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Multi-scale design in layered synthetic biological systems

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Abstract— **Synthetic biologists rely on mathematical modelling to predict and tune the behaviour of experimentally implementable biomolecular systems. In addition to electronic engineering and control/system theoretic ideas, other characteristic properties of biological systems can be used to achieve complex behaviours. This paper considers the use of timescale separation and layered architectures in synthetic biomolecular systems. We discuss how, by constructing a biomolecular reaction network on two timescales, we are able (a) to implement nonlinear, tunable constraints on a network's state independently of its slow-scale dynamics, and (b) to control the slow dynamics of a network's state in a constrained state space.**

I. INTRODUCTION

The field of synthetic biology is rapidly advancing towards the goal of producing tightly-controlled, predictable behaviour from designed biomolecular networks integrated into living cells [7], [28], [30], [33]. To accelerate the field towards this goal, researchers are currently seeking design frameworks that combine established knowledge of control and systems theory with the domain-specific behaviours and challenges of using biochemical substrates.

There has been a great deal of work developing the practical use of mathematical modelling in the design cycle of synthetic biology [1], [23]. This paper considers tuning parameters to influence the dynamic behaviour of mathematical models. There are many such 'dials' that can be tuned experimentally, and mechanistic mathematical models provide an important tool for predicting the effect of different strategies. These 'dials' can exist at all levels of biological organisation including transcription [11], [22], [37], translation [10], [31], post-translation [6], [15], and global [2], [8] levels. Furthermore, these 'dials' must be tuned subject to the uncertainty underlying the experimental realisation of a mathematical model [7].

A key characteristic of biomolecular networks is the prevalence of timescale separation, seen in particular within natural biochemical reaction networks, shaped by evolutionary processes [16]. Singular perturbation of stiff ODE systems is commonly used to model these networks, producing systems of differential algebraic equations [4], [21], [24] and requiring geometric analyses of invariant manifolds in phase space [13], [34], [35]. However, the use of this strategy in synthetic biology designs has been limited up to now. As one example, scale separation has been used to improve the modularity of synthetic systems [17]. Often, when connecting biochemical designs together in larger systems, the directionality of signal propagation from upstream to downstream modules is compromised by *retroactivity*, where a downstream load affects upstream dynamics [9], [14]. This effect can be attenuated by exploiting timescale separation to construct 'buffer' devices that insulate modules from one another [9], [17].

This paper proposes a synthetic biology design approach that exploits the layered structure of timescale-separated systems [26]. Section II summarises our previous work, in which we derive approximated models for such systems, expressed in the original state space [27]. In Section III we consider how timescale separation can be used to achieve two closely-related design goals: to impose new, tuneable, nonlinear constraints on the dynamics of a biomolecular network; and the dual task of how to design the slow dynamics of a timescale-separated system, given fixed nonlinear constraints imposed by its fast dynamics. Applications of this design approach include, for example, the use of genetic networks to regulate and adapt metabolic processes in cells [3], [20].

II. APPROXIMATING TIMESCALE-SEPARATED SYSTEMS

This paper considers Ordinary Differential Equation (ODE) systems modelling biomolecular reaction networks that consist of N species X_i taking part in M reactions R_j. The ODE state is denoted x and takes values $x(t) \in \mathbb{R}^N$. Each element $x_i(t)$ of $x(t)$ denotes the concentration of species X_i at time $t \geq 0$. The dynamics of x are:

$$\dot{x}(t) = Sv(x(t)). \tag{1}$$

In (1), each component $v_j(x(t))$ of the flux vector $v(x(t)) \in \mathbb{R}^M$ represents the rate, in units of concentration over time, of each reaction j at time t, which is in general dependent on the concentration vector $x(t)$. Each element S_{ij} of the stoichiometric matrix $S \in \mathbb{Z}^{N\times M}$ represents the number of molecules of X_i produced or consumed by reaction v_j. Hence S maps reaction rates to the rates of concentration change.

We assume that the reaction networks separate in timescale, such that the set of reactions can be partitioned into two groups, representing a set of slow and fast reactions. In terms of the flux vector (reordering the reaction index w.l.o.g.) this assumption is equivalent to the existence of a parameter $0 < \varepsilon \ll 1$ such that the partition

$$v(x(t)) = \begin{bmatrix} v^s(x(t)) \\ v^f(x(t)) \end{bmatrix} = \begin{bmatrix} \bar{v}^s(x(t)) \\ \bar{v}^f(x(t))/\varepsilon \end{bmatrix}$$

holds with $\bar{v}^s$ and $\bar{v}^f$ taking maximum values on the same scale. In other words, the values of v^f are on the scale $1/\varepsilon$ times the values of v^s. One situation where this assumption applies is when the binding and unbinding of a transcription

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factor to a DNA promoter region is fast, relative to the rate of transcription of its downstream mRNA product.

