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**Abstract**

The eukaryotic heat shock response is a highly conserved defense mechanism against cellular stress. We propose in this paper a quantitative Petri net-based representation of the heat shock response model introduced in [14]. We consider both a continuous and a stochastic representation, and analyze the properties of the networks. We extend the models to account for the heat-induced degradation of the self-defense mechanism itself, as well as its ability to repair itself. We conclude with an analysis of the simulation results, and a discussion on how modeling biological systems with Petri nets scales with further expansions of the model.

**Keywords:** Petri nets, reaction-based models, heat shock response, P-invariants, T-invariants.

**TUCS Laboratory**
Computational Biomodeling Laboratory

# 1 Introduction

A growing momentum has been witnessed recently in biological research in integrating more profoundly computational methods, both in data acquisition and analysis, but especially in constructing computational models. This is supported by developments in the computing infrastructure and by advances in quantitative experimental techniques. The general goal of computational modeling in biology is two-fold. On one hand, it aims to *represent* in a formal, mathematical language the existing knowledge and data about the studied system. On the other hand, it opens the way to mathematical verification of (suitably formulated) biological properties, numerical simulations, predictions, identification of incomplete/missing knowledge, etc. This has a profound transformational effect on biological research shifting its focus from *laboratory experiments* towards *computational analysis*.

A typical computational biomodeling project consists of several iterations of the following main stages:

- *Biological model.* One first chooses the underlying biological model, including the main variables of interest and the interactions among them. The stress on this level is to capture the main aspects that are of interest and to ignore the less relevant ones.

- *Mathematical model.* A mathematical model is associated to the biological model. Regardless of the type of mathematics the model is based on (deterministic or stochastic; continuous or discrete), there are many different ways of building the model, possibly leading to different end results. Modeling principles include mass-action kinetics, Michaelis-Menten enzymatics, Hill kinetics, competitive and non-competitive inhibition, etc.

- *Qualitative model checking.* The mathematical model is then subject to a qualitative check, including logical consistency, mass conservation relations, elementary modes, etc.

- *Model fit.* This step involves solving a multi-parametric optimization problem where the unknown numerical values of the model parameters are sought so that the model predictions fit well with the available experimental data.

- *Quantitative model validation.* The model predictions are compared with other available (quantitative and qualitative) experimental data and observations.

Throughout this paper we consider reactive models consisting of a list of reactions of type $A_1 + A_2 + ... + A_m \to B_1 + B_2 + ... + B_n$, with $m, n \geq 0$,

where $A_i, B_j$ are molecular species. The mathematical semantic for such a model can be defined both in terms of continuous mathematics, where the variables are modeled as nonnegative real numbers (e.g., in terms of molecular concentrations), or in terms of discrete mathematics, where the variables are modeled as nonnegative integers (e.g., in terms of particle numbers). Moreover, one very often adds a higher level of abstraction to the model in terms of Petri nets, Bayesian networks, timed automata, process algebra, etc. We focus in this paper on a Petri net approach, with the goal of observing the structural properties of our models, such as mass conservation relations (in terms of P-invariants) and elementary cycles (in terms of T-invariants).

Our case study in this paper is the heat shock response, a highly conserved cellular response to elevated temperatures. A Petri net representation of the heat shock response model introduced in [14] has been discussed in [2] in terms of standard, non-deterministic Petri nets. In contrast, we take a quantitative approach in this paper and construct both a continuous and a stochastic Petri net model for the heat shock response. Moreover, we then extend the model to account how the cellular repair mechanism is affected by the heat shock and include its ability to repair itself. Our focus is on the ability of the Petri net framework to scale up with model extensions.

The paper is organized as follows: we start with a short overview of Petri nets formalism and their use in modeling biological systems, in Section 2. We continue with the biological semantics of the eukaryotic heat shock response, our case study, in Section 3. We also present here the molecular model for the heat shock response mechanism proposed in [14], and its extension that accounts for the ability of the mechanism to repair itself. In Section 4 we introduce our Petri net models for the heat shock response, and perform structural analyses and quantitative simulations. We discuss in Section 5 our results and the ability of extending models in the Petri net framework.

# 2 Modeling with Petri nets

In this section, we introduce the formalism of Petri nets, and explain its semantics in a biological setup. We then briefly present the software tools that we used to build and analyze our models.

## 2.1 Petri nets formalism

Petri nets are a sound formalism for representing systems with concurrency and resource sharing. They can also be viewed as a simple, graphical modeling language represented as bipartite graphs. The language was defined by Carl Adam Petri with the purpose of describing chemical processes in [15]. Many extensions of Petri nets have been developed, but they are not of in-

terest for this paper.

