2014 American Control Conference (ACC)
June 4-6, 2014. Portland, Oregon, USA

Signal propagation across layered biochemical networks

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Abstract— **Biochemical reaction networks typically consist of a complicated structure with many interacting species and components. Techniques for the analysis of such complex systems commonly use decompositions into simpler subsystems. These decompositions are often modular, representing the state vector as a concatenation of component vectors. Without transformation, modular decompositions may lead to system parameters directly influencing the dynamics of many subsystems at once. When parameters are the control inputs, this complicates analysis and design. This paper investigates an alternative decomposition, termed layering, which partitions parameters between layers. This allows for hierarchical analysis, where the steady state response of the integrated system to the perturbation of a parameter is calculated in stages. The first stage is to calculate the local response of the steady state of a layer, considered in isolation from other layers; the second is to calculate the perturbed layer's effect on the others when connected back into the full system. This analysis results in a strategy for detecting the layered structure of a biochemical network based on preserving cycles of mass flow within layers. Additionally, by expressing how the local response propagates through the system we uncover the paths by which the direct control of a certain layer may indirectly control others, giving insights into how to exploit their dependencies.**

I. INTRODUCTION

Biochemical reaction networks are characterised by their scale and structure [7], [1]. They are highly complicated, with many interacting components and often several temporal and spatial scales. Direct mathematical analysis of such networks is difficult, and therefore current techniques focus mainly on their decomposition into subsystems, each of which is simpler to analyse than the whole.

A key goal of systems biology is to gain an understanding of large systems through analysing them as systems of interconnecting subsystems [20], called modules. The chemical species in the network are grouped such that each module describes the evolution of a group of chemical species. However, there are conflicting views on the degree of modularity in such systems [2]. For example, while modular decomposition implies that the behaviour of the isolated components is unchanged upon their interconnection, biochemical systems demonstrate retroactivity [12], [13], whereby the mechanism of interconnection changes the behaviour of each component.

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We gratefully acknowledge the constructive comments of our five anonymous reviewers, and the financial support received from EPSRC through the University of Oxford LSI Doctoral Training Centre. A. Papachristodoulou was supported in part by the EPSRC projects EP/J012041/1, EP/I031944/1 and EP/J010537/1.

Rather than seek a modular decomposition, the approach of this paper is to use a layered decomposition of biochemical reaction networks. Our previous work [22] focused on layers in systems exhibiting timescale separation. This paper generalises layering to consider systems without a scale separation of their parameters. Assigning each parameter to exactly one layer ensures that parameter-actuated control actions will directly affect one layer only. By integrating the layer into the system we can then observe how those direct effects propagate.

Layering and model decomposition in engineered networks has received a lot of attention [14]. A key paper [10] considers layering in communication networks as the decomposition of a large optimisation problem into subproblems. Other methods [6], [4], [3] consider decomposition into interacting subsystems with various coupling strengths. Another interesting approach is that of overlapping subsystems [17] coupled such that the overlapping components are equal.

Modular and hierarchical control analysis [8] are systems-biological approaches to the analysis of reaction networks of interconnected components. Kholodenko *et al.* [18] use a control analysis approach [16] to the "top-down" problem [9] of reverse-engineering the interconnection of modules from experimental data and steady-state analysis. After stating the layered decomposition of biochemical networks in Section II, this paper uses a layered analysis to consider the complementary "bottom-up" problem of finding the steady state response to external parameter perturbations.

In Section IV we give two examples of layered steady-state analysis. Section V discusses this analysis from three perspectives: first, as giving a strategy for choosing a layered decomposition of a given network; second, understanding how the direct control of a layer results in the indirect control of other layers; and third, the dynamic nature of the dependencies between layers.

II. LAYERING BIOCHEMICAL NETWORKS

A biochemical network is a collection of N chemical species $\{X_1,\dots,X_N\}$ taking part in M reactions. The evolution of the concentrations x_i of each species may be modelled as a system of ODEs [19]. Denote the number of molecules of species i produced by reaction j as S_{ij}, which is negative if it is consumed by the reaction. Each reaction j proceeds at a rate $v_j(x)$, depending on the reactants' concentrations. The dependence is often modelled using the Law of Mass Action, resulting in polynomial functions of concentrations x_i. Forming the matrix $S=(S_{ij})$ and vectors $x=(x_i)$ and $v=(v_j)$, the ODEs are written compactly as

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

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where the dot corresponds to differentiation with respect to time t, and the initial conditions are $x(0) = x_0$.

