Sensitivity Analysis in Biological Modeling: an Application in the Model Development of Staphylococcal Enterotoxin B Pre-Apoptotic Pathways

Rudiyanto Gunawan[†], *Stephanie R. Taylor*[‡] and *Francis J. Doyle III*[†*]
[†] *Department of Chemical Engineering*
[‡] *Department of Computer Science*
University of California Santa Barbara
Santa Barbara, CA 93106

Abstract

One of the challenges in a system-level approach in systems biology is the development of in silico models from experiments that can accurately capture the cellular behavior. The hurdles in this effort, known as reverse engineering, are multiple and include network size and complexity, and quantity and quality of measurements. System analysis can help unraveling the complexity in cellular networks. One such method is sensitivity analysis, which shows the dependence of system behavior on model parameters. For the measurement aspect of modeling, information theoretic approach such as the Fisher information matrix (FIM) can provide a measure of the degree of information content in noisy measurement data for estimating the accuracy of parameter estimates. These tools are included in a MATLAB-based graphical user interface (GUI) named BioSens, for ease-of-use by non-experts in systems theory. The utility of sensitivity analysis and the Fisher information matrix is demonstrated in the model development of staphylococcal enterotoxin-B (SEB) response in kidney cells.

1 Introduction

A system-level understanding of cell behavior requires an accurate representation of the complex interactions of gene/protein networks. Advances in molecular biology have provided a glimpse of such complexity through diverse measurements of cellular activities such as gene expression profiles using DNA microarrays. In systems biology, the goal of network inference or reverse engineering problem is to reconstruct the complex network of regulatory interactions from experiments using a mathematical model. Here, the reverse engineering effort faces two daunting problems: network size and complexity, and incomplete and inaccurate measurements. Network inference from experiments has been extensively investigated in the field of engineering, which is known as system identification. In addition, many concepts in engineering, such as modularity, robustness, and optimality, have been observed in many biological systems. For these reasons, engineering approaches have been instrumental in the reverse engineering effort.

The size and complexity of cellular networks make intuition inadequate for deducing cellular behavior from the underlying gene and protein interactions. Systems analysis can help to

[*] Corresponding Author: Email: frank.doyle@icb.ucsb.edu. Phone: 805- 893-8133. Fax: 805-893-4731

unravel this complexity. One such method is sensitivity analysis [1], in which linear sensitivities quantify how much the system behavior changes as the parameters are varied. In cellular networks, high sensitivities point to the weakest links in the system on which cellular behavior strongly depends. Perturbations to these links can potentially lead to a large disruption in the network behavior, *i.e.*, the network is not robust (fragile) to the uncertainty in these pathways. By mapping critical pathways back to the genome, one can point to the set of genes and interactions that control the cellular behavior. Such information can be used for guiding data-fitting and model refinement in the reverse-engineering of cellular networks.

BioSens [2] provides a sensitivity analysis toolkit that also includes a Fisher information matrix based sensitivity measure and optimal measurement selection. Sensitivity analysis in reverse engineering guides parameter estimation and model refinement, focussing on the critical mechanisms of system behavior. The Fisher information matrix (FIM) conveys the information content in (noisy) measurement data for parameter estimation. This matrix defines the upper bound for achievable accuracy in the parameter estimates, given particular experiment and measurement noise. In reverse engineering, the FIM can also be used for designing new experiments or selecting system variables to be measured that maximize the informativeness of data for better parameter accuracy and/or identifiability. In addition, the FIM carries multiple interpretations as sensitivity measures that consolidate the dynamical sensitivities into coefficients that are easy to compare.

This article presents the background theory underlying the tools in BioSens and the application of these tools in the model development of staphylococcal enterotoxin-B (SEB) pre-apototic pathways in kidney cells. Section 2 reviews the concept of sensitivity analysis and its computation. Section 3 discusses the Fisher information matrix and its dual roles as information measure and sensitivity ranking. An overview of the BioSens toolkit is presented in Section 4. Finally, Section 5 describes the application of the sensitivity analysis and the Fisher information matrix in the model development of SEB response.

2 Sensitivity Analysis

Sensitivity analysis elucidates the dependence of a system behavior on the parameters that affect its dynamics. First-order sensitivity coefficients provide the simplest measure of this dependence by quantifying the variations in the system outputs due to perturbations in the parameters:

$$S_{i,j} = \frac{\partial y_i}{\partial p_j} \tag{1}$$

where $S_{i,j}$ is the sensitivity coefficient of the i-th system output y_i with respect to the j-th parameter p_j. Although this definition implicitly assumes the continuity of the output with respect to the parameters, sensitivity analysis has been developed for systems in which this assumption does not hold, such as discrete stochastic systems [3]. The system outputs are typically comprised of the states or some functions of the states.

The biological system of interest is described by coupled ordinary differential equations:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}, t, \mathbf{p}) \tag{2}$$

where $\mathbf{x} \in \mathbf{R}^n$ denotes the states, $\mathbf{p} \in \mathbf{R}^m$ the parameters, t the time, and $\mathbf{f}$ consists of (nonlinear) functions of the states, time, and parameters. There exist several methods to compute the state sensitivities from Eq. (2) such as Direct, Green's function, and finite difference methods [1]. The output sensitivities in Eq. (1) can be directly computed from the state sensitivities. The Direct and Green's function methods obtain the state sensitivities by solving the derivative of (2) with respect to each parameter

$$\frac{d}{dt}\frac{\partial \mathbf{x}}{\partial p_j}(t) = \mathbf{J}(t)\frac{\partial \mathbf{x}}{\partial p_j}(t) + \frac{\partial \mathbf{f}}{\partial p_j}(t) \tag{3}$$

where $\mathbf{J}(t)$ is the Jacobian matrix of $\mathbf{f}$ with respect to $\mathbf{x}$ (*i.e.*, $J_{i,j} = \partial f_i / \partial x_j$). The initial conditions are typically zero except when p_j is an initial condition of (2). The latter method solves a different differential equation for the Green's function matrix $\mathbf{\Gamma}(t, t')$

$$\frac{d}{dt}\mathbf{\Gamma}(t, t') = \mathbf{J}(t)\mathbf{\Gamma}(t, t'), \quad t \geq t' \tag{4}$$

with the initial condition $\mathbf{\Gamma}(t', t') = I$. The Green's function matrix provides the sensitivities according to

$$\frac{\partial \mathbf{x}}{\partial p_j}(t) = \mathbf{\Gamma}(t, 0)\frac{\partial \mathbf{x}}{\partial p_j}(0) + \int_0^t \mathbf{\Gamma}(t, t')\frac{\partial \mathbf{f}}{\partial p_j}(t')dt' \tag{5}$$