Petri nets are represented as directed bipartite graphs, with four main components: places, transitions, arcs and tokens. **Places** are represented as circles, and they stand for the "states" of the system. In a biological context, they may represent species, proteins, complexes, etc. **Transitions** are depicted as squares, and they stand for the transition of the system from one state to another. In a biological model, they encode reactions. A transition has several pre-places (places that are required in order for a reaction to fire) and several post-places (places that are modified once the reaction occurs) that are connected to it by arcs. It also encodes the kinetics of the reaction (mass action, Michaelis-Menten, etc.) and the rate constants. **Arcs** represent the connection between places and transitions. The direction of an arc may be from a place into a transition, to denote that the place is a pre-place for that particular transition (the species denoted by that place is consumed in the reaction), or from a transition into a place, denoting that the place is a post-place of the transition (or, equivalently, the species denoted by that place is produced in the reaction). Arcs may have an associated multiplicity (stoichiometry, in a biological context), denoting how many elements of the preceding (following) place are consumed (produced). **Tokens** represent the amount of substance for a particular place (be it the number of particles or the concentration of a species). They can be represented as dots inside a place, if the value is discrete, or as real numbers when they represent the concentration of a species. For more details on Petri nets, we refer the reader to [17], [18].

We exemplify the elements of a Petri net for the reversible reaction $\mathsf{hsf} + \mathsf{hsf}_2 \leftrightarrows \mathsf{hsf}_3$ in Figure 1.

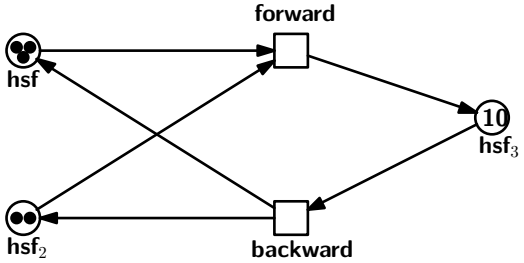


Figure 1: A Petri net representation of a reversible trimerization reaction $\mathsf{hsf} + \mathsf{hsf}_2 \leftrightarrows \mathsf{hsf}_3$. Circles represent places, while squares represent the transitions. The markings inside places represent the number of tokes for each place.

## 2.2 Petri nets in biomodeling

Biological reaction-based models can be easily translated into Petri nets representations. They are bipartite, i.e. they consist of species and the interactions between them. Some of the interactions are independent, and could fire in parallel, thus biological systems exhibit concurrent behavior. These characteristics make them suitable for modeling within the Petri nets formalism. The advantages of using this formalism lie in the ability of analysing both structural and behavioral properties of a network, integrating quantitative and qualitative analysis techniques.

A reaction-based biological system consists of a set of reactions having the following general form: $\mathsf{S_1} + \mathsf{S_2} + ... + \mathsf{S_n} \rightarrow \mathsf{P_1} + \mathsf{P_2} + ... + \mathsf{P_m}$, where species $\mathsf{S_1}$ through $\mathsf{S_n}$ represent the substrate of the reaction, and species $\mathsf{P_1}$ through $\mathsf{P_m}$ represent its products. All species can be modeled as places in the Petri nets framework, and each reaction can be represented as a transition that encodes the kinetics of the reaction, and has all substrates as pre-places, and all products as post-places.

## 2.3 Snoopy and Charlie modeling tools

Snoopy [20] is a well-documented [11], [9], [3] tool for designing and running Petri nets. It supports basic Petri nets, as well as many extensions of Petri nets, and can run both stochastic and deterministic simulations. In our implementation, we used the latest(02-05-2012) stable version of Snoopy under Windows.

In order to qualitatively analyze a network, Snoopy offers support for Charlie, a tool specially designed for analyzing structural (e.g. connectedness) and behavioral (e.g. boundness, liveness) properties of Petri nets. Two important properties for a Petri net are the places and transitions invariants (P- and T-invariants respectively). The P-invariants are sets of places with the property that their weighted sum of tokens is constant throughout the simulation, and thus they encode the mass conservation relations. T-invariants are sequences of transitions whose ordered firing can reproduce a start-point state, thus encoding the elementary modes of e system.

# 3 The heat shock response

In this section, we describe the regulatory mechanism of heat shock response, then present a biochemical reactions model of this process, as proposed in [6]. We discuss the behavior of the system in steady state conditions ($37°C$), and under thermic stress ($42°C$).

## 3.1 The regulatory mechanism of heat shock response

The heat shock response (HSR) is a highly conserved regulatory mechanism among eukaryotes, crucial for the survival of cells under stress conditions. Exposing a cell to a temperature greater than its optimum leads to a heat shock. In order to restor proper functioning of cellular mechanisms, HSR reduces the shock effects through the activity of specialized proteins that re-establish the equilibrium in the over-heated cell.

In an over-heated environment, proteins misfold (with a rate depending on temperature) and tend to form large aggregates, with destructive effects on the cell leading to apoptosis, see [1]. To counter this, cells produce heat shock proteins (hsp's), whose role is to assist misfolded proteins in their correct refolding (a process called chaperoning), see [5]. Temperature is not the only possible shock factor. Cells also react in a similar manner when being exposed to toxins, oxidants, viral infections etc, see [12].

All the genetic information in a cell is encoded in genes. Genes can be active or not. When a gene is active, it means it is synthesizing the corresponding protein (process also known as gene expression, since the gene is being expressed as a protein). A cell can change its gene expression in response to internal or external stimuli. Stimuli alert some specialized proteins, called transcription factors, which either promote (they act as an activator, process called upregulation) or block (they act as a repressor, process called downregulation) RNA polymerase from binding to the necessary gene and initiating the expression of that gene. This cellular response mechanism activates when cells are subject to external stress, e.g. heat shock. For detailed aspects on cellular biology, we refer the reader to [1].

