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Quantitative Steady-State Bounds in Biomolecular Circuits Due to Bounded Multi-Parametric Perturbations

Gunjan Chorasiya, Rudra Prakash and Shaunak Sen.

Abstract— **Biomolecular circuit performance depends upon the reaction rate parameters. Any perturbation in these parameters can propagate to the output of the system, altering its performance. A rigorous quantitation of the extent of such deviations in response to variations in one or more parameters, especially in nonlinear settings, is generally unclear. To address this, we developed theoretical frameworks based on the Banach Contraction Theorem and Interval Analysis. We used a parametrized version of the Banach Contraction Theorem to develop a method to bound steady-state variations in terms of variations in multiple parameters, in the linearized and in the nonlinear settings. We extended this to propose a solution to the design problem of obtaining parametric bounds given steady-state bound specifications. We developed a complementary method based on Interval Analysis to rigorously obtain steady-state intervals given parametric intervals. These methods are illustrated using benchmark biomolecular circuit examples. These results contribute to the analysis and design of biomolecular systems in the presence of uncertainties.**

I. INTRODUCTION

Determining how the solutions $x(t)$ of

$$\dot{x} = f(x,u), x \in \mathbb{R}^{n\times 1}, u \in \mathbb{R}^{m\times 1}, \tag{1}$$

depend on the parameters u is important for analysis and design in multiple contexts. For a general nonlinear system, even the solution at steady-state ($\dot{x} = 0$) can change qualitatively and quantitatively as the parameters are varied.

For biomolecular systems, where f is a polynomial or rational function of the states and the parameters, the parametric dependence of the steady-state, particularly in a qualitative sense, has received much attention. Theoretical methods have been used to identify constraints on the dynamics that would guarantee the existence of the number of steady-states [1], [2], [3], [4] as well as to structurally assess and tightly bound the ratio between the changes in the output and the changes in the input at steady-state for specific classes of systems [5]. Robustness of the steady-state value to bounded variations in a particular input parameter has also been much investigated, with mechanisms such as integral feedback control and incoherent feedforward loops being identified as mechanisms that can make the steady-state independent of the input parameter [6], [7], [8]. Numerical simulations and linearized analyses have served as important methods to support these studies. These have also underpinned efforts to quantitate the variation in the steady-states when one or more parameters are perturbed [9], [10], [11], [12].

There are at least three striking aspects in obtaining the parametric dependence of the quantitative variations in the steady-state. One is the importance of this relation in the design problem of finding parameter intervals to achieve given steady-state specifications. Two is the nature of typical parametric perturbations, where a parameter value may change relative to a nominal value and multiple parameter values could change simultaneously. Three is the nonlinearity that generally characterizes the relations between the parameters and the steady-states. Typically, analysis is done for a linearized system around one of the possibly many steady-states complemented with a random sampling of parameter space. A rigorous estimate of the steady-state variations in response to the variations in multiple parameters would help address the analysis and design challenges.

Here we aim to develop rigorous quantitative bounds on the steady-states of the system for bounded multi-parametric perturbations. We addressed this using methods based on the Banach Contraction Theorem and Interval Analysis. We used a parametric version of the Contraction Mapping Theorem to develop a method to express the steady-state bounds in terms of the parametric bounds. We linearized around the steady-state(s) and presented bounds on the deviations from the steady-state in terms of the deviations around the parameters. This naturally extends to the design of parametric bounds for given steady-state bound specifications in the linear and the nonlinear cases. We developed the Interval Newton Algorithm to obtain steady-state intervals given the parametric intervals, and, in the linear case, related these intervals to each other, offering a complementary perspective to the above analysis and design problems. These methods are illustrated on benchmark biomolecular systems with feedback and feedforward loops, including negative autoregulation, incoherent feedforward, and positive autoregulation with multiple steady-states, which also highlight the salient features of the methods.

II. CONTRACTION-BASED ANALYSIS

A. Background

The Banach Contraction Theorem can be used to obtain bounds on the parametric dependence of the fixed point of a contractive mapping.

Lemma 1 ([13]): Given a complete metric space (X, d_X) and a metric space (U, d_U), let $T : X \times U \to X$ be

- uniformly contracting in its first argument, that is, uniformly Lipschitz in its first argument,

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The authors are with the Department of Electrical Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India (email: Gunjan.chorasiya@ee.iitd.ac.in, Rudra.Prakash@ee.iitd.ac.in, shaunak.sen@ee.iitd.ac.in).

$d_X(T(x,u),T(y,u)) \leq l_X d_X(x,y)$ for all $x,y \in X$ and $u \in U$, with $0 < l_X < 1$, and
- uniformly Lipschitz in its second argument, $d_X(T(x,u),T(x,v)) \leq l_U d_U(u,v)$ for all $x \in X$ and $u,v \in U$, with $l_U > 0$.

Then the solution $x^* : U \to X$ to the parametrized fixed point problem

$$x = T(x,u) \tag{2}$$

exists and is Lipschitz with constant $l_U(1-l_X)^{-1}$, that is:

$$d_X(x^*(u),x^*(v)) \leq \frac{l_U}{1-l_X} d_U(u,v) \text{ for all } u,v \in U. \tag{3}$$

For continuously differentiable mappings, the Lipschitz constant can be obtained by taking the supremum of the norm of the derivative mapping [14].

