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Structured Singular Value Analysis of the Fas Apoptosis Pathway

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Abstract

A systems level understanding of the genetic protocol controlling apoptosis, programmed cell death, is critical to the development of multi-targeted therapies for complex diseases such as cancers and autoimmune diseases. While several methods exist to evaluate system robustness, structured singular values offer the advantage of measuring the robustness of specific biological performances to multiple, simultaneous parametric perturbations. In this paper, the Fas-induced apoptosis network, whose failure has been cited in several forms of cancer [14], is analyzed for robust stability to parametric uncertainty. Analysis reveals apoptotic signalling is most sensitive to perturbations in degradation rates, while the system is robustly stable to perturbations in complex formation and catalytic reactions. Correlation analysis shows that the robustness of the interaction of caspase 8 with the mitochondria is restricted by the robustness (or allowable variation) of other elements in the apoptotic pathway. The robustness trends discovered via SSV analysis provides another measure for model (in)validation, and the predicted uncertainty bounds have direct experimental implications.

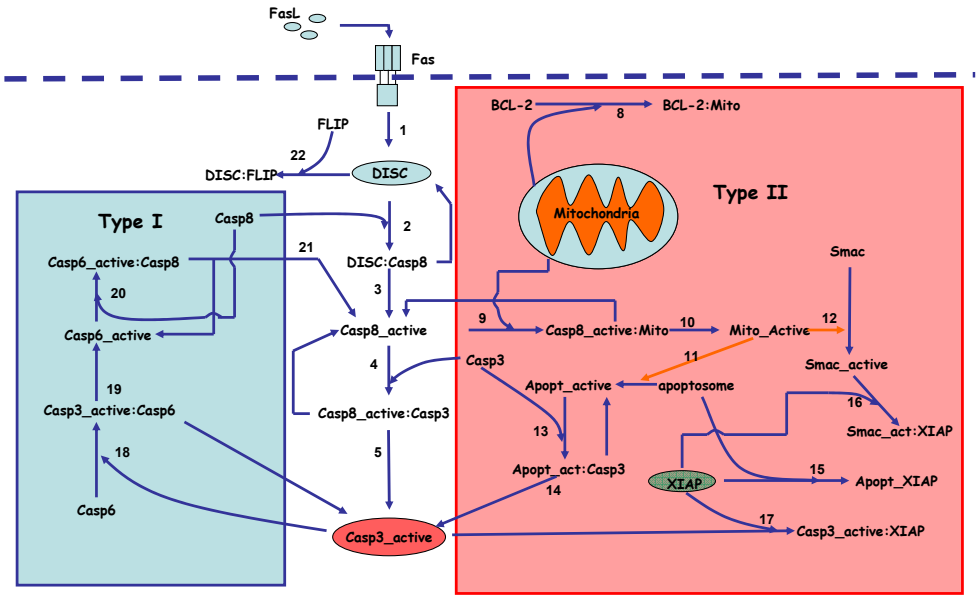
1 Introduction

A systems level understanding of the genetic protocol controlling apoptosis, programmed cell death, is essential to understanding the maintenance of homeostasis of the immune system [15] and to the development of multi-targeted therapies for complex diseases such as cancers and autoimmune diseases. Despite years of effort, cancer therapy remains broadly ineffective [7], and is generally detrimental to malignant and healthy cells alike. Cancers acquire a variety of traits via the suppression of critical anti-growth signals and the hijacking of cellular machinery (*e.g.* angiogenesis) so as to promote unmitigated growth. The failings in the complex machinery allowing for tumorigenesis require a systematic understanding of the networks governing cellular behavior. Mathematical models provide a means of understanding cellular behavior at a systems level, and, as such, systems level phenomena (cellular performance, system robustness, *etc.*) can be explored. In this work, an integrated mechanistic and data-driven model of FasL-induced apoptosis is explored for parametric robustness through sensitivity and structured singular values analysis.

