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Model Validation and Robust Stability Analysis of the Bacterial Heat Shock Response Using SOSTOOLS

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Abstract

The complexity inherent in gene regulatory network models, as well as their nonlinear nature make them difficult to analyze or validate/invalidate using conventional tools. Combining ideas from robust control theory, real algebraic geometry, optimization and semi-definite programming, SOSTOOLS provides a promising framework to answer these robustness and model validation questions algorithmically. We adopt these tools in the study of the heat shock response in bacteria. For this purpose, we use a reduced order model of the bacterial heat stress response. We study the robust stability properties of this system to parametric uncertainty, and address the model validation/invalidation problem by proving the necessity for the existence of certain feedback loops to reproduce the known time behavior of the system.

1 Introduction

One of the predominant goals of systems biology is to uncover how all of the genetic information is organized in regulatory systems that control life, health, and disease. The first step in this direction is to build accurate computer models that give reliable, both qualitative and quantitative, descriptions of the mechanisms under study. An intrinsic problem with this approach revolves around model validation/invalidation. In biological modeling, model validation is usually carried out by comparing the model predictions to data. Implicitly underlying this task is the assumption that models could be unambiguously compared with data, when in fact this comparison is even more computationally challenging than modeling and analysis itself. Specifically, given a model with a large number of unknown parameters, simulation plus local sensitivity analysis and search can sometimes produce parameter values that fit data or are locally maximally likely to fit. If this fails, however, there may be no short proof that the model is incompatible with the data, mirroring the NP versus coNP distinction. Furthermore, with sufficiently complex models, it is well-known that almost any finite amount of data can be fit. Furthermore, even if a model has been shown to agree well with the measured data, the ability to assess the robustness of such a model is still important for understanding the function of the overall system and the contribution of its component subsystems. Combining notions from dynamical systems theory, real algebraic geometry and semi-definite programming, SOSTOOLS [9, 10] provides an ideal framework to handle these issues in an algorithmic way. The procedure is based on the construction of *Lyapunov functions* as certificates that guarantee stability for a system which may contain uncertain parameters, as well as *barrier functions* as invalidation certificates for proposed models. These *barrier functions* separate the evolution of the model from measured data. The positivity conditions in these methodologies, such as those in Lyapunov's stability theorem, can be relaxed computationally to Sum of Squares conditions, as the former are in general NP-hard to test whereas the latter can be verified in polynomial time using Semi-Definite Programming. Several constraints on these conditions can be adjoined to the system using the general framework of *Positivstellensatz*, a central theorem in Real Algebraic Geometry.

In this work, we approach the validation/invalidation and robustness analysis of a model of the bacterial heat stress response in the context of SOSTOOLS. The bacterial heat shock response is a fairly complex, highly conserved regulatory network that is of crucial importance in the survival of most organisms. This system possesses a hierarchy of feedforward and feedback loops that serve different functions, ranging from increasing robustness to parametric uncertainty, to achieving fast transients and rejecting intrinsic cellular noise. We report the results of a preliminary test pertaining to the validity of a reduced order model of the HS response, in addition to results relevant to its robust stability features to parametric uncertainty, all using SOSTOOLS.

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2 The Heat Shock Response in *E. coli*

