Employing optimization and sensitivity analyses tools to generate and analyze mathematical models of T cell signaling events

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Abstract. Approaches using optimization and sensitivity analysis to generate and analyze mathematical models relating to T cell signaling are reviewed and extended. Two approaches to rationally evolve a mathematical model of the signaling events associated with T cell receptor engagement are compared: one relies upon expert knowledge and the other is directed by sensitivity analyses. Both approaches extensively employ optimization to address model structure and parametric uncertainty through exogenous inputs and parameter identification. In isolation, the sensitivity analyses driven approach failed to converge to a model interpretable within the context of known signaling pathway information. This work suggests that expert knowledge supplemented with sensitivity analyses results may effectively guide model evolution through use of constrained optimization techniques. These methods are equally applicable to other cellular types even though they are presented within the context of T lymphocytes.

Keywords: Uncertain model structure and parameters, parameter identification, extended FAST sensitivity analysis, adjoint optimization methods, genetic algorithms, Erk MAPK pathway
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1. INTRODUCTION

Future advances in biomedicine will be directed, discovered, and supplemented through the use of optimization and systems analysis of mathematical models of cellular processes from biological systems. The current method of developing new drugs or therapies by pharmaceutical companies will eventually be cost prohibitive [1]. A recent study found that less than 0.02% of screened compounds become marketed drugs [1]. These drugs take an average of 15 years to develop and over 800 million dollars [1]. A new approach that utilizes a system-level understanding of the body, organs, tissues, and cells is required to efficiently design drugs with the highest impact and fewest side effects [2]. Important components of this approach are quantitative mathematical models. Some initial efforts have utilized models to pinpoint intervention points for drugs; future work will lead to their application for de novo drug design with a broader consideration of their systemic effects [2, 3].

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As our awareness of the inherent complexity associated with cellular systems increases, the limitations of intuition and exhaustive, exploratory experimental approaches become more obvious. Clearly, a quantitative approach would be beneficial to help systematically understand and engineer cellular processes. To date, numerous studies have focused on generating mathematical models of cellular processes and analyzing these models to understand behaviors. Many of these models have successfully predicted the response of cells to different stimuli. However, comparatively few have utilized these models for cellular engineering to manipulate the complex dynamics associated with cellular processes.

Mathematical models of the cellular processes are highly nonlinear and uncertain and may be time varying, hybrid and/or stochastic; these properties impede the systems analysis and to some extent prohibit the engineering design. To support systems analysis and engineering design, mathematical model genesis should consider the available analysis and optimization tools. For example, the construction of mathematical models for engineering applications routinely utilizes simple models and neglects unwarranted details. Although it is entirely possible to generate a very accurate model with hundreds of equations, the utility of the model may be limited. Analysis of overly complex models relies heavily upon numerous simulations to generate an understanding of the processes. Most analytical tools are not applicable to large, complex models. Simple models are preferred; in fact most engineering tools support the systems analysis and optimization for ordinary differential equation (ODE) models that are linear lumped-parameter mathematical models. Thus the genesis of the model must consider the desired application and evolve to provide sufficient accuracy for engineering design and accurate systems analysis.

This chapter describes a system analysis and optimization approach for generating mathematical models of T cell signaling that are suitable for supporting engineering analysis and design. The second section of this chapter briefly reviews the biological principles and events within the T cell that are initiated upon engagement of their receptors to provide a context for the rest of the document. The third section summarizes previously published modeling and analysis work related to these events. The subsequent section provides the theoretical background for the optimization and analysis techniques that support the genesis of appropriate ODE models. The fifth section provides an example of the evolution of a mathematical model of the early signaling events associated with T cell signaling utilizing embedded optimization and sensitivity analyses. The final section summarizes the findings and illustrates how the results and approaches are equally applicable to other cellular types and processes even though they are presented and discussed within the context of T lymphocytes.

2. T CELL SIGNALING AND ACTIVATION

The T cell (or T lymphocyte) will serve as the vehicle to illustrate and study the application of systems analysis and optimization techniques to mathematically model biological systems and cellular processes. The T cell is a crucial element of the human immune system that utilizes membrane bound receptors (TCRs) to identify and detect the presence of foreign particles (antigens), see Fig. 1. When antigenic peptides bind to these membrane-bound receptors, the T cells are activated by the biomechanical

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stimulus, initiating a sequence of biochemical events involving protein tyrosine kinase activation, and resulting in protein phosphorylation. This cascade of biochemical events is paralleled in many cells that are stimulated through membrane bound receptors. In fact, there is a nearly identical, analogous cascade, activated upon membrane-antibody binding on B cells, another element of the immune system. Portions of this T-cell signaling pathway, including the mitogen-activated protein kinases (MAPK), are ubiquitous to most cells, serving as a precursor to gene activation. Thus this document encompasses several highly conserved mechanisms that future mathematical models of the cell can build upon.

