transfer function paradigm of Figure 1(a) is replaced by the bond graph based paradigm of Figure 1(b).

Figure 1(b) is based on the notation for modular bond graphs [22]. The two bond graph modules are CON and SYS; CON represents the controller and has three ports: [Act] (activation), [Inh] (inhibition) and [Con] (control signal) and SYS represents the system and has two ports:
[S] (substrate) and [P] product. In the sequel, the controller module will be instantiated by three modules in turn: an enzyme catalysed reaction with competitive activation and inhibition (§ 2.2), cyclic flow modulation (§ 3) and cyclic flow modulation with integral action (§ 3.1).

The → symbol indicates an energetic connection between two subsystems; the half-arrow indicates the direction corresponding to positive energy flow. In the biomolecular context, each such bond is associated with two covariables: chemical potential $\mu$ J mol$^{-1}$ and flow $v$ mol/sec. The key point is that the product of $\mu$ and $v$ is power $p = \mu v$ W. Alternatively, it is possible to scale these co-variable by Faraday’s constant $F$ C mol$^{-1}$ to give $\phi = \frac{1}{F} \mu V$ and $f = Fv A$ where J C$^{-1}$ has been replaced by the more convenient unit V and C/ sec has been replaced by the more convenient unit A [23]. The components Ce:P and Ce:S represent the product and substrate species respectively and the components Ce:P0 and Ce:D represent the reference species and product disturbance respectively; because Ce:P0 and Ce:D represent exogenous variables, they are chemostats [21, 13].

As shown in the sequel, the bond graph modelling approach can make use of the transfer function approach to understand the dynamic properties of feedback systems of the form of Figure 1(b). In particular, as shown in § 2.3, the fundamental control systems concept of loop-gain can be retrieved from the bond graph modelling paradigm. But first, linearisation must be considered.

2.1 Linearisation

Biomolecular systems are nonlinear and must be linearised before applying transfer function techniques. Linearisation of biomolecular systems in a biomolecular context, together with a discussion on retroactivity, is given by [13]. In particular, the non-linear system equations are:

$$\frac{d}{dt}x = N f$$

$$f = F(x, x_{ch})$$ (3)

In systems biology terms: the $n_x$ vector $x$ represents the amount of each non-chemostatted species (mol), the $n_x \times n_f$ matrix $N$ is the system stoichiometric matrix, the $n_f$ vector $f$ represents the flow in each reaction (mol s$^{-1}$). The $n_{x_{ch}}$ vector $x_{ch}$ represents the amount of each chemostatted species (mol). $F(x, x_{ch})$ is a nonlinear function of both arguments. Because of thermodynamic constraints, $F$ has a particular structure dependent on the stoichiometric matrix $N$ [24, 25] and is automatically generated from the bond graph representation. In standard control system terms, $x$ is the system state, $f$ is the system output and $x_{ch}$ the system input.

The corresponding linearised equations are:

$$\frac{d}{dt}\tilde{x} = N \tilde{f}$$ (4)

$$\tilde{f} = C\tilde{x} + D\tilde{x}_{ch}$$ (5)

where $\tilde{x} = x - \bar{x}$

$$\tilde{x}_{ch} = x_{ch} - \bar{x}_{ch}$$ (7)

$$\tilde{f} = f - \bar{f}$$ (8)

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where the $n_f \times n_x$ matrix $C$ and the $n_f \times n_{x_{ch}}$ matrix $D$ are given by the partial derivatives:

\begin{align*}
C &= \frac{\partial f}{\partial x} \\
D &= \frac{\partial f}{\partial x_{ch}}
\end{align*}

(9)

evaluated at the steady-state values $\bar{x}$ and $\bar{f}$ of state and flow respectively corresponding to the constant chemostat state $x_{ch} = \bar{x}_{ch}$:

\begin{align*}
N\bar{f} &= 0 \\
\bar{f} &= F(\bar{x}, \bar{x}_{ch})
\end{align*}

(10)

Linearisation has two steps: finding the steady-state state $\bar{x}$ and flow $\bar{f}$ and then computing the linearisation matrices $C$ and $D$. The first is simply accomplished by numerically simulating the system until a steady-state is reached ($\frac{dx}{dt} \approx 0$). The second is achieved symbolically within BondGraphTools (https://pypi.org/project/BondGraphTools) using the symbolic derivative functions of the sympy library (https://www.sympy.org). The Python Control Systems Library (https://pypi.org/project/control/) is used to convert the linearised system from state-space form to transfer function form, manipulate transfer functions and to generate time and frequency responses.