We can identify the dimension of (1) as $r = \text{rank}(S)$. Given a decomposition of $S = UC$ for $U \in \mathbb{R}^{N\times r}$ and $C \in \mathbb{R}^{r\times M}$, a minimal realisation of (1) is

$$\dot{\xi} = Cv(x(0) + U\xi), \tag{2}$$

for $\xi \in \mathbb{R}^r$, with $x = x(0) + U\xi$. If $\ker(S) = \{0\}$, this matches the reaction coordinates realisation in [5].

The analysis of large-scale biochemical networks is typically simplified by their modular decomposition into interconnections of simpler subsystems, which corresponds to partitioning $x \in \mathbb{R}^N$ into L sub-vectors $x_l \in \mathbb{R}^{n_l}$, with $\sum n_l = N$. Examples of such decompositions can be seen in signal transduction networks [23], genetic networks [18], the heat shock response [15], and cell cycle networks [11].

Assume that (1) is controlled through its parameters (for example, reaction rates). In the modular decomposition, the parameters of the system, and hence the control effect, may be distributed across multiple subsystems. To simplify the analysis and design of controllers for such systems, decompositions should instead ensure that a control input affects one subsystem only.

This paper will neglect modular decomposition, and instead enforce the partition of parameters using *layered* decompositions. Associated with each reaction j is a reaction rate $v_j(x)$, often characterised by a reaction rate constant k_j determining the relative speed of the reaction. Suppose that, rather than partitioning species, the reactions (and hence the parameters) are partitioned into L groups, so that $v = (v^1, v^2, \ldots, v^L)^T$. Note that superscript indices denote layers while subscripts denote vector components. When necessary, exponents will be written outside brackets. Partitioning the columns of $S = (S^1, S^2, \ldots, S^L)$ conformally with the reactions, the system dynamics are

$$\dot{x} = \sum S^i v^i(x).$$

A layer, with state $x^i \in \mathbb{R}^N$, is defined through its dynamics

$$\dot{x}^i = S^i v^i(x), \tag{3}$$
$$x = \sum x^i, \tag{4}$$

for each $i = 1, \ldots, L$. We are free to distribute the initial conditions x_0^j of each layer in any way, subject to $x_0 = \sum x_0^j$. Then x as defined in (4) has dynamics $\dot{x} = Sv(x)$, recovering the system behaviour. In general, the ith component of the jth layer's state x_i^j corresponds to that layer's contribution towards the total concentration x_i of X_i. As will be seen in the following example, this contribution may be negative if layer j consumes species i.

Consider the example nonlinear reaction network

$$U \xrightarrow{\alpha} X, \tag{5}$$
$$X + Y \xrightarrow{\beta} Z, \tag{6}$$

the two-dimensional dynamics of which can be written

$$\begin{bmatrix} \dot{u} \\ \dot{x} \end{bmatrix} = \begin{bmatrix} -1 & 0 \\ 1 & -1 \end{bmatrix} \begin{bmatrix} \alpha u \\ \beta x(u + x + y_0 - u_0 - x_0) \end{bmatrix},$$

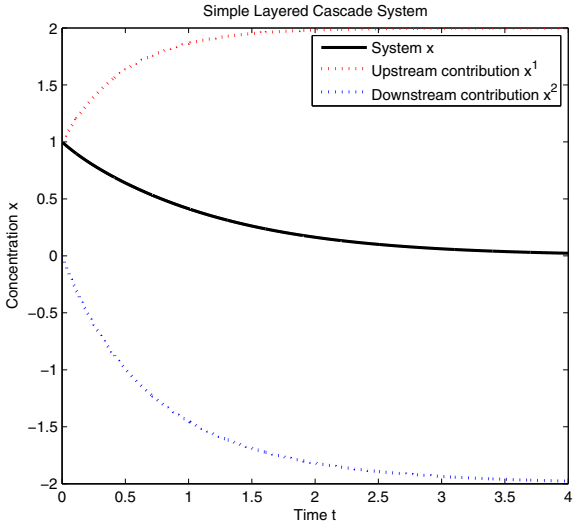

Fig. 1: Plotting the trajectories of x and the layered contributions x^1 and x^2 with parameters $[\alpha, \beta] = [2, 1]$. The system has initial conditions $[u(0), x(0)] = [1, 1]$ with $y_0 = 3$.