B. Results

Method: We combined these in the following method to obtain quantitative bounds on the steady-state of (1) to variations in the parameters,

Step 1: Define the parametrized fixed point problem $T(x,u) = x + Cf(x,u)$, where $C \neq 0$. If $C \equiv f_x^{-1}$, where $f_x = \frac{\partial f}{\partial x}$, then this is the Newton's method.

Step 2: Compute the Lipschitz constants l_X and l_U. Choose C to ensure that $l_X < 1$.

Step 3: Apply Lemma 1 to obtain the bounds.

The following theorem gives a sufficient condition for the existence of such a C.

Theorem 1: If $\det(f_x) \neq 0$, a C can be found such that $l_X = \|I + Cf_x\| < 1$.

Proof: Set $I + Cf_x = A$ where $A \in \mathbb{R}^{n \times n}$ such that $\|A\| < 1$ (for example, $I/2$). Then $C = (A-I)f_x^{-1}$ is a unique solution that exists when $\det(f_x) \neq 0$. ∎

Remark 1: If $\det(f_x) = 0$, then a (minimum norm) solution for the underdetermined equations may be found for $n > 1$.

Linear Analysis: A linearization around a steady-state x^* of (1) may be used to perform a linear analysis. Assuming that the steady-state has been calculated for a default parameter set u^*, the linearization gives a relation between the deviation in the states around the steady-state $\Delta x = x - x^*$ and the deviation in the parameters around their default values $\Delta u = u - u^*$,

$$f_{x*}\Delta x + f_{u*}\Delta u = 0, \tag{4}$$

where $f_u = \frac{\partial f}{\partial u}$. Assuming f_{x*}^{-1} exists, this implies $\Delta x = -f_{x*}^{-1} f_{u*} \Delta u$ and

$$\|\Delta x\|_X \leq \left\| f_{x*}^{-1} f_{u*} \right\|_{U \to X} \|\Delta u\|_U, \tag{5}$$

where $\|\cdot\|_X$ and $\|\cdot\|_U$ are vector norms, and $\|\cdot\|_{U \to X}$ is the induced matrix norm.

Remark 2: The form of the bound (5) is the same as that obtained by applying the above method to (4).

Proof: Let $T(\Delta x, \Delta u) = \Delta x + C(f_x \Delta x + f_u \Delta u)$. Then $l_X = \|I + Cf_x\|$ and $l_U = \|Cf_u\|$. If $\det(f_x) \neq 0$, choose $C = (A - I)f_x^{-1}$, where $A = I/2 \Rightarrow l_X = 1/2 < 1$ and $l_U = \|f_x^{-1} f_u\|/2$. Application of (3) gives (5). ∎

The bound obtained from the linearized analysis is computed using the value of the point of linearization and is valid locally. The bound obtained from the contraction analysis is computed over a larger domain and is more general.

Linear Design: The linearization in (4) naturally provides a way to obtain the bounds on the allowed parameter variations given the specified bounds on steady-state variations through the rearrangement,

$$f_{u*}\Delta u = -f_{x*}\Delta x. \tag{6}$$

The solution of (6) depends on the relation between the number of parameters (m) and the number of states (n). Typically $m > n$, and the least squares solution to the underdetermined system, using the right pseudo-inverse [15], can be used to obtain a design solution.

Design: A method for the nonlinear design problem may be similarly obtained from the above method by interchanging the roles of the states and the parameters. If $n = m$, then the same steps apply. If $m > n$, as is typically the case, then a solution may be obtained as in the underdetermined case. The bound is

$$d_U(u^*(x),u^*(y)) \leq \frac{l_X}{1-l_U} d_X(x,y), \tag{7}$$

where $x,y \in X^*$, the desired steady-state space, and $l_U = \|A\|, l_X = \|Cf_x\|, C = (A-I)f_u^T(f_u f_u^T)^{-1}, \|A\| < 1$.

III. INTERVAL-BASED ANALYSIS

A. Background

The theoretical framework developed above exploits the contraction in a suitably defined fixed point problem to map the variations in the parameters to the variations in the steady-states, and vice-versa for design. The resulting bounds are essentially relations between intervals of possible values of the steady-states and the parameters. Next, we developed a complementary framework, based on Interval Analysis [16], that emphasizes the interval nature of the steady-states and the parameters in obtaining the bounds.

Interval methods are a family of set-based methods that offer a complementary perspective to point-based methods with many applications such as approximating the range of functions and solving equations, both linear and nonlinear, as well as several conceptual advantages such as theoretical constructions and validated numerical computations [17].

