2013 American Control Conference (ACC)
Washington, DC, USA, June 17-19, 2013

Layering in networks: The case of biochemical systems

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Abstract— **Networked systems are characterised by their scale and structure. In particular, biochemical reaction networks involve complicated interconnections of chemical reaction pathways and cycles, occurring on a number of different time and space scales even within a cell. This paper seeks to formalise a method of layering the dynamics of a biochemical network by decomposing its stoichiometric matrix into a sum of stoichiometric matrices, each of which we identify with a layer. We derive a condition to test when a given layer directly communicates with another. We also examine singular perturbation by considering decomposition into fast and slow layers, characterising the approximate dynamics through the quasi-steady state approximation in terms of a perturbation of the dynamics of the slow layer.**

I. INTRODUCTION

Networked systems are characterised by their scale and structure [3]. They are structured, but also highly complicated, operating on many time and space scales and with many interacting functional components. Complexity provides robustness to most environments, although it also allows for catastrophic failures in unexpected conditions [1], [29]. In order to understand such networks, the idea of *layering* is a useful abstraction. By decomposing the system into layers, then the problem of understanding the system decomposes into understanding the functionality of its layers, and the protocols with which these communicate [10], [11].

Biological systems, such as biochemical networks, are complicated systems currently under extensive research. These exist on many scales with complicated interconnections and feedback loops. A layering approach is important for understanding such complexity. For example, in [12], [22], the authors decompose a model of the heat shock response (hsr) within a single *E. coli* cell into modules of chemical species with various functions and identify layers (termed "flux modules") of interconnecting feedback and feedforward loops between these functional modules. Other biochemical models, such as those for glycolysis [6] and the *lac* operon [31] are similarly layered.

However, when considering the cell as a whole, each biochemical model is itself part of a much larger cellular architecture. For simplicity it is analysed (and layered) independently of the other cellular functions, but it is also important to appreciate that any model of a biochemical pathway or function is itself a single layer within the complex interconnection of other layers which make up the cell. A key challenge for systems biology is to understand how these layered models are integrated.

There have been a number of different conceptual approaches to layering. In terms of mathematical formulations, a key concept is that of a multislice network [24]. These are made up of a collection of network slices, where inter-slice connections between corresponding nodes connect the slices together. This approach of overlaying networks, anchored by inter-slice connections, has a parallel in the approaches in [13] and [15] to modelling layered networks, with applications to overlaid rail and air transport systems.

Layering has also been approached from the perspective of optimisation decomposition [7]. If a network is modelled as solving an optimisation problem, layering corresponds to the partition of the problem into sub-problems at lower layers, coordinated by a master problem at a higher layer. Such coordination is seen in the hierarchical layering of networks of communicating autonomous systems [28], [30], [32]. Here a large communication network is partitioned into sub-networks, each of which coordinate a subset of agents, and which also communicate with one another (through one leader, or some other low-rank information) via a higher-layer communication network.

In addition to these formulations of layers in complex networks, models of biochemical reaction networks have had similar approaches to layered decompositions, using the same vocabulary [17], [5], [4]. These approaches give a specific definition of a layer in a biochemical network such that mass flow along reactions can only occur within it, but not between layers. This results in a natural layering: transcription and translation do not consume DNA or mRNA, so there is no mass flow from these layers to metabolic and protein layers. Similarly, gene promoters are not consumed when promoting a certain gene's transcription, so remain in another layer.

In this paper we will define layers in a biochemical network without assuming a given structure of mass flow. Time-scale separation will be used as a motivating example, and we will examine how singular perturbation can be approached through layering.

NOTATION

In describing biochemical networks of n chemical species, X_i will denote the ith species for $i = 1,\dots,n$ and x_i will denote the concentration of X_i. The concentration vector is $x = (x_1,\dots,x_n)^T$ where T denotes transpose. When layering, x^k will denote the state of the kth layer, and x_i^k the ith component of the kth layer. Similarly, superscript natural numbers k (such as f^k, S^k and v^k and so on) will refer to the kth layer. In summary: subscripts refer to components, and superscripts to layers. We will write "Lk" as shorthand for "layer k". Approximated system states will be denoted by $\tilde{x}$.