The reactions corresponding to v^f are 'fast', while those corresponding to v^s are 'slow'. The ODE (1) can thus be decomposed into the sum of fast and slow vector fields

$$\dot{x}(t) = S^s \bar{v}^s(x(t)) + \frac{1}{\varepsilon} S^f \bar{v}^f(x(t)), \tag{2}$$

where the partition of $S = \begin{bmatrix} S^s & S^f \end{bmatrix}$ matches that of $v(x)$.

Traditional singular perturbation techniques for timescale separation focus on a modular decomposition of $x = [x_s, x_f]^T \in \mathbb{R}^N$ into slow and fast components $x_s \in \mathbb{R}^{N_s}$ and $x_f \in \mathbb{R}^{N_f}$, with $N_s + N_f = N$ [18], [19], [24]. This often requires a state transformation before the transformed state admits this decomposition. In contrast, our approach assumes that any variable x_i can take part in both slow and fast processes [4], [21]. This allows us to approximate (2) in the limit as $\varepsilon \to 0$ in the originally modelled coordinates, without any state transformation [26], [27] .

Definition 1: Given the ODE system (2) define the *layered ODE system* for the states $x^s, x^f \in \mathbb{R}^N$ in the original state space with dynamics

$$\dot{x}^s(t) = S^s \bar{v}^s(x^s(t) + x^f(t)) \tag{3a}$$

$$\varepsilon \dot{x}^f(t) = S^f \bar{v}^f(x^s(t) + x^f(t)) \tag{3b}$$

with initial conditions $x^s(0) = x(0)$ and $x^f(0) = 0$.

Consider the solutions x^s and x^f to (3). Their sum $x = x^s + x^f$ clearly satisfies the timescale separated ODE (2), and hence solves the original ODE (1) exactly. Hence, the decomposition (3) of (2) is essentially an expansion of (2) into a new state-space $(x^s, x^f) \in \mathbb{R}^{2N}$.

It is possible to apply Tikhonov's theorem to (3) and reduce the system. The goal of Tikhonov's theorem is to find an appropriate approximation $\tilde{x}^f = \phi(\tilde{x}^s)$ for the fast variable as a static function of the slow variable. This can then be substituted into (3a).

Lemma 2: Given the fast layer's stoichiometric matrix $S^f \in \mathbb{R}^{N \times M^f}$, there exists a decomposition $S^f = U^f C^f$ for the full column-rank and row-rank matrices $U^f \in \mathbb{R}^{N \times r^f}$ and $C^f \in \mathbb{R}^{r^f \times M^f}$ respectively. The constant $r^f = \operatorname{rank}(S^f)$ represents the underlying dimension of the fast layer (3b).

Proof: The existence of such a decomposition follows from the compact form of the singular value decomposition of $S^f = U^f \Sigma^f (V^f)^T$, for the $r^f \times r^f$ diagonal matrix Σ^f of non-zero singular values, setting $C^f = \Sigma^f (V^f)^T$. ∎

The following results are independent of the specific choice of U^f and C^f in the decomposition $S^f = U^f C^f$ [27].

Theorem 3: Denote the Jacobian of $\bar{v}^f(x)$ evaluated at x by $\mathrm{d}\bar{v}^f(x)/\mathrm{d}x$, and write $S^f = U^f C^f$ as described in Lemma 2. Assuming that $C^f[\mathrm{d}\bar{v}^f(x)/\mathrm{d}x]U^f$ is non-singular for all possible values of x, there exists a function ϕ satisfying the implicit relationship

$$0 = S^f \bar{v}^f(x^s + \phi(x^s)), \tag{4a}$$

such that the solution to

$$\dot{\tilde{x}}^s(t) = S^s \bar{v}^s(\tilde{x}^s(t) + \phi(\tilde{x}^s(t))) \tag{4b}$$

with $\tilde{x}^f := \phi(\tilde{x}^s)$, converges to the solution to (3) as $\varepsilon \to 0$.

