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Observability Based Parameter Identifiability for Biochemical Reaction Networks

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Abstract— **In systems biology, models often contain a large number of unknown or only roughly known parameters that must be identified. This work examines the question of whether or not these parameters can in fact be estimated from available measurements. We consider identifiability of unknown parameters in biochemical reaction networks obtained from first-principles-modeling of metabolic and signal transduction networks. Such systems consist of continuous time, nonlinear differential equations. Several methods exist for answering the question of identifiability for such systems; many of which restate the question of identifiability as one of observability. We consider the application of such methods to a representative biological system: the NF-κB signal transduction pathway. It is shown that existing observability based strategies, which rely on finding an analytical solution, require significant simplifications to be applicable to systems biology problems which are often not feasible. For this reason, a new method based on the use of an 'empirical observability Gramian' for checking identifiability is proposed. This method is demonstrated through the use of a simple biological example.**

I. Introduction

In systems biology, models generally contain a large number of unknown or only roughly known parameters. Accurate knowledge of these parameter values is important for describing and analyzing the dynamics and behaviour of biological systems. This can be done using one of several existing parameter identification strategies (see eg. [1–3]) which all involve the fitting of measurement data. However, these methods are often difficult to apply in practice and offer no guarantee that available measurements will yield meaningful values for the desired parameters. For this reason, it is important to first consider the question of whether or not parameters can in fact be determined (uniquely) from a given model and choice of measurements. This identifiability analysis is required to ensure the mathematical significance of the estimated parameter values and can also be a valuable tool for experimental design.

We focus on identifiability of nonlinear systems in continuous time as this is generally the result of first-principles-modeling of biochemical reaction networks. Several methods exist for checking identifiability of this type of system such as those found in [4–6]. However, such methods were not developed with biological systems in mind so do not necessarily address the specific challenges posed by this type of problem.

Identifiability methods can be roughly divided into two categories: those that check for functional relationships between parameters through simulation, optimization and parameter estimation such as [7–9] and those which restate the question of identifiability as one of observability by extending the state space to include parameters, see eg. [4–6, 10–12].

In this work, we specifically consider observability based methods. We look at existing methods in the context of a representative systems biology problem: the NF-κB signal transduction pathway. This example serves to highlight some of the limitations of the use of previous methods for biological systems. To overcome the specific challenges presented by existing methods, we propose a new approach for checking identifiability based on the use of the 'empirical observability Gramian' presented in [13, 14]. This method is simulation based so does not require an analytical solution to be found as is the case for the other methods. Ideally, this will allow for identifiability analysis of larger and more complex systems such as those found in biology. This approach is demonstrated on the relatively simple biological example of microbial growth with Michaelis-Menten kinetics as described in [15–17].

This paper is structured as follows. Section II contains a review of existing observability based parameter identifiability methods as well as their application to a representative systems biology problem. This provides the motivation for the following sections. In Section III, a new method for checking identifiability based on the use of an 'empirical observability Gramian' is proposed to overcome the challenges highlighted in Section II. Section IV deals with the application of this new method to a simple biological example as well as other computational considerations. Conclusions and directions of future work are discussed in Section V.

II. Background and Motivation

There are many specific challenges to consider when dealing with parameter identification and identifiability of biological systems. For example, in traditional systems theory, systems are often linearized or worked with in discrete time for

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analysis purposes. This is not a desirable approach for biological systems as parameters have physical significance, such as describing reaction rates, which would be lost. For this reason, methods dealing with nonlinear systems in continuous time are considered. Since most methods were not developed with this specific purpose in mind, they are discussed here in the context of their relevance to systems biology problems.

A. Review of Identifiability Methods

We are specifically concerned with structural or *a priori* identifiability: whether or not parameters can be identified from a given model structure and outputs without considering possible measurement noise (as opposed to 'practical identifiability' in [7–9] which considers the quality of the data as well). Many of these methods are based on restating the question of identifiability as one of observability by expanding the state space to include parameters. For this reason, these are the methods focused on here.