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978-1-4799-0178-4/$31.00 ©2013 AACC

We will use $\dot{z} = \mathrm{d}z/\mathrm{d}t$ for differentiation w.r.t. time t. For a function $\phi : \mathbb{R}^p \to \mathbb{R}^q$ we will denote the partial derivatives of $\phi(x)$ by $\partial\phi_i/\partial x_k$, and denote the Jacobian matrix $J\phi = \partial\phi/\partial x = (\partial\phi_i/\partial x_k)_{ik}$ interchangeably.

For $S \in \mathbb{R}^{n\times m}$ we will use $\ker(S) = \{v \in \mathbb{R}^m \mid Sv = 0\}$ to refer to the kernel of S, and $\mathrm{im}(S) = \{Sv \mid v \in \mathbb{R}^m\}$ for the image of S. For any subspace V of $\mathbb{R}^n$, we denote the perpendicular complement $V^\perp = \{w \in \mathbb{R}^n \mid w^T v = 0 \ \forall v \in V\}$.

II. DECOMPOSITION INTO LAYERS

A. Biochemical network representation

Consider a biochemical reaction network which can be described using a stoichiometric matrix S and flux vector $v(x)$ [26]. To illustrate these structures, consider a single reaction transforming one species to another: $X_1 \xrightarrow{k} X_2$. The rate of this reaction, using the law of mass action, is proportional to the concentration x_1 of species X_1 so that the single component in the flux vector is $v(x) = kx_1$. In this reaction, for every one molecule of species X_1 that is consumed, one molecule of X_2 is produced. Thus the stoichiometry is the matrix with a single column $S = [-1,1]^T$. Writing $x = [x_1, x_2]^T$, the ODE system describing this reaction network is

$$\dot{x}_1 = -kx_1$$
$$\dot{x}_2 = kx_1$$

or $\dot{x} = Sv(x)$. This representation applies to any network made up of n species taking part in m reactions.

Let $x(t) \in \mathbb{R}^n$ be the vector of concentrations x_i of n species X_i. Consider reaction j, which can be written

$$\sum_i \alpha_{ij} X_i \to \sum_i \beta_{ij} X_i$$

for integer-valued α_{ij} and β_{ij}. If $\alpha = (\alpha_{ij})_{ij}$ and $\beta = (\beta_{ij})_{ij}$, then the stoichiometric matrix $S = \beta - \alpha$. In the example above, $\alpha = [1,0]^T$ and $\beta = [0,1]^T$, giving $S = [-1,1]^T$. The jth column of S therefore describes the net result of reaction j by totalling how many molecules of each species are consumed or produced by the reaction.

Finally we construct $v(x)$, where $v(x)_j$ is the rate of the jth reaction. By the law of mass action, $v(x)_j$ is proportional to $\prod (x_i)^{\alpha_i}$. However, the reaction rate may also depend on the concentration of some species not involved in the mass flow of the reaction itself, for instance through catalysis by enzymes, or competitive exclusion by other proteins. For an example of this, see reaction 4 in Figure 1.

In summary: the time evolution of the biochemical system is modelled by the system of ODEs

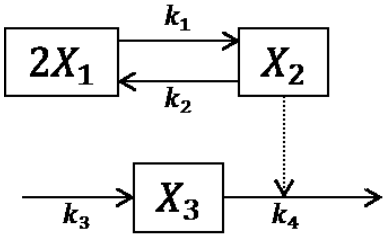
$$\dot{x}(t) = Sv(x(t)) \tag{1}$$

where initial conditions $x(0) = x_0$ are also specified.

B. Layering a system

We will define a decomposition of a general biochemical network with dynamics (1) into L layers by decomposing the stoichiometry S into a sum of matrices $S = S^1 + \cdots + S^L$. With each layer $i = 1,\ldots,L$ we associate a state vector $x^i \in \mathbb{R}^n$ and define its dynamics as

$$\dot{x}^i(t) = S^i v(x(t)) \tag{2}$$



$$S = \begin{bmatrix} -2 & 2 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \qquad v(x) = \begin{bmatrix} k_1(x_1)^2 \\ k_2 x_2 \\ k_3 \\ k_4 x_2 x_3 \end{bmatrix}$$

Fig. 1: Four reactions with three species. Species X_3 is created and degrades from the environment, while species X_1 and X_2 are reversibly formed from one another. Reaction 1 takes two molecules of X_1 into one of X_2, giving the first column of S, and so on. Species X_2 catalyses reaction 4 without any mass flow. Note that S is block diagonal. The system ODE is $\dot{x} = Sv(x)$.