2.2 Example: Enzyme-catalysed reaction

The modified enzyme-catalysed reaction module of Figure 2(b) and the pathway module of Figure 2(c) are embedded in the feedback loop of Figure 1(b) and used for the purposes of illustration; the parameters are given in Figure 3.

The non-linear system equations were derived from the modular bond graph of Figure 1(b) using BondGraphTools and simulated to give the steady-state condition corresponding to the parameters of Figure 3. The linearised equations were then extracted and the transfer function relating the disturbance $\bar{x}_D$ to the product $\bar{x}_P$ generated. The corresponding closed-loop step response appears in Figure 3.

However, simulation does not provide an explanation of why the steady-state is the particular value shown nor why the dynamics are as shown. The explanation is provided by the analysis of the following section.

2.3 Open-loop analysis

As discussed above, the loop-gain $L(s)$ is a key transfer function in the classical control systems analysis of Figure 1(a). This section indicates how the loop-gain $L(s)$ can be derived from the bond graph of Figure 1(b).

The closed-loop system of Figure 1(b) includes two chemostats Ce:P0 and Ce:D which make the corresponding states $x_{P0}$ and $x_D$ independent variables; the product state $x_P$ remains a dependent variable which evolves with time as in Figure 3. To create an open loop system, the component Ce:P representing the product is also made a chemostat thus making $x_P$ an independent variable.
Figure 2: Enzyme-catalysed reaction (ECR). The Bond Graph notation is: $\rightarrow$ energy connection; $\text{Ce}$ species; $\text{Re}$ reaction; 0 common potential connection; 1 common flow connection [26]. (a) Enzyme-catalysed reaction with competitive activation and inhibition. $\text{C}:A$, $\text{C}:B$, $\text{C}:E$, $\text{C}:C$, $\text{C}:\text{Act} \& \text{C}:\text{Inh}$ represent the substrate, product, enzyme, enzyme-substrate complex, activation and inhibition respectively. $\text{C}:F \& \text{C}:G$, provide the driving energy. Example species appear in Figure 7. (b) A simple model of cooperativity is included by specifying that 4 activation and 4 inhibition species interact with the enzyme; this is achieved in a modular fashion. (c) The controlled system of the module $\text{Path:sys}$ of Figure 4 is, in this example, a simple path of 3 reactions represented by $\text{Re}:r1$–$\text{Re}:r3$ with intermediate species $\text{C}:I1$ and $\text{C}:I2$. 
Figure 3: Non-linear and linearised closed-loop step response. The asymptotic value \( g_D \) is indicated by a dashed line. The amplitude of the disturbance step is given for the non-linear simulations and the resultant response is divided by the step amplitude. The normalised nonlinear response is close to the linear case for an amplitude of 0.1 and differs slightly for an amplitude of 1.0. For the purposes of illustration, all parameters are unity except \( K_F = 10^3, K_G = 10^{-3}, \kappa_{con} = 2 \) and \( \kappa_{sys} = 10 \). The steady-state values were \( x_S = 12.85 \) and \( x_P = 10.12 \).

Figure 4: Loop analysis. (a) The block diagram corresponding to opening the bond graph feedback loop by setting the product \( CeP \) to be a chemostat. \( x_P \) is the amount of product and \( v_P \) the product flow. \( G_P(s), G_D(s) \) and \( G_{pP_0}(s) \) are transfer functions relating \( x_P, x_D \) and \( x_{P0} \) to \( f_P \). (b) The transfer function \( G_P(s) \) is split into two terms: \( G_{act}(s) \) and \( G_{pas}(s) \) corresponding to active and passive feedback.
The linearised flow $\tilde{f}_P$ into the chemostat $\text{Ce:P}$ is given by the sum of three terms corresponding to the three chemostats $\text{Ce:P}$, $\text{Ce:P0}$ and $\text{Ce:D}$ respectively:

$$\tilde{f}_P = -G_P(s)\tilde{x}_P + G_{P0}(s)\tilde{x}_{P0} + G_D(s)\tilde{x}_D$$  \hspace{1cm} (11)$$

where $-G_P$, $G_{P0}$ and $G_D$ are the transfer functions relating $\tilde{f}_P$ to $\tilde{x}_P$, $\tilde{x}_{P0}$ and $\tilde{x}_D$ respectively. The minus sign associated with $G_P$ is to give compatibility with standard definitions of loop gain in a negative feedback context.