One such method based on a power series expansion as described in [6, 11]. It involves determining whether or not one can express the system outputs and its derivatives in terms of the unknown parameters using a Taylor series expansion. The complexity of the method has been found to increase greatly with system size [11] and it generally only provides local identifiability results.

In [10, 11] a method using local state isomorphism theory is employed. This method consists of solving a set of partial differential equations for a set of parameters and determining the uniqueness of the solution.

Differential algebra methods, such as those found in [4, 5, 12, 18], are based on using differentiation and algebraic manipulation to find an observable representation of the system in a structured manner. This can then be used to evaluate if it is in fact possible to express parameters in terms of measurable quantities through rank considerations. This method does not require previous knowledge of the system such as initial conditions which would need to be determined experimentally. This condition is especially important for *a priori* identifiability. However, the downside is that the computation becomes increasingly complex with more states and parameters which could pose a problem for biological systems.

B. Motivation for New Approach

We use the NF-κB signal transduction pathway from [19] as a framework by which to discuss and evaluate existing observability based identifiability strategies and to offer motivation for the new Gramian based approach.

The original model from [19] consists of 15 states and 29 parameters. In order to facilitate analysis using existing methods, a model reduction is required. The pathway of the reduced model examined is shown in Fig. 1.

This model contains 8 states and 15 parameters of which, as an additional simplification, only 9 are considered to be unknown and in need of being identified. The system is described by a set of ordinary differential equations derived

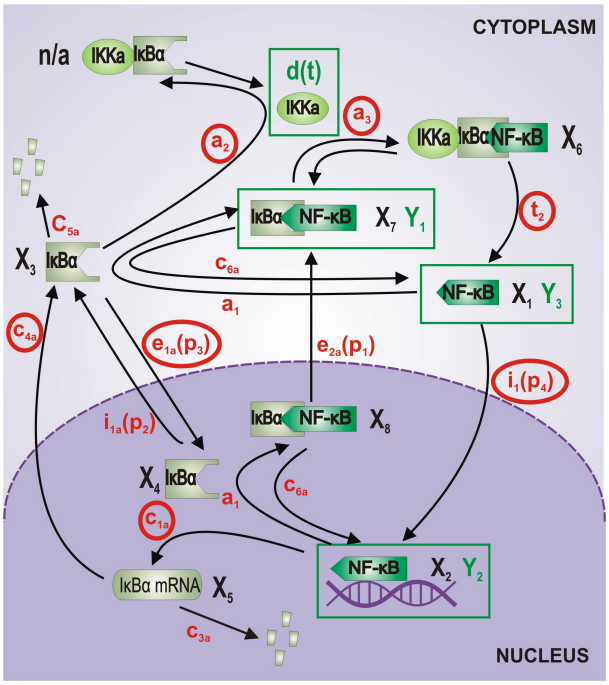

Fig. 1. Schematic pathway representation of the reduced NF-κB model with unknown parameters circled.

from mass action kinetics which are linear in terms of the parameters and nonlinear (bilinear) in terms of the states. As is the case for many biological systems, the system is limited in terms of potential inputs that will sufficiently excite the system (in the NF-κB case no inputs are considered). This is in stark contrast to traditional systems theory in which most identification methods are based on the input-output map of the system [20].

Details of the full algebraic identifiability analysis of the reduced NF-κB model are presented in [21]. It turns out that the identifiability analysis can only be carried out after making significant simplifications to the model. The number of states have to be reduced through model reduction and finding a solution is highly dependent on the choice of outputs. As shown in [21] for this example, addition of a third output is required in order to compute the observability map of the system. Due to the presence of the bilinear terms, the highest order derivative that can be taken before the system of algebraic equations can no longer be solved analytically is $y^{(2)}$ imposing a restriction that a solution can only be found for systems $\leq 3p$ states where p is the number of outputs. However, in biological systems one is extremely limited in terms of what can be measured experimentally so the addition of more outputs is not necessarily feasible. One must also consider that this system is still of only moderate size and complexity in comparison to the range of systems biology problems available. This suggests existing observability based methods are not well suited for analyzing identifiability of biological systems.