We briefly summarize the basics in a real-valued setting. Points in $\mathbb{R}$ generalize to intervals, $\mathbb{IR} = \{[\underline{a},\overline{a}] : \underline{a} \leq \overline{a}; \underline{a},\overline{a} \in \mathbb{R}\}$. For an element $A = [\underline{a},\overline{a}] \in \mathbb{IR}$, the *radius* and *midpoint* are defined as $\text{rad}(A) = (\overline{a} - \underline{a})/2$ and $\text{mid}(A) = (\underline{a} + \overline{a})/2$, respectively. Arithmetic is defined based on the endpoints $A + B = [\underline{a} + \underline{b}, \overline{a} + \overline{b}]$, $A - B = [\underline{a} - \overline{b}, \overline{a} - \underline{b}]$, $A \times B = [\min\{\underline{ab}, \overline{ab}, \underline{a}\overline{b}, \overline{a}\overline{b}\}, \max\{\underline{ab}, \overline{ab}, \underline{a}\overline{b}, \overline{a}\overline{b}\}]$, $A \div B = A \times [1/\overline{b}, 1/\underline{b}]$, if $0 \notin B$. For division by intervals containing 0, an *extended* version of interval arithmetic can be used.

The next step in interval analysis is the notion of functions over interval variables. A *natural* extension is one where each instance of a real variable x is replaced by an interval X. Briefly, interval extensions of standard functions are defined

and these are used to obtain interval versions of functions that are combinations of the standard functions. Typically, the arithmetic defined above leads to the issue of overestimation, called *interval dependency*. For example, if $g(x) = x^2$ and $X = [-1,2]$, then the natural interval extension $G(X) = X \times X = [-2,4]$ whereas a sharper range enclosure is $[0,4]$.

We state three key elements of Interval Analysis that are useful in addressing our problem. The first element relates to the approximation of the range of function $R(g;X) = \{g(X) : x \in X\}\}$ as tightly as needed by subdividing the domain X.

Theorem 2 ([17]): Consider a real-valued function g whose subexpressions are all Lipschitz and let G be an interval extension of g that is well-defined for some interval X in the domain of g. Then there exists a positive real number K, depending on G and X, such that, if $X = \cup_{i=1}^{k} X^{(i)}$, then $R(g;X) \subseteq \cup_{i=1}^{k} G(X^{(i)}) \subseteq G(X)$ and $\text{rad}(\cup_{i=1}^{k} G(X^{(i)})) \leq \text{rad}(R(g;X)) + K \max_{i=1,\ldots,k} \text{rad}(X^{(i)})$.

The second element relates to bounding solutions of a linear system of equations.

Theorem 3 (Oettli & Prager, [18]): Let $A \in \mathbb{IR}^{m\times n}$, $b \in \mathbb{IR}^{m\times 1}$. The solution set $\Sigma(A,b) = \{z \in \mathbb{R}^{n\times 1} | \tilde{A}z = \tilde{b}$ for some $\tilde{A} \in A, \tilde{b} \in b\}$ satisfies

$$z \in \Sigma(A,b) \Leftrightarrow |\text{mid}(A)z - \text{mid}(b)| \leq \text{rad}(A)|z| + \text{rad}(b). \quad (8)$$

The third element relates to finding the zeroes of functions. Interval Analysis offers a generalization of the Newton method to find the zeroes of a function [17]. The *interval Newton operator* is defined as

$$N(X) = \text{mid}(X) - \frac{g(\text{mid}(X))}{G_x(X)}, \quad (9)$$

where g is a real-valued continuously differentiable function with a zero $x^* \in X$, G_x is an interval extension of the derivative of g with $0 \notin G_x(X)$. Starting with an initial enclosure X_0 of x^*, a sequence of intervals are obtained,

$$X_{k+1} = N(X_k) \cap X_k, \text{ for } k = 0,1,2,\ldots. \quad (10)$$

The following summarizes the properties of this iteration.

Theorem 4 (Interval Newton Method, [17]): Assume that $N(X_0)$ is well-defined. If X_0 contains a zero x^* of g, then so do all iterates X_k, $k \in \mathbb{N}$. Furthermore, the intervals X_k form a nested sequence converging to x^*.

The assumption that $N(X_0)$ is well-defined includes the condition that $0 \notin G_x(X)$.

B. Results

Method: In our problem, the steady-state depends on the parameters and the parameters are in intervals, $u \in U$. Therefore, we extended Theorem 4 to the case where the function depends on the parameters. We considered a modification of the operator in (9) to find the steady-states of (1),

$$N(X,U) = \text{mid}(X) - \frac{F([\text{mid}(X),\text{mid}(X)],U)}{F_x(X,U)}, \quad (11)$$

where F and F_x are the interval extensions of f and f_x, respectively.

Some properties of this modified operator are summarized in the following theorem. For simplicity, we restricted to the real-valued setting.

Theorem 5: If X_0 contains an interval of zeroes $X^* = x^*(U)$ of f, then so do all iterates X_k, $k \in \mathbb{N}$. Furthermore, the intervals X_k form a nested sequence that contain X^*.