with initial conditions $x^i(0) = 0$. Putting $x(t) = x_0 + \sum_i x^i(t)$, where x_0 are the system initial conditions, recovers the dynamics in (1). The problem in this case is therefore how to decompose S into a meaningful matrix sum, so that the resulting layers enable us to understand the underlying structure of the network.

a) Layering by species: We may define layers by partitioning the chemical species into groups. If we partition the components of $x = \begin{bmatrix}\hat{x}^1 & \cdots & \hat{x}^L\end{bmatrix}^T$ then the rows of S will split conformally into $S = \begin{bmatrix}\hat{\Sigma}^1 & \ldots & \hat{\Sigma}^L\end{bmatrix}^T$. We can then define each layer's stoichiometry by $S^i = \begin{bmatrix}0 & \hat{\Sigma}^i & 0\end{bmatrix}^T$ and put this S^i into equation (2). It is clear that the vectors $\dot{x}^i$ are then in orthogonal subspaces of $\mathbb{R}^n$, and that the dynamics of each subset of species in the partition of x is associated with a unique layer. Therefore (2) reduces to

$$\dot{\hat{x}}^i = \hat{\Sigma}^i v(x)$$

for $i = 1,\ldots,L$ in this case.

b) Layering by fluxes: Alternatively, we may define layers by partitioning the reactions into groups. If we partition the components of $v(x) = \begin{bmatrix}v^1(x),\ldots,v^L(x)\end{bmatrix}^T$ for $v^i(x)$ taking values in $\mathbb{R}^{m^i}$, then we can conformally group together the columns of $S = [\Sigma^1,\ldots,\Sigma^L]$. We can then define each layer's stoichiometry by $S^i = [0,\Sigma^i,0]$ and put this S^i into (2). The fluxes are partitioned by this construction of S^i, so that the dynamics for each layer can be written

$$\dot{x}^i = S^i v(x) = \Sigma^i v^i(x). \tag{3}$$

Remark 1: Our decomposition method has some similarity to the methods of expansion [18], [19], [33], reviewed in [2]. Both approaches increase the state space dimension of the system to create interconnected subsystems. However, the expansion methods use the principle of system inclusion to go between the expanded and original system, forcing overlapping variables to take equal values, while we simply sum the state vectors to retrieve the original dynamics. This

formulation also generalises layers from the requirement in [20], [5] that the stoichiometric matrix is block diagonal, to allow retroactive coupling [8] between layers (see Section IV-B).

III. LAYER CONNECTION TOPOLOGY

Whether we choose $\{S^i\}$ to layer S by flux or by species, we can write the system dynamics as

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

$$x(t) = x_0 + \sum_{i=1}^{L} x^i(t) \tag{5}$$

Each layer's dynamics (4) may depend also on the state of any other layer. The aim of this section is to make clear the conditions under which some layers have no influence on others, defining an interconnection topology.

To work out whether a given layer Lk talks to Li we can rewrite (4) slightly differently to give

$$\dot{x}^i(t) = S^i v(x^k(t) + \xi^k(t))$$

where $\xi^k(t) = x(t) - x^k(t)$. If, for arbitrary $\xi \in \mathbb{R}^n$, we have

$$S^i v(x^k(t) + \xi) = S^i v(\xi) \tag{6}$$

for all allowed values of x^k, then it is clear that Lk does not talk to Li. To test (6) we need to clarify which values of x^k are allowed. As $\dot{x}^k$ is in the image space of S^k, and $x^k(0) = 0$, we know that $x^k(t) \in \mathrm{im}\left(S^k\right)$. Thus a more precise formulation of condition (6) is

$$v(x^k + \xi) - v(\xi) \in \ker\left(S^i\right) \quad \forall x^k \in \mathrm{im}\left(S^k\right) \tag{7}$$

for any $\xi \in \mathbb{R}^n$. The result below expresses this condition in terms of the Jacobian of v and the two stoichiometric matrices S^i and S^k.