High temperatures cause cell proteins unfold from their normal shapes, resulting in malfunctioning and eventually death of the cell. Cells have evolved gene regulatory mechanisms to counter the effects of heat shock by expressing specific genes that encode heat shock proteins (hsps) whose role is to help the cell survive the consequence of the shock. Many hsps serve as molecular chaperones that assist in the refolding of denatured proteins; others are proteases that degrade and remove the denatured proteins. In *E. coli*, the heat shock (HS) response is implemented through an intricate architecture of feedback loops centered around the σ- factor that regulates the transcription of the HS proteins under normal and stress conditions. The enzyme RNA polymerase (RNAP) bound to this regulatory sigma factor, σ^{32}, recognizes the HS gene promoters and transcribes specific HS genes. The HS genes encode predominantly molecular chaperones (DnaK, DnaJ, GroEL, GrpE, etc.) that are involved in refolding denatured proteins and proteases (Lon, FtsH, etc.) that function to degrade unfolded proteins. At physiological temperatures (30°C to 37°C), there is very little σ^{32} present and hence little transcription of the HS genes. When bacteria are exposed to high temperatures, σ^{32} first rapidly accumulates, allowing increased transcription of the HS genes and then declines to a new steady state level characteristic of the new growth temperature. There are two mechanisms by which σ^{32}levels are increased when the temperature is raised. First, the translation rate of the rpoH mRNA (encoding σ^{32}) increases immediately, resulting in a fast 10-fold increase in the concentration of σ^{32} [11]. This mechanism implements what we refer to as the *feedforward control loop*. Second, during steady state growth, σ^{32} is rapidly degraded ($t_{1/2} = 1$ minute), but is stabilized for the first five minutes after temperature upshift, so that its concentration rapidly increases. *In vivo* evidence is consistent with the following titration model for the HS response. DnaK, and its cochaperone DnaJ are required for the rapid degradation of σ^{32} by the HS protease FtsH. Raising the temperature produces an increase in the cellular levels of unfolded proteins that then titrate DnaK/J away from σ^{32}, allowing it to bind to RNA polymerase (resulting in increased transcription) and stabilizing it in the process. Together, increased translation and stabilization lead to a transient 15-20 fold increase in the amount of σ^{32} at the peak of the HS response. The accumulation of high levels of HS proteins leads to the efficient refolding of the denatured proteins thereby decreasing the pool of unfolded protein, freeing up DnaK/J to sequester this protein from RNA polymerase. This implements what is referred to as a *sequestration feedback loop*. Furthermore, this sequestration itself promotes the degradation of σ^{32} and results in feedback regulated degradation, mainly by the protease FtsH. We refer to this as the *FtsH degradation feedback loop*. The overall result is a decrease in the concentration of σ^{32} to a new steady state concentration that is dictated by the balance between the temperature-dependent translation of the rpoH mRNA and the level of σ^{32} activity modulated by the hsp chaperones and proteases acting in a negative feedback fashion.

3 A Reduced Order Model for the HS Response

In a previous work, we have developed a detailed deterministic mathematical model for the heat stress response in *E. coli* [2, 4] The dynamics described above were modeled using differential rate equations, and the full model takes the form of a set of 31 Differential-Algebraic Equations (*DAEs*), which are of the form:

$$\begin{aligned} \dot{X}(t) &= F(t;X;Y) \\ 0 &= G(t;X;Y) \\ X(t=t_0) &= X_0 \\ Y(t=t_0) &= Y_0, \end{aligned}$$

where X is a 11-dimensional vector whose elements are the differential variables and Y is a 20-dimensional vector whose elements are algebraic variables. This form is known as a *semi-explicit* DAE. The model possesses 27 kinetic rate parameters. It was simulated using the specialized software *DASSL* [7]. Validation of this model against biological data has been successfully carried out. Subsequently, a reduced order model was derived using insight into the system's architecture and separation principles in time and concentrations (in preparation). As in the full model, this reduced model involves the dynamics of the basic building blocks of the HS response, namely the σ factor (S), the chaperones (D), and the protein folding mechanism. The model equations are as follows

$$\begin{aligned} \frac{dD_t}{dt} &= K_d S_f - \alpha_d D_t \\ \frac{dS_t}{dt} &= \eta(T) - \alpha_0 S_t - \alpha_s S:D \\ \frac{dU_f}{dt} &= K(T) P_{folded} - K_{fold} U:D \\ S:D &= K_s.S_f.D_f \\ U:D &= K_u.U_f.D_f \\ D_t &= D_f + U:D + S:D \\ S_t &= S_f + S:D \\ P_t &= P_{folded} + U_f + U:D \end{aligned} \quad (1)$$

where $U:D$ is the complex formed by the binding of the unfolded proteins U_f to D, $S:D$ is the complex formed by the binding of S to D, and P_t is the total