The heat shock response has been extensively studied in the past decades, as its main actors, the heat shock proteins, play a central role in many other regulatory processes, in signalling and in cancer cells resilience, see [16, 7, 4, 10]. Studies have shown that there are several types of such proteins (HSP70, HSP90 etc., named after their size), and they are present in every sought organism, from bacteria to plants and animals. hsp's or very similar proteins are also present in cells at normal temperatures and play an important role in proper cell functioning. In the presence of a stress factor, the synthesis of hsp's is very intensive, as it is an emergency response. Some of the first identified functions of hsp's are transport of proteins across membranes, disruption of protein-protein interactions and establishment of proper interactions, DNA replication. In some cases, it was observed that HSPs may induce thermotolerance. That is, if temperature is increased gradually, the number of HSPs in the cell increases progressively, and the cell starts to function normally even at higher temperatures. Further research results and in-depth HSP gene analysis reviews can be found in [8].

The transcription of the hsp-encoding genes is promoted by some proteins

called heat shock factors (hsf's), see [21], that have been proven to have a trimeric structure, [22]. Thus, in order to perform their function three hsf molecules must bind together first, forming a trimer. hsf trimers ($\mathsf{hsf}_3$'s) have a binding affinity to heat shock promoters (nucleotide sequence of a gene indicating the start point for RNA synthesis), called heat shock elements (hse's), see [13]. The bond between an $\mathsf{hsf}_3$ and an hse signals DNA transcription to begin. At normal temperatures, hsf's in a cell are present in monomeric state due to sequences that suppress hsf trimerization. More on hsf structure, trimerization and hse binding can be found in [23]. For a graphical representation of the heat shock response, we refer the reader to http://combio.abo.fi/projects/heat_shock.

## 3.2 A molecular model for the heat shock response

During the heat shock response process, hsf monomers in inactive state are transported to the cell nucleus, where they form trimers and bind onto DNA heat shock genes, expressing heat shock proteins. When the number of hsp's is sufficient, they will negatively regulate the reaction, binding to hsf active trimers and causing them to detach from DNA and dissociate into inactive monomers. The chaperon activity of hsp's is to assist the correct refolding of unfolded proteins. The first mathematical model of the heat shock response was proposed in [19]. The model contains 15 species, and models the HSR-specific interactions using ODEs. A new molecular model and its mathematical representation was proposed in [6], and a simplified version of it can be found in [14]. These are the two models we consider in this paper.

The species considered in the system are listed in Table 1. A few simplifications have been made: although there exist several types of heat shock proteins, they are all treated as one species, namely hsp. The reduction of several types of proteins with the same function to a single general-featured protein also applies for hsf's. Although cells contain a myriad of different proteins (other than heat shock-related ones), the only relevant information for the modeled process is whether these proteins are correctly folded (prot) or not (mfp). The misfolding of the proteins driving the response to stress has to be explicitly modeled. The compound species containing hsp and a misfolded protein (mfp, mhsf or mhsp) stand for the fusion where hsp's assist denaturated proteins to refold correctly. The simplified HSR model in [14] contains only species 1 to 10.

The molecular model describing the heat shock response consists of 18 reactions (or, in its simplified version, only 12 - reactions 1 through 12 in Table 2). They cover the trimerization of heat shock factors in two steps (reactions 1 and 2), $\mathsf{hsf}_3$ binding to heat shock elements (reaction 3), transcription of DNA and translation of hereby synthesized RNA into heat shock proteins(reaction 4). Negative regulation of the response is accomplished

Table 1: The species of the heat shock response model proposed in [6].

| No. | Species | Meaning |
|---|---|---|
| 1 | hsf | heat shock factor in a monomeric state |
| 2 | $\mathsf{hsf}_2$ | heat shock factor in a dimeric state |
| 3 | $\mathsf{hsf}_3$ | heat shock factor in a trimeric state |
| 4 | hse | heat shock element on a heat shock gene |
| 5 | $\mathsf{hsf}_3\mathsf{:hse}$ | an hsf bound to an hse |
| 6 | hsp | heat shock protein |
| 7 | hsp: hsf | compound containing hsp and hsf |
| 8 | prot | correctly folded protein |
| 9 | mfp | misfolded protein |
| 10 | hsp: mfp | compound containing hsp and mfp |
| 11 | mhsf | misfolded hsf |
| 12 | hsp: mhsf | compound containing hsp and mhsf |
| 13 | mhsp | misfolded hsp |
| 14 | hsp: mhsp | compound containing hsp and mhsp |

with reactions 5-8, as hsp's bind to hsf's and stop their promoter activity. Degradation of hsp's is modeled with reaction 9; although all proteins die, the level of hsf's and other proteins in cell is maintained constant, thus both synthesis and degradation of these proteins is not present in the model. Protein misfolding and chaperon activity of hsp's are modeled throughout reactions 10-18. The complete list of reactions is presented in Table 2. For the simpler model with only 12 reactions and 10 species, refer to [14].