Proof: The proof follows, with some modifications, the proof of Theorem 4 [17]. $X^* \subseteq X_0 \Rightarrow X^* \subseteq X_k$ for all $k \in \mathbb{N}$ by mathematical induction. The intervals X_k form a nested sequence and contain X^* from the definition (10). ■

Unlike in Theorem 4, the intervals X_k in Theorem 5 do not converge to the interval X^*. This can be shown by means of a **counterexample**. Consider $f(x,[\alpha,\gamma]) = \alpha - \gamma x$, where $\alpha \in [100,300]$ and $\gamma \in [1,3]$. For an initial interval $X_0 = [0,800]$, $X_k = [0,366.667], k \in \mathbb{N}$. This does not converge to the desired $X^* = [100/3,300]$ even though it is a rigorous bound, $X^* \subset X_k$, $k \in \mathbb{N}$.

The main challenge here is the overestimation in the calculation of $N(X,U)$ in (11) due to interval dependency. In order to obtain tighter bounds, we subdivided the parameter intervals. We proved that the solution set can be approximated as tightly as needed by this subdivision.

Theorem 6: Assume that f and f_x are continuously differentiable. For the subdivision $U = \cup_{i=1}^{m} U_i$, the solution set $X^*(U) = \{x^* : f(x^*,u) = 0$ for some $u \in U\}$ satisfies $X^*(U) \subseteq \cup_{i=1}^{m} \lim_{k\to\infty} X_k(U_i) \subseteq \lim_{k\to\infty} X_k(U)$ and $\text{rad}(\bigcup_{i=1}^{m} \lim_{k\to\infty} X_k(U_i)) \leq \text{rad}(X^*(U)) + K \max_{i=1\ldots m} \text{rad}(U_i)$, where K is a positive real number.

Proof: For $k \in \mathbb{N}$, the set $R_k(U) = \{x : x \in X_k([u,u]), u \in U\}$ satisfies $R_k(U) = R_k(\bigcup_{i=1}^{m} U_i) = \bigcup_{i=1}^{m} R_k(U_i) \subseteq \bigcup_{i=1}^{m} X_k(U_i) \subseteq X_k(\bigcup_{i=1}^{m} U_i) = X_k(U)$ by the inclusion principle. Taking the limit and using Theorem 4 proves the first part.

We used Theorem 2 on the operator in (11) to show that $\text{rad}(\bigcup_{i=1}^{m} X_1(U_i)) \leq \text{rad}(R_1(U)) + K_1 \max_{i=1\ldots m} \text{rad}(U_i)$. An inductive argument extends this to $\text{rad}(\bigcup_{i=1}^{m} X_k(U_i)) \leq \text{rad}(R_k(U)) + K_k \max_{i=1\ldots m} \text{rad}(U_i)$, where $k \in \mathbb{N}$. A limit of this expression proves the second part. ■

Based on these modified results, we formulated the following method to obtain bounds on the steady-state when the parameters are in given intervals,

Step 1: Choose initial enclosure X_0 that contains the steady-state.

Step 2: Iterate as per (10) and (11). Use subdivision.

Step 3: End iteration as per a stopping criteria, such as on $\text{rad}(X_k)$, if required.

We note that the existence of such an X_0 is typically guaranteed by the bounded nature of the systems under consideration. Conceptually, the subdivision in the interval framework provides a seamless and rigorous link to linearization.

If $0 \in F_x(X,U)$, the extended arithmetic needs to be used together with bisection. This is important in situations with multiple steady-states that are pervasive in biomolecular systems. Division by a signed zero in (11) leads to two disjoint intervals, each of which can be processed separately in the spirit of the bisection method. Another layer of standard

bisection at the mid-point of X is needed when $0 \in F_x(X,U)$ and $0 \in F([\text{mid}(X),\text{mid}(X)],U)$. We incorporated these in Step 2 above.

Linear Analysis: In the linearized setting, (4) is an interval linear equation,

$$F_{x*}\Delta X = -F_{u*}\Delta U, \tag{12}$$

where, ΔX and ΔU are interval vectors, and F_{x*} and F_{u*} are interval extensions of f_{x*} and f_{u*}, respectively. Equation (8) can be directly used to obtain bounds on ΔX given the parameter intervals ΔU.

Linear Design: In a similar fashion, using (8), given the desired steady-state intervals, the positions of the states and parameters in (12) can be interchanged to obtain the allowed parameter intervals.

IV. EXAMPLES

The utility of the above methods is demonstrated using benchmark biomolecular system examples [6], [7]. These case studies also serve to highlight important features of the methods.

Example 1 (Open loop): The open loop model is a standard model to represent the constant production of a protein and its degradation through a first-order process. Here, $f(x,u) = \alpha - \gamma x$, with the state $x \in \mathbb{R}$ and $u = [\alpha,\gamma]^T \in \mathbb{R}^{2\times 1}$.

The linear analysis in (5) gives the variation $|\Delta x| \leq \|[\frac{1}{\gamma^*}, \frac{-x^*}{\gamma^*}]\| \|\Delta u\|$ around a steady-state x^* for default parameters $[\alpha^*,\gamma^*]^T$. For the nonlinear analysis in (3), we set $T(x,u) = x + c(\alpha - \gamma x)$. For $c = \frac{1}{2\gamma}$, $l_X = \frac{1}{2}$ and $l_U = \sup \|\frac{[1,-x]}{2\gamma}\|$, where the supremum is over the parameter space $\alpha \in [\underline{\alpha},\overline{\alpha}], \gamma \in [\underline{\gamma},\overline{\gamma}]$. The bound is $l_U(1-l_X)^{-1} = \sup \|\frac{[1,-x]}{\gamma}\|$. The expression coincides with the one obtained with the linearization, although it is valid over the entire parameter space.

