

OPTIMAL PERFORMANCE OF THE HEAT-SHOCK GENE REGULATORY NETWORK¹

Hana El Samad,^{*} M. Khammash^{*}
Chris Homescu^{**} and Linda Petzold^{**}

^{*} *Mechanical Engineering, UC Santa Barbara*

^{**} *Computer Science, UC Santa Barbara*

Abstract: In this work, we study the optimality principles in the heat shock system, a cellular response that refers to the mechanism by which organisms react to a sudden increase in the ambient temperature. The consequence of such a temperature increase at the cellular level is the unfolding of cell proteins, which threatens the life of the cell. This increase in damaged proteins is usually counteracted by a simultaneous increase in the level of the so called heat shock proteins that are necessary for protein refolding. Their production, however, is metabolically costly and the benefits should be constantly balanced with the cost. Building on a heat shock model we have developed in previous work (El-Samad *et al.*, 2005), we formulate and solve a multi-objective optimization problem where both the cost of producing and maintaining HSPs and the cost of unfolded proteins are considered as optimization criteria. *Copyright ©2005 IFAC*

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1. INTRODUCTION

Stress-inducing agents, including heat, have a deleterious effect on cellular function as they usually increase the level of misfolded proteins in the cell. Misfolded proteins lose most of their biological activities and cause failures in the cellular networks where they operate. At the molecular level, the cellular response to stress is represented by the synthesis of heat shock proteins (HSPs), of which molecular chaperones and proteases represent two well characterized families. Molecular chaperones function in protein fold-

ing, translocation, and refolding, while proteases ensure that damaged proteins are degraded efficiently. In order to achieve basic heat disturbance rejection, cells need to sense the temperature and counteract its effect by increasing the production of chaperones and proteases. Indeed, a simple and operational heat shock system would simply consist of a temperature sensor and transcriptional/translational apparatus that responds appropriately to temperature changes. This open loop design would, however, result in a fragile and metabolically inefficient system that will quickly be at a disadvantage in the noisy environment of the cell. As a consequence, most organisms have evolved complex heat shock systems that involve intricate feedback and feedforward mechanisms.

In previous work, we have developed a detailed mechanistic model describing the dynamics of the

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heat shock response in the bacterium *E. coli* (El-Samad *et al.*, 2005). We used this model to provide valuable insight into the system, explaining dynamic phenomena exhibited by wild type and mutant heat shock responses, corroborating experimental data and guiding novel biological experiments. We further demonstrated, through the careful control analysis of the model, several design principles that appear to have shaped the feedback structure of the heat shock system. Specifically, we itemized the roles of the various feedback strategies and demonstrated their necessity in achieving performance objectives such as efficiency, robustness, stability, good transient response, and noise rejection. In this work, and in addition to these important characteristics, we study the principles of optimality in the heat shock system. We specifically assess the metabolic cost imposed on the cell by the requirement of achieving an acceptable level of folded proteins. The motivation for addressing this problem is as follows. Since the objective of the heat shock response is to refold unfolded proteins, a first glance at the heat shock response system suggests that overproducing HSPs in anticipation of heat is the best strategy. This would be the case when the control effort in the system, i.e. the chaperones, is cheap and unconstrained. This is certainly not true for the heat shock system where the production of HSPs' necessitates the use of the cell's limited resources. It is therefore apparent that an excess of HSPs can be expensive. Low levels of chaperones and unfolded proteins in the cell are however two mutually conflicting objectives. If the objective is to achieve simultaneous minimization of both costs, the solution would necessarily depend on how important is one objective versus the other. To tackle this issue in this work, we formulate an optimization problem, and generate a Pareto optimal curve which gives the optimal cost associated with these two objectives as their relative importance is varied. We then investigate the optimality of the heat shock response by measuring its distance from this optimal curve.