The difficulty with the current observability based methods examined, is that finding the observability of nonlinear

systems in continuous time is a highly challenging problem in itself without even considering the parameter values. In fact, the limitations of these methods lay in the number of states they can handle as they all require an analytical solution to be found. For this reason, it is necessary to develop methods which are not as dependent on the size and nonlinearity of the system for their computation. In the next section, we propose a new, simulation based identifiability method considering the observability Gramian as a measure for identifiability.

III. Empirical Gramian for Identifiability

The concept of empirical observability and controllability Gramians was introduced in [13] for the purpose of model reduction for nonlinear systems. It is introduced as a 'data based' approach making use of data either from simulation or experiments in order to avoid some of the computational complexity associated with other methods. It has been successfully applied to systems with dozens of states [22] for purposes of model reduction. In [14] the use of such Gramians for observability (and controllability) analysis is proposed and compared to the use of linear Gramians and Lie algebra based methods. Currently there are no results of using these Gramians for the purpose of identifiability analysis as is proposed here. The hope is that since this method is simulation based, it can overcome some of the constraints on the system size and complexity imposed by the other observability based methods previously discussed and thus can be used for biological systems.

A. System Description

Consider a nonlinear dynamic system of the form:

$$\Sigma : \begin{cases} \dot{x} = f(x,\theta)\ ,\ x(0) = x_0, \\ y = h(x,\theta), \end{cases} \tag{1}$$

where $x \in X \in \mathbb{R}^n$, $y \in \mathbb{R}^p$, and $\theta \in P \in \mathbb{R}^q$ are the states, outputs, and unknown time-invariant parameters respectively. P is a simply connected open subset of $\mathbb{R}^q$ of feasible parameter values and X is a simply connected open subset of $\mathbb{R}^n$ of feasible states. $X \times P$ together form the operating region of the system. Note that we do not consider inputs for simplicity of presentation however the results are easily expandable to this case.

We consider the following definition of structural identifiability:

Definition 1: (Structural identifiability)
A given parameter θ_i is (*a priori* or structurally) globally identifiable if there exists, for all possible measurement trajectories, a unique solution to (1) for θ_i. A parameter with a countable or uncountable number of solutions is considered locally identifiable or unidentifiable.

For the purpose of our approach, the question of identifiability is restated as one of observability by including parameters as states. Considering time invariant parameters the following augmented system is obtained from that in (1):

$$\tilde{\Sigma} = \begin{cases} \dot{\tilde{x}} = \begin{bmatrix} \dot{x} \\ \dot{\theta} \end{bmatrix} = \begin{bmatrix} f(x,\theta) \\ 0 \end{bmatrix}\ ,\ \tilde{x}(0) = \begin{bmatrix} x_0 \\ \theta_0 \end{bmatrix}\ , \\ \\ y = h(x,\theta), \end{cases} \tag{2}$$

where $\tilde{x} \in \mathbb{R}^{\tilde{n}}$, $\tilde{n} = n + q$ is the augmented state vector containing both the parameters and original states.

B. Empirical Identifiability Gramian

The following definition is adapted from that of the empirical observability Gramian found in [13, 22] by considering the augmented system.

Definition 2: (*Empirical identifiability Gramian)*

$$W_I = \sum_{l=1}^{r} \sum_{m=1}^{s} \frac{1}{rsc_m^2} \int_0^\infty T_l \Psi^{lm}(t) T_l^T\ dt \tag{3}$$

where $\Psi^{lm}(t) \in R^{\tilde{n}\times\tilde{n}}$ is given by:

$$\Psi_{ij}^{lm}(t) = (y^{ilm}(t) - y_{ss}^{ilm})^T (y^{jlm}(t) - y_{ss}^{ilm}). \tag{4}$$