Since t' is the integrating variable, the adjoint equation of (4) is a more practical system to solve:

$$\frac{d}{dt'}\mathbf{\Gamma}^\dagger(t', t) = -\mathbf{\Gamma}^\dagger(t', t)\mathbf{J}(t), \quad t' \leq t \tag{6}$$

where $\mathbf{\Gamma}^\dagger(t', t) = \mathbf{\Gamma}(t, t')$ and the initial condition is $\mathbf{\Gamma}^\dagger(t, t) = I$. Thus the adjoint Green function $\mathbf{\Gamma}^\dagger(t, t)$ must be solved backwards in time t'. The Green's function method becomes more efficient than the Direct method when the number of parameters exceeds that of the states. On the other hand, the finite difference method uses a black-box approach by approximating the derivative of Eq. (1) using a finite difference. A second-order accurate finite difference approximation of the sensitivity is given by:

$$S_{i,j} = \frac{y_i(\mathbf{p} + \Delta p_j \mathbf{e}_j) - y_i(\mathbf{p} - \Delta p_j \mathbf{e}_j)}{2\Delta p_j} \tag{7}$$

where $\mathbf{e}_j \in \mathbf{R}^m$ is a vector of zeros except for the j-th element which equals 1, and Δp_j denotes the magnitude of j-th parameter perturbations. This magnitude is selected to be small enough to limit the approximation error, but large enough to avoid dependence on simulation tolerance.

3 Fisher Information Matrix

One of the biggest challenges in reverse engineering of cellular networks is the availability of data required to completely identify the chosen model structure. The selection of model structure (*e.g.*, Boolean networks, differential equations) determines the types and amount of data necessary to completely reverse-engineer the network [4]. For example, to identify p parameters in a set of nonlinear differential equations, one theoretically needs $2p+1$ randomly chosen experiments (assuming zero measurement noise) [5]. Problems arise because the available (noisy) data do not have enough information to uniquely and/or accurately identify the model parameters. This is known as the parameter identifiability problem in the field of system identification. Here, approaches from information theory such as the Fisher information matrix can provide a mathematical measure of the informativeness of measurements. Such measures allow for the estimation of parameter accuracy from a particular experiment, as well as the design of experiments for maximizing the information content in measurements.

The Fisher information matrix (FIM) represents a measure of the information content in noisy measurements for the identification of model parameters. When the measurement noise follows the Gaussian distribution, the FIM reduces to [6]

$$\mathbf{FIM} = \mathbf{S}^T \mathbf{V}_\mu^{-1} \mathbf{S} \tag{8}$$

where $\mathbf{V}_\mu$ is the measurement covariance matrix. Using the Cramer-Rao inequality [7], the upper bound for the parameter accuracy can be derived from the FIM

$$\mathbf{V}_p \geq \mathbf{FIM}^{-1} \tag{9}$$

where $\mathbf{V}_p$ denotes the parameter covariance matrix. The diagonal elements of the matrix $\mathbf{V}_p$ give the parameter variances. The 95% confidence interval for each parameter p_i can be defined as

$$[p_i - 1.96\sigma_{p_i}, p_i + 1.96\sigma_{p_i}] \tag{10}$$

where σ_{p_i} denotes the standard deviation of the i-th parameter (*i.e.*, the square root of parameter variance). In BioSens, a parameter is called *practical identifiable* when its estimated value is non-zero within a 95% confidence.

The Gaussian assumption may not apply for gene expression as this process involves chemical species that have very low concentrations (nanomolar level), that is, the gene expression behaves as a discrete stochastic system. In such a case, the noise in the system can become non-Gausssian (*e.g.*, log-normal or bimodal distributions). Nevertheless, the FIM can still be evaluated using a direct analysis of the chemical master equation using the general formula [3]

$$\mathbf{FIM} = E\left[\left(\nabla_{\mathbf{p}} \log \rho \right) \left(\nabla_{\mathbf{p}} \log \rho \right)^T \right] \tag{11}$$

where ρ is the probability density function of the states. For discrete stochastic systems, the FIM can be evaluated by simulating the chemical master equation (CME) for the joint probability density function of the states ρ. This simulation uses a Monte Carlo approach such as the stochastic simulation algorithm [8] or its approximate accelerated algorithm [9].

As a measure of information, the FIM provides an avenue for designing experimental protocols that maximize the utility of measurement data for parameter identification. One aspect of this design is the selection of measurements that will have the most information for parameter identifiability and accuracy. Given a particular input-output experiment, this process consists of two steps; first, the removal of parameters that are not *a priori* identifiable, and finally, the optimization of some measures of information based on the FIM. *A priori* identifiability relates to the ability to uniquely identify parameters from perfect measurements. In BioSens, the first step uses the orthogonal procedure proposed by MacAuley and coworkers [10], which is a geometric based approach where the number of *a priori* identifiable parameters correlates with the rank of the orthogonalization of the scaled sensitivity matrix $\hat{\mathbf{S}}$:

$$\hat{S}_{i,j} = \frac{\partial y_i}{\partial p_j} \frac{p_j}{y_i} \tag{12}$$

The parameters corresponding to the columns of orthogonalized sensitivity matrix are deemed unindentifiable if the norms are smaller than a given tolerance. The measurement selection then chooses the system variables (states) that can maximize some measures of information with respect to the *a priori* identifiable parameters. Figure 1 illustrates the two most effective FIM-based optimality criteria, D-optimal and A-optimal, in designing experiments [11]. D-optimal design aims to maximize the degree of informativeness in data by maximizing the determinant of FIM, which corresponds to the area/volume of information hyperellipsoid (Figure 1a). On the other hand, A-optimal design is equivalent to reducing the hyperellipsoid of uncertainty in parameter estimates (Figure 1b). BioSens includes both of these optimality criteria in the measurement selection tool.