by exploiting conservation relations $y = u + x + y_0 - u_0 - x_0$ and $z = z_0 + u_0 + x_0 - u - x$ for initial conditions $[u_0, x_0, y_0, z_0]$. We will decompose this example by putting each reaction in a single layer. Setting $\phi = u_0 + x_0 - y_0$, the dynamics are

$$\begin{bmatrix} \dot{u}^1 \\ \dot{x}^1 \end{bmatrix} = \begin{bmatrix} -1 \\ 1 \end{bmatrix} \alpha(u^1 + u^2),$$
$$\begin{bmatrix} \dot{u}^2 \\ \dot{x}^2 \end{bmatrix} = \begin{bmatrix} 0 \\ -1 \end{bmatrix} \beta(x^1 + x^2)(u^1 + u^2 + x^1 + x^2 - \phi).$$

Each layer is a one-dimensional system, and $\dot{u}^2 = 0$ implies that layer 1 is independent, and can be thought of as "upstream", of layer 2. Taking a control action through changing k_1 will directly affect only the trajectory of the state of layer 1, but this effect will pass to the downstream layer through the perturbed layer 1 trajectory $[u^1, x^1]$.

To illustrate the physical intuition behind layered states, consider the two layers' trajectories x^1, x^2, and the system trajectory x plotted in Figure 1. The broken red trajectory x^1 corresponds to the concentration of X in the isolated upstream layer (5) initiated from $[1, 1]$. The downstream layer (6) shares a species with the upstream layer. Therefore the connection of the downstream layer to give the full system causes retroactivity [12], where the behaviour of X is changed upon connection. The full system's dynamics result in the black curve x. The retroactive effect is equal to the broken blue trajectory x^2, which has been found by plotting (6) initiated from $[0, 0]$ with input x^1. This trajectory is the negative contribution of the downstream layer to the total concentration of X. Thus, the dynamics of x resulting from retroactivity can be interpreted as the overlaying of the layers' trajectories $x^1 + x^2$.

In previous work [22], the magnitude of the reaction rate parameters separated in scale, forming a natural decomposition into fast and slow layers. When the reactions are on a

common timescale there is no such partition. Given a large reaction network, the existence of the layering framework begs the question of how to layer.

Similarly to (2), a layer can be minimally realised through the decomposition $S^i = C^i U^i$ as

$$\dot{\xi}^i = C^i v(x^i(0) + U^i \xi^i), \tag{7}$$

for $x^i = x^i(0) + U^i \xi^i$. It is necessary that $\text{rank}(S^i) < \text{rank}(S)$ to ensure the layers are simpler systems, but there may be many decompositions satisfying this requirement. In the following section we find a formula for the steady-state dependencies between layers, and in the subsequent discussion we will identify two ways in which this will help to develop a decomposition strategy.

III. STEADY STATE ANALYSIS

Any steady state $\bar{x}$ of (1) satisfies $0 = Sv(\bar{x})$. We will now investigate how the effect of a parameter change on the steady state can be decomposed as a combination of direct and indirect effects on the layers' states, which will uncover inter-layer dependencies.

A. Layered Steady States

Consider the layered decomposition (3)–(4). In what follows, we assume the following:

Assumption 1: The layered stoichiometries S^i are chosen such that their column spaces intersect only at 0.

Proposition 1: Assuming Condition 1, and given a fixed distribution of the initial conditions $x(0)$ into the layers' initial conditions $x^i(0)$, there is a unique decomposition of the steady state into $\bar{x} = \sum \bar{x}^i$ where $x \to \bar{x}$ implies $x^i \to \bar{x}^i$.