Proof: This result is an application of Tikhonov's theorem [18] to (3). For the algebraic relationship $0 = S^f \bar{v}^f(\tilde{x}^s + \tilde{x}^f)$ to imply $\tilde{x}^f = \phi(\tilde{x}^s)$, we need to satisfy the conditions of the Implicit Function Theorem [32].

The fast state x^f takes values in the column space of S^f, denoted $\mathrm{Col}(S^f)$ and spanned by the columns of U^f. Thus $x^f = U^f \xi^f$ for the uniquely determined state $\xi^f \in \mathbb{R}^{r^f}$, and hence (3b) reduces to

$$\varepsilon \dot{\xi}^f = C^f \bar{v}^f(x^s + U^f \xi^f).$$

We therefore aim to find ξ^f as a function of x^s to satisfy the implicit algebraic relation

$$0 = C^f \bar{v}^f(x^s + U^f \xi^f) = F(x^s, \xi^f),$$

where the function F maps $\mathbb{R}^N \times \mathbb{R}^{r^f} \to \mathbb{R}^{r^f}$. According to the IFT, a function $\xi^f = \psi(x^s)$ exists if the derivative

$$\frac{\partial F}{\partial \xi^f} = C^f \frac{\mathrm{d}\bar{v}^f(x)}{\mathrm{d}x} U^f$$

is non-singular, as assumed, where $x = x^s + U^f \xi^f$. Since $x^f = U^f \xi^f$, we then define $\phi(x^s) = U^f \psi(x^s)$. ∎

Corollary 4: In the limit as $\varepsilon \to 0$, the solution to (2) can be approximated by the solution $\tilde{x}$ to the system

$$\dot{\tilde{x}} = \left[I - M^f(\tilde{x})\right] S^s v^s(\tilde{x}), \tag{5a}$$

where $M^f(\tilde{x})$ is defined as

$$M^f(\tilde{x}) = U^f \left(C^f \frac{\mathrm{d}\bar{v}^f(\tilde{x})}{\mathrm{d}\tilde{x}} U^f \right)^{-1} C^f \frac{\mathrm{d}\bar{v}^f(\tilde{x})}{\mathrm{d}\tilde{x}}, \tag{5b}$$

for initial conditions $\tilde{x}(0) = x(0) + \phi(x(0))$.

Proof: The system (2) is expanded into (3), which is in turn approximated according to Theorem 3. We then construct the resulting approximations $\tilde{x}^s$ and $\tilde{x}^f = \phi(\tilde{x}^s)$ for x^s and x^f respectively. Summing these variables gives an approximation $\tilde{x} = \tilde{x}^s + \phi(\tilde{x}^s)$ to the original solution $x = x^s + x^f$ of (2).

Taking the derivative of $\tilde{x}$ with respect to t, we find

$$\dot{\tilde{x}} = \left[I + \frac{\mathrm{d}\phi(\tilde{x}^s)}{\mathrm{d}\tilde{x}^s} \right] \dot{\tilde{x}}^s$$

for the Jacobian $\mathrm{d}\phi(\tilde{x}^s)/\mathrm{d}\tilde{x}^s$. A standard corollary to the IFT holds that the Jacobian of $\xi^f = \psi(\tilde{x}^s)$ implied by $F(\tilde{x}^s, \xi^f) = 0$ satisfies

$$\begin{aligned} \frac{\mathrm{d}\psi(\tilde{x}^s)}{\mathrm{d}\tilde{x}^s} &= -\left(\frac{\partial F}{\partial \xi^f} \right)^{-1} \frac{\partial F}{\partial \tilde{x}^s} \\ &= -\left(C^f \frac{\mathrm{d}\bar{v}^f(\tilde{x})}{\mathrm{d}\tilde{x}} U^f \right)^{-1} C^f \frac{\mathrm{d}\bar{v}^f(\tilde{x})}{\mathrm{d}\tilde{x}}. \end{aligned}$$

As $\phi = U^f \psi$, then $\mathrm{d}\phi(\tilde{x}^s)/\mathrm{d}\tilde{x}^s = U^f \mathrm{d}\psi(\tilde{x}^s)/\mathrm{d}\tilde{x}^s$, from which it follows that

$$\frac{\mathrm{d}\phi(\tilde{x}^s)}{\mathrm{d}\tilde{x}^s} = -M^f(\tilde{x})$$

for M^f given in (5b). ∎

Corollary 4 is the key result used in this paper. From a system (2) where slow and fast reactions have been

identified, we construct the approximation (5a), valid on the slower timescale. The approximation pre-multiplies the isolated slow layer by a factor $P^f := I - M^f$ depending only on the structure and dynamics of the fast layer. Furthermore, $(M^f)^2 = M^f$, implying that P^f is a projection.