Lemma 1: For each i let $U^i \in \mathbb{R}^{n \times r^i}$ be any matrix with columns which form a basis of the column space $\mathrm{ColSp}(S^i)$, which has dimension r^i. Let $C^i \in \mathbb{R}^{m^i \times r^i}$ be any matrix with columns which form a basis of the row space $\mathrm{RowSp}(S^i)$ (which is also of dimension r^i). Condition (7) is equivalent to

$$(C^i)^T (Jv) U^k = 0 \tag{8}$$

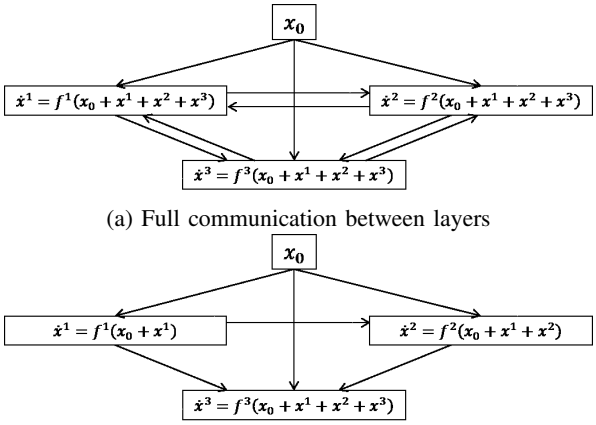
for the Jacobian $Jv(z)$ of $v(z)$, for all possible values of $z \in \mathbb{R}^n$.

Proof: By the definition of U^k, any $x^k \in \mathrm{im}(S^k)$ can be uniquely described by a vector $\lambda \in \mathbb{R}^{r^k}$ through the relation $x^k = U^k \lambda$. Then (7) is true for all $x^k \in \mathrm{im}(S^k)$ if and only if it is true for all $\lambda \in \mathbb{R}^{r^k}$.

Since, from the definition of C^i, its columns form a basis for the space $\mathrm{RowSp}(S^i) = (\ker(S^i))^\perp$, any vector $y \in \ker(S^i)$ if and only if $(C^i)^T y = 0$. Thus we can re-write (7) by taking $y = v(x^k + \xi) - v(\xi)$. Rewriting $x^k = U^k \lambda$, we therefore require that $(C^i)^T (v(U^k \lambda + \xi) - v(\xi)) = 0$.

Suppose we construct the function

$$\theta_{ik}(\xi, \lambda) = (C^i)^T \left(v\left(\xi + U^k \lambda\right) - v(\xi) \right)$$



(a) Full communication between layers

(b) Inferred communication structure between layers

Fig. 2: Suppose a system has three layers, with initial conditions feeding into all three. Figure 2a shows the general interconnection between any three layers. Figure 2b is an example where the each layer's dynamics have been shown to depend on only a subset of other layers. The resulting topology means that the layers can be analysed in sequence from 1 to 3, with no feedback loops.

for $\xi \in \mathbb{R}^n$ and $\lambda \in \mathbb{R}^{r^k}$. Then we can combine the two facts above to deduce that (7) is equivalent to requiring $\theta_{ik}(\xi, \lambda) = 0$ for all $\lambda \in \mathbb{R}^{r^k}$.

If we can prove that $\theta_{ik}(\xi, \lambda)$ is constant as λ varies, then since $\theta_{ik}(\xi, 0) = 0$ the result will follow. Differentiating θ_{ik} with respect to λ means that we require

$$\frac{\partial \theta_{ik}}{\partial \lambda} = (C^i)^T \frac{\partial}{\partial \lambda} v(\xi + U^k \lambda) = 0$$

for all values of λ and ξ. Using the chain rule,

$$(C^i)^T \frac{\partial}{\partial \lambda} v(\xi + U^k \lambda) = (C^i)^T (Jv) U^k$$

and (8) follows. ■

Once we have determined the interconnection topology we can analyse the system as a whole by treating layers and their interconnections separately. This is particularly true if we have a hierarchical layering, such as in Figure 2. If there are no (directed) cycles of communication then the layers can be analysed in "order": there is an indexing of the layers such that the inputs to any layer Li will depend only on the outputs of layers Lj with $j < i$. Thus any perturbations will propagate through this sequence in cascade, without any complicating feedback loops.

IV. TIME-SCALE SEPARATION

Singular perturbation is a standard analytical tool with which large, complicated systems are simplified [14], [21]. It is very commonly employed on biochemical network models to enable simulation or analytical insight [25]. Standard singular perturbation, through the separation of two time scales, classifies variables (i.e. species) as either fast or slow by the dynamics

$$\dot{x} = f(x, y)$$
$$\varepsilon \dot{y} = g(x, y)$$

where f and g are of the same order ~ 1 and the parameter $\varepsilon \ll 1$ is small. Then y is fast, since $\dot{y} \sim 1/\varepsilon$ is large in comparison to $\dot{x}$. However, as noted in [23], in biochemical networks it is not necessarily species which are fast or slow, but *reactions*: each species can take part in both fast and slow reactions. Applying standard singular perturbation techniques often requires a transformation of the state space in order to separate out fast and slow variables.