Robustness is the relative insensitivity of a system to parametric and, at times, structural uncertainties [17]. Robust systems maintain their state and behavior regardless of internal or external disturbances, and, in highly robust systems, even structural disturbances produce small to negligible effects in system performance. Robustness is a key characteristic of biological processes [9], *e.g.* it is generally believed that the human genome has approximately 120 irreparable mutations each generation, but these mutations typically have no affect on the health or appearance of the individual. This is because the human body uses positive and negative feedback, modularity, and redundancy to ensure robust performance (in this case defined as health). Cellular signaling networks use the same tactics to maintain signal sensitivity and accuracy in a noisy intracellular environment.

The necessary counterpart to system robustness is fragility. Any system designed to be robust to a particular set of disturbances must become fragile to other often more complex, exotic perturbations. The inherent trade-off between robustness and fragility has been long known in engineering [9, 16]. In biology, systems evolve to be robust to routine external and internal perturbations, and as such, will remain fragile to unconventional disturbances. By applying sensitivity analysis and SSVs to the Fas-induced apoptosis model, the parameters to which successful apoptotic signalling is fragile can be discovered.

Several methods have been developed to measure system robustness to parametric uncertainty. When a system is limited to one or two uncertain parameters, bifurcation analysis provides the greatest insight into the relationship between system behavior and parameter variation. For larger systems from systems biology such as circadian rhythms [18, 19] and signal transduction networks [8], sensitivity analysis has been used to evaluate the effect of parameter variation on system behavior. Sensitivity analysis evaluates the response of the system to an infinitesimal perturbation in a single parameter [22]. Sensitivity is a local measure, and should be calculated over a grid in parameter space. As such, important correlations between parameters may elude discovery. Furthermore, sensitivity analysis is valuable for identifying the set of disturbances that most affect the system response, but it is difficult to relate the results to specific performance criteria. Structured singular values (SSVs) offers a method to succinctly analyze system performance



(a)

			Individual Perturbations		
Parameter	#	Function	wieght	μ	AV
J1_f	1	Formation of the DISC complex	1	0.101	987
J1_r	2	Deactivation of the DISC complex	1	8.99	11.3
J2_f	3	DISC and Casp8 complex formation	1	0.0856	1170
J2_r	4	DISC and Casp8 complex deformation	1	0.881	113.4
J3_k	5	DISC activation of Casp8	1	0.118	843
J4_f	6	Casp3 binding of activated Casp8	1	0.122	816
J4_r	7	Casp3 unbinding of activated Casp8	1	0.853	117
J5_k	8	Catalytic activation of Casp3 via Active Casp8	1	0.147	681
J9_f	11	Active Casp8 interaction with mitochondria	1	1.00	100
J11_k	12	Effect of activated mitochondria on apoptosome formation	1	1.01	99.2
J12_k	13	Mitochondrial catalytic activation of SMAC	1	1.00	100
J14_k	17	Casp3 activation via activated apoptosome interaction	1	0.88	114
J10_k	32	Catalytic activation of the mitochondria by active Casp8	1	1.00	100

AV= allowable variatation

(b)

Figure 1: (a)The FasL induced apoptotic network, adapted from Hua, *et al.* [7]. Each interaction is identified with an integer, and each interaction, i, may be associated with a forward reaction, reverse reaction, and/or a catalytic reaction, labeled Ji_f, Ji_r, and Ji_k, respectively. (b) The most sensitive parameters and their allowable variations (AV) calculated via SSV when each uncertainty is considered independently.

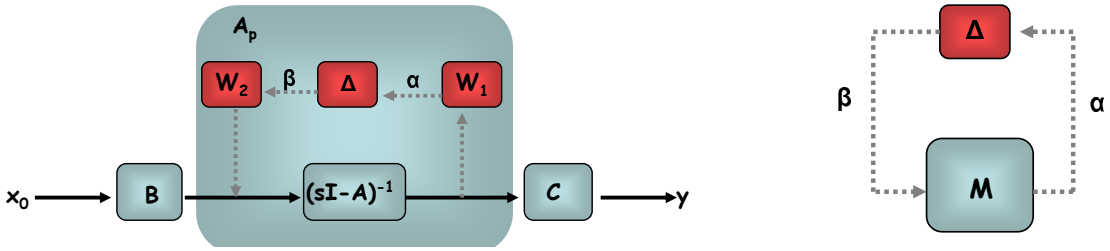
robustness to parametric uncertainty.