III. Layered Design

Given a biomolecular reaction network (1) with reactions classified as slow or fast, we have derived an approximate model (5), re-written with the tilde notation suppressed:

$$\dot{x} = \left[I - M^f(x)\right] S^s v^s(x),$$
$$M^f(x) = U^f \left(C^f \frac{\mathrm{d}v^f(x)}{\mathrm{d}x} U^f \right)^{-1} C^f \frac{\mathrm{d}v^f(x)}{\mathrm{d}x},$$

with initial conditions $x(0) = x_0 + \phi(x_0)$. The validity of this model improves as the scale separation parameter $\varepsilon \ll 1$ approaches zero, and can be quantified in terms of $\mathscr{L}_2$ norm of the output trajectory using dissipativity theory [25].

In this section we consider two consequences of this model, both arising from the independent effects of the slow and fast layers. Given a fast layer with associated projection $P^f = I - M^f$, a slow layer can be designed, with respect to P^f, to implement a desired behaviour. Conversely, given an arbitrary slow layer with vector field $S^s v^s(x)$, we can constrain the system's dynamics by constructing a fast layer to implement a desired projection P^f. We begin with the second of these consequences.

A. Using fast layers to impose nonlinear constraints

Consider an arbitrary reaction network, with state $x \in \mathbb{R}^N$, stoichiometric matrix $S \in \mathbb{R}^{N \times M}$, and flux vector $v(x) \in \mathbb{R}^M$. Assume that all of the reaction rates are on the same 'slow' scale, giving dynamics (1) with initial condition $x(0) = x_0$.

The state evolves in its stoichiometric compatibility class [12], such that $x(t) - x_0 \in \mathrm{Col}(S)$, for the column space $\mathrm{Col}(S)$. Equivalently, for any vector $\lambda \in \mathbb{R}^N$ such that $\lambda^T S = 0$, the conservation relation $\lambda^T x(t) = \lambda^T x_0$ holds for all t. If $r = \mathrm{rank}(S)$, then there exists a set of $N - r$ independent $\lambda_i \in \mathbb{R}^N$ that span the left-null space $\mathrm{LNS}(S)$. Thus, given the stoichiometric matrix S, there are $N - r$ independent conservation relations $\lambda_i^T x(t) = K_i$ for $i = 1, \ldots, N - r$, with the constants $K_i = \lambda_i^T x_0$ defined by the initial conditions.

From this point, a classical synthetic biology design strategy is to tune parameters of the functions $v_j(x)$, representing the reaction rates of the network, in order to achieve a specific design goal. However, the approximation (5) suggests a new design technique: we can overlay additional reactions on a much faster timescale, that can be designed to project the slow network's dynamics into a new vector field [29].

The following results summarise the effect of a new fast layer on the linear conservation relations $\lambda_i^T x(t) = K_i$ of the isolated slow network. The stoichiometric matrix of the fast layer is denoted $S^f = U^f C^f$, and the fast flux vector $v^f(x)$.

Theorem 5: For a given state value $x(t)$, define the set $\Lambda(x) = \left\{ \lambda \in \mathbb{R}^N \mid \lambda^T \dot{x}(t) = 0 \right\}$ of vectors lying perpendicular to $\dot{x}(t)$, which we assume is non-zero. Then

$$\Lambda(x) = \left(\mathrm{LNS}(S) \cap \mathrm{LNS}(U^f)\right) \oplus \mathrm{Row}(M^f(x)),$$

where $\oplus$ denotes the disjoint sum of vector spaces.

Proof: Since $P^f(x) = I - M^f(x)$ is a projection, the underlying vector space can be written $\mathbb{R}^N = \mathrm{Row}(P^f(x)) \oplus \mathrm{Row}(M^f(x))$. The following equalities are standard:

$$\mathrm{Row}(P^f) = \mathrm{Col}((P^f)^T) = \ker((M^f)^T) = \mathrm{LNS}(M^f),$$
$$\mathrm{Row}(M^f) = \mathrm{Col}((M^f)^T) = \ker((P^f)^T) = \mathrm{LNS}(P^f).$$

Furthermore, M^f in (5b) satisfies $\mathrm{LNS}(M^f) = \mathrm{LNS}(U^f)$.