An important property of the HSR model is its three mass conservation relations. The amounts of heat shock factors, proteins and heat shock elements are constant in a cell:

$$\begin{cases} [\mathsf{hsf}] + 2[\mathsf{hsf}_2] + 3[\mathsf{hsf}_3] + 3[\mathsf{hsf}_3\mathsf{:hse}] + [\mathsf{hsp:hsf}] = K_1 \\ [\mathsf{prot}] + [\mathsf{mfp}] + [\mathsf{hsp:mfp}] = K_2 \\ [\mathsf{hse}] + [\mathsf{hsf}_3\mathsf{:hse}] = K_3, \end{cases}$$

for some constants $K_1, K_2, K_3$.

As previously mentioned, some reactions are temperature dependent. The following formula for the denaturation rate of proteins with temperature was proposed in [6]:

$$\phi_T = \left(1 - \frac{0.4}{e^{T-37}}\right) \cdot 1.4^{T-37} \cdot 1.45 \cdot 10^{-5} \quad s^{-1}, \tag{1}$$

where $T$ is the temperature in Celsius degrees and it may vary between $37°C$ and $45°C$. This is a very important factor in the model, since it gives the

Table 2: The molecular model for the eukaryotic heat shock response proposed in [6].

| | |
|---|---|
| 1. | $2\,\mathsf{hsf} \leftrightarrows \mathsf{hsf}_2$ |
| 2. | $\mathsf{hsf} + \mathsf{hsf}_2 \leftrightarrows \mathsf{hsf}_3$ |
| 3. | $\mathsf{hsf}_3 + \mathsf{hse} \leftrightarrows \mathsf{hsf}_3\colon \mathsf{hse}$ |
| 4. | $\mathsf{hsf}_3\colon \mathsf{hse} \rightarrow \mathsf{hsf}_3\colon \mathsf{hse} + \mathsf{hsp}$ |
| 5. | $\mathsf{hsp} + \mathsf{hsf} \leftrightarrows \mathsf{hsp}\colon \mathsf{hsf}$ |
| 6. | $\mathsf{hsp} + \mathsf{hsf}_2 \rightarrow \mathsf{hsp}\colon \mathsf{hsf} + \mathsf{hsf}$ |
| 7. | $\mathsf{hsp} + \mathsf{hsf}_3 \rightarrow \mathsf{hsp}\colon \mathsf{hsf} + 2\,\mathsf{hsf}$ |
| 8. | $\mathsf{hsp} + \mathsf{hsf}_3\colon \mathsf{hse} \rightarrow \mathsf{hsp}\colon \mathsf{hsf} + 2\,\mathsf{hsf} + \mathsf{hse}$ |
| 9. | $\mathsf{hsp} \rightarrow \emptyset$ |
| 10. | $\mathsf{prot} \rightarrow \mathsf{mfp}$ |
| 11. | $\mathsf{hsp} + \mathsf{mfp} \leftrightarrows \mathsf{hsp}\colon \mathsf{mfp}$ |
| 12. | $\mathsf{hsp}\colon \mathsf{mfp} \rightarrow \mathsf{hsp} + \mathsf{prot}$ |
| 13. | $\mathsf{hsf} \leftrightarrows \mathsf{mhsf}$ |
| 14. | $\mathsf{hsp} + \mathsf{mhsf} \leftrightarrows \mathsf{hsp}\colon \mathsf{mhsf}$ |
| 15. | $\mathsf{hsp}\colon \mathsf{mhsf} \rightarrow \mathsf{hsp} + \mathsf{mhsf}$ |
| 16. | $\mathsf{hsp} \rightarrow \mathsf{mhsp}$ |
| 17. | $\mathsf{hsp} + \mathsf{mhsp} \leftrightarrows \mathsf{hsp}\colon \mathsf{mhsp}$ |
| 18. | $\mathsf{hsp}\colon \mathsf{mhsp} \rightarrow 2\,\mathsf{hsp}$ |

amount of denaturated proteins. Simulations of cellular behavior outside this temperature range can be made, but they are not relevant.

The model is considered to be in a steady state at $37°C$, when no regulatory activity occurs. At $42°C$, $\mathsf{hsf}_3$'s bind to $\mathsf{hse}$'s located on DNA strands and promote RNA replication of heat shock genes. Their activity is very intense and prompt, and as time passes $\mathsf{hsp}$'s assist proper folding of misfolded proteins. As the number of $\mathsf{mfp}$'s decreases, heat shock proteins will react with $\mathsf{hsf}_3$'s, breaking the $\mathsf{hsf}_3\colon \mathsf{hse}$ bound and thus inhibiting $\mathsf{hsp}$ synthesis. The concentration of DNA binding activity eventually returns to basal levels. At small shocks, the response DNA binding activity is mild, but as the shock gets sharper, $\mathsf{hsf}_3$'s activity intensifies. The plot in Figure 2 shows the DNA binding activity at $42°C$, as reported in [6].

# 4 Petri nets for the heat shock response

Here, we perform both quantitative and qualitative analyses on two Petri net implementations of the heat shock response model, namely continuous and stochastic. We then consider the HSR model that accounts for the misfolding of HSR-driving proteins proposed in [6], and study its behavior for different misfolding rates.