Parameter	Value
K_d	3
α_d	0.015
$\eta(T)$	10 @ T_1 & 60 @ T_2
α_0	0.03
α_s	3
K_s	0.05
K_u	0.0254
$K(T)$	40 @ T_1 & 80 @ T_2
K_{fold}	6000
P_t	2×10^6

Table 1: Parameter Values for Heat Shock model

number of proteins in the cell, considered here to be constant. The parameters used in this model are given in Table 1. We replace the algebraic constraints into the initial system (1), then use the facts that $S_t \ll D_t$ and that $U_f \gg 1$ in the wild type bacterial HS response and simplify the expression for S_f and D_f. Simple algebraic manipulations yield a compact description for the reduced order HS model:

$$\begin{aligned} \frac{dD_t}{dt} &= f_1(D_t, U_f, S_t) - \alpha_d D_t \\ \frac{dS_t}{dt} &= \eta(T) - \alpha_0.S_t - f_2(D_t, U_f, S_t) \\ \frac{dU_f}{dt} &= K(T)[P_t - U_f] - [K(T) + K_{fold}]D_t \end{aligned} \quad (2)$$

As in the original equations, the feedforward control is achieved by the temperature dependent function $\eta(T)$ in the ODE describing the dynamics of S_t. $f_1(D_t, U_f, S_t) = K_d \frac{S_t}{1+\frac{K_s D_t}{1+K_u U_f}}$ and $f_2(D_t, U_f, S_t) = \alpha_s \frac{\frac{K_s D_t}{1+K_u U_f}}{1+\frac{K_s D_t}{1+K_u U_f}} S_t$ describe the various feedback strategies implemented in the HS response. f_1 is the effect of the sequestration of S by D, while f_2 reflects the effect of the regulated degradation of S through the action of the sequestration itself. The dynamics of the third state U_f are much faster than those of S_t and D_t. Such stiffness (large differences in time scales with the fastest stable) is also strongly present in the full model and creates ill-conditioning and algorithms that don't exploit stiffness are almost certainly doomed to suffer from it; thus the need for *DASSL* [7] to simulate the full system. However, stiffness can also be exploited to robustly produce simplified models by singular perturbation, as was done in deriving the 3-state from the full model. By further setting $\frac{dU_f}{dt} = 0$ to obtain a quasi-steady state approximation, the third equation is then replaced by an algebraic one, and the result is again a differential-algebraic equation (DAE). The validity of this approximation has been verified by simulation which showed virtually no difference in the solution of the ODE as compared to that of the DAE.

4 Robustness Analysis of the Reduced HS Model

4.1 Robustness Analysis Methodology Using SOSTOOLS

Here we present how robustness analysis can be addressed in general, and then specialize the analysis to the example of the HS response in *E-coli*. Consider the nonlinear system

$$\begin{aligned} \dot{x} &= f(x,p), & (3)\\ P &= \{p \in \mathbb{R}^m : q_{k_1}(p) \leq 0, k_1 = 1, \dots K_1; \\ &\quad r_{l_1}(p) = 0, l_1 = 1, \dots L_1\} & (4)\\ D &= \{x \in \mathbb{R}^n : q_{k_1}(x) \leq 0, k_1 = K_1 + 1, \dots K_2\} & (5) \end{aligned}$$

where $x \in \mathbb{R}^n$ is the state of the system, constrained to D, $p \in \mathbb{R}^m$ is a set of unknown parameters in a set P and f is a polynomial vector field. Without loss of generality, we assume that the origin of the state space is the equilibrium of interest of the system. We are first interested in proving that this equilibrium is stable for all parameters $p \in P$ and all states $x \in D$. A standard method is to construct a Lyapunov function V [3] according to the following theorem.

Theorem 1 *Suppose that for the above system description we can find a function $V(x,p)$ such that:*

$$\begin{aligned} V(x,p) &> 0 \qquad \forall x \in D \setminus \{0\}, p \in P \\ -\frac{\partial V(x,p)}{\partial x} f(x,p) &\geq 0 \qquad \forall x \in D, p \in P \end{aligned}$$

Then the origin of the state space is a stable equilibrium of the system.