In Section II we discussed two decompositions of $\dot{x} = Sv(x)$ into layers, either by flux or by species, but not how to choose the decomposition. Whether we have fast reactions or fast variables, time-scale separation presents itself as a natural decomposition of the network. If fast reactions, we can partition the flux vector (and therefore the columns of S) into groups with a common scale; if fast species, we simply partition the rows of S accordingly.

Since in general it is reactions that are fast or slow, to formulate the system in terms of fast and slow species requires a transformation of the state space. In a biochemical setting, the transformed variables may then be physically meaningless quantities. We will instead consider the singular perturbation of fast and slow fluxes, which does not require any transformations.

A. Two time scales

Suppose we have a biochemical network whose flux vector v can be partitioned into

$$v(x) = \begin{bmatrix} v^1(x) \\ v^2(x)/\varepsilon \end{bmatrix}$$

where $v^i \sim 1$ are both of the same order. Since v^i are both functions of x, we will assume that the system has been non-dimensionalised so that each component x_k is of the same order too. If we group the columns of $S = [\Sigma^1, \Sigma^2]$ conformally with the partition of v, the system dynamics can be written

$$\dot{x} = Sv(x) = \Sigma^1 v^1(x) + \frac{1}{\varepsilon}\Sigma^2 v^2(x). \tag{9}$$

Standard singular perturbation techniques would attempt to transform the vector x to determine fast and slow variables. We will instead create two layers: one fast, and one slow.

As we are layering by flux, we can apply (3) to the two-time-scale decomposition (9) to give

$$\dot{x}^1 = S^1 v(x_0 + x^1 + x^2) = \Sigma^1 v^1(x_0 + x^1 + x^2) \tag{10}$$

$$\varepsilon \dot{x}^2 = \varepsilon S^2 v(x_0 + x^1 + x^2) = \Sigma^2 v^2(x_0 + x^1 + x^2) \tag{11}$$

where x_0 is the initial condition, and $x^i(0) = 0$. Standard singular perturbation requires us to partition $x \in \mathbb{R}^n$, into two orthogonal subspaces with *total* dimension n. However, (10)–(11) gives fast and slow variables, where instead each $x^i \in \mathbb{R}^n$. Nevertheless, since $x = x^1 + x^2$, we lose no meaning with the new variables: instead, x^i_k is simply the amount of the species x_k in layer i.

Remark 2: Note that, in the remainder of this section, we will be working with $\Sigma^i \in \mathbb{R}^{n \times m^i}$ as opposed to the general layered stoichiometry $S^i \in \mathbb{R}^{n \times m}$.

As with standard singular perturbation, the system (10)–(11) with state x can be approximated by letting $\varepsilon \to 0$, known as the quasi-steady state approximation (QSSA), to give an approximate layered state $\tilde{x} = x_0 + \tilde{x}^1 + \tilde{x}^2$. This gives $\Sigma^2 v^2(x_0 + \tilde{x}^1 + \tilde{x}^2) = 0$ or, equivalently, $v^2(x_0 + \tilde{x}^1 + \tilde{x}^2) \in \ker(\Sigma^2)$. This, together with requiring $\tilde{x}^2 \in \mathrm{im}(\Sigma^2)$, means that the output $\tilde{x}^2$ from L2, given inputs x_0 and $\tilde{x}^1$, belongs to the set

$$\tilde{x}^2 \in \left\{ z \in \mathrm{im}\left(\Sigma^2\right) \mid v^2\left(x_0 + \tilde{x}^1 + z\right) \in \ker\left(\Sigma^2\right) \right\}. \tag{12}$$

We want to show that (12) defines a function ϕ mapping the input $\tilde{x}^1 + x_0$ to the unique output $\tilde{x}^2$ of the QSSA approximation of L2. The result below shows that, as $\varepsilon \to 0$, the (local) existence of $\tilde{x}^2 = \phi(x^1 + x_0)$ depends on the Jacobian Jv^2, through the application of the Implicit Function Theorem.