To reclose the loop, $\text{Ce:P}$ is restored to non-chemostatted dynamics using the transfer function relating $\tilde{x}_P$ to $\tilde{f}_P$:

$$\tilde{x}_P = \frac{1}{s} \tilde{f}_P$$  \hspace{1cm} (12)$$

The block diagram corresponding to Equations (11) and (12) is shown in Figure 4(a). Using Equation (2), the loop gain $L(s)$ is given by:

$$L(s) = \frac{G_p(s)}{s}$$  \hspace{1cm} (13)$$

From the block diagram of Figure 4(a), or from Equations (11) and (12), the closed-loop system can be explicitly written as

$$\tilde{x}_P = \frac{1}{s + G_P(s)} [G_{P0}(s)\tilde{x}_{P0} + G_D(s)\tilde{x}_D]$$  \hspace{1cm} (14)$$

The steady state value $\bar{\tilde{x}}_P$ of $\tilde{x}_P$ is obtained by setting $s = 0$ to give

$$\bar{\tilde{x}}_P = \frac{1}{G_P(0)} [G_{P0}(0)\bar{\tilde{x}}_{P0} + G_D(0)\bar{\tilde{x}}_D]$$  \hspace{1cm} (15)$$

In particular, the steady-state disturbance gain $g_D$ is given by:

$$g_D = \frac{\bar{\tilde{x}}_P}{\bar{\tilde{x}}_D} = \frac{G_D(0)}{G_P(0)}$$  \hspace{1cm} (16)$$

With the parameters given in Figure 3:

$$G_D(0) = 1.00$$  \hspace{1cm} (17)$$

$$G_P(0) = 4.44$$  \hspace{1cm} (18)$$

hence $g_D = 0.23$  \hspace{1cm} (19)$$

This corresponds to Figure 3.
2.4 Split Loop analysis

The previous section shows how the loop gain $L(s)$ may be derived from the closed-loop system in the bond graph form of Figure 1(b). This section expands this analysis by dividing the loop gain $L(s)$ into two parts: an active part $L_{\text{act}}(s)$ and a passive part $L_{\text{pas}}(s)$ so that

$$L(s) = L_{\text{act}}(s) + L_{\text{pas}}(s) \quad (20)$$

The active part arises mainly from the properties of the controller (CON); the passive part arises mainly from the properties of the system (SYS) appearing in the closed-loop bond graph of Figure 1(b).

The split-loop procedure is based on removing the feedback bond linking the controlled product $\text{Ce:P}$ to the inhibition port ([Inh]) of the controller. This is depicted in Figure 5 where the bond has been removed and the chemostat $\text{Ce:Inh}$ has been added. To focus on the loop gain, the chemostats $\text{Ce:P0}$ and $\text{Ce:D}$ are held at the steady state values ($\bar{x}_D = \bar{x}_{P0} = 0$) for the rest of this section. The linearised flows $\tilde{f}_{PP}$ into the chemostat $\text{Ce:P}$ and $\tilde{f}_{II}$ into the chemostat $\text{Ce:I}$ are each given by the sum of two terms corresponding to the two variable chemostats $\text{Ce:Inh}$ and $\text{Ce:P}$ respectively:

$$\tilde{f}_{PP} = -G_{PI}(s)\bar{x}_{Inh} - G_{PP}(s)\bar{x}_P \quad (21)$$
$$\tilde{f}_{II} = -G_{II}(s)\bar{x}_{Inh} - G_{IP}(s)\bar{x}_P \quad (22)$$