Structured singular value (or μ) analysis presents the advantage of measuring the robustness of specific biological performances to multiple, simultaneous parametric perturbations. Given a specific performance objective, μ analysis determines the ranges parameters can fluctuate prior to performance failure. Furthermore, the interdependencies between robust elements can be explored, ultimately identifying the set of parameters upon which system behavior is most dependent. SSV analysis has been used in flight simulator design and other engineering application to identify parameters which must be accurately measured and maintained [12].

In this work, sensitivity and SSV analysis are used to analyze the robust stability of the Fas-induced apoptosis pathway [7]. Sensitivity analysis is used to identify the set of parameters to which executioner caspase production is most sensitive. Once this subgroup is identified, SSV analysis is used to elucidate both fragile and robust groupings within the signalling network. Furthermore, the interdependencies between the robust elements are explored to determine which robust parameters are dependent on the uncertainty ranges of others.

2 Fas-induced Apoptosis

The model developed by Hua *et al.* incorporates a hybrid approach, integrating mechanistic and data driven modelling, to accurately map apoptotic behavior due to FasL exposure in Jurkat cells over a large set of initial conditions [7]. Currently, several research groups are working to unravel the cellular networks controlling Fas-induced apoptosis. Proper control of apoptosis is critical for normal development, maintenance of homeostasis in the immune system, and adaptation. As such, dysregulation of cellular apoptosis is associated with several degenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis [1, 11]. The Fas-induced apoptotic network is extracellularly activated by the binding of the Fas ligand (FasL) to the transmembrane Fas receptor protein (See Figure 1a). The Fas receptor protein belongs to the tumor necroses factor (TNF) superfamily, and is ubiquitously expressed in cells. Conversely, FasL is mainly present in activated T lymphocytes, natural killer (NK) cells, and macrophages [10]. The binding of Fas and FasL results in the formation of the death inducing signaling complex (DISC). DISC activates two pathways, both resulting in the activation of executor caspase 3 [7]. In Type I activation, significant levels of caspase 8 are required for caspase 3 activation. Yet, in Type II cells, only a small amount of caspase 8 is sufficient to induce apoptosis as the death signal is indirectly amplified by the mitochondrial activity [1]. Each of the interactions seen in Figure 1a is identified with an integer, and each interaction, i, may be associated with a forward reaction, reverse reaction, and/or a catalytic reaction, labeled Ji_f, Ji_r, and Ji_k, respectively. All reactions have first order, mass-action kinetics.



(a) (b)

Figure 2: (a)The block diagram of a state space system with uncertainty in the **A** matrix. (b) The **MΔ** configuration.

3 Structured Singular Value Analysis

The structured singular value, introduced by John Doyle in 1982, is a generalization of the singular value, $\bar{\sigma}$, which provides a measure of the smallest "size" a perturbation block can attain before instabilities occur in the system [16]. First, the system is linearized about a desired steady state, and uncertain parameters are assigned multiplicative perturbations in the form of $k_i = k_i(1 + \delta_i w_i)$ such that $\delta_i \in$[-1 1] and w_i weights the perturbation. All perturbations are then collected into the Δ block,

$$\Delta = \begin{pmatrix} \delta_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & \delta_n \end{pmatrix}, \tag{1}$$

and the system is rearranged into the **MΔ** configuration (see Figure 2b). Properly configured, SSV analysis determines the smallest **Δ**, measured in terms of its maximum singular value, required to shift one of the system's stable eigenvalues to the imaginary axis. Thus, μ is defined as

$$\mu(\mathbf{M})^{-1} \triangleq \min_{\Delta} \{\bar{\sigma}(\Delta) | \det(\mathbf{I} - \mathbf{M}\Delta) = 0 \text{ for structured } \Delta\}. \tag{2}$$

If μ <1 for all frequencies, then, within the uncertainty bounds defined, the system is stable for all possible parameter combinations. If, at some frequency, $\mu > 1$, then the fluctuation ranges on some parameters must be restricted.