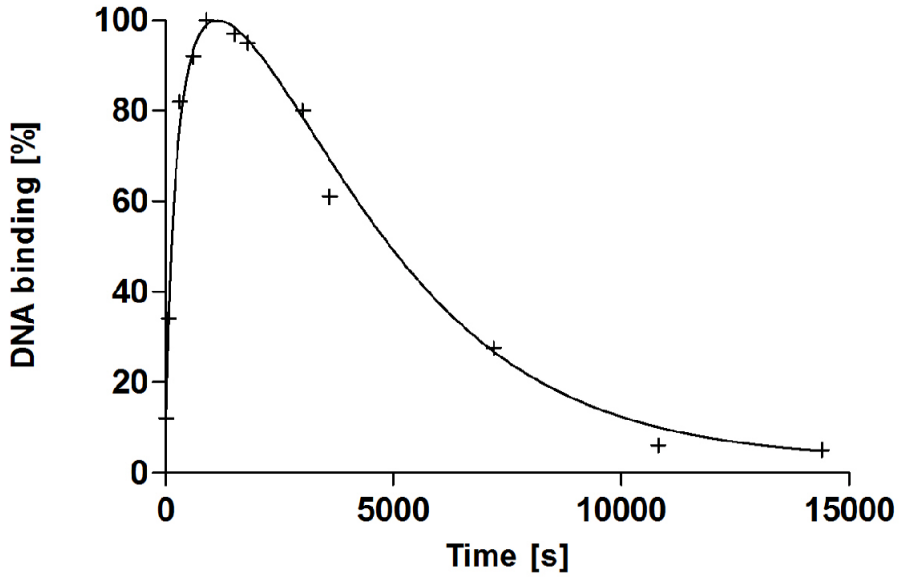


Figure 2: Regulation of the heat shock response

## 4.1 Petri net for the simplified HSR model

Our Snoopy implementation of the heat shock response model presented in [6]can be seen in figure 3. For the numerical setup of the model, in terms of initial concentrations of species and reaction rate constants, we refer the reader to [14] and [6]. In order to compare the predictions of the study model with our Snoopy implementation, the simulation time was set to 14400s, and the quantitative behavior of the system is similar with the one reported in the paper, see Figure 6, black line. An average over 100 stochastic simulations is shown in Figure 4, green line.

The P-invariants reported by Charlie (see Table 3) correctly encode the mass conservation relations reported in [14]. The T-invariants for this model are invariants 1 through 9 and 14 in Table 5, and they encode all successions of transitions whose overall effect is leaving the system unchanged.

The last property of the network that was checked in Charlie is the reachability graph. In the heat shock response model, all species are interconnected via the execution of a certain number of reactions (graphically, from each place one can reach any other place by following successive arcs). This property is validated by the reachability graph analysis. Charlie predicts a reachability graph containing one single place, which means that all places are interconnected.