Lemma 2: Let $U \in \mathbb{R}^{n \times r^2}$ be a matrix, the columns of which form a basis for $\mathrm{ColSp}(\Sigma^2) = \mathrm{im}(\Sigma^2)$. Also let the matrix $C \in \mathbb{R}^{m^2 \times r^2}$ have columns which form a basis for $\mathrm{RowSp}(\Sigma^2) = (\ker(\Sigma^2))^\perp$. Suppose we know a point $x = c$ such that $\Sigma^2 v^2(c) = 0$. Assume that the Jacobian $Jv^2(c)$ evaluated at c is such that

$$C^T Jv^2(c) U \in \mathbb{R}^{r^2 \times r^2}$$

is non-singular.

Then for each $\xi^\star \in c + \mathrm{im}(\Sigma^2)$ there exists an open set A containing $\xi^\star$, and a unique differentiable function $\phi : A \to \mathrm{im}(\Sigma^2)$ such that $\tilde{x}^2 = \phi(\tilde{x}^1 + x_0)$ is on the manifold defined by (12) for all $\tilde{x}^1 + x_0 \in A$.

Proof: Denoting $\xi = x_0 + \tilde{x}^1$ as the external input into L2, we want to show that there exists a function $\tilde{x}^2 = \phi(\xi)$ defined by the manifold (12). The set in (12) is a subset of $\mathrm{im}(\Sigma^2)$, which has dimension $r^2 = \mathrm{rank}(\Sigma^2)$. The columns of $U \in \mathbb{R}^{n \times r^2}$ form a basis for $\mathrm{im}(\Sigma^2)$. Then we can write $\tilde{x}^2 = U\lambda$ for a unique $\lambda \in \mathbb{R}^{r^2}$.

The columns of $C \in \mathbb{R}^{m^2 \times r^2}$ form a basis for $(\ker(\Sigma^2))^\perp$. Then the condition $v^2 \in \ker\left(\Sigma^2\right)$ is equivalent to $C^T v^2 = 0$.

We want to prove the existence of a function ϕ such that $\tilde{x}^2 = \phi(\xi)$ or, equivalently since $\tilde{x}^2 = U\lambda$, we want to find $\lambda = \Lambda(\xi)$. Define the function $\theta : \mathbb{R}^n \times \mathbb{R}^{r^2} \to \mathbb{R}^{r^2}$ such that

$$\theta(\xi, \lambda) = C^T v^2(\xi + U\lambda). \tag{13}$$

where $\xi = x_0 + x^1$ is the independent input. Requiring that $v^2(\xi + \tilde{x}^2) \in \ker\left(\Sigma^2\right)$ is equivalent to requiring that $\theta(\xi, \lambda) = C^T v^2(\xi + U\lambda) = 0$. We will use the Implicit Function Theorem to show that this relation admits $\lambda = \Lambda(\xi)$. Since $C^T v^2(c) = 0$ by the assumption on c, then for any $\lambda^\star \in \mathbb{R}^{r^2}$ we can put $\xi^\star = c - U\lambda^\star$ to fix $\theta(\xi^\star, \lambda^\star) = 0$.

Then if $\frac{\partial \theta}{\partial \lambda}$ evaluated at $(\xi^\star, \lambda^\star)$ is invertible there exists an open set $A(\xi^\star) \subset \mathbb{R}^n$ containing (and dependent on) $\xi^\star$ and open $B(\lambda^\star) \subset \mathbb{R}^{r^2}$ containing (and dependent on) $\lambda^\star$, and unique $\Lambda : A \to B$, such that $\theta(\xi, \Lambda(\xi)) = 0$, and therefore $\tilde{x}^2 = U\Lambda(\xi)$ is on the manifold (12) for all $\xi \in A$.

We now need to determine when $\frac{\partial \theta}{\partial \lambda}$ evaluated at $(\xi^\star, \lambda^\star)$ is invertible. This matrix, by application of the chain rule, is

given by

$$\frac{\partial \theta}{\partial \lambda} = C^T \frac{\partial}{\partial \lambda} v^2(\xi + U\lambda) = C^T \left(Jv^2\right) U \tag{14}$$

for the Jacobian Jv^2 evaluated at $\xi^\star + U\lambda^\star = c$. By assumption, this matrix is invertible, and holds for any $\xi^\star = c - U\lambda^\star \in c + \mathrm{im}(\Sigma^2)$. ∎

Using this lemma we can show that when x^2, under the QSSA, can be approximated by a function $\tilde{x}^2 = \phi(x_0 + \tilde{x}^1)$ we have

$$\dot{\tilde{x}}^1 = \Sigma^1 v^1(x_0 + \tilde{x}^1 + \phi(x_0 + \tilde{x}^1))$$

now autonomous. Then $\tilde{x} = x_0 + \tilde{x}^1 + \phi(x_0 + \tilde{x}^1)$ forms the approximated trajectory. A quantification of the error of the approximation, for example $\int_0^\infty (x - \tilde{x})^2 \, \mathrm{d}t$, can be calculated using Sum of Squares methods as discussed by our previous work [27].