The value of μ is calculated using the MUSSV Toolbox in Matlab [2]. For real, scalar perturbations, μ is calculated by finding the first hypercube in uncertain parameter space for which unstable systems exist. As such, re-weighting must be performed to scale parameters which are most fragile, allowing for greater mobility in the direction of more robust parameters. Properly weighting fragile parameters can provide insights into

- which uncertain elements dominate the fragile behaviors
- which elements are interrelated,
- and which elements weakly affect system stability.

To truly understand the nature of the stable parameter subspace, a series of re-weightings is performed to determine parameter correlations.

4 Results

4.1 Sensitivity Analysis

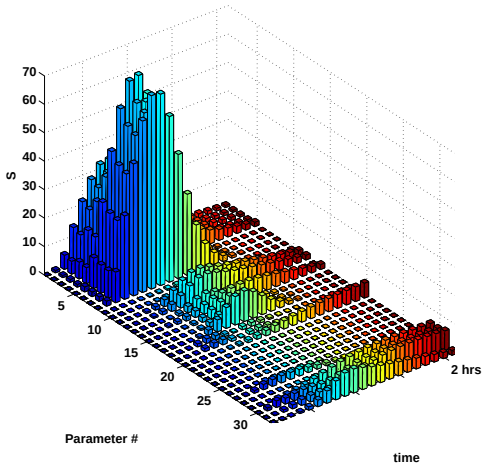


Figure 3: The sensitivity of activated caspase 3 production to parameter perturbations. S is the absolute value of the sensitivity.

The sensitivity of executioner caspase production to parameter perturbations can be seen in Figure 3. As has been observed in other biological models, the sensitivity distributions were generally robust to variations in parameter values [3], and the sensitivities generally cluster into modules within the pathway. The most sensitive module is the direct activation of caspase 3 via caspase 8. The interaction of caspase 8 and the mitochondria during mitochondrial amplification and apoptosome formation is the second most sensitive group, and the XIAP sequestering and deactivation of active executioner caspase is the third most sensitive cluster. The Jurkat cells upon which the model is based exhibit Type II behavior, and the model's insensitivity to parameters in the Type I activation pathway is not surprising.

4.2 SSV Analysis of the FasL Apoptosis Pathway

The system is linearized about its active stable steady-state (FasL= 10 ng/mL), and weighting blocks are created for each of the sensitive parameters identified via sensitivity analysis. Initially, all parameters are weighted equally, allowing the parameters to vary between 0 and twice its nominal value. Then a series of re-weighting is performed to find the largest subspace, in parameter space, in which the system remains stable.

Figure 1b shows the percent of allowable variation (in percentages with respect to the parameter's nominal value) before instability occurs when each parameter fluctuates in isolation. All parameters exceed the requirement of allowing approximately 100% variation about their nominal value except that of J1_r which can only tolerate 11.3% uncertainty in its nominal value before instability. J1_r is the rate of deactivation for the Death Inducing Signaling Complex (DISC). DISC formation involves the aggregation of oligomeric CD95,