Proof: At any time t, (1) implies that $x(t) - x_0 \in \text{Col}(S)$. In particular, $\bar{x} - x_0 \in \text{Col}(S)$. Condition 1 implies a unique decomposition of $x - x(0) = \sum z^i$ for $z^i \in \text{Col}(S^i)$, and similarly for $\bar{x}$ and $\bar{z}^i$. Noting that the layered dynamics (3) imply that $x^i(t) - x^i(0) \in \text{Col}(S^i)$, define $x^i = x^i(0) + z^i$ and similarly for $\bar{x}^i$ and $\bar{z}^i$. We want to show that $z^i \to \bar{z}^i$, which implies $x^i \to \bar{x}^i$.

Write $x - \bar{x} = \sum (z^i - \bar{z}^i)$. Recalling $S^i = U^i C^i$ gives

$$x - \bar{x} = \sum U^i \xi^i = \begin{bmatrix} U^1 & \cdots & U^L \end{bmatrix} \xi \to 0.$$

Assumption 1 implies that the matrix is full column-rank, which in turn implies that each $\xi^i \to 0$. As $z^i - \bar{z}^i = U^i \xi^i$, the result follows. ∎

Condition 1 can be compared to the conditions for layered analysis required in [8]. There the authors require S to be block diagonal, so that modular and layered decompositions coincide, automatically satisfying Condition 1. However, in Section IV it will be seen that non-block diagonal stoichiometric matrices may be decomposed into layers amenable to the subsequent steady-state analysis.

B. Inter-layer dependencies

Define $\bar{u}^i = \bar{x} - \bar{x}^i$ as the input to layer i from the other layers. Then the steady state condition for each layer is

$$0 = S^i v^i(\bar{x}^i + \bar{u}^i). \tag{8}$$

This equation implicitly defines the dependence of $\bar{x}^i$ on $\bar{u}^i$. The following proposition formulates an expression for how perturbations to $\bar{x}^j$ in layers $j \neq i$ affect $\bar{x}^i$.

Proposition 2: Consider the layered system at steady state (8), and decompose $S^i = U^i C^i$. The dependence of $\bar{x}^i$ on $\bar{x}^j$ is such that

$$\frac{\partial \bar{x}^i}{\partial \bar{x}^j} = -U^i \left[C^i \partial_x v^i(\bar{x}) U^i \right]^{-1} C^i \partial_x v^i(\bar{x}) =: M^i, \tag{9}$$

where $\partial_x v^i(\bar{x})$ denotes the Jacobian of v^i evaluated at $\bar{x}$.

Proof: Recalling (7), condition (8) can be written

$$0 = C^i v^i(U^i \bar{\xi}^i + \bar{u}^i) =: F^i(\bar{\xi}^i, \bar{u}^i) \tag{10}$$

where the bar denotes steady state as usual. The Implicit Function Theorem implies the existence of an explicit dependence of $\bar{\xi}^i$ on $\bar{u}^i$.

A standard corollary of the IFT is that

$$\frac{\partial \bar{\xi}^i}{\partial \bar{u}^i} = -\left[\frac{\partial F^i}{\partial \bar{\xi}^i} \right]^{-1} \frac{\partial F^i}{\partial \bar{u}^i}.$$

Each derivative of F^i can easily be calculated through applying the chain rule to (10). Since $\bar{x}^i = U^i \bar{\xi}^i$ and $\bar{u}^i = \sum_{j \neq i} \bar{x}^j$, this derivative is pre-multiplied by U^i to give (9). ∎

C. Isolated response

The aim of this section is to decompose the steady-state response to a parameter perturbation of (1) into a layered response. For this, we first need to calculate how each layer responds. Consider layer i at steady state $0 = S^i v^i(\bar{x}^i + u^i; \pi)$, where the notation makes explicit that v^i, and therefore the steady state $\bar{x}^i$, has a direct dependence on a parameter vector π. Although the other layers' steady states $\bar{x}^j$ will also depend on π in the integrated system, their dependence is achieved indirectly through their dependence on $\bar{x}^i$.

The direct dependence of $\bar{x}^i$ on π can now be calculated. The method is similar to that used in the proof of Proposition 2 by using the Implicit Function Theorem, to give

$$\frac{\partial \bar{x}^i}{\partial \pi} = -U^i \left(C^i \partial_x v^i(\bar{x}) U^i \right)^{-1} C^i \partial_\pi v^i(\bar{x}), \tag{11}$$

where $\partial_x v^i$ is the Jacobian of v^i with respect to its x coordinates, and $\partial_\pi v^i$ the Jacobian with respect to its dependence on π.