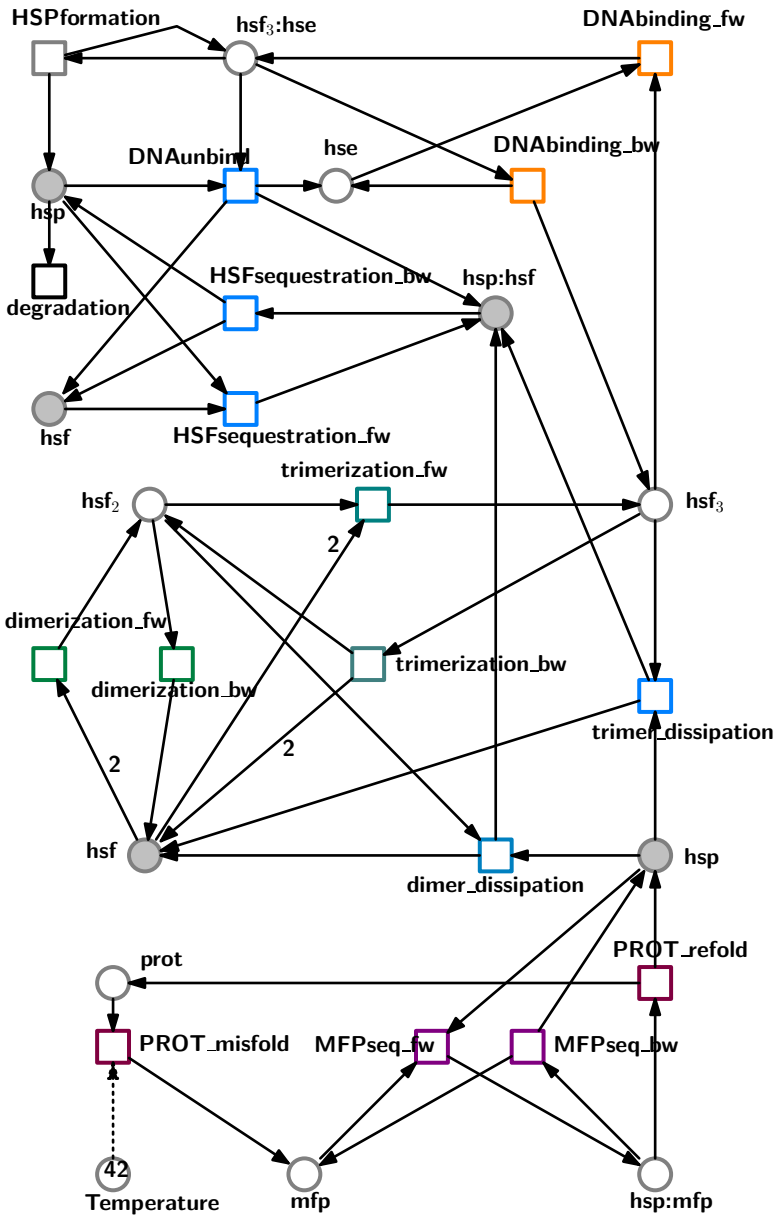


Figure 3: Snoopy representation of the simplified heat shock response model

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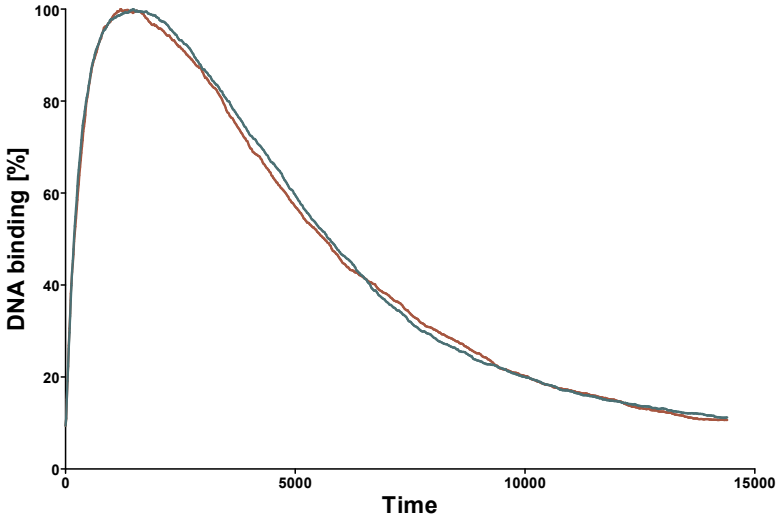


Figure 4: DNA binding activity for the simple (green line) and extended (red line) HSR models, average over 100 stochastic runs.

## 4.2 Petri net for the HSR model with misfolding of HSR-driver proteins

The P-invariants reported by Charlie (see Table 4) are consistent with the mass conservation relations reported in [14]. The only difference from the previous model is in the mass conservation of hsf's. Since hsf's misfold under thermal stress, their total amount in the cell includes also the misfolded variant mhsf and hsp: mhsf. The detailed mass conservation relation becomes $[\mathsf{hsf}]+2[\mathsf{hsf}_2]+3[\mathsf{hsf}_3]+3[\mathsf{hsf}_3\colon \mathsf{hse}]+[\mathsf{hsp}\colon \mathsf{hsf}]+[\mathsf{mhsf}]+[\mathsf{hsp}\colon \mathsf{mhsf}] = \text{constant}$.

The T-invariants for the model are presented in Table 5. They all validate the model, in the sense that all successions of reactions that are able to balance each-other out are present in a T-invariant. Invariants 1-4, 8, 10, 12 and 14 represent reversible reactions, while the others encode more complex sequences of reactions whose ordered execution returns the system to the starting state. For example, the T-invariant 6 denotes a sequence of reactions that is needed in order to first produce (via first producing an $\mathsf{hsf}_2$, then consuming it to form an $\mathsf{hsf}_3$, then dissipating the latter to produce a molecule of hsp: hsf) and then consume one token of hsp: hsf (via the HSFsequestration_bw reaction).

We studied the behavior of the model for different misfolding rates of hsf's and hsp's. First of all, including the reactions 13-16 from Table 2 in the model presented in Figure 3 induced a slight increase in the DNA binding activity. This agrees well with the expected behavior. Because hsp's should

Table 3: The p-invariants for the eukaryotic heat shock response proposed in [14], as reported by Charlie.

| No. | Components | Multiplicity |
|---|---|---|
| 1 | Temperature | 1 |
| 2 | hse | 1 |
| | $\mathsf{hsf}_3$: hse | 1 |
| 3 | hsf | 1 |
| | $\mathsf{hsf}_2$ | 2 |
| | $\mathsf{hsf}_3$ | 3 |
| | $\mathsf{hsf}_3$: hse | 3 |
| | hsp: hsf | 1 |
| 4 | mfp | 1 |
| | hsp: mfp | 1 |
| | prot | 1 |

also chaperon the correct refolding of mhsf's and mhsp's, their concentration should be greater than in the previous model. Thus, the DNA binding activity peaks at a slightly increased concentration (27.61).

Increasing the hsp and hsf misfolding rate 100 times did not change the numerical behavior of the model significantly. This is explained by the extremely small misfolding rate in Equation 1. Considering a 1000-fold denaturation rate, the DNA binding activity is noticeably more intensive, and the repression of the heat shock effect on the cell takes longer, see Figure 6, green line.

At 10000 times the initial misfolding rate of hsf's and hsp's, the response is limited by the available hse's in the system. Almost instantaneously, all hsp-encoding genes are activated, and the cell undergoes a sustained effort in countering the shock, being able to survive (red line in Figure 6).

For larger misfolding rates, the cell is unable to cope with the shock. Although the hsp translation is extensive and sustained, most of the newly synthesized hsp's misfold rapidly, being unable to exert their chaperon role. In the attempt to reduce the level of misfolded proteins, the cell floods itself with misfolded chaperons and dies. The simulation results for this scenario are presented in Figure 7.