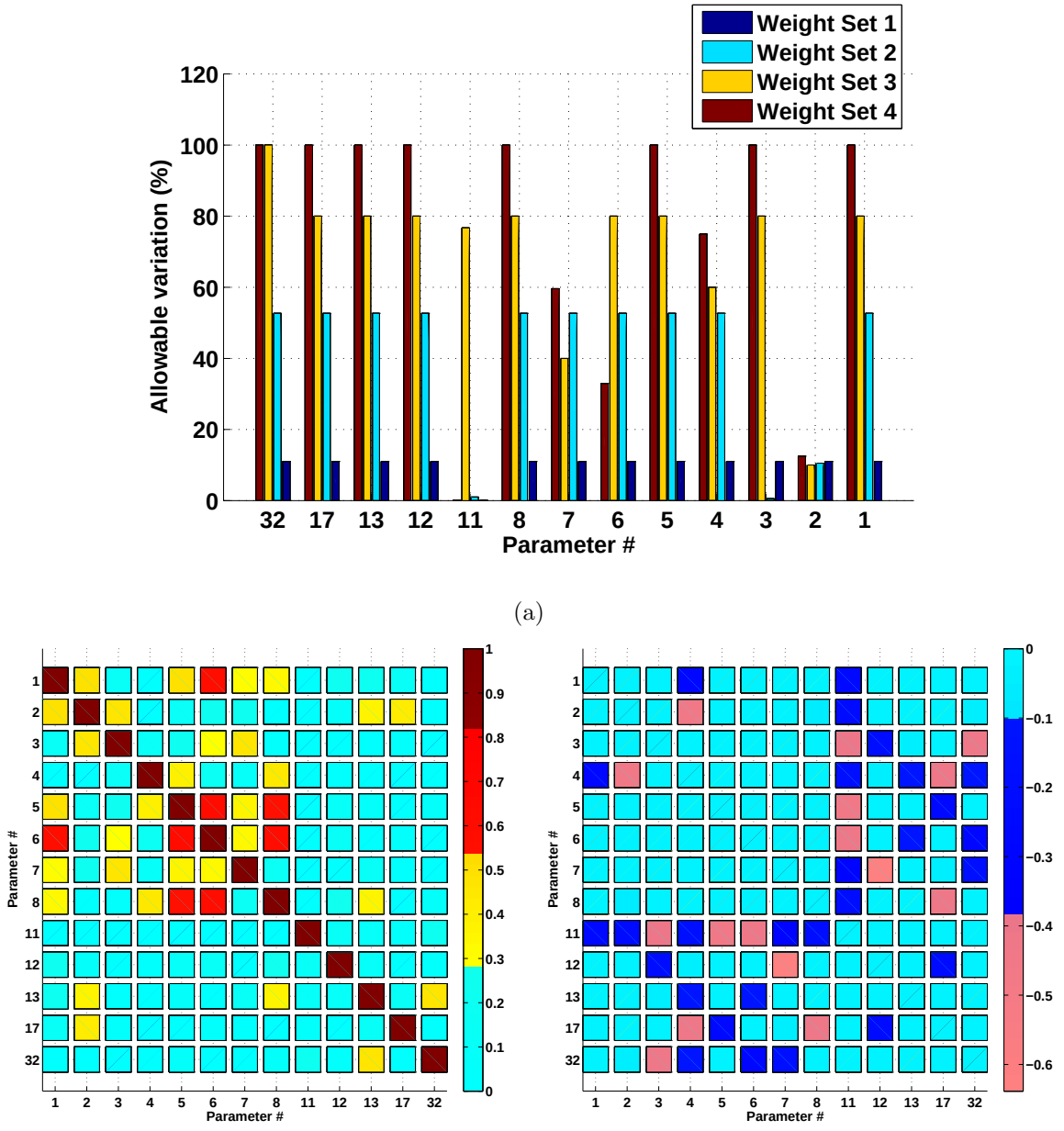


Figure 4: For 4 different weight sets, the allowable variation (AV) prior to destabilization is shown. Weight set 3 shows the best overall attainable distribution of parameter robustness. Attempts to increase the AV for any parameter beyond that of weight set 3 effects the AV of other parameters in the system (weight set 4 for example). (b) The correlation matrix depicting parameters whose AV is positively correlated.(c) The correlation matrix depicting parameters whose AV is negatively correlated.

the death domain (DD) containing FADD (an adaptor protein), procaspase 8, procaspase 10, and c-Flip[4], and as such, the first order rate constant for DISC formation (J1_f) must be robust to the noise inherit in such a complicated aggregation process. Conversely, DISC degradation is a simple zeroth order reaction and, as perturbations in the degradation rate are unlikely, the system need not be robust in perturbations to J1_r.

Figure 4a shows the percent each parameter can vary simultaneously before system instability occurs. Due to the nature of the algorithm used to find μ, the parameters are re-weighted several times to discover the largest subspace in uncertain parameter space for which stability is guaranteed. In the initial weight set, all parameters are weighted equally. J1_r, the parameter accounting for DISC formation, limits the size of the stable hypercube in uncertain parameter space and, as such, J1_r's corresponding uncertainty bounds must be re-weighted. A series of re-weightings are performed, and as seen for weighting set 3, although J1_r is always limited to 11% variability, most parameters are robust up to 60% or 80% above or below their nominal values. The two most sensitive parameters are J1_r and J4_r. J4_r accounts for the interaction of activated caspase 8 and caspase 3. In weighting set 4, attempts to re-weight J4_r to allow for greater robustness of the caspase 8/caspase 3 binding mechanism requires sacrificing robustness in mitochondrial activation (J9_f).