For the design problem in the linearized setting (6) $\Rightarrow [1,-x^*][\Delta\alpha,\Delta\gamma]^T = \gamma^*\Delta x$, which is an underdetermined system of equations. A solution is $[\Delta\alpha,\Delta\gamma]^T = [\frac{x^*}{1+(x^*)^2}, \frac{-1}{1+(x^*)^2}]^T \frac{\gamma^*}{x^*}\Delta x \Rightarrow \|[\Delta\alpha,\Delta\gamma]^T\| \leq \|[\frac{1}{1+(x^*)^2}, \frac{-x^*}{1+(x^*)^2}]^T\| \gamma^* |\Delta x|$. The design problem in the nonlinear setting (7) also has the same form $l_X(1-l_U)^{-1} = \sup \|[\frac{1}{1+x^2}, \frac{-x}{1+x^2}]^T\gamma\|, x \in X^*$ where we chose $C = \frac{[-1,x]^T}{2(1+x^2)}$.

In the Interval Analysis approach, we directly considered the parameter space $\alpha \in [\underline{\alpha},\overline{\alpha}], \gamma \in [\underline{\gamma},\overline{\gamma}]$. The natural interval extensions are $F(X,U) = [\underline{\alpha},\overline{\alpha}] - [\underline{\gamma},\overline{\gamma}]X$ and $F_x(X,U) = -[\underline{\gamma},\overline{\gamma}]$. For $X_0 = [0,x_0]$, we carried out the iteration in (11). The convergence of this iteration depended on a proper choice of x_0. Typically, a large enough value of x_0 is justifiable because of the bounded nature of these systems. A rigorous bound for the steady-state is obtained as [33.05, 300] (Fig.1), which is quite close to the analytically expected value of [100/3, 300]. To make the bound tighter, the parameter intervals can be further subdivided.

The Contraction Analysis above gives a bound where the maximum radius of the steady-state interval is 30100, which is quite conservative relative to the bounds obtained from the Interval Analysis. The conservativeness originates from the determination of the Lipschitz constants using the supremum of the first derivative, which can increase as the parameter ranges increase, as well as the form of the contraction used. For a smaller parameter range of 0.1% around the nominal values, the maximum radius of the steady-state interval for the Contraction Analysis is 10.13. The corresponding interval is $[89.87, 110.13]$, which is close to $[99.80, 100.20]$, the steady-state interval from Interval Analysis.

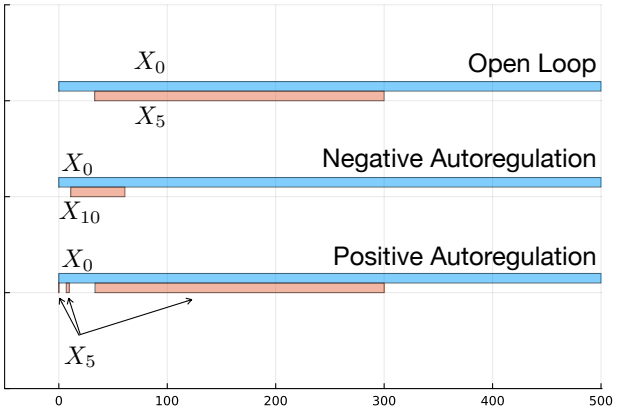

Fig. 1. Steady-state bounds. Parameters for open loop: $\alpha \in [100,300]$, $\gamma \in [1,3]$, $m = 10$ subdivisions. Additional parameters for negative autoregulation: $K \in [20/3, 15]$, $m = 5$. Additional parameters for positive autoregulation: $K = 10$, $n = 10$, $m = 10$.

For the linearized versions, the steady-state for default parameters needs to be calculated, for example, using the standard Interval Newton Algorithm. The interval of variation of steady-states for given variations in the parameters satisfies $|\text{mid}(\gamma)\Delta x - \text{mid}(\Delta\alpha - x^*\Delta\gamma)| \leq \text{rad}(\gamma)|\Delta x| + \text{rad}(\Delta\alpha - x^*\Delta\gamma)$. Complementarily, if the desired bounds on the steady-state are known, allowed parameter intervals satisfy $|\text{mid}([1,-x^*])[\Delta\alpha,\Delta\gamma]^T - \text{mid}(\gamma^*\Delta x)| \leq \text{rad}([1,-x^*][|\Delta\alpha|,|\Delta\gamma|]^T) + \text{rad}(\gamma^*\Delta x)$.

Example 2 (Negative Autoregulation): The negative autoregulation model is another standard model, in which a negative feedback on the expression of a protein is implemented by the repression of its own production. Here, $f(x,u) = \frac{\alpha}{1+x/K} - \gamma x$, with state $x \in \mathbb{R}$ and $u = [\alpha,\gamma,K]^T \in \mathbb{R}^{3\times 1}$.