The paper is organized as follows. In the next section, we give a detailed account of the molecular components in the heat shock system. We then briefly describe the type of equations which are used to model the dynamics of the system. In the fourth section, we provide a brief overview of Pareto optimality, and formulate our optimization problem in that context. We subsequently describe the methods used to numerically tackle the problem, and give its solution. We end with some general conclusions, and a synopsis of our future work.

2. THE BIOLOGY OF HEAT SHOCK

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} (Straus *et al.*, 1989). 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 and 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 MODEL OF THE HEAT SHOCK SYSTEM

The mathematical model that we proposed in (El-Samad *et al.*, 2005) to describe the dynamics of the heat shock response uses mass-action first order kinetics to describe both the synthesis of new proteins (σ factors, chaperones, and proteases), and the association/dissociation activity of molecules as described in the previous section. This modelling approach produces a set of ordinary differential equations. Upon simulation, the equations exhibited numerical stiffness. Usually, this behavior is due to the interaction of some fast and slow dynamics and is a manifestation of rate constants different by several orders of magnitude. It is a common practice, corroborated by experimental data, to assume that the binding rates (association and dissociation) between proteins or between proteins and specific DNA promoters are fast compared to the rate of synthesis and degradation of *mRNAs* and proteins. Therefore, we assumed that the binding dynamics reach their steady-state very fast compared to other reactions in the system. We also used mass-balance equations to relate the total quantity of a species in the system to its free concentration and the concentration of the different compounds where it appears. The resulting model was a set of *Differential Algebraic Equations (DAEs)*, which are of the form

$$\dot{X}(t) = F(X, Y, \theta; t) \quad (1)$$

$$0 = G(X, Y, \theta; t) \quad (2)$$

where \mathbf{X} is a 11-dimensional vector whose elements are the differential variables, \mathbf{Y} is a 20-dimensional vector whose elements are algebraic variables, and θ is a 38-dimensional vector whose elements are the model parameters. This form is known as a *semi-explicit* DAE, with (2) being the constraint equation. If we differentiate (2) with respect to time, we get the following

$$0 = G_X(X, Y, \theta; t)\dot{X} + G_Y(X, Y, \theta; t)\dot{Y} + G_\theta(X, Y, \theta; t).$$

If $G_Y(X, Y, \theta; t) = \frac{\partial G(X, Y, \theta; t)}{\partial Y}$ is nonsingular, the system is an implicit ODE. Therefore, the DAE system is of index one (Ascher and Petzold, 1998) and is solvable by *Backward Differentiation Formulas* as implemented in specialized software packages such as *DASSL* (Petzold, 1983). This is exactly the case of the heat shock response model whose equations and parameter values are given in (El-Samad *et al.*, 2005).

4. PARETO OPTIMALITY IN THE HEAT SHOCK RESPONSE

We are now in position to introduce the concepts of Pareto optimality, and formulate the optimization problem relevant to the heat shock system.

4.1 Multi-Objective or Pareto Optimality

Suppose that in a general setting, we are interested in solving the following problem

$$\begin{aligned} & \underset{\theta}{\text{Minimize}} \quad \phi_i(X(\theta; t), Y(\theta; t)), \quad i = 1 \dots p \\ & \text{subject to} \quad \begin{cases} f_j(X(\theta; t), Y(\theta; t)) \leq 0, \quad j = 1 \dots m \\ \theta \geq 0 \\ \dot{X} = F(X, Y, \theta; t) \\ 0 = G(X, Y, \theta; t) \end{cases} \end{aligned} \quad (3)$$

where $[X \ Y]^T \in R^n$ and $\phi_i : R^n \rightarrow R$ and $f_j : R^n \rightarrow R$ are convex functions. The above optimization problem is well posed if we define the concept of Pareto optimality as follows.