When the split-loop is reconnected

$$\bar{x}_{Inh} = \bar{x}_P \quad (23)$$
$$\bar{f}_P = \tilde{f}_{PP} + \tilde{f}_{II} \quad (24)$$
$$= -[G_{PI}(s) + G_{PP}(s) + G_{II}(s) + G_{IP}(s)]\bar{x}_P \quad (25)$$

$G_{PI}(s)$ is the transfer function from the inhibition port of the controller to the product and is thus
the active part of the control. Hence the previous equation is rewritten as:

\[ \hat{f}_P = - [G_{act}(s) + G_{pas}(s)] \hat{x}_{P} \]  

(26)

where \( G_{act}(s) = G_{PI}(s) \)  

(27)

\( G_{pas}(s) = G_{PP}(s) + G_{II}(s) + G_{IP}(s) \)  

(28)

Once again, the minus signs associated with \( G_{act} \) and \( G_{pas} \) are to give compatibility with standard definitions of loop gain in a negative feedback context.

To allow comparison with Equation (11), the transfer functions appearing Equation (21) are evaluated with the same steady states as those of the closed-loop system and, in addition, reconnection of the split loop implies

\[ \hat{x}_{inh} = \hat{x}_{P} \quad \hat{x}_{inh} = \hat{x}_{P} \]  

(29)

Comparing Equations (11 and Equation (21), it follows that:

\[ G_{P} = G_{act} + G_{pas} \]  

(30)

Further, defining

\[ L_{act}(s) = \frac{G_{act}(s)}{s} \quad L_{pas}(s) = \frac{G_{pas}(s)}{s} \]  

(31)

Equation (20) follows from Equation (30). Thus the block-diagram of Figure 4(a) can be expanded to give the block-diagram of Figure 4(b).

The conventional approach to feedback control in the engineering context would regard \( L_{pas} \) as an unwanted artefact to be eliminated by correct design; similarly, in the life-sciences context, \( L_{pas} \) would be regarded as due to retroactivity and therefore undesirable [6]. A theme of this paper is that both these attitudes are incorrect. In the engineering context, using such interactions to improve control are well established as physical-model based control [7–12]. In the systems biology context, this paper will show that \( L_{pas} \) has a stabilising influence on the control system.

The closed-loop system is given in terms of \( G_{P}(s) \) by Equation (14). Because of the decomposition (30), it is possible to see how the control system would, in principle, behave with only passive or only active control. In particular, if \( \hat{x}_{pass} \) and \( \hat{x}_{act} \) are the product concentration deviations in the two cases:

\[ \hat{x}_{pass} = \frac{1}{s + G_{pass}(s)} \left[ G_{P0}(s) \hat{x}_{P0} + G_{D}(s) \hat{x}_{D} \right] \]  

(32)

\[ \hat{x}_{act} = \frac{1}{s + G_{act}(s)} \left[ G_{P0}(s) \hat{x}_{P0} + G_{D}(s) \hat{x}_{D} \right] \]  

(33)