BioSens also adopts the Fisher Information Matrix as a measure of the sensitivities. The formulation in Eq. (8) motivates a new use of the FIM as a consolidation of (weighted) sensitivities. In general, the FIM captures the sensitivity of the (log) distribution with respect to the parameters as shown in Eq. (11). The use of the FIM as a sensitivity measure requires novel interpretations of the properties of this matrix. The FIM captures not only the first order sensitivities of the system, but also the effects of parametric interactions (second order sensitivities). Three sensitivity measures can be derived based on the FIM - the diagonal elements, the eigenvalues, and the inverse of standard deviations (*i.e.*, the inverse of the diagonals of $V_{\mathbf{p}}$).

The diagonal elements of the FIM represent the magnitudes of the sensitivities with respect to each individual parameter. Under the Gaussian assumption, these elements are equal to the weighted norms of the first order sensitivities:

$$\mathrm{FIM}_{i,i} = \mathbf{S}_i^T \mathbf{V}^{-1} \mathbf{S}_i = ||\mathbf{S}_i||^2_{\mathbf{V}^{-1}} \tag{13}$$

where $\mathbf{S}_i$ is the i-th column of the sensitivity matrix. The eigenvalues of the FIM represent the magnitudes of the sensitivities with respect to simultaneous parameter variations whose relative magnitudes and directions are given by the corresponding eigenvectors. The product of the eigenvalues presents an index of the information content for use in the design of optimal experiments, which is the aforementioned D-optimality. Here, each eigenvalue is assigned as the sensitivity measure with respect to the parameter that corresponds to the element of the eigenvector with the largest magnitude. Thus, a parameter may have more than one sensitivity

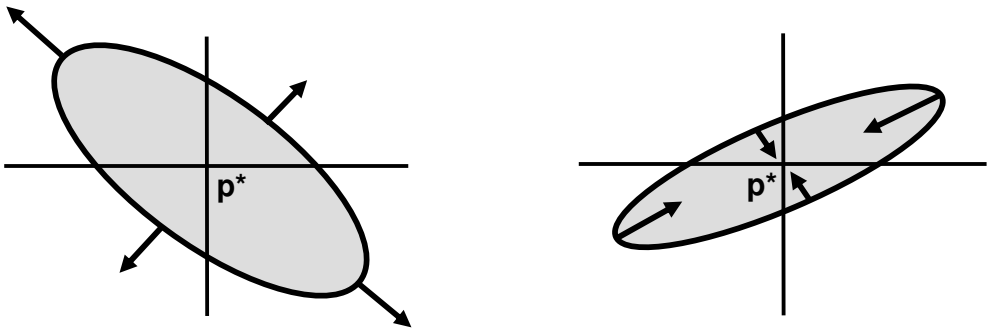

Fig. 1: Fisher information matrix-based optimality criteria. The axes represent the model parameters where the origin describes the best parameter estimates. For simplicity, only two parameters are shown. In a system with three of more parameters, these ellipses are projections of the higher dimensional ellipses (hyperellipsoids) onto two-parameter axes. (a) The ellipse of information. The ellipsoidal axes are defined by the FIM eigenvalues and eigenvectors. The area quantifies the amount of information, while the shape indicates the distribution of information for each parameter. D-optimality design aims to maximize the area/volume of information (as indicated by the arrows), which is proportional to the determinant of FIM. (b) The ellipse of parameter uncertainty. The lengths of the ellipsoidal axes equal to the inverse of the eigenvalues of FIM. A-optimal design aims to reduce the region of parameter uncertainty (shown by the arrows), which is measured by the sum of the parameter variances.

measure, while others may not have an assigned measure (*i.e.*, there may not be a one-to-one correspondence between the eigenvalues and the parameters). Finally, the diagonal elements of the matrix $\mathbf{V}_p$ are the square of the standard deviations of the parameters. The sum of the standard deviations is used in the A-optimal design of experiments. Based on Eq. (9), the standard deviations inversely correlate with the sensitivity of the system. As with the eigenvalue measures, the standard deviations incorporate the parametric interactions, but without the problematic one-to-one correspondence. The computation of standard deviation, however, is more prone to numerical inaccuracy in matrix inversion. These new interpretations of the diagonal elements, eigenvalues, and standard deviations of FIM provide sensitivity measures with different attributes, and thus should be utilized and compared accordingly. In BioSens, the sensitivity ranking is based on the diagonal elements of the FIM due to the difficulties with the eigenvalue- and standard deviation-based measures, *i.e.*, one-to-one correspondence and matrix inversion issues, respectively.

4 BioSens Overview

BioSens is a part of Bio-SPICE program, in which the term SPICE stands for Simulation Program for Intra- and Inter-Cell Processes. Bio-SPICE contains a suite of tools for model development (including data mining and analysis), analysis, and simulation of biological systems (freely available at http://www.biospice.org). BioSens is implemented with a Matlab GUI and can

be run from the Bio-SPICE Dashboard via the BioMat Bridge, or directly from Matlab. It accepts SBML and XPP (*.ode) model files as input, which are then parsed to an intermediate ODE-based format conducive to fast loading. Using the GUI, the user is able to run simulations of the model, perform sensitivity analysis (Sensitivity Tool), rank the parameters according to their relative sensitivities (FIM Tool), and determine the optimal states to measure in an experiment (Measurement Selection Tool). Figure 2 illustrates the data flow for BioSens.