Consider first $\lambda \in \Lambda(x)$. It can be decomposed uniquely into $\lambda = \lambda_P + \lambda_M$ for $\lambda_P \in \mathrm{Row}(P^f(x)) = \mathrm{LNS}(U^f)$ and $\lambda_M \in \mathrm{Row}(M^f(x)) = \mathrm{LNS}(P^f(x))$. Then the condition

$$0 = \lambda^T P^f S v(x) = \lambda_P^T P^f S v(x) + \lambda_M^T P^f S v(x)$$
$$= \lambda_P^T S v(x)$$

implies that $\lambda_P \in \mathrm{LNS}(S)$, and hence $\Lambda \subseteq \left(\mathrm{LNS}(S) \cap \mathrm{LNS}(U^f)\right) \oplus \mathrm{Row}(M^f(x))$.

To show the reverse inclusion, we first note that for $\lambda_M \in \mathrm{Row}(M^f(x)) = \mathrm{LNS}(P^f(x))$, we have $\lambda_M^T P^f(x) S v(x) = 0$. Furthermore, for $\lambda_P \in \left(\mathrm{LNS}(S) \cap \mathrm{LNS}(U^f)\right)$ we have $\lambda_P^T P^f(x) S v(x) = \lambda_P^T S v(x) = 0$. Hence any linear combination of λ_P and λ_M is a member of Λ, and the result follows. ■

The key consequence Theorem 5 is that $\dot{x}$ defined by (5) lies perpendicular to two sets of vectors: a globally-valid set $\mathrm{LNS}(S) \cap \mathrm{LNS}(U^f)$, and a state-dependent set $\mathrm{Row}(M^f(x))$. The globally-valid set is the subspace of conservation relations $\mathrm{LNS}(S)$ for the isolated slow layer that are also valid conservation relations $\mathrm{LNS}(U^f)$ for the isolated fast layer.

The state-varying perpendicular space $\mathrm{Row}(M^f(x))$ corresponds to the slow manifold defined by $C^f v^f(x) = 0$. Compared to the fixed, linear conservation relations of $\mathrm{LNS}(S)$, we now have a set of $r^f = \mathrm{rank}(S^f)$ potentially nonlinear conservation relations. These are also (in principle) tuneable, in that they depend on parameter values used in v^f, and are independent of the slow layer.

1) Conservation Relations in Gene Regulation Networks: We illustrate this approach through implementing conservation relations onto an arbitrary gene regulation network. Consider N genes producing products X_i with concentrations $x_i(t)$, according to dynamics

$$\dot{x}_i(t) = \alpha_i - \delta_i x_i(t) + R_i(x(t)). \tag{6}$$

Each function $R_i(x(t))$ models the regulatory effect of all X_j on the production of X_i. For example, if X_j is a promoter of gene i then this may be modelled by

$$R_i(x(t)) = \frac{\gamma_i x_j(t)^m}{K_i^m + x_j(t)^m}.$$

A typical synthetic biology task is to design the parameters in each R_i to produce a desired behaviour.

We now aim to couple the dynamics of two gene products (indexed as X_1 and X_2 without loss of generality) such that $x_2 = K x_1^2$ for a specified constant K. Rather than design the genetic regulatory dynamics via the parameters in some or all of the functions R_i, we instead propose to design an overlaid *fast* protein interaction network.

One approach to implementing the nonlinear conservation $x_2 = K x_1^2$ might be to choose the products X_i such that two

enzymes E_+ and E_- exist (at constant concentrations e_+ and e_-) which catalyse the following reactions:

$$X_1 + X_1 \underset{E_-}{\overset{E_+}{\rightleftharpoons}} X_2. \tag{7}$$

The forward and backward reactions in this protein interaction network proceed at rates $k_\pm e_\pm$ proportional to the concentrations $e_\pm$ of enzymes $E_\pm$, and are assumed to be on a much faster timescale than the genetic regulatory interactions [36]. Hence we can consider the model (5) for this network, constructing M^f from the stoichiometric matrix and flux vector corresponding to the fast reactions (7).