Although Lyapunov functions are extremely important tools for stability and robust stability analysis of nonlinear and hybrid systems, even testing the positivity conditions in Theorem 1 is NP-hard, and until recently no general methodology existed. A computational relaxation to testing positivity is the existence of a Sum of Squares decomposition, which can be verified algorithmically in polynomial time by solving a Semi-Definite Program (SDP). We state without proof the following useful theorem:

Theorem 2 *Given a polynomial $p(x)$, $x \in \mathbb{R}^n$, a necessary and sufficient condition for $p(x)$ to be a Sum of Squares, i.e. $p = \sum_{i=1}^m f_i^2(x)$ for some polynomials $f_i(x)$ is that $p(x)$ can be written as*

$$p(x) = Z(x)^T Q Z(x) \quad (6)$$

where $Z(x)$ is a vector of all monomials of degree less than or equal to $\frac{degree(p)}{2}$ and Q is a positive semi-definite matrix.

The search for Q in Theorem 2 is in fact a search in the cone of positive semi-definite matrices under affine constraints between its entries and the coefficients of the monomials of p, and is thus a Semi-Definite program [6]. Based on Theorem 2 an effective method to test the conditions in Theorem 1 is:

Proposition 3 *For the sets P and D defined earlier, the two conditions in Theorem 1 can be tested as follows:*

$$\left(V(x,p)+\sum_{i=1}^{K_2} a_{1i}(x,p)q_i(x,p)+\sum_{i=1}^{L_1} b_{1i}(x,p)r_i(x,p)-\varphi(x)\right)$$

is a Sum of Squares, and

$$\left(-\frac{\partial V(x,p)}{\partial x}f(x,p)+\sum_{i=1}^{K_2} a_{2i}(x,p)q_i(x,p)+\sum_{i=1}^{L_1} b_{2i}(x,p)r_i(x,p)\right)$$

is a Sum of Squares. $V(x,p)$, $b_{1i}(x,p)$ and $b_{2i}(x,p)$ are polynomials in (x,p), $\varphi(x)$ is positive definite and $a_{1i}(x,p)$, $a_{2i}(x,p)$ are Sum of Squares.

This proposition is a generalization of the S-procedure [1] and duality, in that equality constraints on conditions are adjoined using polynomial multipliers, and inequality constraints using Sum of Squares (non-negative) multipliers. Note that the above reduces to the S-procedure when the multipliers are constants; when they are polynomials, the resulting conditions are at least as powerful as the S-procedure.

4.2 Robustness Analysis of the HS system

For the heat shock model, we can consider the problem of proving robust stability for the system under parametric uncertainty. Note that the vector field is rational, but this case can be treated by multiplying out the derivative condition in Theorem 1 by the (non-vanishing) common denominator of the vector field. In the spirit of Theorem 1, robust stability analysis is achieved by constructing parameter-dependent Lyapunov functions. We proceed by non-dimensionalizing the states of (2) by their equilibrium values $(D_{t_0}, S_{t_0}, U_{f_0})$, followed by a shifting of the equilibrium of the system to the origin. We then obtain a system with states (x_1, x_2, x_3) that is better conditioned, in the sense that the states are of the same order of magnitude:

$$\begin{aligned}\frac{dx_1}{dt} &= \tilde{f}_1(x_1,x_2,x_3)-\alpha_d x_1\\ \frac{dx_2}{dt} &= \tilde{\eta}(T)-\alpha_0 x_2-\tilde{f}_2(x_1,x_2,x_3) \qquad (7)\\ \frac{dx_3}{dt} &= K(T)[\tilde{P}_t-x_3]-K_{Tot}x_1\end{aligned}$$