The DNA binding activity predicted by an average over 100 stochastic simulations for the initial misfolding rate is shown in Figure 4, red line. The stochastic simulation results show a behavior similar to the expected one, as shown in Figure 2.

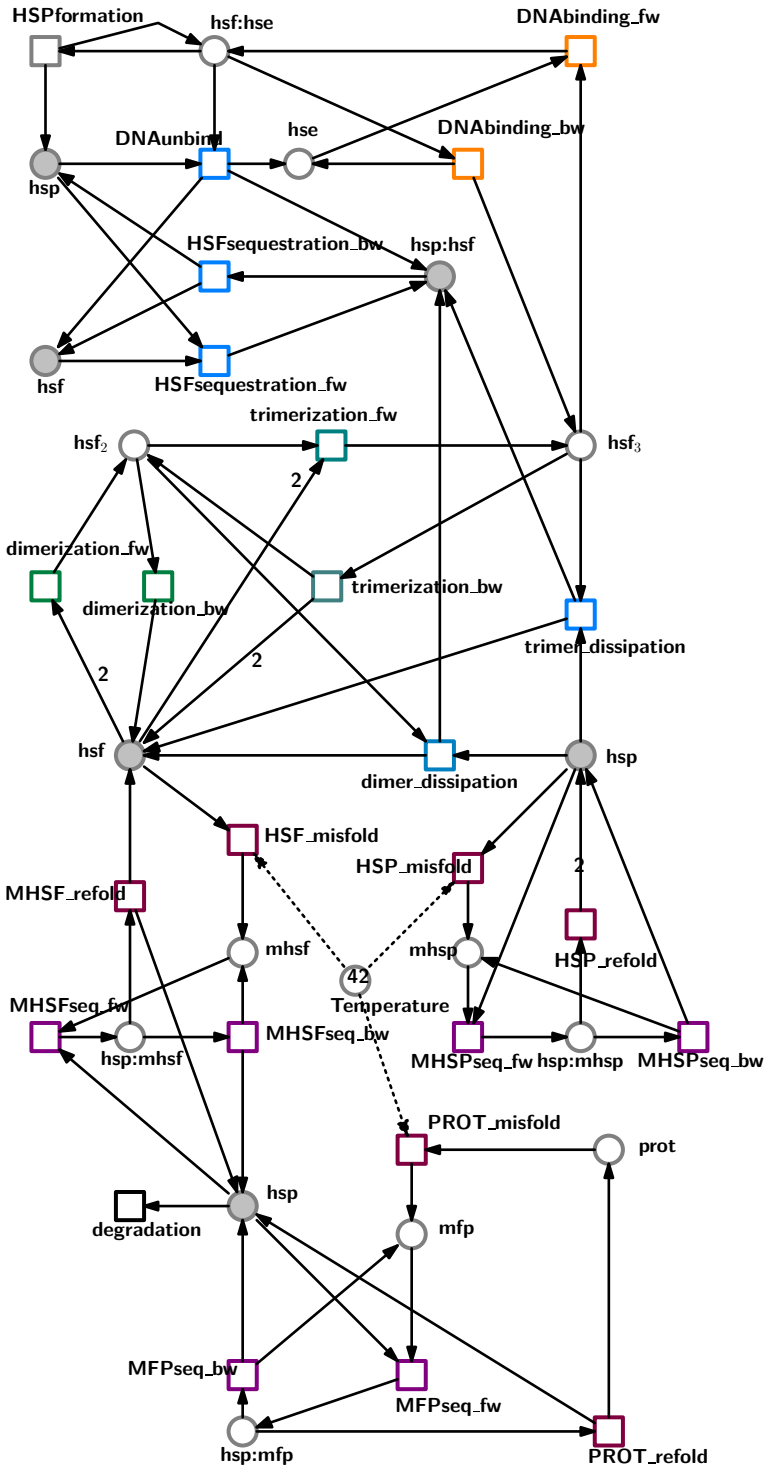


Figure 5: Snoopy representation of the heat shock response model with misfolding of hsf's and hsp's

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Table 4: The p-invariants for the eukaryotic heat shock response proposed in [6], as reported by Charlie.

| No. | Components | Multiplicity |
|---|---|---|
| 1 | Temperature | 1 |
| 2 | hse | 1 |
| | $hsf_3$: hse | 1 |
| 3 | hsf | 1 |
| | $hsf_2$ | 2 |
| | $hsf_3$ | 3 |
| | $hsf_3$: hse | 3 |
| | hsp: hsf | 1 |
| | mhsf | 1 |
| | hsp: mhsf | 1 |
| 4 | mfp | 1 |
| | hsp: mfp | 1 |
| | prot | 1 |