Inspired by the trade-off in the robustness of mitochondrial activation and caspase 8/caspase 3 binding, the correlation of robust elements is explored by slightly perturbing the weights about weight set 3, and calculating the correlation coefficients over 1000 randomly generated weighting sets. The random weighting blocks are created by allowing the weights from weighting set 3 to vary randomly within 5.0% of their nominal values. Correlation coefficients relate the variation of the allowable variation (AV) between two parameters as their robustness ranges vary. Two parameters whose AV has a correlation of 1 are perfectly correlated while 0 correlation implies the robustness of the two elements are independent. Negative correlation is of particular interest in that the robustness of one parameter is restricting the AV of another.

Figures 4b and 4c show the correlation matrices for positively and negatively correlated parameters, respectively. From Figure 4b, it is apparent that the robustness of caspase 3 binding of caspase 8 (J4_f) is directly proportional to the AV of DISC aggregation (J1_f), DISC activation of caspase 8 (J3_k), and the catalytic activation of caspase 3 via activated caspase 8 (J5_k). And the robustness of DISC activation of caspase 8 (J3_k) is directly proportional to the robustness of the activation of executioner caspase via caspase 8 (J5_k). Figure 4c shows that the AV of active caspase 8 interacting with the mitochondria (J9_f) is restricted by the AV in the DISC activation of caspase 8 and caspase 3 binding of activated caspase 8 (J3_k and J4_f, respectively). Furthermore, the AV in the executioner caspase activation via activated apoptosome (J14_k) is limited by the AV in the degradation of the caspase 8/DISC complex (J2_r) and the catalytic activation of caspase 3 via active caspase 8 (J5_k).

5 Discussion

Elucidating the control mechanisms maintaining apoptotic signaling is critical to cancer therapeutic development and to understanding the maintenance and operation of the

immune system. The Fas induced apoptosis (FIA) network is an attractive system as it has been heavily studied [1, 3, 5, 6, 7, 20], and cancer cultures in which the death signal is attenuated despite Fas-FasL bindings have been observed [10, 14]. Parameter robustness analysis of the FIA network can provide insight into the genetic algorithm responsible for accurate control of the apoptotic machinery.

Here, SSV analysis of the FIA reveals significant insights into the robustness of caspase 3 production that was not apparent via sensitivity analysis. While sensitivity analysis assigned approximately equal significance in parameter robustness for parameters #1 through #8, SSV analysis reveals that the system is far less robustly stable to the degradation of the DISC complex than any other element in the direct activation of the executioner caspase. And in reference to Figure 4a, apoptotic signalling tends to be more fragile to perturbations in degradation/reverse reactions (parameters #2,4, 7, and 11). Degradation reactions are generally zeroth order, independent of secondary species, and are expected to be less noisy. Thus, one may infer that during the evolution of the apoptotic network, cellular machinery was dedicated to protect elements prone to greater fluctuation and uncertainty. Elements less prone to fluctuation were not allotted machinery to guarantee robust performance as it would be both unessential and uneconomical.

Furthermore, SSV analysis provides insight into the trade-offs between robust elements within the FIA network. The result that the robust stability of DISC formation, DISC activation of caspase 8, caspase 3 binding of caspase 8 and the catalytic activation of caspase 3 via caspase 8 are directly related is not surprising as they appear in series in the apoptotic signaling network, and the ability to allow robustness in any one element of the series will provide more flexibility to the remaining elements. Contrarily, the restrictions placed on the robustness of the interaction of caspase 8 and the mitochondria by the allowable variations of the parameters associated with the DISC activation of caspase 8 and caspase 3 binding for caspase 8 implies a certain conservation of robustness between these elements. These insights would not have been feasible through sensitive analysis alone, and the results of this analysis is directly applicable to future experimentation. As experimental protocol is developed to analyze the system's robustness (such as the genetic tug-of-war [13]), matching the trends in parameter robustness predicted in this work provides a means of model (in)validation separate from predicting apoptotic output.