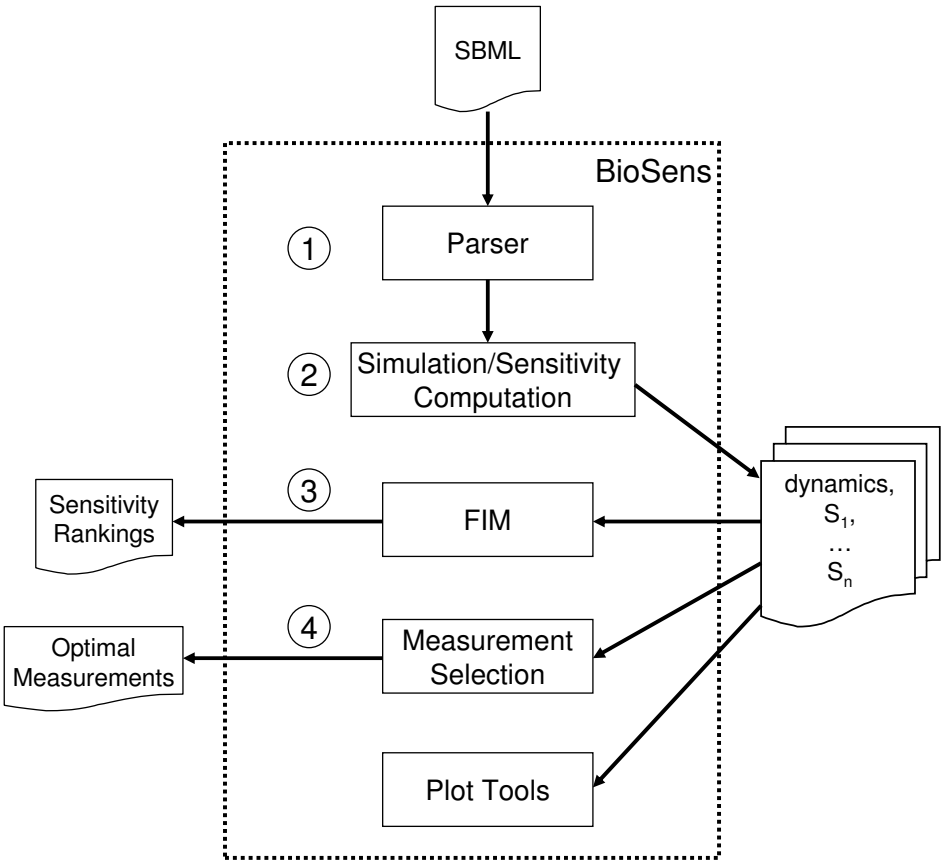

Fig. 2:

Layout of BioSens

1) Parser: Load an SBML file into BioSens and converting it to an internal format of ODEs.
2) Simulator: Simulate the model and perform sensitivity analysis on selected parameters. Outputs are saved in Matlab data format (.mat).
3) FIM: Give ranking of the system sensitivities according to the diagonal entries of the FIM.
4) Measurement Selection: Determine the optimal measurement set that maximizes the informativeness of experimental data.

The Sensitivity Tool uses one of two simulation engines - XPP [12] or DASPK [13]. In this work, we focus on the DASPK implementation because it computes the sensitivities more efficiently and accurately than the implementation that uses finite difference approximations and XPP. The FIM Tool then computes the FIM using the computed sensitivities and a covariance

matrix provided by the user. The diagonal entries are used to rank the parameters from least to most sensitive.

The Measurement Selection Tool then allows the user to eliminate parameters that are not practically identifiable (which can be done with minimal effort because the settings from the FIM Tool are used as the default settings for this tool), and then obtain an optimal set of measurements using either the A-optimality or D-optimality criterion. The computation is performed in Matlab and the output is both displayed to the user and saved to a file.

5 SEB Modeling Application

The sensitivity analysis and the Fisher information matrix tools in BioSens have been applied in the model development of staphylococcal enterotoxin B (SEB) response in kidney cells, as part of the pre-apoptosis use-case in Bio-SPICE. Initially, BioSens was used to simulate early versions of the SEB models to check for the correct behavior, *e.g.*, apoptosis in the presence of SEB, and vice versa, no apoptosis in the absence of SEB. The simulations were also used for calibrating the time scales of key processes such as nf-κb and akt activations. Here, the FIM-based sensitivity analysis was able to guide the parameter and initial value refinements for the model to give the desired behavior [14].

Subsequent use of BioSens focused on the model refinement. Sensitivity analysis coupled with a comparative study of the diagonal elements of the FIM, provided the information for iteratively refining the model. This iterative process involved incorporating more details in the subnetworks where the system is highly sensitive, using literature searches and database such as GENEWAYS [15] (performed by Dr. Zhang at the University of California Berkeley). Additional details were included in the SEB model to describe: mitochondria control switch through Bcl-2, negative feedback on ERK through MKP, post-translational regulation of transcription factors fkhr and nf-κb, export of death ligands and TNF-α with subsequent activation of death receptor apoptosis pathway, and numerous cross-talks between the upstream kinase cascades, central transcription factors and downstream apoptotic modules [14]. Figure 3 shows an example of sensitivity analysis of the SEB model in the two regimes: with and without SEB treatment. The information from such analysis guided the model refinement to include more details in the model (in this case, to the pathways activating ERK and c-MYC and their downstream cross-talks). The differences between the analysis in the presence and absence of SEB also indicated regime-dependent sensitivities, which suggested a divergence in the signalling routes between the normal (no-SEB) and SEB-induced toxic shock responses. During the iterative model refinement, the model evolved from 77 states and 179 parameters, to a more detailed system with 117 states and 356 parameters. Figure 4 show the evolution of SEB response model during the model refinement process.

Figures 5 and 6 show the dynamic and sensitivity responses of the latest SEB pre-apoptosis model, respectively. The transient response was localized early (within 2000 seconds) and followed by steady state behavior. The sensitivities also exhibited similar response in which the sensitivities to different parameters peaked at different times. This behavior motivates using a temporal sensitivity analysis to investigate the dependence of behavior in different dynamic