The linear analysis (5) gives the variation $|\Delta x| = \left\| \frac{\left[\frac{K^*}{x^*+K^*} \;\; -x^* \;\; \frac{\alpha^* x^*}{(x^*+K^*)^2}\right]}{\frac{\alpha^* K^*}{(x^*+K^*)^2} + \gamma^*} \right\| \|\Delta u\|$ around a steady-state x^* for the default parameters $[\alpha^*,\gamma^*,K^*]^T$. For the nonlinear analysis in (3), we set $T(x,u) = x + c(\frac{\alpha}{1+\frac{x}{K}} - \gamma x)$. For $c = (2(\gamma + \frac{\alpha K}{(x+K)^2}))^{-1}$, $l_X = 1/2$ and $l_U = \sup \|c\left[\frac{K}{x+K} \;\; -x \;\; \frac{\alpha x}{(x+K)^2}\right]\|$, where the supremum is over the parameter space $\alpha \in [\underline{\alpha},\overline{\alpha}], \gamma \in [\underline{\gamma},\overline{\gamma}], K \in [\underline{K},\overline{K}]$. The bound is $l_U(1-l_X)^{-1}$.

For the design problem in the linearized setting, (6) may be solved using the right pseudo-inverse to obtain a bound on the parametric variations given the bounds on the steady-state specifications, $\|[\Delta\alpha,\Delta\gamma,\Delta K]^T\| \leq \|\frac{\lambda^*}{\sigma^*}[\frac{K^*}{x^*+K^*}, -x^*, \frac{\alpha^* x^*}{(x^*+K^*)^2}]^T\| |\Delta x|$, where $\lambda = \frac{\alpha K}{(x+K)^2} + \gamma$ and

$\sigma = (\frac{K}{x+K})^2 + x^2 + (\frac{\alpha x}{(x+K)^2})^2$. The design problem in the nonlinear setting (7) also has the same form $l_X(1-l_U)^{-1} = \sup\|\frac{\lambda}{\sigma}[\frac{K}{x+K}, -x, \frac{\alpha x}{(x+K)^2}]^T\|, x \in X^*$ where we chose $C = \frac{1}{2\sigma}[\frac{-K}{x+K}, x, \frac{-\alpha x}{(x+K)^2}]^T$.

We implemented the modified Interval Newton Approach to obtain steady-state bounds [10.99, 60.94] (Fig.1), as in the example above. The linear analysis and design inequalities are $\left|\text{mid}(F_{x*})\Delta X - \text{mid}(-F_{u*}^T\Delta U)\right| \leq \text{rad}(F_{x*})|\Delta X| + \text{rad}(-F_{u*}^T\Delta U)$ and $\left|\text{mid}(F_{u*}^T)\Delta U - \text{mid}(-F_{x*}\Delta X)\right| \leq \text{rad}(F_{u*}^T)|\Delta U| + \text{rad}(-F_{x*}\Delta X)$, respectively. For the same parameter ranges, the linear analysis gives the steady-state interval as $[-71.93, 88.73]$, where the steady-state for the linear analysis was calculated from the standard Interval Newton algorithm. This is expected as the linear analysis would be valid only locally. For a 10% variation around the nominal parameters the linear and nonlinear analyses give the steady-state intervals as $[21.31, 32.71]$ and $[22.95, 31.62]$, respectively, which are closer to each other.

To assess the conservativeness of the bounds, we performed a numerical comparison with a random sampling of the parameter space. The parameters were sampled uniformly ($N = 10^6$) and this sampling was repeated $M = 10^3$ times. The mean and standard deviation of the upper and lower bounds were 59.54 ± 0.16 and 12.06 ± 0.04, respectively. These compare favourably with the bound [10.99, 60.94] obtained from the Interval Analysis (Fig. 1).

Motivated by the Interval Analysis approach, we investigated the steady-state bound for the case of perturbations due to temperature changes, which typically result in bounded changes in all parameters. For perturbations due to temperature changes, it is customary to quantitate the bounds using a temperature co-efficient Q_y, which is the ratio of the values of $y(T)$ ten degrees apart, $Q_y = y(T+10)/y(T)$ [9]. The following gives Q_{x^*} for the negative autoregulation example.

Proposition 1: If $Q_\alpha = [2,3]$, $Q_\gamma = [2,3]$, and $Q_K = [2/3, 3/2]$, then $Q_{x^*} = [2/3, 3/2]$.

Proof: The steady-state is a solution to $\frac{\alpha}{1+x/K} - \gamma x = 0 \Rightarrow x^2 + Kx - K\frac{\alpha}{\gamma} = 0 \Rightarrow x^2 + K_0Q_Kx - K_0Q_K\frac{\alpha_0Q_\alpha}{\gamma_0Q_\gamma} = 0$. We note that $Q_K = [2/3, 3/2] = Q_\alpha/Q_\gamma$. Therefore, the equation is $x^2 + K_0x[\frac{2}{3}, \frac{3}{2}] - K_0\frac{\alpha_0}{\gamma_0}[\frac{2}{3}, \frac{3}{2}]^2 = 0$. We set $x = y[2/3, 3/2] \Rightarrow (y^2 + K_0y - K_0\frac{\alpha_0}{\gamma_0})[\frac{2}{3}, \frac{3}{2}]^2 = 0$. So, solution of y only depended on α_0, γ_0, and K_0 and $Q_{x^*} = [2/3, 3/2]$. ∎

Remark 3: A direct computation can be used to show that Q_{x^*} for the open loop example is $[2/3, 3/2]$ when $Q_\alpha = [2,3]$ and $Q_\gamma = [2,3]$.