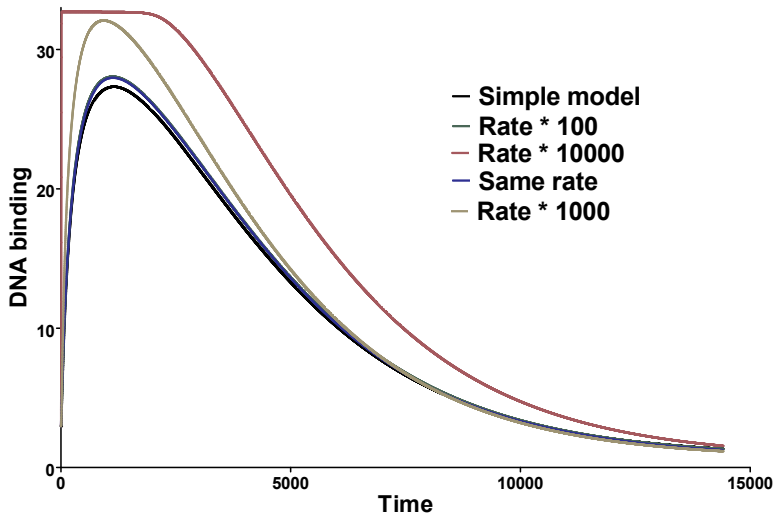


Figure 6: DNA binding activity for different rates of hsf and hsp misfolding.

Table 5: The t-invariants for the eukaryotic heat shock response proposed in [14], as reported by Charlie.

| No. | Transition | Invariants |
|---|---|---|
| 1 | DNAbinding_bw | 1 |
| | DNAbinding_fw | 1 |
| 2 | trimerization_fw | 1 |
| | trimerization_bw | 1 |
| 3 | dimerization_fw | 1 |
| | dimerization_bw | 1 |
| 4 | HSFsequestration_bw | 1 |
| | HSFsequestration_fw | 1 |
| 5 | dimerization_fw | 1 |
| | HSFsequestration_bw | 1 |
| | dimer_dissipation | 1 |
| 6 | dimerization_fw | 1 |
| | trimerization_fw | 1 |
| | HSFsequestration_bw | 1 |
| | trimer_dissipation | 1 |
| 7 | dimerization_fw | 1 |
| | trimerization_fw | 1 |
| | HSFsequestration_bw | 1 |
| | DNAunbind | 1 |
| | DNAbinding_fw | 1 |
| 8 | MFPseq_fw | 1 |
| | MFPseq_bw | 1 |
| 9 | MFPseq_fw | 1 |
| | PROT_refold | 1 |
| | PROT_misfold | 1 |
| 10 | MHSFseq_fw | 1 |
| | MHSFseq_bw | 1 |
| 11 | HSF_misfold | 1 |
| | MHSFseq_fw | 1 |
| | MHSF_refold | 1 |
| 12 | MHSPseq_bw | 1 |
| | MHSPseq_fw | 1 |
| 13 | HSP_misfold | 1 |
| | MHSPseq_fw | 1 |
| | HSP_refold | 1 |
| 14 | degradation | 1 |
| | HSPformation | 1 |

# 5 Conclusions

We have introduced two quantitative models for the heat shock response, using Petri nets as the modeling framework. The first model contains 10

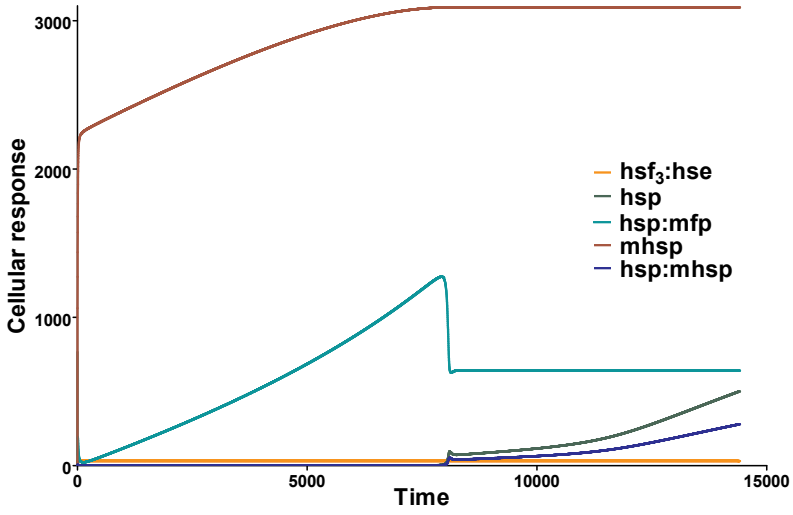


Figure 7: For misfolding rates, the cell cannot cope with thermal stress. The concentration of hsp's has to be increased to a level that incurs unsustainable cost for the cell.

species and 12 reactions, as in [14]. The second model is built as an extension of the first one, by including additional reactions and species that describe the misfolding of HSR-driving proteins (namely hsf's and hsp's).

Modeling with Petri nets allows for both qualitative and quantitative analysis of models. As structural properties of the networks, we studied the P- and T-invariants of our implementations, and showed they correspond well with reported biological data (e.g. mass conservation relations, and elementary modes).

In order to extend the first model, we introduced a place for each new species in the model, and a transition for each newly included reaction. This representation can be compressed further more in the framework of colored Petri nets, where species and reactions with similar behavior and kinetics can be grouped together using properly defined data structures (color sets). This extended framework could further be used for a compact representation of a HSR model containing all different types of hsf's and hsp's modeled as different colors of the same color set. This is not in the scope of this paper, but it is an important argument in favor of modeling large biological systems within the Petri net framework.

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