Definition 1. A Pareto optimal point θ^* is a point satisfying the following property: If θ^0 is feasible and $\phi_i(X(\theta^0; t), Y(\theta^0; t)) \leq \phi_i(X(\theta^*; t), Y(\theta^*; t))$ for $i = 1 \dots p$, then $\phi_i(X(\theta^0; t), Y(\theta^0; t)) = \phi_i(X(\theta^*; t), Y(\theta^*; t))$ for $i = 1 \dots p$. In other words, a point θ^* is Pareto optimal if and only if it is feasible and there is no better feasible point in the sense that for all θ , $\phi_i(X(\theta^*; t), Y(\theta^*; t)) \leq \phi_i(X(\theta; t), Y(\theta; t))$, $\forall i = 1 \dots p$ and, for at least one i , $\phi_i(X(\theta^*; t), Y(\theta^*; t)) < \phi_i(X(\theta; t), Y(\theta; t))$.

Based on this definition of Pareto optimal points, we now present the following theorem.

Theorem 2. Consider the scalar optimization problem:

$$\begin{aligned} & \underset{\theta}{\text{Minimize}} \quad \alpha^T \phi(X(\theta; t), Y(\theta; t)) \\ & \text{subject to} \quad \begin{cases} f_j(X(\theta; t), Y(\theta; t)) \leq 0, \quad j = 1 \dots m \\ \theta \geq 0 \\ \dot{X} = F(X, Y, \theta; t) \\ 0 = G(X, Y, \theta; t) \end{cases} \end{aligned} \quad (4)$$

where $\phi = [\phi_1, \dots, \phi_p]^T$, $\alpha \in R^p$ and $\alpha_i > 0$, $i = 1 \dots p$. Suppose that θ^* is an optimal point for (4). Then θ^* is Pareto optimal for the vector optimization problem (3).

The proof of this theorem is standard and can be found in (Boyd and Vandenberghe, 2004).

The vector α is called the weight vector. It is a free parameter; by varying it, we obtain (usually)

different Pareto optimal solutions for the vector optimization problem (3). Roughly speaking, the method of scalarization as in Theorem 2 yields all the Pareto optimal points as the weight α is varied over the set of nonnegative scalar vectors. Therefore, as opposed to single objective optimization, there is no single optimal solution in multi-objective optimization with conflicting objectives. The interaction among different objectives gives rise to a set of compromised solutions, largely known as the Pareto-optimal solutions. Each solution of the Pareto optimal set is not dominated by any other solution. In going from one solution to another, it is not possible to improve on one objective without making at least one of the other objectives worse.

4.2 Multi-Objective Optimization Formulation for the Heat Shock Problem

Using the framework of multi-objective optimization, the heat shock problem can be formulated as

$$\min_{\theta} \mathbf{F}_{\alpha}(\theta) \equiv \int_0^T x_1^2(t, \theta) dt + \alpha \int_0^T x_2^2(t, \theta) dt, \quad (5)$$

where x_1 and x_2 are the levels of unfolded proteins and, respectively, chaperones. For every value of the weight α , the parameters of the model, lumped in the control vector θ , should be determined in order to minimize the cost function $F_{\alpha}(\theta)$. α should be interpreted as the relative weight (importance) for one of the objectives versus the other. Different values of α yield different optimal solutions. We denote by θ_{α}^{OPTIM} the values of the parameter vector that generate the optimal solution for the α 's considered. By plotting

$$\int_0^T x_1^2(t, \theta_{\alpha}^{OPTIM}) dt \quad \text{vs.} \quad \int_0^T x_2^2(t, \theta_{\alpha}^{OPTIM}) dt$$

we obtain a Pareto optimal curve. Using this curve, we can then answer our questions about the optimality of the heat shock system by inspecting where the point of operation of the wild type system falls with respect to that curve.

4.3 Numerical Methods and Results

The mathematical model describing the heat shock problem is a set of DAEs. General solvers for the formulated optimization problem are not available, neither commercially nor for academic purposes. Therefore, to tackle this problem we use a modification (Meeker, 2004) of the large-scale DAE solver *DASPK3.1* (Li and Petzold, 2000), which has a facility for sensitivity analysis. This modified version of *DASPK*, denoted

DASPKmod, is combined with the derivative-based optimizer KNITRO (Waltz and Nocedal, 2003).