The lemma above only proves existence. The following corollary is used to deduce information about the derivative of the implicit function.

Corollary 1: Under the QSSA, when $\tilde{x}^2 = \phi(x_0 + \tilde{x}^1) = \phi(\xi)$, the Jacobian of $\tilde{x}^2$ when differentiated by ξ is given by

$$\frac{\partial \tilde{x}^2}{\partial \xi} = -U \left(C^T \left(Jv^2\right) U\right)^{-1} C^T \left(Jv^2\right). \tag{15}$$

Proof: A corollary of the Implicit Function Theorem is that, if $\theta(\xi, \lambda) = 0$ gives $\lambda = \Lambda(\xi)$, then

$$\frac{\partial \lambda}{\partial \xi} = -\left(\frac{\partial \theta}{\partial \lambda}\right)^{-1} \frac{\partial \theta}{\partial \xi}.$$

Since $\tilde{x}^2 = U\lambda$ we have

$$\frac{\partial \tilde{x}^2}{\partial \xi} = U \frac{\partial \lambda}{\partial \xi}$$

and noting (14) and, similarly deriving $\frac{\partial \theta}{\partial \xi} = C^T(Jv^2)$, we find (15). ∎

Remark 3: Note that the Jacobian (15) is invariant to the basis chosen to construct both U and C.

B. Layers and retroactivity

We can approach the concept of retroactivity [8], [9], [16] using the framework of layering. Suppose we have two isolated systems $\dot{x}^i = f^i(x^i)$. If the first system is upstream of the second, and the two are connected, the principle of retroactivity is that the interconnection of the systems may cause the upstream system dynamics to change from the isolated case. The example in [8] considers the upstream system with isolated dynamics

$$\dot{X} = k - \delta X$$

and the interconnected dynamics

$$\dot{X} = k - \delta X + \frac{\delta}{\varepsilon} C - \frac{\delta}{k_d \varepsilon} (p_{TOT} - C) X$$
$$\dot{C} = -\frac{\delta}{\varepsilon} C + \frac{\delta}{k_d \varepsilon} (p_{TOT} - C) X$$

for small $\varepsilon \ll 1$. Clearly, this system can be partitioned into two layers such that

$$\dot{x}^1 = \begin{bmatrix} 1 & -1 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} k \\ \delta x_1 \end{bmatrix} \tag{16}$$

$$\varepsilon \dot{x}^2 = \begin{bmatrix} 1 & -1 \\ -1 & 1 \end{bmatrix} \begin{bmatrix} \delta x_2 \\ \frac{\delta}{k_d}(p_{TOT} - x_2) x_1 \end{bmatrix}, \tag{17}$$

where $x_1 = [X]$ and $x_2 = [C]$ are the species concentrations. The authors then take the limit as $\varepsilon \to 0$ to quantify the effect of L2 on L1: the "retroactivity to the output".

More generally, suppose we have an isolated slow upstream system $\dot{z}^1 = f^1(z^1)$, a much faster isolated fast downstream system $\dot{z}^2 = f^2(z^2)/\varepsilon$, and the interconnected system $\dot{x} = f^1(x) + f^2(x)/\varepsilon$ with $f^i(x) = \Sigma^i v^i(x)$. Decomposing the full system gives

$$\dot{x}^1 = \Sigma^1 v^1(x_0 + x^1 + x^2) \tag{18}$$

$$\varepsilon \dot{x}^2 = \Sigma^2 v^2(x_0 + x^1 + x^2) \tag{19}$$

and, in the limit as $\varepsilon \to 0$, we can use the arguments of Section IV-A to find $\tilde{x}^2 = \phi(\tilde{x}^1)$ using the QSSA. Since $\tilde{x} = \tilde{x}^1 + \tilde{x}^2$ we have that

$$\dot{\tilde{x}} = \dot{\tilde{x}}^1 + \dot{\tilde{x}}^2 = \left(I + \frac{\partial \phi}{\partial x^1}\right) \Sigma^1 v^1(\tilde{x}) \tag{20}$$

for the identity matrix I and Jacobian $\frac{\partial \phi}{\partial x^1}$. Thus, through the Jacobian, we can quantify the retroactivity from the fast layer to the slower layer.