Figure 6(a) shows \( G_{P}(j\omega), G_{pass}(j\omega) \) and \( G_{act}(j\omega) \) plotted on a Bode diagram [3]. The magnitude of \( G_{act}(j\omega) \) is large at low frequencies and small at high frequencies whereas the magnitude of \( G_{pass}(j\omega) \) is small at low frequencies and high at high frequencies. Hence the \( G_{P}(j\omega) \) is close to \( G_{pass}(j\omega) \) at high frequencies yet retains the high gain at low frequencies due to \( G_{act}(j\omega) \).
Figure 6: ECR: Split-loop analysis. (a) Components of $G_P(s)$. $G_{PI} \approx 0$ and is not shown. Of the two remaining components of $G_{pas}$, $G_{II}$ is small at low frequencies and so $G_{pas} \approx G_{PP}$ at low frequencies; at mid and high frequencies, $G_{pas}$ provides phase advance. $G_{act}$ is large at low frequencies and small at high frequencies. Thus $G_{P}(j\omega) \approx G_{act}(j\omega) + G_{PP}(j\omega)$ at low frequencies and $G_{P}(j\omega) \approx G_{pas}(j\omega)$ at high frequency. Thus $G_{act}(j\omega)$ provides high gain (and thus low steady-state error) at low frequencies and $G_{pass}(j\omega)$ provides stabilising phase advance at mid frequencies. (b) Open-loop frequency response. The loop gain $L(j\omega) = \frac{G_{P}(j\omega)}{s}$ and its two components $L_{act}(j\omega)$ and $L_{pas}(j\omega)$ are plotted on a Nyquist diagram. $L_{act}(j\omega)$ passes close to the $-1$ point and thus, without the term $L_{pas}(j\omega)$, $L(j\omega)$ would correspond to closed-loop system close to instability. However, at the relevant frequencies, $L(j\omega) \approx L_{pass}(j\omega)$ and is well away from the $-1$ point and thus corresponds to a stable closed-loop system. (c) Closed-loop disturbance step response. The hypothetical closed-loop responses corresponding to $L_{pass}(j\omega)$ and $L_{act}(j\omega)$ are well damped with large steady-state error and oscillatory with small steady-state error respectively. The actual response corresponding to $L(j\omega)$ combines the best of both.
In classical control theory, the frequency response of the loop gain reveals dynamical properties – including stability – of the closed-loop system. In particular, $s$ is replaced by $j\omega$ where $j = \sqrt{-1}$ and $\omega$ is frequency in rad s$^{-1}$. One such frequency-based approach is based on the Nyquist diagram [3] where the imaginary part of $L(j\omega)$ is plotted against the real part of $L(j\omega)$ for a range of frequencies. The phase $\angle L(j\omega)$ when the modulus $|L(j\omega)| = 1$ is of interest, hence the unit circle is plotted on the Nyquist diagram of Figure 6(b). There are three frequency responses plotted: $L_{\text{pass}}(j\omega)$ shows that, as the frequency response is well away from the $-1$ point, the time response is well-damped; $L_{\text{act}}(j\omega)$ shows that, as the frequency response passes close to the $-1$ point, the time response is oscillatory; $L(j\omega)$ is similar to $L_{\text{pass}}(j\omega)$ near the unit circle and therefore also has a well-damped response.

The corresponding unit step responses appear in Figure 6(c) along with the step response of $\dot{x}_P$ corresponding to Equation (14). The disturbance response of the passive-only system is well-behaved but the steady-state value is large; in contrast, the disturbance response of the active-only system is oscillatory but the steady-state value is small. The overall controller combines the best of both responses: it is well behaved with a small steady-state value. The numerical steady-state values for the overall controller are given in Equation (19); in a similar fashion:

$$g_{\text{pass}} = \frac{1}{G_{\text{pass}}(0)} = 1/1.0 = 1$$

(34)

$$g_{\text{act}} = \frac{1}{G_{\text{act}}(0)} = 1/3.44 = 0.29$$

(35)

Thus the small steady-state value is largely due to the active part of the control.

3 Cyclic flow modulation (CFM)

“The parallel existence of two irreversible reactions is of the greatest importance in metabolic regulation: it means that the direction of flux between two metabolites is determined by differential regulation of the activities of the two enzymes” [16]. A bond graph interpretation of this mechanism appears in Figure 7(a) and this will be used as the basis replacing the CON component in the bond graph feedback loop of Figure 1(b) by a more sophisticated control actuator.

The use of such cyclic flow modulators is motivated by the pair of key metabolic reactions discussed by Cornish-Bowden [16]:

$$F_6P + ATP \underset{\text{PFK}}{\overset{\text{PFK}}{\Rightarrow}} F_{10}P + ADP$$

(37)

$$F_{10}P + H_2O \underset{\text{PPB}}{\overset{\text{PPB}}{\Rightarrow}} F_6P + Pi$$

(38)