with $\tilde{f}_1(x_1,x_2,x_3)=\tilde{K}_d\frac{x_2}{1+\frac{\tilde{K}_s x_1}{1+\tilde{K}_u x_3}}$ and $\tilde{f}_2(x_1,x_2,x_3)=\alpha_s x_2\frac{\frac{\tilde{K}_s x_1}{1+\tilde{K}_u x_3}}{1+\frac{\tilde{K}_s x_1}{1+\tilde{K}_u x_3}}$, and where $\tilde{K}_d = K_d S_{t_0}/D_{t_0}$, $\tilde{K}_s = K_s D_{t_0}$, $\tilde{K}_u = K_u U_{f_0}$, $\tilde{\eta} = \eta/S_{t_0}$, $\tilde{P}_t = P_t/U_{f_0}$ and $K_{Tot} = D_{t_0}(K(T)+K_{fold})/U_{f_0}$. We then use $\frac{dx_3}{dt}=0$ to get a 2-D state-space (x_1,x_2). Now we can investigate robust stability of system (7) using the results in Theorem 1. For this, we need to define the region D:

$$D=\{x_i\in\mathbb{R}:(x_i-x_{i_0})^2-\gamma_i^2\le 0, i=1,2\} \qquad (8)$$

with $\gamma_i = 0.2$ defining a square around the equilibrium where stability is to be proven, and x_{i_0} is the equilibrium of the i-th state. For robust stability analysis purposes, we pick two crucial parameters, $\tilde{\eta}$ and α_s. $\tilde{\eta}$ depicts the feedforward gain, while α_s is a crucial component of the feedback gain. We ask whether the system (2) is stable for all values of $\tilde{\eta}$ and α_s in a certain range for $T = T_1$:

$$\begin{aligned}P=\{\tilde{\eta},\alpha_s\in\mathbb{R}^2:&(\tilde{\eta}-\tilde{\eta}_0)^2-(\gamma_3\tilde{\eta}_0)^2\le 0,\\ &(\alpha_s-\alpha_{s_0})^2-(\gamma_4\alpha_{s_0})^2\le 0\}\end{aligned}$$

with γ_3 and γ_4 measuring the percentage variation. This case can be handled using Theorem 1. The procedure that one follows is to propose an upper bound on the degree of V and of the multipliers a and b, and then test whether the conditions in Proposition 3 are satisfied. The decision variables in the Semi-Definite programme are the coefficients of these unknown polynomials; when it is feasible, the various polynomials are constructed, and form *proofs* of Robust stability of the system as per Theorem 1. This is done easily using SOSTOOLS [10]. For this example, a parameter-dependent Lyapunov function $V(x_1,x_2;\tilde{\eta},\alpha_s)$ was constructed in the region D under the uncertainty defined by P. In particular, by constructing a quadratic Lyapunov function we were able to prove stability for $\gamma_3 = \gamma_4 = 0.14$. The region P can be increased further if one considers quartic Lyapunov functions. Figure 1 shows the level curves of the Lyapunov function for two sets of parameters in a parameter set with $\gamma_3 = \gamma_4 = 0.44$. We see that by increasing the order of the certificate, we can prove robustness for a larger parameter range. The computational complexity increases in a *polynomial* manner with the number of the parameters, in contrast to any other method that involves simulation, where it increases exponentially. Dynamic uncertainty is another type of uncertainty that is usually described by Integral Quadratic Constraints (IQCs). IQCs are a powerful tool in Robust Control analysis; simulation of such uncertainty however is not possible, whereas such uncertainty descriptions can be incorporated in the search for a Lyapunov function using this methodology in a unified way. See [5] for more details.