The Gramian W_I is constructed using output values obtained through perturbations of the initial states of the system. In this case $y^{ilm}(t)$ and y_{ss}^{ilm} are the time varying and steady state output of the system for the particular 'experiment' considered by the initial state given by:

$$\tilde{x}(0)^{ilm} = c_m T_l e_i + \tilde{x}_{nom}. \tag{5}$$

These initial states are obtained through perturbations around a nominal value $\tilde{x}_{nom}$. The following sets describe the perturbations:

$$\begin{aligned} &T^{\tilde{n}} = \left\{T_1, \ldots, T_r;\ \ T_l \in \mathbb{R}^{\tilde{n}\times\tilde{n}},\ T_l^T T_l = I,\ l = 1, \ldots, r\right\}, \\ &M = \left\{c_1, \ldots, c_s;\ \ c_m \in \mathbb{R},\ c_m > 0,\ m = 1, \ldots, s\right\}, \\ &E^{\tilde{n}} = \left\{e_1, \ldots, e_n;\ \ \text{standard unit vectors in } \mathbb{R}^{\tilde{n}}\right\}. \end{aligned}$$

Here r is the number of matrices that describe the direction of the perturbations and s is the number of different perturbation magnitudes for each direction. The perturbations reflect the reasonable or desired operating range of the system.

Remark 1: Note that the augmented system is only neutrally stable. However, in order for the empirical Gramian to be applied it is typically required that the complete system be exponentially stable (at least locally for the region considered). This does not pose a problem here though because the neutrally stable parts are resulting from the system parameters that are adjoint to the original system. Since the parameters are constant, they are canceled out by the subtraction of the steady state values.

Based on the empirical observability Gramian, the following two statements can be made:

- Firstly, the augmented system (2) is locally observable over the operating range considered if the resulting Gramian is of full rank.

- As a direct consequence of this, it follows that since the states of the augmented system consist of the states and parameters of the original system, that if the augmented system is observable then the parameters are locally identifiable over the operating range considered.

This provides a means to check identifiability by employing the observability Gramian. One basically considers the observability Gramian for the expanded system and checks if it is of full rank.

If one can make further assumptions or show that the system being examined is observable (the observability Gramian of the original system is invertible) additional simplifications can be made to the Gramian of the augmented system so that the part dealing only with identifiability can be isolated. This is beneficial as it eliminates the effect of the cross terms (for example, the effect of changing the parameters on the time course of the states which can indirectly effect the output). In this case, W_I of the augmented system can be decomposed as follows:

$$W_I^{(\tilde{n}\times\tilde{n})} = \left[\begin{array}{c|c} W_X^{(n\times n)} & W_{X\theta}^{(n\times q)} \\ \hline W_{\theta X}^{(q\times n)} & W_\theta^{(q\times q)} \end{array}\right].$$

The $(q\times q)$ empirical identifiability Gramian can then be defined as:

$$W_I = W_\theta - W_{\theta X} W_X^{-1} W_{X\theta}. \tag{6}$$

If this matrix has full rank (rank$=q$ where q is the number of unknown parameters) then those parameters are identifiable. Since the matrix is obtained using numerical approximations, eigenvector/ eigenvalue decomposition is used as a measure of rank deficiency.

IV. Application of Empirical Identifiability Gramian

The empirical Gramian can be computed from experimental or simulation data from within a region where the process is to be operated. While this helps overcome the restrictions on system size of the other observability based methods, it also introduces complications in terms of the number of degrees of freedom one is presented with for computation. One must select nominal values for the states and parameters, a region of interest for the system, and define the directions and magnitudes of the perturbations.

Remark 2: It is also important to note that the system should be scaled prior to perturbation. One can include the scaling considerations when determining the perturbations by rewriting (5) as:

$$\tilde{x}(0)^{ilm} = c_m S^{-1} T_l e_i + \tilde{x}_{nom}$$

where S is the scaling matrix. The scaling can also reflect the different feasible operating regions of the states and parameters.

We discuss computational considerations and demonstrate the application of empirical Gramians for identifiability analysis through the use of a simple biological example.