D. Signal propagation

Putting the two sections above together allows the formulation of how the steady state of a system reacts to a perturbation in π by calculating $\mathrm{d}\bar{x}/\mathrm{d}\pi$ in stages. Denote the isolated response of a layer with $\partial \bar{x}^i / \partial \pi$ as in (11), and denote the integrated response of each layer (i.e. what actually happens) with $\mathrm{d}\bar{x}^i/\mathrm{d}\pi$. The latter incorporates all of the direct and indirect responses to the change in π. A simple application of the chain rule gives

$$\frac{\mathrm{d}\bar{x}^i}{\mathrm{d}\pi} = \frac{\partial \bar{x}^i}{\partial \pi} + \sum_{j \neq i} \frac{\partial \bar{x}^i}{\partial \bar{x}^j} \frac{\mathrm{d}x^j}{\mathrm{d}\pi}$$

for each i. Equation (9) gives a formula for the matrices $M^i = \partial \bar{x}^i / \partial \bar{x}^j$. We can therefore rewrite this set of equations compactly as

$$\begin{bmatrix} I & -M^1 & \dots & -M^1 \\ -M^2 & I & \dots & -M^2 \\ \vdots & \vdots & \ddots & \vdots \\ -M^L & -M^L & \dots & I \end{bmatrix} \begin{bmatrix} \mathrm{d}\bar{x}^1/\mathrm{d}\pi \\ \vdots \\ \mathrm{d}\bar{x}^L/\mathrm{d}\pi \end{bmatrix} = \begin{bmatrix} \partial \bar{x}^1/\partial \pi \\ \vdots \\ \partial \bar{x}^L/\partial \pi \end{bmatrix}. \tag{12}$$

Solving this equation gives a map from the isolated responses to the integrated responses of the layers' steady states. Since $x = \sum x^i$, the resulting system-level response $\mathrm{d}\bar{x}/\mathrm{d}\pi$ is equal to $\sum_i \mathrm{d}\bar{x}^i/\mathrm{d}\pi$.

In summary, the layered approach to the steady-state response of (1) to disturbances in π proceeds as follows. First calculate the local response of each layer to a perturbation in π through (11). If v^i does not depend on π, the local response will be 0. To calculate the integrated response of each layer, solve (12). The aggregated system response is the sum of the layers' integrated responses.

IV. Examples of Layering

A. Closed linear system

We will first illustrate the methods in the preceding section on a linear example $U \underset{1}{\overset{\pi}{\rightleftharpoons}} Y \underset{3}{\overset{2}{\rightleftharpoons}} Z$ with initial conditions $(1,1,1)$ and dynamics

$$\begin{bmatrix} \dot{u} \\ \dot{y} \\ \dot{z} \end{bmatrix} = \begin{bmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \pi u - y \\ 2y - 3z \end{bmatrix}.$$

Denoting the state $x = (u,y,z)$, the steady state $\bar{x}$ is $\frac{3}{3+5\pi}(3, 3\pi, 2\pi)$. Choose the layered decomposition so that each reversible reaction is in its own layer:

$$\begin{bmatrix} \dot{u}^1 & \dot{y}^1 & \dot{z}^1 \end{bmatrix}^T = \begin{bmatrix} -1 & 1 & 0 \end{bmatrix}^T (\pi u - y), \tag{13}$$

$$\begin{bmatrix} \dot{u}^2 & \dot{y}^2 & \dot{z}^2 \end{bmatrix}^T = \begin{bmatrix} 0 & -1 & 1 \end{bmatrix}^T (2y - 3z). \tag{14}$$

Here $\bar{x}$ has been found analytically as a function of π, but in larger complicated networks it may only be found through numerical methods. We will use a layered analysis to describe the response of $\bar{x}$ to a perturbation in π without using its analytic expression.