5 Validation/Invalidation of the HS Model

The new methodology in conjunction with SOSTOOLS can be used to address the critical issue of model valida-

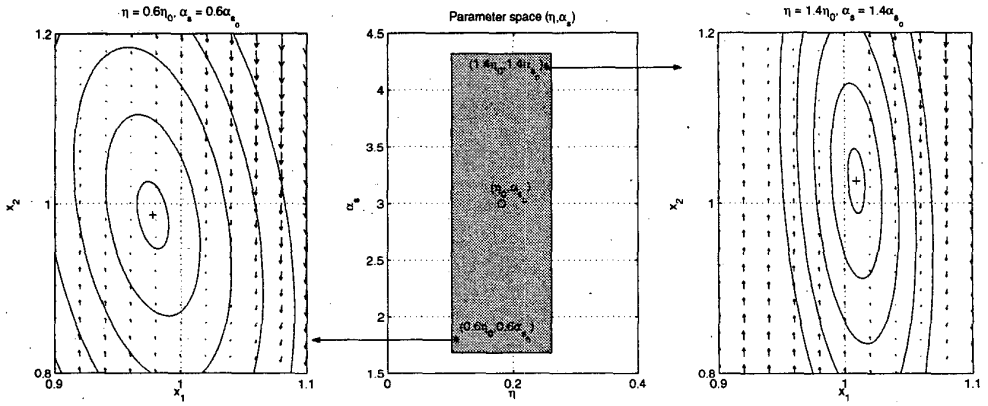

Figure 1: Level curves for the parameter-dependent Lyapunov function $V(x_1, x_2; \tilde{\eta}, \alpha_s)$ for system (2) for two sets of parameters.

tion/invalidation in biological modeling. The key ideas of this methodology can be illustrated in the context of the heat-shock example, where at least two feedback loops are involved in the regulation scheme. We will show rigorously that each loop adds its own important function to the overall system and that both are necessary to explain the phenotypic behavior of the heat shock system. In previous work, we have used sensitivity analysis and confirmed that these feedback loops indeed increase the robustness to parametric uncertainty [2]. However, upon disabling the degradation (FtsH) feedback loop, one observes in simulation that the transient response to a temperature increase becomes considerably slower. Achieving a faster transient response in the absence of this (FtsH) feedback loop necessitates a substantial increase in the protein synthesis rate, and therefore, produces a larger number of chaperones. Therefore, it is reasonable to conjecture that the (FtsH) feedback loop is instrumental in achieving a fast response to the heat disturbance while using a relatively modest number of chaperones. To illustrate how we might actually prove such a conjecture using our new invalidation scheme, we would perform an experiment with the system to obtain some data that will be used in the construction of a barrier function to invalidate a model. Consider the system (3) with uncertain parameter $p \in P$ as before. Additional information about the system (for example: the state variables cannot be negative, cannot be too large, etc.) may be available. We include this information in D, defined in the same manner as before. Next, we assume that from experiments performed at time $t = 0$ and $t = T_f$, we obtain the following measurement results:

$$Y_0 = \{x \in \mathbb{R}^n : q_0(x) \leq 0, r_0(x) = 0\} \quad (9)$$

$$Y_{T_f} = \{x \in \mathbb{R}^n : q_{T_f}(x) \leq 0, r_{T_f}(x) = 0\}. \quad (10)$$

With this notation, our problem can be stated as follows: given a priori information on the state space model of the system, admissible parameter P, provide a proof (if possible) that the measurement Y_0, Y_{T_f} is inconsistent with the a priori information. If such a proof is found then there is no parameter $p \in P$ for which the model considered produces measurements Y_0, Y_{T_f}, and thus the model is invalidated. For this, we need the following theorem (see [8]).

Theorem 4 *Assume that there is a function* $B : \mathbb{R}^{n+m+1} \to \mathbb{R}$ *such that*

$$B(x_{T_f}, p, T_f) - B(x_0, p, 0) > 0, \quad \forall\, x_{T_f} \in Y_{T_f},\ x_0 \in Y_0,\ p \in P, \quad (11)$$

$$\frac{\partial B}{\partial x}(x,p,t) f(x,p,t) + \frac{\partial B}{\partial t}(x,p,t) \leq 0, \quad \forall\, t \in [0, T_f],\ x \in D,\ p \in P. \quad (12)$$

Then the measurements $\{Y_0, Y_{T_f}\}$ *is inconsistent with the system (3) and the parameter set* P.