Biological Example

As a biological example we look at a model of microbial growth from [16, 17]. The structural identifiability of this system was previously analyzed in [15] using a different approach based on several existing methods so can be used to confirm the results of the Gramian approach. The system description is as follows:

$$\begin{cases} \dot{x} = \frac{\mu_m b_2 l(t) x(t)}{K_s + b_2 l(t)} - K_d x(t) \\ \dot{l} = -\frac{\mu_m l(t) x(t)}{Y(K_s + b_2 l(t))} \\ x(0) = x_0 \ , \ l(0) = 1, \end{cases} \tag{7}$$

where $l(t) = \frac{1}{b_2} s(t)$. The system is derived using Michaelis-Menten kinetics where $s(t)$ and $x(t)$ are the concentration of substrate and product respectively and μ_m, K_d, b_2, K_s, Y are the reaction parameters. Note that the system is nonlinear in continuous time.
We examine the question of whether or not the unknown parameter set:

$$\theta = (\mu_m, K_d, b_2, K_s, Y)^T$$

is identifiable over the range of system states and parameters considered. The measurable output in this case is given by:

$$y(t) = x(t).$$

Comments on Computation

The Gramian calculations are done using nominal state and parameter values and by considering perturbations within a region of biological interest; similar to what would be examined experimentally for the purpose of parameter estimation. The nominal values for the biological example are [16]:

$$\tilde{x}_{nom} = \begin{bmatrix} x_0 \\ l_0 \\ \mu_m \\ K_d \\ b_2 \\ K_s \\ Y \end{bmatrix}_{nom} = \begin{bmatrix} 1 \\ 1 \\ 0.5 \\ 0.025 \\ 10 \\ 3.0 \\ 0.6 \end{bmatrix}.$$

With respect to the perturbations, one would want to consider a larger range of parameter values as the exact nominal values are not known. In this case, perturbations of $\pm 10\%$ about the nominal values are considered for the states and $\pm 50\%$ for the parameters.

In order to specify the perturbation directions, we use a $2^{\tilde{n}}$ factorial design to generate the T matrix. This leads to an $(\tilde{n}\times 2^{\tilde{n}})$ matrix made up of ± 1 which is made orthogonal by dividing it by $\sqrt{2^{\tilde{n}}}$.

There are several reasons for utilizing this approach, as opposed to the ones presented in [13, 14], when considering parameter identifiability. In [13] the use of $T = [I, -I]$ is suggested while the perturbation directions in [14] are evenly distributed around a unit circle. The use of randomly generated orthogonal $(n \times n)$ matrices is also considered. It is important to note that in both [13, 14], the use of a steady state point in the system for the nominal state values is proposed. The use of $\pm I$ to define the perturbation directions essentially translates into 'experiments' in which only one state or parameter is perturbed at a time. This approach proves to be disadvantageous when considering parameter identifiability of biological systems as the steady state value for at least some of the states is often zero. This makes it impossible to obtain information about the parameters associated with these states because when the parameter is perturbed the state will remain at zero. The use of random perturbation directions gives a T matrix that contains not only direction but also magnitude to a certain degree. The use of a factorial design for T not only allows for the consideration of every perturbation combination, it facilitates the use of an equilibrium point of the system as the nominal states and has no magnitude associated with it as well.

Remark 3: In [13, 14] it is specified that T is an $(n \times n)$ orthogonal matrix and multiple $(l = 1, \ldots, r)$ T matrices are used to define the perturbation directions considered. However, these are not requirements as long as the following adjustments are made (proof not included here). The set M describing the perturbation magnitude remains the same although $T^{\bar{n}}$ becomes $T \in \mathbb{R}^{\bar{n} \times 2^{\bar{n}}}, T^T T = I$, the l index is no longer required and $E^{\bar{n}}$ becomes: $E^{2^{\bar{n}}} = \{e_1, \ldots, e_{2^{\bar{n}}};$ standard unit vectors in $\mathbb{R}^{2^{\bar{n}}}\}$. The Ψ matrix also changes dimensions to become $(2^{\bar{n}} \times 2^{\bar{n}})$. Despite these changes the dimensions of the empirical Gramian remain the same.

The perturbations are used to define the initial conditions for each 'experiment' and the resulting outputs are used to calculate W_I according to (3).