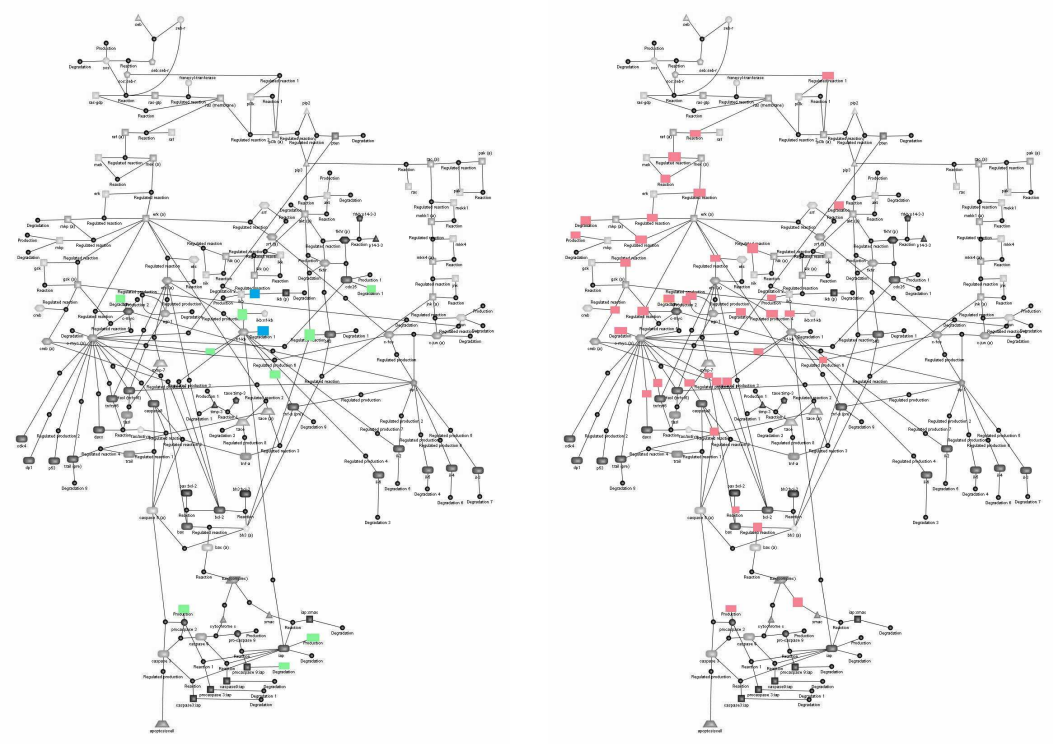

Fig. 3: Sensitive pathways in two dynamic regimes: without SEB (left) and with SEB (right). The colored boxes represent the pathways to which the system behavior is highly sensitive.

regions, which is currently under development for BioSens. Such temporal analysis can be used in the determination of the optimal time window(s) to administer drugs for SEB. The FIM-based sensitivity analysis of this model using BioSens suggests that the sensitive pathways in the network are highly localized (*i.e.* there are hot spots) around the ERK, NF-κB and PI3K modules as indicated in Figures 7 and 8. The transcription factor NF-κB is highly conserved among many organisms (from *Drosophila* to human) and primarily responsible for controlling immune and inflammatory responses [16]. In apoptosis, NF-κB has been found to exert both pro- and anti-apoptotic effects [17]. The phosphoinositide 3-kinases (PI3K) is a family of signal transducers which is responsible in regulating several transduction pathways for various cellular functions including apoptosis [18]. The ERK belongs to mitogen activated protein kinases (MAPK) and participates in the signalling cascades for growth factor stimulations via tyrosine receptor kinases [19].

These hot spots have several implications. First, further model refinements on the hot spots in the network may be necessary. Second, when the model is sufficiently accurate, the differences between the analysis with and without SEB treatment, such as shown in Figures 7 versus 8 and in Figure 3, can have physiological relevance and provide the candidate pathways in the network for targeted detection and/or therapy of the SEB toxin response. On the other hand, where there exist cold spots (*i.e.*, insensitive pathways) in the network, the model size can be reduced by eliminating or combining the corresponding pathways. In Figure 7, these “cold” spots concentrated on the pathways downstream of NF-κB (regulated production of several species by NF-κB), and the interconversions among RASGRP, PK, and NF-κB species. Possible model

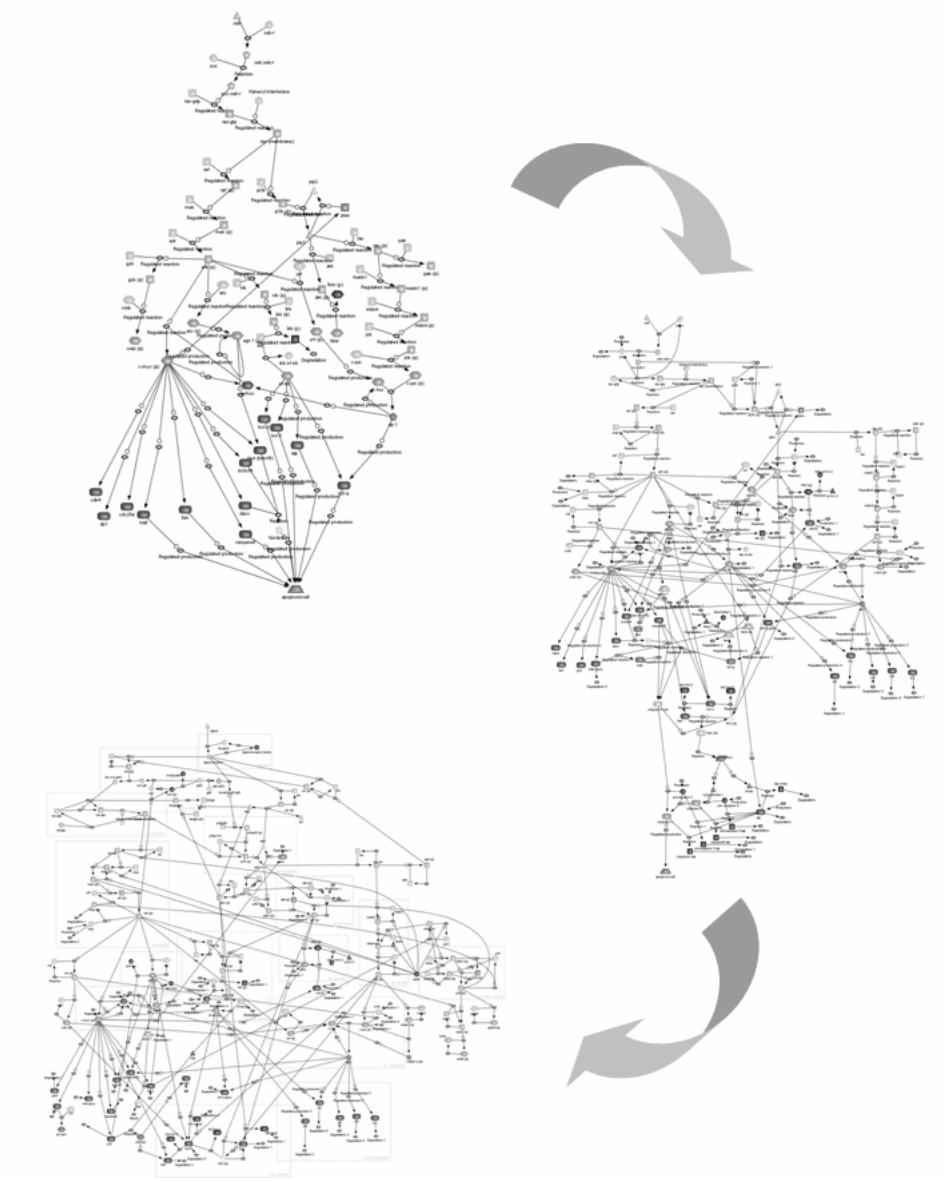

Fig. 4: The evolution of SEB response model.