The slow manifold defined by the quasi-steady state relation $C^f v^f(x) = 0$ satisfies

$$0 = \begin{bmatrix} 1 & -1 \end{bmatrix} \begin{bmatrix} k_+ e_+ x_1^2 \\ k_- e_- x_2 \end{bmatrix},$$

where the stoichiometric matrix has been decomposed into

$$S^f = U^f C^f = \begin{bmatrix} -2 \\ 1 \\ 0 \\ \vdots \end{bmatrix} \begin{bmatrix} 1 & -1 \end{bmatrix},$$

and a simple first-order linear model is used for the effect of the enzymes $E_\pm$. We then can deduce that $x_2 = K x_1^2$ in the model (5). Here the constant $K(e_+, e_-) = k_+ e_+ / k_- e_-$ can be tuned by varying the concentrations $e_\pm$ of the enzymes $E_\pm$. Thus the conservation relation implemented by the protein interaction layer is nonlinear and can be tuned experimentally. For parameters chosen such that $K = 1/2$ and three uncoupled genes (i.e. $R_i = 0$), a simulation of the behaviour of this layered network is shown in Figure 1. The trajectory quickly equilibrates to the slow manifold in $\mathbb{R}_3$, before evolving on a slower timescale according to the nonlinear conservation relation.

2) Multiple Implementations: Given a specific conservation relation $p(x) = 0$, there may be multiple implementations available to the designer. Practical considerations (such as the availability of biochemicals that interact in the desired way) play an important role in the choice, but different implementations also result in vastly different dynamics on the same slow manifold.

Consider again the conservation relation $x_2 = K x_1^2$ implemented above. An alternative approach might be to consider implementing a fast network consisting of two reversible reactions:

$$X_1 + X_3 \underset{E_{-1}}{\overset{E_1}{\rightleftharpoons}} X_2, \tag{8a}$$

$$X_1 \underset{E_{-2}}{\overset{E_2}{\rightleftharpoons}} X_3, \tag{8b}$$

again with reaction rates $k_{\pm i} e_{\pm i}$ proportional to the concentrations $e_{\pm i}$ of enzymes $E_{\pm i}$. In this case, there are two independent conservation relations given by the equation

$$0 = C^f v^f(x) = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & -1 & 1 \end{bmatrix} \begin{bmatrix} k_1 e_1 x_1 x_3 \\ k_{-1} e_{-1} x_2 \\ k_2 e_2 x_1 \\ k_{-2} e_{-2} x_3 \end{bmatrix},$$

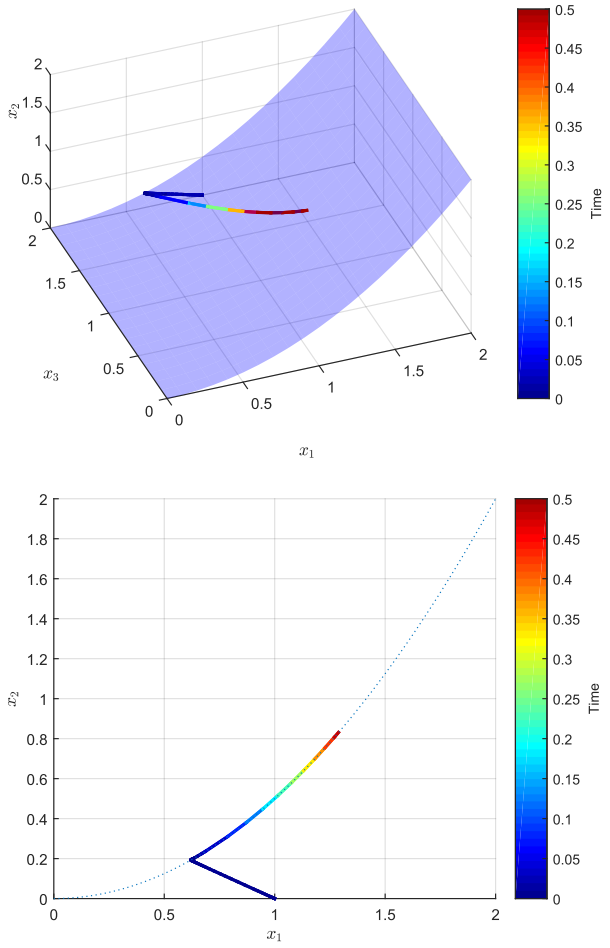

Fig. 1. Simulation of (5) with arbitrary slow layer (6) and fast layer (7), for parameters chosen such that $K = 1/2$. The upper plot projects the dynamics into (x_1, x_2, x_3) space, showing the slow manifold in blue. The lower plot projects into (x_1, x_2). The colour of the trajectory corresponds to the time of the simulation.