This pair of reactions can be related to the CFM bond graph of Figure 7(a) (with reference to Figure 2) as follows. The enzyme corresponding to $\text{ECR:Fwd}$ is PFK (phosphofructokinase) and the enzyme corresponding to $\text{ECR:Rev}$ is FBP (fructose biphosphatase). The substrate corresponding to $\text{Ce:A}$ is $F_6P$ (fructose-6-phosphate) and the product corresponding to $\text{Ce:A}$
Figure 7: Cyclic flow modulation. (a) The two components $\text{ECR}:\text{Fwd}$ and $\text{ECR}:\text{Rev}$ are instances of the modulated ECR of Figure 2(b). The $\text{Ce}:A$ component represents both the substrate of $\text{ECR}:\text{Fwd}$ and the product of $\text{ECR}:\text{Rev}$; the $\text{Ce}:B$ component represents both the substrate of $\text{ECR}:\text{Rev}$ and the product of $\text{ECR}:\text{Fwd}$. Component $\text{Ce}:\text{Act}$ both activates $\text{ECR}:\text{Fwd}$ and inhibits $\text{ECR}:\text{Rev}$; component $\text{Ce}:\text{Inh}$ both inhibits $\text{ECR}:\text{Fwd}$ and activates $\text{ECR}:\text{Rev}$. (b) $\text{CFM}:\text{P}$ gives proportional (P) action whereas $\text{CFM}:\text{I}$ gives integral (I) action by driving the species $\text{Ce}:\text{Int}$ which activates $\text{CFM}:\text{P}$. To exemplify strong activation, three activation bonds are used.
is F\textsubscript{16}P (fructose-1,6-biphosphate). Within ECR:F\textsubscript{wd}, Ce:F corresponds to ATP (Adenosine triphosphate) and Ce:G corresponds to ADP (Adenosine diphosphate); within ECR:Rev, Ce:F corresponds to H\textsubscript{2}O and Ce:G corresponds to Pi (inorganic phosphate).

Species which activate PFK and inhibit FBP include AMP (Adenosine monophosphate) and F\textsubscript{26}P (fructose-2,6-phosphate); species which inhibit PFK and activate FBP include ATP and Cit (citrate).

This section examines the effect of replacing the ECR based control of the feedback loop of Figure 1(b) by a CFM based controller. The ECR module of Figure 2 has four visible chemostats Ce:A, Ce:B, Ce:Act, and Ce:Inh the latter three of which are used as ports ([B], [Act], [Inh]) in the feedback loop of Figure 1(b); the same chemostats (Ce:A, Ce:B, Ce:Act, and Ce:Inh) are visible in the CFM module of Figure 7(a) and can be used as ports in the same way. Thus the CFM module can directly replace the ECR module in the feedback loop of Figure 1(b) (which was analysed in Figure 6); this CFM-based feedback loop is analysed in Figure 8.

The linearised response of the ECR and CFM are similar for the parameters chosen. However, there is a significant difference: the CFM controller is bidirectional, the ECR is not. In both cases, the constant low-frequency gain corresponds to the proportional (P) controller of classical control. In contrast, the next section shows that two CFMs can be combined to give the classical proportional+integral by endowing the controller with integral action.

### 3.1 Integral action

**Integral action** is an important concept in classical control theory [3] and endows a control system with zero steady-state error. In section 3.5 *Integral feedback in energy metabolism: the forgotten side reaction* of their paper Cloutier and Wellstead [5] discuss the role of F\textsubscript{26}P (fructose-2,6-biphosphate), a strong activator of PFK (phosphofructokinase). In particular, F\textsubscript{26}P interconverts with F\textsubscript{6}P (fructose-6-phosphate) via the reaction cycle:

\[
\begin{align*}
F_6P + ATP &\rightleftharpoons_{\text{PFK2}} F_{26}P + ADP \\
F_{26}P + H_2O &\rightleftharpoons_{\text{F26BP}} F_6P + Pi
\end{align*}
\]

catalysed by the enzymes PFK2 (phosphofructokinase-2) and F\textsubscript{26}BP (fructose-2,6-biphosphatase). The species which simultaneously activate PFK2 and inhibit F26BP include AMP and F\textsubscript{6}P. Hence this pair of reactions is a further example of Cyclic Flow Modulation (CFM).

Moreover, the PFK CFM and the PFK2 CFM strongly interact: the PFK CFM is positively modulated by the product of the PFK2 CFM: F\textsubscript{26}P and both are positively modulated by AMP.