Remark 4: While Definition 2 considers the integral from zero to infinity, a finite end time, t_f, must be used for computational purposes. As long as $t_F >$ the time it takes for the outputs to reach steady state, the results will not be effected.

Identifiability Result

In the case of the microbial growth example, the system is observable with the considered output so W_I, as defined in (6), can be used for the identifiability analysis. This leads to the following $(q \times q)$ identifiability Gramian:

$$W_I = \begin{bmatrix} 0.0946 & 0.1871 & -0.0024 & -0.0554 & -0.0446 \\ 0.1871 & 6.081 & 0.0059 & -0.1328 & -0.0916 \\ -0.0024 & 0.0059 & -0.0020 & 0.00001 & -0.0030 \\ -0.0554 & -0.1328 & 0.00001 & 0.0357 & 0.0272 \\ -0.0446 & -0.0916 & -0.0030 & 0.0272 & 0.0188 \end{bmatrix}.$$

An eigenvalue/eigenvector decomposition of the W_I matrix is used to determine its rank and thereby whether or not the unknown parameters are identifiable. The eigenvalues, λ_i, are expressed as relative values or as a percentage of the total norm. For the microbial example we obtain:

$$\begin{aligned} \lambda_1 &= 0.978 \\ \lambda_2 &= 0.0223 \\ \lambda_3, \lambda_4, \lambda_5 &= \mathcal{O}(10^{-4}) \end{aligned}$$

with the corresponding eigenvector matrix:

$$v = \begin{bmatrix} 0.032 & -0.797 & -0.266 & 0.535 & -0.082 \\ 0.999 & 0.041 & 0.0007 & 0.003 & -0.001 \\ 0.001 & 0.007 & -0.704 & -0.427 & -0.567 \\ -0.022 & 0.474 & 0.076 & 0.656 & -0.582 \\ -0.015 & 0.372 & -0.653 & 0.319 & 0.577 \end{bmatrix}.$$

In this case λ_3, λ_4, and λ_5 are $2-3$ orders of magnitude smaller than the other eigenvalues and can be considered negligibly small (as noise due to the numerical approximation). This suggests that $3/5$ of the unknown parameters from (7) are unidentifiable. By looking at the eigenvectors of W_I one can see that these unidentifiable parameters correspond to b_2, K_s, and Y. This is consistent with the results from [15] obtained using a different parameter identifiability approach.

V. Conclusions and Outlook

Identifying parameters for biochemical reaction networks is a challenging task. We look at existing observability based methods for structural or *a priori* identifiability, originally developed for general nonlinear systems, in the context of a typical systems biology problem: the NF-κB signal transduction pathway. It is found in [21] that the identifiability analysis itself can only be carried out after significant simplifications are made. The number of states must be reduced through model reduction and whether or not identifiability can be analyzed is found to depend greatly on the choice of outputs. One must also consider that this example is still of only moderate size and complexity compared to the range of systems biology problems available, suggesting that such methods are not well suited for this purpose.

The difficulties with the current observability based methods stem from the fact that they all require an analytical solution to be found imposing limitations on the size and complexity of systems that can be handled. In order to overcome this, we propose the use of an 'empirical observability Gramian' for identifiability analysis. Since this method is data/simulation based it should be able to avoid computational complexities associated with the previous methods and thereby be used on biological systems. The

empirical observability Gramian has already been shown to be able to handle dozens of states for the purpose of model reduction in [22]. The use of this Gramian for identifiability purposes is demonstrated on a biological example: a model of microbial growth. Results from [15] for the same system state that the unknown parameters b_2, K_s, and Y are unidentifiable which verifies the results of our method.

Since the empirical Gramian is generally used for model reduction purposes, many questions remain regarding the analysis in the identifiability case; namely how to extract as much information from the Gramian as possible. This becomes increasingly important when dealing with larger systems. Future work will look at applying this method to larger biological examples, such as the NF-κB pathway, to be better able to compare and contrast it with the previous analytical based approaches shown. In this work, only time invariant parameters are used. Since when using the Gramian for identifiability purposes the parameters are included as states by considering their time derivatives, this method could easily be extended to the case of time variant parameters as well.

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