so that $x_3 = k_2 e_2 x_1 / k_{-2} e_{-2}$ and $x_2 = k_1 e_1 x_1 x_3 / k_{-1} e_{-1}$. These conservation relations combine to give $x_2 = K x_1^2$ for

$$K = \frac{k_1 k_2}{k_{-1} k_{-2}} \times \frac{e_1 e_2}{e_{-1} e_{-2}}.$$

Hence, although the same conservation relation holds, the matrices $P^f(x)$ in each implementation (7) and (8) differ in their effect on the slow dynamics. The different dynamics of these two systems can be seen in the simulations shown in Figure 1 and Figure 2, corresponding to fast layers (7) and (8) respectively. Furthermore, different choices of $e_{\pm i}$ in (8) that give the same conservation relation can result in distinct outputs. This is shown in Figure 3, which simulates the system with a different set of $e_{\pm i}$ to those in Figure 2, albeit chosen such that $K = 1/2$.

Notwithstanding the practical constraint of selecting biologically realistic fast reactions, the design of the implemented fast layer should be informed by the subsequent task of tuning the slow dynamics $S^s v^s(x)$ given the nonlinear constraints. The following section discusses this dual task.

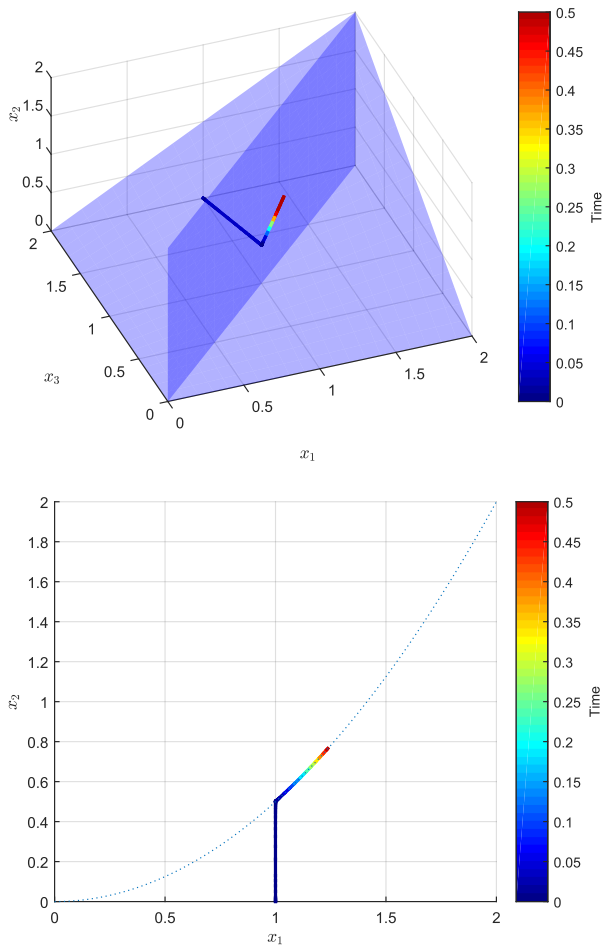

Fig. 2. Simulation of (5) with arbitrary slow layer (6) and fast layer (8), for parameters $k_{\pm i}$ and $e_{\pm i}$ chosen with $K = 1/2$. The upper plot projects the dynamics into (x_1, x_2, x_3) space, showing both slow manifolds in blue. The lower plot projects into (x_1, x_2). The colour of the trajectory corresponds to the time of the simulation.