1) Isolated response: Since the layer 2 dynamics in (14) have no dependence on π, the isolated response $\partial \bar{x}^2/\partial \pi = 0$. Substituting S^1 and v^1 from (13) into (11) results in the layer 1 isolated response:

$$\frac{\partial \bar{x}^1}{\partial \pi} = \frac{\bar{u}}{1+\pi} \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix}.$$

2) Inter-layer communication: The matrices M^i in (9) determine how perturbations propagate to layer i from other layers. Substituting S^i and v^i from (13) and (14) into (9) gives

$$M^1 = \begin{bmatrix} -\gamma & \gamma - 1 & 0 \\ \gamma & 1-\gamma & 0 \\ 0 & 0 & 0 \end{bmatrix}, \quad M^2 = \frac{1}{5} \begin{bmatrix} 0 & 0 & 0 \\ 0 & -2 & 3 \\ 0 & 2 & -3 \end{bmatrix}$$

for $\gamma = \pi/(1+\pi)$.

3) Integrated response: Suppose we perturb π around a nominal value $\pi = 3$. Solving (12) for the integrated responses gives

$$\begin{bmatrix} \mathrm{d}\bar{x}^1/\mathrm{d}\pi \\ \mathrm{d}\bar{x}^2/\mathrm{d}\pi \end{bmatrix} = \begin{bmatrix} -0.139 & 0.139 & 0 & 0 & -0.056 & 0.056 \end{bmatrix}^T.$$

Note that the integrated response of layer 1 is of greater magnitude than the isolated response $\partial \bar{x}^1/\partial \pi = \begin{bmatrix} -1/8 & 1/8 & 0 \end{bmatrix}^T$, while that of layer 2 is non-zero even though $\partial \bar{x}^2/\partial \pi = 0$. Layer 2 therefore amplifies the isolated perturbation to u in layer 1, but also absorbs some of the perturbation to y in z.

B. Open linear system with nonlinear layer

Open systems are those in which there is mass flow in and out of the system. For example, consider the open system

$$\overset{\pi}{\rightarrow} \quad U \quad \overset{\delta_u}{\rightarrow} \tag{15}$$

$$U \overset{k_{uy}}{\rightarrow} \quad Y \quad \overset{\delta_y}{\rightarrow} \tag{16}$$

$$Y + p \quad \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \quad Y^\star, \tag{17}$$

representing transcription of DNA into mRNA U at rate π, the subsequent translation into protein Y at rate k_{uy}, and the reversible phosphorylation of Y into $Y^\star$, where each of U and Y also degrade.

The dynamics of this system, with state $[u,y,p,y^\star]$, has stoichiometry

$$S = \begin{bmatrix} 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}.$$

Our layered decomposition takes the first four columns, corresponding to (15)–(16), as S^1 and the final two, describing (17), as S^2. The column spaces of S^i are disjoint, so that the steady state analysis can be carried out.

Put each of the reaction rates equal to 1, and fix the conserved value of $p + y^\star = 3$. Then the steady state of the system is $\begin{bmatrix} 1/2 & 1/2 & 2 & 1 \end{bmatrix}$. The inter-layer communication matrices are found through (9) as

$$M^1 = \begin{bmatrix} -I_2 & 0 \\ 0 & 0 \end{bmatrix}, \quad M^2 = \frac{1}{7} \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & -4 & -1 & 2 \\ 0 & -4 & -1 & 2 \\ 0 & 4 & 1 & -2 \end{bmatrix}.$$

A perturbation to $\pi = 1$ produces the isolated response in layer 1 of

$$\frac{\partial \bar{x}^1}{\partial \pi} = \begin{bmatrix} 0.5 & 0.25 & 0 & 0 \end{bmatrix}^T,$$

with $\partial \bar{x}^2/\partial \pi = 0$. By solving (12) the integrated responses of each layer are

$$\mathrm{d}\bar{x}^1/\mathrm{d}\pi = \begin{bmatrix} 0.5 & 0.583 & 0 & 0 \end{bmatrix}^T,$$

$$\mathrm{d}\bar{x}^2/\mathrm{d}\pi = \begin{bmatrix} 0 & -0.333 & -0.333 & 0.333 \end{bmatrix}^T.$$

Note again that the response of layer 1 is amplified upon integration with layer 2. However, overlaying $\mathrm{d}\bar{x}^2/\mathrm{d}\pi$, we find that this is compensated by the response of layer 2.