To illustrate how this could apply to heat shock, we will assume that the "real system" is just the model with the degradation (FtsH) loop (2), and compare it to a hypothesized model lacking this feedback. If we denote the state variables (D_t, S_t, U_f) by (x_1, x_2, x_3), then the hypothesized model will just be $\dot{x} = f(x,p)$, where the vector field are defined by (2), without the degradation loop. The parameters p will be defined below. We perform an experiment with the "real system" and observe that a typical time response of this system satisfies the following conditions (the notation is the same as in Theorem 4) :

$$Y_0 = \{(x_1, x_2, x_3) \in \mathbb{R}^3 : 0.9D_0 \leq x_1 \leq 1.5D_0 \quad 0.9S_0 \leq x_2 \leq 1.5S_0, 2.9U_0 \leq x_3 \leq 3.1U_0\} \quad (13)$$

$$Y_{T_f} = \{(x_1, x_2, x_3) \in \mathbb{R}^3 : 1.5D_0 \leq x_1 \leq 2.5D_0, \quad 2S_0 \leq x_2 \leq 3S_0, 0.5U_0 \leq x_3 \leq 1.5U_0\} \quad (14)$$

where D_0, S_0, and U_0 denote their steady state values at low temperature, and the final time $T_f = 25$. Note that we use intervals here to take into account the effects of measurement uncertainty, variation of initial conditions, and so on. In addition, we also observe that between time $t = 0$ and $t = T_f$, the state variables satisfy the following conditions, which define D:

$$D = \{(x_1, x_2, x_3) \in \mathbb{R}^3 : 0.9D_0 \leq x_1 \leq 2.5D_0, \\ 0.9S_0 \leq x_2 \leq 8S_0, 0.2U_0 \leq x_3 \leq 4U_0\}. \quad (15)$$

As for the parameters, we will focus on three parameters $p = (K_d, \alpha_0, \eta(T))$. Plausible ranges for these parameters define P:

$$P = \{(K_d, \alpha_0, \eta(T)) \in \mathbb{R}^3 : 0.5\overline{K}_d \leq K_d \leq 5\overline{K}_d, \\ 0.5\overline{\alpha}_0 \leq \alpha_0 \leq 1.5\overline{\alpha}_0, 0.5\overline{\eta(T)} \leq \eta(T) \leq 1.5\overline{\eta(T)}\}, \quad (16)$$

where $\overline{K}_d$, $\overline{\alpha}_0$, and $\overline{\eta(T)}$ denote their nominal values. We deliberately make the upper bound for K_d quite large, since one obvious way for obtaining a fast response is to increase the number of chaperones, corresponding to increasing this parameter. With our method, we can effectively prove that this nevertheless cannot be achieved without violating the other constraints, namely that the model without the degradation (FtsH) loop with parameters K_d, α_0, $\eta(T)$ satisfying (16) cannot possibly generate a time response that satisfies (14)–(15). This indicates that an inherent mechanism is missing from this model. When the FtsH mechanism is included, obviously there are values for parameters K_d, α_0, and $\eta(T)$ (e.g. the nominal values $\overline{K}_d$, $\overline{\alpha}_0$, and $\overline{\eta(T)}$) such that the model has a time response that satisfies (14)–(15).

6 Conclusions and Future Work

In this work, we use the procedure introduced in [5] and the software SOSTOOLS to investigate some of the robustness features of the heat shock response in *E. coli*. We also report the results of a model invalidation scheme which suggests the essential role of one of the feedback loops involved in this regulatory system. In addition to being yet another application of SOSTOOLS, such an observation about the *degradation loop* is crucial from a biological perspective. Indeed, we were able to affirm that the existence of such a loop is not necessarily explained by *redundancy* as was suggested in the heat shock literature. Rather, this loop seems to play a crucial role in enhancing the transient response of the system after a heat disturbance. Such conclusions are valuable in guiding experiments and complementing biological knowledge. Finally, while the work reported in this paper is still preliminary, it proved to be very promising. We plan to expand our investigation to include more complex variations of the model, in addition to using the SOSTOOLS machinery in a more systematic model reduction scheme of the original detailed model.

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