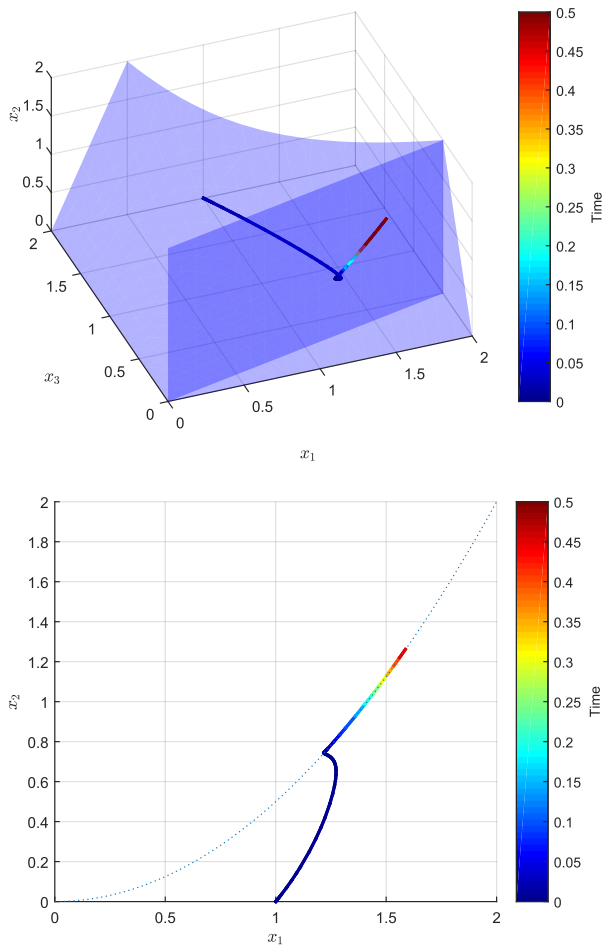

Fig. 3. As Figure 2, for an alternative set of parameters $k_{\pm i}$ and $e_{\pm i}$, chosen again such that $K = 1/2$, with new slow manifolds still in blue.

B. Designing the slow adaptation of a fast layer

The preceding section used (5) to consider the design of a fast layer that could implement specific conservation relations onto an arbitrary slow reaction network. It remains to now consider the closely-related dual task: the ability to design a slow reaction network that evolves the entire system (i.e. the integrated fast and slow layers) according to a specified performance.

The isolated slow dynamics are defined by the vector field $S^s v^s(x)$. In the presence of the fast layer, these vectors are projected by $P^f(x)$ to lie in the tangent space to the slow manifold defined by $0 = C^f v^f(x)$. The goal of layered design in the presence of a fixed fast layer is to design the slow vector field $S^s v^s(x)$ such that the projected vector field $P^f(x) S^s v^s(x)$ satisfies the specification.

Suppose that a parameter change is applied to the slow layer to give a small change $\delta v^s(x)$ to the flux vector. In isolation, the slow layer's vector field is then changed by $S^s(\delta v^s(x))$ in response to the parameter perturbation. The projection P^f is independent of all of the parameters in the slow layer, and therefore its effect will be robust to their perturbation. Therefore the response of the integrated system's vector field to perturbations of the slow layer's parameters is the product of the isolated response $S^s(\delta v^s(x))$ and the projection P^f, to give a response $P^f(x) S^s(\delta v^s(x))$.

Similarly to the proof of Theorem 5, the space $\mathbb{R}^N$ can be decomposed into

$$\mathbb{R}^N = \mathrm{Col}(P^f) \oplus \mathrm{Col}(M^f) = \mathrm{Col}(P^f) \oplus \ker(P^f).$$

In particular, the isolated response $S^s(\delta v^s(x))$ of the slow layer's vector field will have a component in $\ker(P^f)$, which will therefore be mapped to zero, and a component in $\mathrm{Col}(P^f)$ that will be left unchanged by $P^f(x)$. Therefore the controllability of the slow dynamics, in terms of the sensitivity of the vector field, is constrained by the projections $P^f(x)$ to only influence the vector field in the tangent space of the slow manifold. The resulting sensitivity is of a new magnitude, corresponding to the component of $S^s \delta v^s$ in $\mathrm{Col}(P^f(x))$. Hence, the integrated system will be robust to some control actions, and much more sensitive to others.

One topic of current research is the design of genetic networks to regulate metabolic processes using feedback [3],

[20]. In terms of layers, the slow genetic layer needs to be designed and tuned to ensure the slow adaptation of the fast layer performs as required. We envision that the layered design approach, and specifically the structure of the projection $P^f(x)$, will be used to translate knowledge of the controllability of the genetic output (in response to parameter variation) to the controllability of the metabolic output.

IV. CONCLUSIONS

This paper has presented a means by which timescale separation can be exploited in the design of synthetic biomolecular systems. Previous work [27] on the layered decomposition of systems through partitioning reaction sets has been used to model the slow dynamics of systems on multiple timescales. We have discussed how this model can be used for two closely-related design tasks: implementing tunable, nonlinear constraints on the feasible states of the system [29]; and designing the slow adaptation of a quasi-steady state over a long timescale. The latter goal has applications to the genetic regulation of metabolic networks.

Future work will develop these results to further quantify the controllability of the integrated fast–slow system with respect to tuning the slow layer's parameters, and in particular how it relates to that of the isolated slow system. We may be able to apply our layered approach to questions of structural stability in multi-scale systems, through methods introduced in [5]. Other likely directions for this design strategy will be to consider the importance of stochastic modelling and noise on the behaviour of the layered system.

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