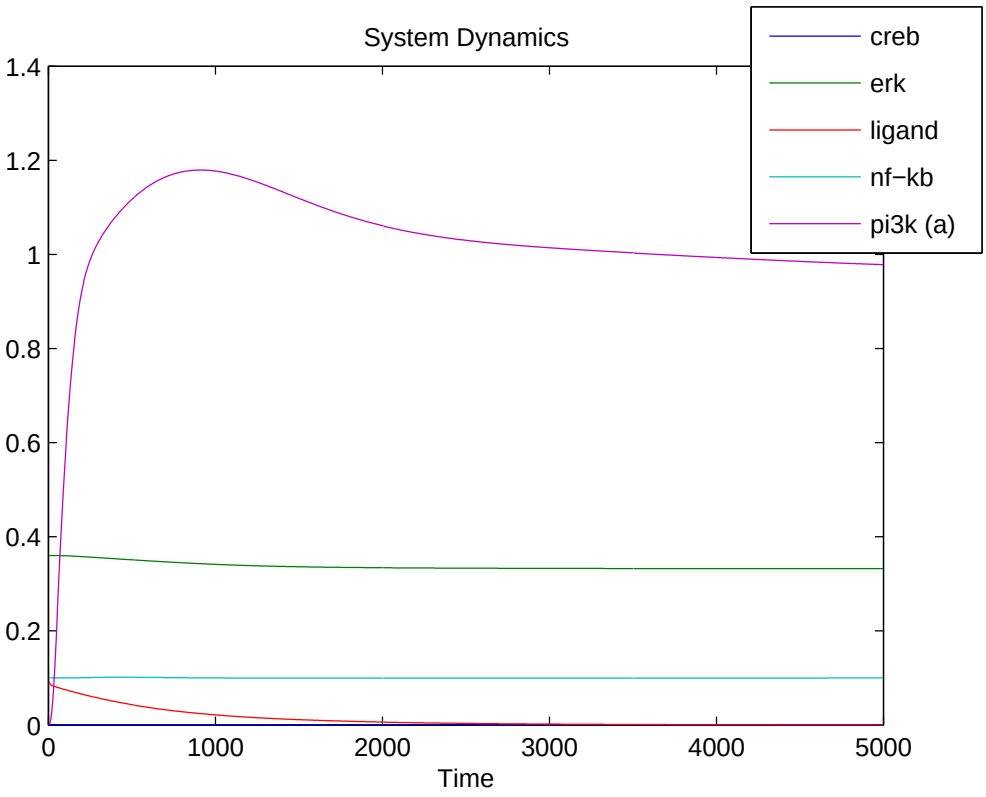

Fig. 5: Simulated SEB dynamic response (ligand input = 0.2).

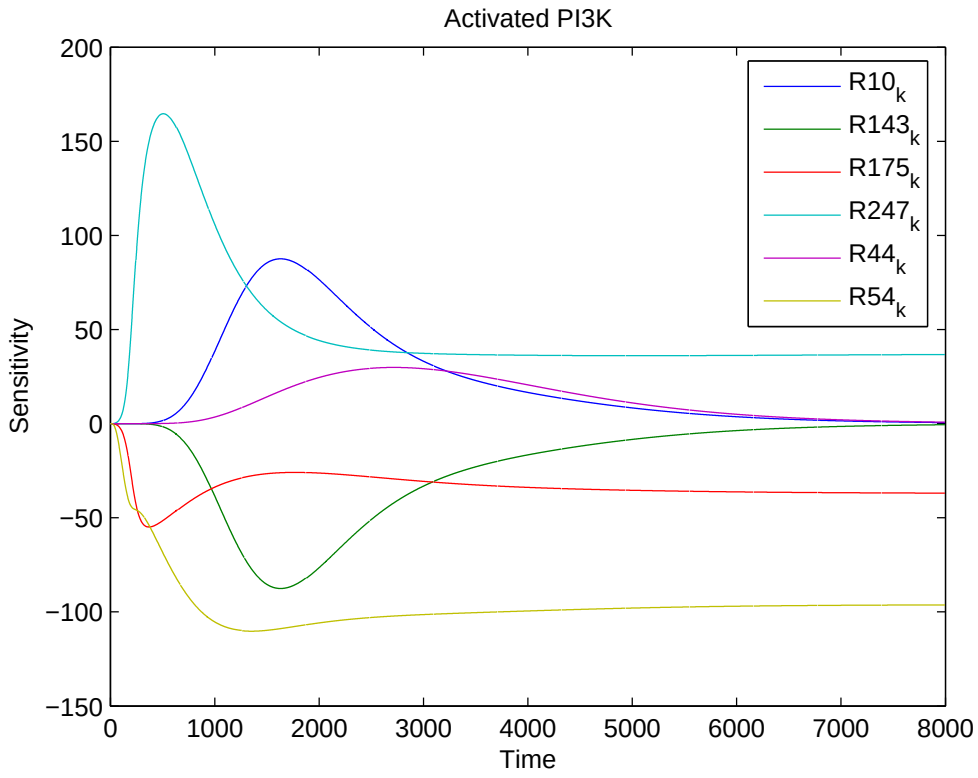

Fig. 6: SEB sensitivity response (ligand input = 0.2).