V. Discussion

A. Layers at steady state

For the analysis of how steady state perturbations propagate, it is assumed that when the system is at steady state, each layer is also at steady state. Proposition 1 characterises the set of layers' stoichiometries S^i for which this is true. The condition that the column spaces are disjoint is equivalent to requiring that the summed dimensions of each layer $\sum \mathrm{rank}(S^i)$ is equal to the dimension of the original system $\mathrm{rank}(S)$. We now consider the implications of this assumption for finding a layered decomposition.

Consider layer 1 of the system (15)–(17) with dynamics

$$\begin{bmatrix} \dot{u}^1 \\ \dot{y}^1 \\ \dot{p}^1 \\ \dot{y}^{\star 1} \end{bmatrix} = \begin{bmatrix} 1 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \pi \\ \delta_u u \\ k_{uy} u \\ \delta_y y \end{bmatrix},$$

where $\mathrm{rank}(S^1) = 2$. Suppose we try to further decompose this layer into two sub-layers, 11 and 12. There is no further partition of the columns of S^1 into two groups to form S^{11} and S^{12} which will result in $\mathrm{rank}(S^{11}) = \mathrm{rank}(S^{22}) = 1$. Therefore any further decomposition is not one in which we can use the steady-state methods described above.

Note that in this example the cycle of mass flowing in and out of the layer is broken across the two sub-layers. In fact, mass flow cycles correspond to linear dependencies in the columns of S. Therefore, in order to ensure the layers are at steady state when the system is at steady state, it is sufficient that all mass-flow cycles (including mass flow in and out of the system) are not broken across layers, so that linearly dependent columns of S are not in different layers.

B. Structure in the steady state response

Consider a system decomposed into two layers, both at steady state (e.g. both examples in Section IV). Assuming the only non-zero isolated response is in layer 1, the integrated response is the closed-loop map from the isolated response as an input, as shown in Figure 2. The outputs $\mathrm{d}\bar{x}^i/\mathrm{d}\pi$ are interpreted as the sum of all alternating projections of $\partial\bar{x}^1/\partial\pi$ through M^1 and M^2 in the feedback loop. The alternating products of M^1 with M^2 therefore determine how the initial isolated response propagates.

Now let $L \geq 2$, and consider the complete graph on L nodes. Given any path $p = (i, l_1, \ldots, l_{r-1}, j)$ of r steps from node i to node j, define the matrix $M(p) = M^j M^{l_{r-1}} \ldots M^{l_1}$ as the projection of a perturbation to layer i onto layer j through the path p. Denoting the set of all paths p from i to j by Π_{ji}, then the ji-th block P_{ji} of the matrix

$$P = (P_{ji}) = \begin{bmatrix} I & -M^1 & \ldots & -M^1 \\ -M^2 & I & \ldots & -M^2 \\ \vdots & \vdots & \ddots & \vdots \\ -M^L & -M^L & \ldots & I \end{bmatrix}^{-1}$$

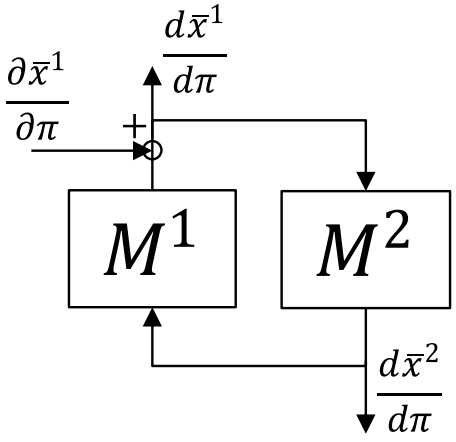

Fig. 2: Suppose the parameter π is perturbed. The map from the response $\partial\bar{x}^1/\partial\pi$ in the isolated layer to the integrated responses $\mathrm{d}\bar{x}^i/\mathrm{d}\pi$ is the closed loop system with static gains M^i given by (9).

is given by the sum $P_{ji} = \sum_{p\in\Pi_{ji}} M(p)$, assuming it converges.