DASPK3.1 is designed to solve DAE systems of the form

$$\begin{aligned} \mathbf{F}(t, \mathbf{y}, \mathbf{y}', \mathbf{u}) &= 0, \\ \mathbf{y}(t_0, \mathbf{u}) &= \mathbf{y}_0(\mathbf{u}), \end{aligned} \quad (6)$$

where \mathbf{u} are the parameters of the problem. The k -step backward differentiation formula (BDF) method employed by *DASPK3.1* approximates the derivative \mathbf{y}' using k past values of the solution \mathbf{y}

$$\mathbf{F}\left(t_{n+1}, \mathbf{y}_{n+1}, \frac{1}{h\beta_0} \sum_{i=0}^k \alpha_i \mathbf{y}_{n+1-i}, \mathbf{u}\right) = 0, \quad (7)$$

where α_i and β_0 are the coefficients of the BDF formulas. The order of the approximation is varied by changing the number of past solution values used. A modified Newton method is used to solve the implicit equation for \mathbf{y}_{n+1} at each time step. The linear system in Newton's method may be solved by direct methods, or by preconditioned Krylov iteration.

DASPK3.1 adaptively selects the stepsize to achieve an efficient simulation. It is well-known among software developers that the timestep selection procedures in modern ODE and DAE solvers do not usually lead to numerical solutions which are smooth with respect to small perturbations in the problem parameters. Since this can be a problem in the context of dynamic optimization, where the optimizer is assuming a certain amount of smoothness in the problem, we have implemented *DASPKmod*, which is able to achieve a much smoother numerical result.

In *DASPKmod* the time step is selected using a new digital filter stepsize controller (Söderlind, 2000), according to

$$h_{n+1} = \left(\frac{\epsilon}{\hat{r}_n}\right)^{\beta_1} \left(\frac{\epsilon}{\hat{r}_{n-1}}\right)^{\beta_2} \left(\frac{h_n}{h_{n+1}}\right)^{-\alpha_2} h_n, \quad (8)$$

where $k\beta_1 = k\beta_2 = \alpha_2 = \frac{1}{4}$, $k = \hat{p} + 1$ and \hat{p} is the order of convergence. In the above formula h_{n+1} is the next stepsize, ϵ is a fraction of the desired error tolerance, \hat{r}_n is the estimated local error, and k is the order of BDF employed for the current step. It was demonstrated (Meeker, 2004) that this modification results in many fewer optimization iterations for the heat shock problem.

The derivative-based optimizer that we employ requires the computation of derivatives of the state variables with respect to the problem parameters. These derivatives are computed by the addition of sensitivity equations to the system being solved.

Using the notation $s_i = \frac{\partial \mathbf{y}}{\partial u_i}$, the new system is given by

$$\mathbf{F}(t, \mathbf{y}, \mathbf{y}', \mathbf{u}) = 0, \quad (9)$$

$$\frac{\partial \mathbf{F}}{\partial \mathbf{y}} s_i + \frac{\partial \mathbf{F}}{\partial \mathbf{y}'} s'_i + \frac{\partial \mathbf{F}}{\partial \mathbf{u}} = 0, \quad i = 1 \dots n_u. \quad (10)$$

The sensitivity equations are generated using the automatic differentiation software ADIFOR (Carle and Fagan, 2002). The staggered corrector method in *DASPK3.1* solves the entire system in two steps. First it computes the approximation to the solution \mathbf{y} to (9) at the next time step using the BDF and Newton iteration. Then it solves the sensitivity equations (10) over the same time step using the BDF discretization and a second Newton iteration. The Jacobian matrix used to solve the original system and the sensitivity system are dependent only on the DAE solution, so they need only be computed once following the solution of the DAE on each time step.