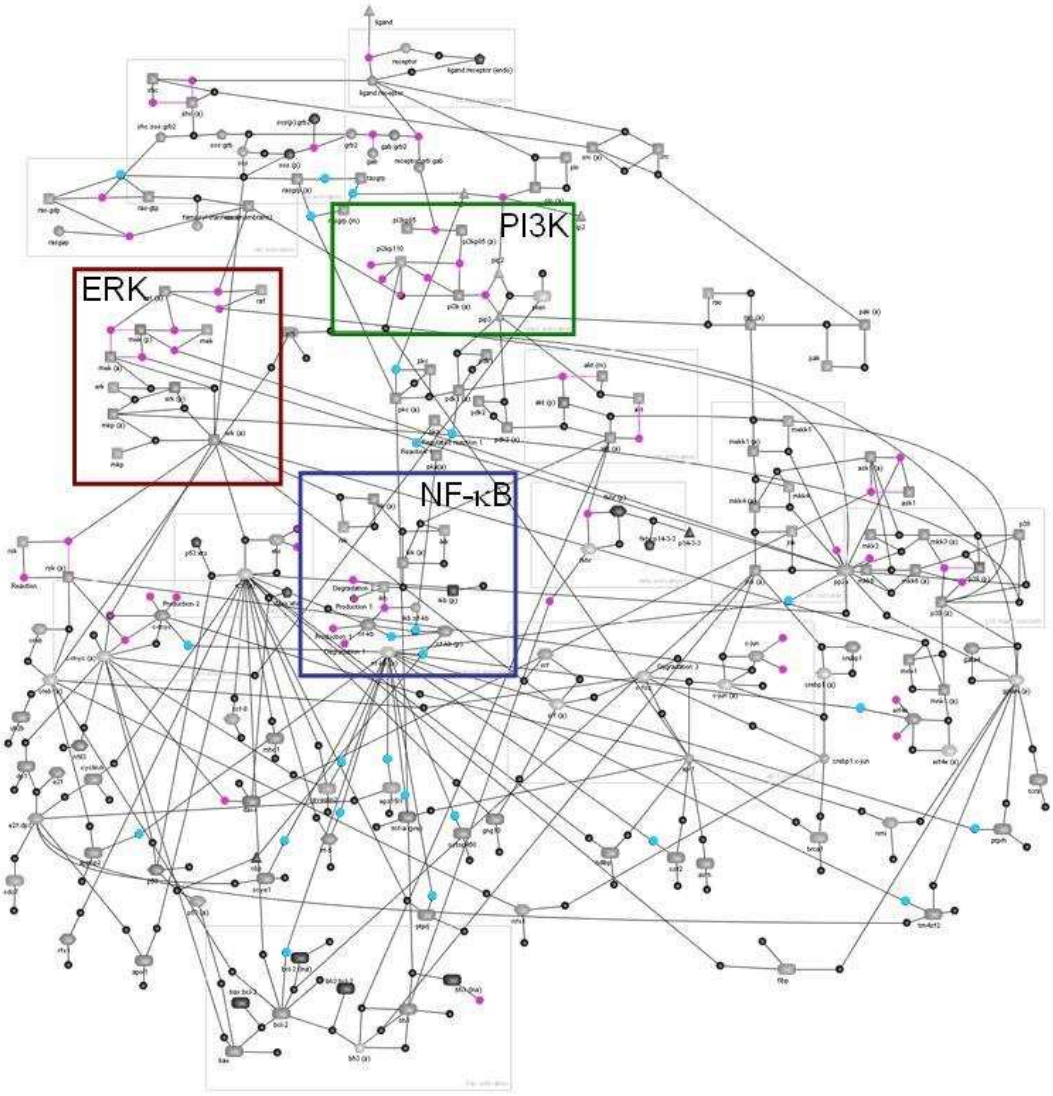

Fig. 7: FIM-based sensitivity analysis of SEB preapoptotic model with SEB treatment (toxic shock response). The analysis shows localized ”hot spots” in the network for sensitivity marked as red dots and ”cold spots” marked as blue dots.

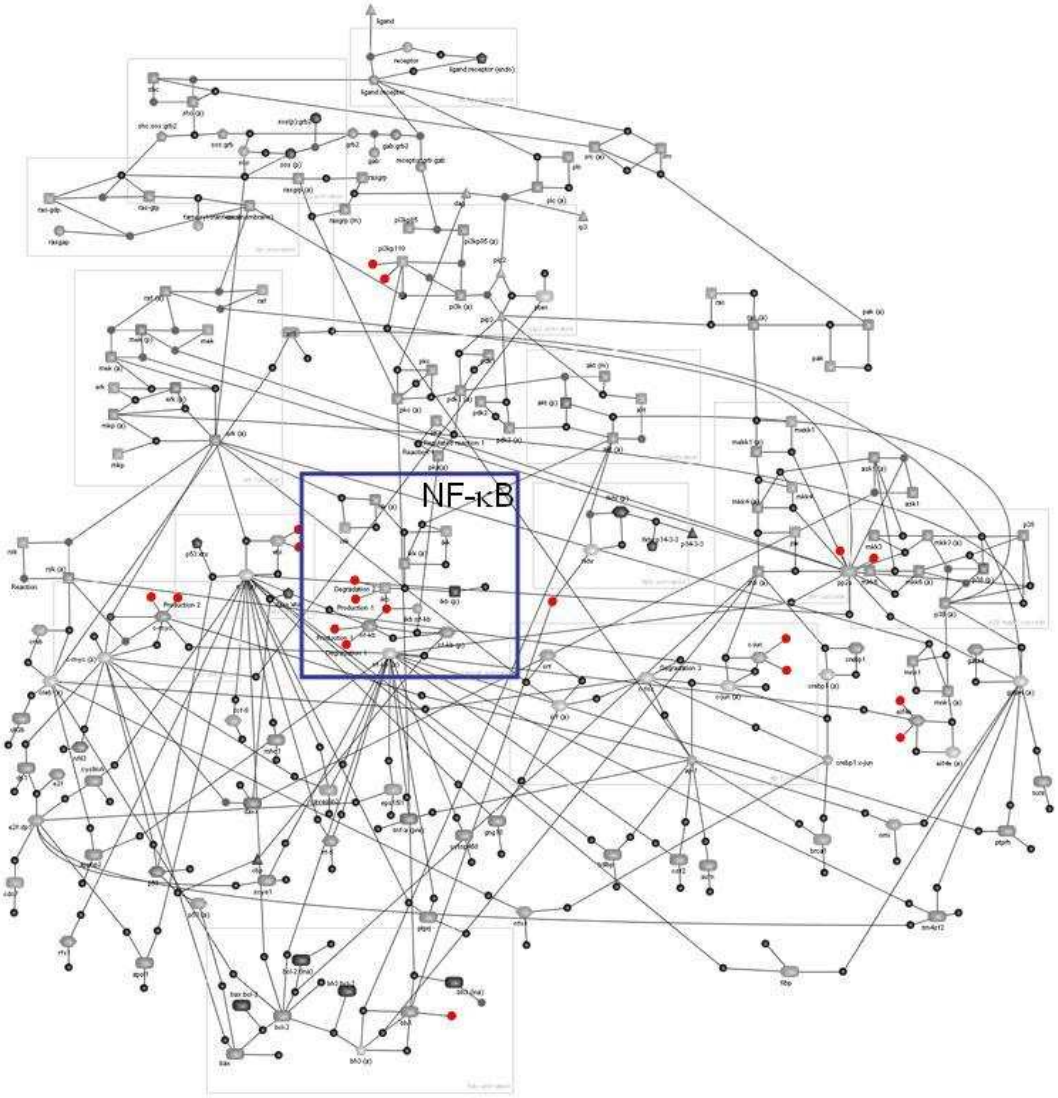

Fig. 8: FIM-based sensitivity analysis of SEB preapoptotic model without SEB treatment (normal response). Again, the analysis shows localized hot spots in the network for sensitivity marked as red dots.

reductions can be done, for example, by representing the different (activated) states of RASGRP, PK, or NF-κB as a single state.