Corollary 1 allows us to write the retroactivity term $\frac{\partial \phi}{\partial x^1}$ in (20) as a function of three quantities: U, whose columns are a basis for the column space of Σ^2; C, whose columns form a basis for the row space of Σ^2); and Jv^2, the Jacobian of the fast dynamics v^2. The results are summarised in the following theorem.

Theorem 1: Consider a biochemical network with n species and m reactions with time-scale separation such that

$$\dot{x} = \begin{bmatrix} \Sigma^1 & \Sigma^2 \end{bmatrix} \begin{bmatrix} v^1(x) \\ v^2(x)/\varepsilon \end{bmatrix}.$$

Choose a matrix $U \in \mathbb{R}^{n \times r^2}$ whose columns form a basis for the column space of Σ^2, and similarly choose $C \in \mathbb{R}^{m^2 \times r^2}$ whose columns form a basis for the row space of Σ^2. Letting Jv^2 represent the Jacobian of v^2, if $C^T(Jv^2(\tilde{x}))U$ is invertible then the dynamics of the system under the quasi-steady state approximation as $\varepsilon \to 0$ are

$$\dot{\tilde{x}} = (I + M(\tilde{x}))\Sigma^1 v^1(\tilde{x}) \tag{21}$$

where

$$M(\tilde{x}) = -U \left(C^T(Jv^2(\tilde{x}))U\right)^{-1} C^T(Jv^2(\tilde{x}))$$

is the derivative of $\tilde{x}^2$ with respect to $\tilde{x}^1$.

Proof: By the results in Section IV-A, we can write $\tilde{x}^2 = \phi(\xi)$ for $\xi = x_0 + \tilde{x}^1$ and we know that $\partial \tilde{x}^2 / \partial \tilde{x}^1 = \partial \tilde{x}^2 / \partial \xi = M$. Equation (20) completes the proof. ∎

Remark 4: In the case of two time scales, by calculating M we can directly analyse the singularly perturbed dynamics under the QSSA without needing to transform variables, and

without needing to explicitly determine the output of the fast layer $\tilde{x}^2$ given its input $x_0+\tilde{x}^1$.

Remark 5: When v^2 is linear, M is constant. When v^2 is nonlinear, M becomes a (potentially nonlinear) function of $\tilde{x}$. Thus, in this case, the retroactivity to the slow dynamics depends not just on parameters but also the state. For example, if the region of state space for which $M(x)$ is very small is also in some sense invariant under the slow dynamics, then this is a low-retroactivity operating region.

Applying this result to the example (16)-(17) gives

$$I+M(x)=\frac{1}{p_{TOT}+k_d+x_1-x_2}\begin{bmatrix} x_1+k_d & x_1+k_d \\ -x_2+p_{TOT} & -x_2+p_{TOT}\end{bmatrix}$$

as the state-dependent perturbation to the dynamics of the slow system which results in the approximated system.

V. CONCLUDING REMARKS

We have presented a framework for decomposing networks into layers. It is applied to biochemical networks, where layers arise through partitioning either the rows or columns of the stoichiometric matrix. We derived a condition to determine how they are interconnected. In the case where layers are formed naturally by time-scale separation, we used this framework to carry out singular perturbation under the quasi-steady state assumption. We derived the system dynamics under the QSSA as a perturbation of the slow dynamics, interpreted as the retroactive effect of downstream fast dynamics on upstream slow dynamics.

The perturbation was found in terms of the row and column spaces of the fast stoichiometry, and the Jacobian of the fast dynamics. Through this result, we expressed the approximated system without transforming the state space, or to determine the output of the fast layer as an explicit function of the slower layer's state. Using (20) for the retroactive perturbation, we have also quantified retroactivity in time-scale separated systems.

Future work will apply these tools to other network dynamics classes (e.g. to network consensus problems) and develop techniques for the control and synthesis of layered biochemical networks by investigating the interconnected dynamics of layers on a common timescale.

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