The optimization problem was solved using KNITRO (Waltz and Nocedal, 2003), a hybrid interior method (also known as barrier method), where the original problem is replaced by a series of barrier subproblems controlled by a barrier parameter. The algorithm uses trust regions and a merit function to promote convergence. It performs one or more minimization steps on each barrier problem, then decreases the barrier parameter, and repeats the process until the original problem has been solved to the desired accuracy. The step computed at every iteration is decomposed into a normal step, whose goal is to improve feasibility, and a tangent step, towards optimality. KNITRO's overall global convergence properties are ensured by the use of trust regions.

The problems solved by KNITRO have the form

$$\begin{aligned} \min_x & f(x) \\ \text{s.t.} & h(x) = 0, \quad g(x) \leq 0, \end{aligned} \quad (11)$$

with $f: \mathbb{R}^n \rightarrow \mathbb{R}$, $g: \mathbb{R}^n \rightarrow \mathbb{R}^m$ and $h: \mathbb{R}^n \rightarrow \mathbb{R}^l$ smooth functions.

A typical iteration computes a primary step by solving primal-dual equations (using direct linear algebra) and performs a line search to ensure decrease in a merit function. In order to obtain global convergence in the presence of nonconvexity and Hessian or Jacobian singularities, the primary step is replaced, under certain circumstances, by a safeguarding trust region step. The second derivatives of the objective function and constraints are approximated using quasi-Newton updating.

With these two powerful numerical schemes, we were able to generate solutions for the heat shock

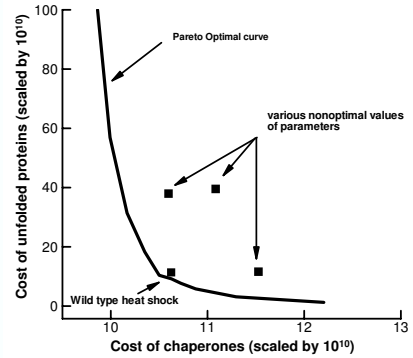


Fig. 1. Pareto optimal design of the heat shock response

optimization problem for a wide range of the weight α . Figure 1 shows the corresponding Pareto optimal curve (red). Superimposed on the same figure is the cost of chaperones and unfolded proteins for wild type heat shock in *E. coli*. The closeness of this point to the optimal curve indicates that the heat shock response is operating in a near-optimal regime, perhaps as a result of a long evolutionary past that converged to a near-optimal solution. To check that our finding is substantial and rule out the possibility that the structure of the model itself is generating near-optimal solutions for all parameter values, we randomly generated combinations of the parameters and computed their costs. These randomly chosen parameter combinations mostly yield operating points well inside the non-optimal region, therefore substantiating our conclusions. Three illustrative points are shown in Figure 1.

5. CONCLUSIONS AND FUTURE WORK

In this work we have studied the optimality of the heat shock response with respect to the cost of unfolded proteins and the heat shock proteins needed to refold them. We formulated an optimization problem with a composite weighted cost of both objectives. The Pareto optimal curve corresponding to different weights for the two objectives was obtained, and the wild type heat shock located with respect to it. We observed that the nominal operating point of the heat shock response falls close to the curve, indicating that for a certain relative importance of the two objectives, the heat shock response is close to optimal. Our future work will include the consideration of other objectives, such as fluxes (production/destruction

regimes of crucial proteins) which are important for the response, but cannot be manipulated without incurring a metabolic cost. We also plan to undertake a thorough analysis of Pareto optimality in heat shock mutants. Special focus will be devoted to *FtsH*-null and feedforward-null mutants. Using the same formulation and similar numerical techniques as those presented in this paper, the Pareto optimal curve for the mutants can be computed and compared to the curve of the wild type. The differences between these curves give concrete indications about the benefits of these loops in alleviating various costs. Such an investigation cannot be carried on experimentally in a realistic time frame, whence the power of mathematical modeling.

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