Example 3 (Positive Autoregulation): The positive autoregulation model is another standard model, in which a positive feedback on the expression of a protein is implemented by the activation of its own production. Here, $f(x,u) = \alpha\frac{x}{x+K} - \gamma x$, with $x \in \mathbb{R}$ and $u = [\alpha, \gamma, K]^T \in \mathbb{R}^{3\times 1}$. Often, co-operativity is included in the model in terms of a Hill co-efficient n in the activation function, $f(x,u) = \alpha\frac{x^n}{x^n+K^n} - \gamma x$. The condition $n > 1$ is useful in the analysis and design of systems with multiple steady-states. In case there are multiple steady-states, the analysis and design methods based on linearization may be performed by linearizing about one of the steady-states. For simplicity, we presented the analysis and design methods for a fixed value of n.

The linear analysis (5) gives the variation $|\Delta x| = \left\|\frac{\left[\frac{(x^*)^n}{(x^*)^n+(K^*)^n} \;\; -x^* - \frac{\alpha^*(x^*)^n n(K^*)^{n-1}}{((x^*)^n+(K^*)^n)^2}\right]}{\frac{-\alpha^*(K^*)^n n(x^*)^{n-1}}{((x^*)^n+(K^*)^n)^2} + \gamma^*}\right\| \|\Delta u\|$ around a steady-state x^* for the default parameters $[\alpha^*, \gamma^*, K^*]^T$. The nonlinear analysis (3) is performed with $T(x,u) = x + c(\alpha\frac{x^n}{x^n+K^n} - \gamma x)$. For $c = (2(\gamma - \frac{\alpha K^n n x^{n-1}}{(x^n+K^n)^2}))^{-1}$, $l_X = 1/2$ and $l_U = \sup\|c\left[\frac{x^n}{x^n+K^n} \;\; -x - \frac{\alpha x^n n K^{n-1}}{(x^n+K^n)^2}\right]\|$, where the supremum is over the parameter space $\alpha \in [\underline{\alpha}, \overline{\alpha}], \gamma \in [\underline{\gamma}, \overline{\gamma}], K \in [\underline{K}, \overline{K}]$. The bound is $l_U(1-l_X)^{-1}$. Care needs to be exercised in the supremum to exclude the region where $f_x = 0$.

For the design problem in the linearized setting, (6) may be solved using the right pseudo-inverse, $\|[\Delta\alpha, \Delta\gamma, \Delta K]^T\| \leq \|\frac{\lambda^*}{\sigma^*}[\frac{(x^*)^n}{(x^*)^n+(K^*)^n}, -x^*, \frac{-\alpha^*(x^*)^n n(K^*)^{n-1}}{((x^*)^n+(K^*)^n)^2}]^T\|\|\Delta x|$, where $\lambda = \gamma - \frac{\alpha K^n n x^{n-1}}{(x^n+K^n)^2}$ and $\sigma = (\frac{x^n}{x^n+K^n})^2 + x^2 + (\frac{\alpha x^n n K^{n-1}}{(x^n+K^n)^2})^2$. The design problem in the nonlinear setting (7) also has the same form $l_X(1-l_U)^{-1} = \sup\|\frac{\lambda}{\sigma}[\frac{(x)^n}{(x)^n+(K)^n}, -x, \frac{-\alpha(x)^n n(K)^{n-1}}{((x)^n+(K)^n)^2}]^T\|, x \in X^*$ where we choose $C = \frac{1}{2\sigma}[\frac{-x^n}{x^n+K^n}, x, \frac{-\alpha x^n n K^{n-1}}{(x^n+K^n)^2}]^T$.

The modified Interval Newton Algorithm naturally overcomes the limitation imposed by $f_x = 0$ to obtain the rigorous bounds for each of the steady-states (Fig. 1). This highlights the importance of the interval-based method in such situations. The linear analysis and design versions are similar to the above examples. We also note that an identical result to Proposition 1 may be obtained for this example.

Example 4 (Incoherent Feedforward Loop): The Incoherent Feedforward Loop model is a benchmark for a variety of phenomena such as pulse generation, fold-change detection and adaptation. A simple version of an Incoherent Feedforward Loop model has $f(x,u) = [f_1(x,u), f_2(x,u]^T = [\alpha - \gamma x_1, \frac{\alpha}{1+x_1/K} - \gamma x_2]^T$ with the state $x = [x_1, x_2]^T \in \mathbb{R}^{2\times 1}$ and the parameters $u = [\alpha, \gamma, K]^T \in \mathbb{R}^{3\times 1}$. The input is lumped with the parameter α in this model.