The sensitivity analysis results, in particular the localized hotspots, confirmed the transcription and clustering analysis (PAINT [20] and NCA [21] analysis) of time-series gene expression data. In addition, the comparison between the analysis of the SEB response in the two dynamic regimes indicated that the presence of SEB evoked different signalling pathways from the normal response. In particular, the pathways involving ERK and PI3K activations were sensitive in the SEB-treated system, but were not sensitive in the absence of SEB (NF-κB pathways was sensitive in both regimes). The use-case effort now focuses on the ERK module to obtain more accurate representation of these critical pathways in the SEB response. By focusing on smaller subnetworks guided by sensitivity analysis, the model development is decomposed into numerically tractable steps.

6 Conclusions

A key aspect in systems biology concerns the reverse engineering of cellular network from experiments to create an accurate *in-silico* representation. The difficulties in this problem stem from the high complexity of cellular systems and the quality of experimental data. Systems theoretic approaches, in particular from the field of model identification, can help unravel the complexity and quantify the data informativeness, using sensitivity analysis and Fisher information matrix. The two methodologies are implemented in BioSens providing ease-of-use for non-experts. The application to the model development of SEB response highlighted the utility of BioSens in guiding model refinement. In addition, the sensitivity analysis of SEB model showed clustering of hot- and cold-spots, indicative of high and low sensitivities. In this case, the hot spots which were in agreement with measurements, gave support to a focused study on the ERK signalling role in SEB response.

Acknowledgments

The authors would like to thank Tingting Zhang, Marti Jett and Adam Arkin for many fruitful discussions. This work was supported by the Institute for Collaborative Biotechnologies through grant DAAD19-03-D-0004 from the U.S. Army Research Office and by the DARPA BioCOMP program.

References

[1] A. Varma, M. Morbidelli, and H. Wu. *Parametric Sensitivity in Chemical Systems*. Oxford University Press, New York, NY, 1999.

[2] S. R. Taylor, K. Gadkar, R. Gunawan, and F. J. Doyle III. Biosens: a sensitivity analysis toolkit for Bio-SPICE. Available at http://doyle.chemengr.ucsb.edu.

[3] R. Gunawan, Y. Cao, L. Petzold, and F. J. Doyle III. Sensitivity analysis of discrete stochastic systems. *Biophys. J.*, 88:2530–2540, 2005.

[4] D. W. Selinger, M. A. Wright, and G. M. Church. On the complete determination of biological systems. *Trends Biotech.*, 21:251–254, 2003.

[5] E. D. Sontag. For differential equations with r parameters, $2r + 1$ experiments are enough for identification. *J. Nonlinear Sci.*, 12:553–583, 2002.

[6] L. Ljung. *System Identification: Theory for the User*. Prentice Hall, Englewood Cliffs, NJ, 1999.

[7] T. M. Cover and J. A. Thomas. *Elements of Information Theory*. John Wiley & Sons, Inc., 1991.

[8] D. T. Gillespie. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. *J. Comput. Phys.*, 22:403–434, 1976.

[9] D. T. Gillespie. Approximate accelerated stochastic simulation of chemically reacting systems. *J. Chem. Phys.*, 115:1716–1733, 2001.

[10] K. Z. Yao, B. M. Shaw, B. Kou, K. B. McAuley, and D. W. Bacon. Modeling ethylene/butene copolymerization with multi-site catalysts: Parameter estimability and experimental design. *Polymer Reaction Eng.*, 11:563–588, 2003.

[11] A. F. Emery and A. V. Nenarokomov. Optimal experimental design. *Meas. Sci. Technol.*, 9:864–876, 1998.

[12] B. Ermentrout. Xpp/xppaut. Available at http://www.math.pitt.edu/ bard/xpp/xpp.html.

[13] S. Li and L. Petzold. Daspk 3.0: Large scale differential algebraic equation solver with sensitivity analysis, 2000. Available at http://www.engineering.ucsb.edu/ cse/software.html.

[14] T. Zhang. Modeling of seb-induced apoptosis. Technical report, University of California Berkeley, 2005.

[15] A. Rzhetsky, I. Iossifov, T. Koike, M. Krauthammer, P. Kra, M. Morris, H. Yu, P. A. Dubou, W. Weng, W. J. Wilbur, V. Hatzivassiloglou, and C. Friedman. GeneWays: a system for extracting, analyzing, visualizing, and integrating molecular pathway data. *J. Biomed. Inform.*, 37(1):43–53, Feb 2004.

[16] T. D. Gilmore. The Rel/NF-kappaB signal transduction pathway: introduction. *Oncogene*, 18(49):6842–6844, Nov 1999.

[17] M. Barkett and T. D. Gilmore. Control of apoptosis by Rel/NF-kappaB transcription factors. *Oncogene*, 18(49):6910–6924, Nov 1999.

[18] M. Osaki, M. Oshimura, and H. Ito. PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis*, 9(6):667–676, Nov 2004.

[19] R. Seger and E. G. Krebs. The MAPK signaling cascade. *FASEB J*, 9(9):726–735, Jun 1995.

[20] R. Vadigepalli, P. Chakravarthula, D. E. Zak, J. S. Schwaber, and G. E. Gonye. PAINT: a promoter analysis and interaction network generation tool for gene regulatory network identification. *Omics*, 7(3):235–252, 2003.

[21] J. C. Liao, R. Boscolo, Y.-L. Yang, L. M. Tran, C. Sabatti, and V. P. Roychowdhury. Network component analysis: reconstruction of regulatory signals in biological systems. *Proc. Natl. Acad. Sci. USA*, 100(26):15522–15527, Dec 2003.