As the number of layers L increases, there are more paths between layers, so that the potential for extremely complex inter-layer interactions will also increase. By decomposing a system into layers, we intend to simplify the analysis. We can keep the various $M(p)$ under control through the following observations. When $M(p)$ is zero, disturbances to layer i cannot propagate to layer j through p. If $\|M(p)\|$ is small, the magnitude of any propagation is limited. When $\mathrm{rank}(M(p))$ is small, only certain types of disturbance to layer i will have an effect in layer j through p.

There may be a number of decompositions of S satisfying the requirement for disjoint column spaces of S^i. To choose between them, we need to define a 'good' layering, so that the system's complexity is distributed amongst layers and as little as possible in the communication between layers. This has an analogy with finding community structure in a network [21]: edge weights within communities are as dense as possible, but sparse between communities. Layering to minimise $\mathrm{rank}(M^j M^i)$ and/or $\|M^j M^i\|$ should ensure that inter-layer communication is kept as simple or weak as possible, respectively. This gives a strategy for future work on the automatic detection of the layered structure of large reaction networks.

C. Dynamic layered architecture

An important feature of (9) is that in nonlinear systems, M^i depends on $\bar{x}$. A layering is therefore not static: layers independent of one another in one operating regime may become tightly coupled in another.

Recall the example (5)–(6). The appropriate steady state of this system depends on the sign of the parameter $\phi = u_0 + x_0 - y_0$. Since we require all concentrations to be non-negative, $(\bar{u}, \bar{x}) = (0, \max(0, \phi))$ is the physical steady state.

Through (9) we can calculate each M^i at this steady state. Independently of ϕ,

$$M^1 = \begin{bmatrix} -1 \\ 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \end{bmatrix}.$$

There are two cases for the other communication matrix:

$$M^2 = \begin{cases} \begin{bmatrix} 0 \\ -1 \end{bmatrix} \begin{bmatrix} 1 & 1 \end{bmatrix} & \text{if } \phi > 0 \\ \begin{bmatrix} 0 \\ -1 \end{bmatrix} \begin{bmatrix} 0 & 1 \end{bmatrix} & \text{if } \phi < 0. \end{cases}$$

If $\phi = 0$, M^2 is not defined due to a singularity in (9).

Independently of ϕ the product $M^1 M^2 = 0$, so that there is no input from layer 2 to layer 1. When $\phi > 0$ we also have $M^2 M^1 = 0$, so that signals will not propagate from layer 1 to 2 either, making the layers independent of each other. However, when $\phi < 0$, the matrix $M^2 M^1 \neq 0$. Then a perturbation to the steady state in layer 1 will propagate to layer 2, giving a cascade layered structure.

This nonlinear example shows that the dependencies of layers are dynamic with respect to the steady state around which it is operating. Perturbations may propagate differently depending on how the layers communicate.

VI. CONCLUSIONS

This paper has developed the concept of layering [22] to apply to biochemical reaction networks without timescale separation. A layered decomposition creates subsystems, called layers, which partition the system's parameters (rather than its state variables) into groups. This generally means grouping reactions, rather than species. All variables may evolve in each layer's state, but each state's contribution is overlaid to give the system behaviour. We have used the layered decomposition to analyse the steady-state response to perturbations in parameters. A condition for this analysis is that each layer's state is stationary at the system's steady state. Having given examples where this assumption is violated, we concluded that a strategy for finding a layered decomposition is to not break cycles of mass flow.

The arguments of the paper centred on the analysis of how the steady state varies with parameter perturbations. The integrated response of each layer's steady state to the parameter perturbation is decomposed into its isolated response through (12). The layers' isolated responses propagate through a sequence of projections M^i to all other layers in closed loop. We have identified features of the closed loop system which may be used in future to develop an algorithmic approach to automatically finding layered decompositions of complicated systems. We have shown that nonlinearity means that the layered architecture of the system is dynamic: different operating regimes change how disturbances propagate through a system. Therefore layering must also take into account the context in which it is operating, and is not a function of stoichiometric structure only.

Other areas for future work include an analysis of dynamic signal propagation, with applications for error estimation and model reduction. For example, consider (5)–(6). Perturbing α by some small amount will not alter the steady state, but the transient signal will be affected. The new signal will propagate to the second layer (6) and result in a new output. Understanding how dynamic signal propagation occurs will give a fuller picture of how the controllability of isolated layers translates to that of the full system.

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