SSV analysis shows important correlations in parameter robustness, and provides insights into general trends that may have evolved in the Fas induced apoptotic network to ensure robust performance. Future work will focus on further model refinement and experimental validation of the apoptotic system's robustness properties. As data becomes available, the natural parameter variation observed in experimentation will be incorporated in the analysis to establish a precedent for the AV used during the analysis. It is unlikely that the AV bounds calculated by SSV at this point of model detail will be accurate, but the trends in robustness properties may be experimentally verifiable. Furthermore, as more data becomes available, performance criteria will be identified and used to further restrict the conservatism of the results presented in this work.

6 Procedure

6.1 Sensitivity Analysis

Sensitivity is the instantaneous response of the system to infinitesimal perturbations in a parameter value. Mathematically defined as

$$S = \frac{dy}{dp} \tag{3}$$

where y is the state of interest (protein concentration, gene expression level, *etc.*) and p is an associated parameter. For multidimensional systems, the sensitivity matrix for an m state and n parameter system is

$$\mathbf{S} = \begin{pmatrix} \frac{dy_1}{dp_1} & \cdots & \frac{dy_1}{dp_n} \\ \vdots & \ddots & \vdots \\ \frac{dy_m}{dp_1} & \cdots & \frac{dy_m}{dp_n} \end{pmatrix}. \tag{4}$$

Sensitivity is direct measure of how much the system behavior shifts via parametric perturbations. The sensitivities are calculated using the software BioSens [21] over varying initial concentrations so as to approximate a semi-global understanding of the system's behavioral dependence on parameter fluctuations. Unlike many other works in systems biology [3, 18, 19] where the sensitivity of all states (proteins, genes, *etc.*) are calculated against all parameters, only the sensitivity of caspase 3 production to parameter uncertainty is considered here. Assuming the pathway's primary objective is to control the production of executioner caspase, evaluating the sensitivities of other states in the system may be misleading when trying to elucidate intracellular design strategies. The sensitivity results are weighted by parameter size, but not by states since only one state is being evaluated.

6.2 Structured Singular Value Analysis

Structured singular value analysis is performed by linearizing the system

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}); \tag{5}$$

and putting the system in the form

$$\begin{aligned} \dot{\mathbf{x}} &= \mathbf{A}\mathbf{x} + \mathbf{B}\mathbf{u} \\ \mathbf{y} &= \mathbf{C}\mathbf{x} \end{aligned} \tag{6}$$

where $\mathbf{A}$ is the system Jacobian, $\mathbf{B}$ is the Jacobian with respect to the inputs, and $\mathbf{C}$ distributes the $\mathbf{x}$ vector between the outputs and inputs. For most of this work, $\mathbf{B}$ is simply the identity matrix, and the only output, $\mathbf{y}$, is active caspase 3.

Parametric uncertainty was incorporated by perturbing $\mathbf{A}$ such that $\mathbf{A_p} = \mathbf{A} + \mathbf{W_2 \Delta W_1}$, where

$$\begin{aligned} \mathbf{W_1} &= \begin{bmatrix} w_1\mathbf{I} \\ \vdots \\ w_p\mathbf{I} \end{bmatrix} \\ \mathbf{W_2} &= \begin{bmatrix} \mathbf{A_1} & \cdots & \mathbf{A_p} \end{bmatrix} \end{aligned} \tag{7}$$

each $\mathbf{A_i}$ is designed to properly distribute the effects of perturbing parameter k_i about the original Jacobian, and each w_i weights the size of the respective perturbation. If each $\mathbf{A_i}$ is full rank, then each perturbation in the Δ block must be repeated rank($\mathbf{A}$) times. For this system, the rank($\mathbf{A}$) is either 1 or 2, thus matrix sizes are significantly reduced.

Once the two weighting blocks are formed, the system is in the form of that seen in Figure 2a. The MatLab μ Analysis and Synthesis Toolbox is used to create the $\mathbf{M\Delta}$ structure seen in Figure 2b. The algorithm to calculate μ for real, parametric perturbations searches parameter space in a hypercube. In order to elucidate which parameters are most robust and which are most fragile, fragile elements must be weighted so as to allow the algorithm to expand more rapidly in more robust directions.

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