Figure 7(b) gives the bond graph abstraction of the two interacting cycles. CFM:P corresponds to the PFK-based CFM giving proportional (P) action whereas CFM:I corresponds to the PFK2-based CFM and, as will be seen, gives integral (I) action. Within each CFM, the interpretation of the species is the same except that the product B of CFM:I corresponds to F\textsubscript{26}P rather than F\textsubscript{16}P. The component Ce:Int corresponds to the product F\textsubscript{26}P which then activates CFM:P. For illustration, and to emphasise the strong activation effect, three bonds represent the activation effect.
Figure 8: CFM: Split-loop analysis. Detailed comments and parameters are given in Figure 6; the low frequency gain is higher leading to a lower steady-state error. Once again, the passive term $G_{pas}$ stabilises the high-gain active control term $G_{act}$. 
Figure 9: CFMI: Split-loop analysis. Detailed comments and parameters are given in Figure 6. Compared to the CFM controller response of Figure 8, the low-frequency gain of the active term $G_{act}$ rises as frequency decreases; this is the behaviour expected of an integrator. However, as the integrator is not perfect, the gain is not infinite at $\omega = 0$. This approximate integral effect gives a lower steady-state error than the CFM controller whilst the passive term $G_{pas}$ continues to act to give a damped response. As the phase of $L_{act}(j\omega_c)$ is below $-180^\circ$ at the critical frequency $\omega_c$ at which magnitude $|L_{act}(j\omega_c)| = 1$, the closed-loop system corresponding to the active part of the controller is unstable.
This section examines replacing the CFM based control within the feedback loop of Figure 1(b) by a CFMI based controller. As in the case of CFM, the same chemostats as ECR are visible in the CFMI module of Figure 7(b) and can be used as ports in the same way. Thus the CFMI module can directly replace the ECR module in the feedback loop of Figure 1(b) analysed in Figure 6; this CFMI-based feedback loop is analysed in Figure 9. Compared to the CFM controller response of Figure 8, the low-frequency gain of the active term \( G_{\text{act}} \) rises as frequency decreases; this is the behaviour expected of an integrator. However, as the integrator is not perfect, the gain is not infinite at \( \omega = 0 \); but this approximate integral effect gives a significantly lower steady-state error than the CFM controller whilst the passive term \( G_{\text{pas}} \) continues to act to give a damped response. The disturbance response of the three controllers in shown in Figure 10; the CFMI controller has a substantially smaller steady-state disturbance error than the other two.

### 3.2 Steady-state values

In the examples so far, the activation chemostat of Figure 1(b) is defined by a unit state \( x_{p0} = 1 \). By analogy with the classical feedback loop of Figure 1(a), it would be expected that \( x_{p0} \) would play a similar role to \( w \). Figure 11(a) indicates that this is approximately true for the CFMI control: \( \bar{x}_P \approx x_{p0} \). Furthermore, varying \( x_{p0} \) changes the steady-state product flow. In this case, as the disturbance reaction gain is \( \kappa_{rd} = 1 \) the product flow \( \bar{f}_P = x_P - x_d = x_P - 1 \). One of the benefits noted for CFM control at the beginning of § 3 is that bidirectional product flow is possible: Figure 11(b) illustrates this for the CFM and CFMI controller; it is not possible for the
Figure 11: Steady-state. (a) The steady-state product state $\bar{x}_P \approx x_{P0}$ for the CFM and CFMI controllers. (b) The steady-state product flow $\bar{f}_P$ is bidirectional in the case of the two CFM-based controllers; this is not possible for the ECR controller.
4 Conclusion

Network thermodynamic modelling via bond graphs has been amalgamated with classical control theory. The dual roles of active and passive feedback have been analysed: active feedback gives good steady state performance whereas passive feedback provides stabilisation.

Cyclic flow modulation (CFM) has been motivated by the phosphofructokinase-fructose biphosphatase reaction metabolic cycle and shown to have a modular bond graph representation. CFM can be used to build the proportional (P) and proportional-integral (PI) controllers of classical control theory, as well as allowing bidirectional flow modulation.

Future work will include building an energy-based model of metabolism with AMP feedback and mitochondrial transduction using existing energy-based models [23, 27].

An important potential result of combining control theory with energy-based modelling is to identify performance/energy trade-offs. This is important to both evolutionary theory [28] and synthetic biology [29].

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