The bounds from the linearized analysis (5) are, $\|\Delta x\| \leq \|-f_x^{-1}f_u\|\|\Delta u\|$ where, $f_x = \begin{bmatrix} -\gamma^* & 0 \\ -\frac{\alpha^*K^*}{(x_1^*+K^*)^2} & -\gamma^* \end{bmatrix}$ and $f_u = \begin{bmatrix} 1 & -x_1^* & 0 \\ \frac{K^*}{x_1^*+K^*} & -x_2^* & \frac{\alpha^*x_1^*}{(x_1^*+K^*)^2} \end{bmatrix}$. For the nonlinear analysis (3), we set $T(x,u) = [x_1, x_2]^T + C[\alpha - \gamma x_1, \frac{\alpha}{1+\frac{x_1}{K}} - \gamma x_2]^T$. For $C = \frac{-1}{2}f_x^{-1}$, $l_X = 1/2$ and $l_U = \sup\|Cf_u\|$. The bound is $l_U(1-l_X)^{-1} = \|f_x^{-1}f_u\|$. For the linearized design problem, (6) may be solved using the right pseudo-inverse, $\|[\Delta\alpha, \Delta\gamma, \Delta K]^T\| \leq \|-f_{u*}^\dagger f_{x*}\||\Delta x|$, where $f_u^\dagger = f_u^T(f_uf_u^T)^{-1}$. The design problem in the nonlinear setting (7) also has the same form $l_X(1-l_U)^{-1} = \sup\|-f_u^\dagger f_x\|, x \in X^*$ where we chose $C = (-I/2)f_u^\dagger$. Interval Analysis can also be used to obtain the bounds (Fig. 2). The linear analysis and design inequalities are similar to the above examples.

V. CONCLUSION

Quantifying the extent of variation in the steady-states of biomolecular circuits in response to parametric perturbations

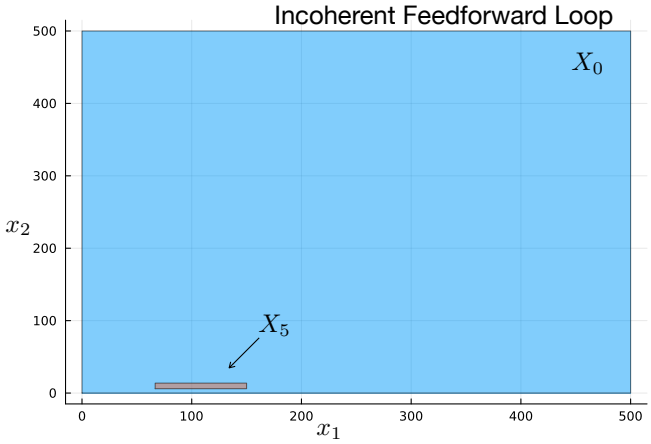

Fig. 2. Steady-state bounds. Parameters for incoherent feedforward loop: $\alpha \in [200, 300]$, $\gamma \in [2, 3]$, $K \in [20/3, 15]$, $m = 100$ subdivisions.

is challenging. We developed a method based on the Contraction Mapping Theorem to compute rigorous steady-state bounds for given changes in the parameters. We linearized at a steady-state and computed bounds on the local steady-state variations for local parametric variations, finding the same form as those obtained by the nonlinear method. These naturally extend to the design problem of bounding parameters for given steady-state bound specifications. We also developed a complementary method based on Interval Analysis, to incorporate the parametric uncertainties directly in the above analyses and linear design. These methods are illustrated on benchmark examples.

Based on the theoretical results and the examples presented here, we found the nonlinear analysis using the interval method to be the most useful. This is strikingly observed in Example 3 where this method could capture multiple steady-states and their intervals. This is a direct consequence of computing using intervals and of the bounded nature of the problem. Further, the bounds computed using Interval Analysis can be as tight as needed. The Contraction Analysis is also rigorous, but its bounds necessarily depend on the contraction used and can be conservative due to the determination of the Lipschitz constant from the supremum of a first derivative, with a larger domain over which the supremum is obtained enhancing the conservativeness. It is interesting to note that the contraction framework is general, with the Newton's method also a form of a contraction. However, in the point-wise sense, the Newton's method does not simultaneously capture all the steady-states, as in Example 3, where the Jacobian is singular. Linear analysis approximates the nonlinear analysis and is contingent on an *a priori* identification of the point of linearization, possibly through other nonlinear methods. However, due to its relative simplicity, linear analysis may be useful in an initial study and in cases where only local analysis is desired.

The method of BDC decomposition can also provide tight bounds for the ratio of the change in steady-state value to the change in the input parameter values for certain classes of systems that satisfy assumptions such as non-singularity of the Jacobian and monotonicity [5]. For Example 1, we found these bounds to be $\frac{[1,-x]}{\gamma} = [[1/3, 1], [-300, -100/9]]$, where the value of steady-state was calculated directly. The similarity in the expression with an intermediate step in the Contraction Analysis was noteworthy. Based on these expressions, we conjectured that the BDC decomposition could be considered as a form of Contraction Analysis where Lemma 1 is applied to each parameter individually. Exploring the connection between these methods would be an interesting subject for future study.

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