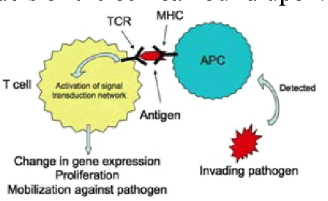

FIGURE 1. Activation of the T cell by an antigen-presenting cell (APC) in the immune system.

A biomechanical stimulus of the membrane receptors on the cell initiates a sequence of biochemical events (signal transduction) that serve to transmit, filter, and amplify the signal to the nucleus. Most signal transduction cascades consist of a sequence of reversible phosphorylation and dephosphorylation events. (Phosphorylation is the replacement of a hydroxl group by a phosphate group within the protein and dephosphorylation is the reversal of this process.) Each step of the cascade effectively integrates signals while transferring them to subsequent stages. Enzymes catalyze the chemical reactions to dramatically increase the rates and thereby increase the amount of substrate reacted upon in a short duration. Thus, enzymes ensure the signal is transmitted in a timely and amplified manner. Also, the specific nature of these enzymes enforces the stepwise process associated with the biochemical cascade. This specificity arises from protein kinases (enzymes that phosphorylate proteins in the presence of adenosine triphosphate, ATP) that act only upon a target serine, threonine, or tyrosine residues within a certain amino acid sequence in proteins. There are additional molecules involved in the biochemical cascade that are not enzymes, but are required for the signal transduction to proceed. These primarily serve as adaptor molecules providing binding sites (scaffolds) that generate the multi-protein complexes necessary for the signaling cascades to proceed.

The biochemical cascade is a carefully coordinated sequence of events, not simply a mixture of proteins that randomly collide. Proteins will not bind and react with just any other protein; there need to be some complementary features that promote binding between certain proteins. For the types of pathways under consideration, the dominant binding motifs identified thus far are the SH2 and SH3 domains on one protein binding to a phosphorylated tyrosine or a proline rich sequence, respectively, on a second protein. These pairings are critical for maintaining an ordered sequence of events and for promoting the next stage of the cascade. Extensive biochemical studies have identified many of the major molecular components and interactions of the T-cell signaling and activation pathways [4-8]. However, this molecular-level knowledge conveys little information on how the entire signaling network operates as a functional

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unit. Systems analysis, optimization, and modeling will help quantitatively understand the interactions and roles of the participating molecules.

Physiologic activation of the T cell signaling pathways begins with the binding of a specific antigen fragment complexed with a MHC (major histocompatibility complex) molecule to the TCR on the surface of the T cell and the activation of a protein kinase that initiates a sequence of biochemical events. (Each TCR is specific for its target antigen; different T cells express different TCRs). Simultaneous engagement of the T cell surface co-stimulatory molecule CD28 results in cell proliferation. Engagement of the TCR in the absence of CD28, results in cell anergy (inactivation) rather than cell proliferation. The Erk pathway is initiated when the TCR binds to its specific antigen. Upon engagement of the TCR, the constitutive receptor internalization, recycling, synthesis, and degradation process is augmented with active receptor phosphorylation and turnover during signaling [9, 10]. The bound TCR (TCRb) rapidly become phosphorylated receptors (TCRp). The phosphorylated receptors are internalized, and the internalized receptors (TCRi) are predominantly recycled back to the membrane while a fraction is degraded. Under saturating stimulation conditions, the recycled receptors are immediately bound and the cycle continues.

Fig. 2 presents a simplified view of the dominant biochemical interactions of the early signaling events and the MAPK pathway. The TCR engagement leads to the activation of Src-family protein tyrosine kinase (SFK) Lck/Fyn. Typically the SFK activation mechanism involves the coordinated interaction of a SFK-phosphorylated transmembrane scaffold protein Cbp, a SFK inhibitory kinase, Csk, and a membrane bound phosphatase, CD45. SFK is activated upon dephosphorylation at an inhibitory site followed by phosphorylation at an activation site. Activated SFK phosphorylates the TCR cytoplasmic tails. Phosphorylated TCR ζ binds to Zap70, allowing it to be phosphorylated by activated SFK at multiple tyrosine residues one of which is the activation site while another mediates its interaction with SFK [7]. A SFK-activated protein tyrosine phosphatase (PTP), SHP-1, can dephosphorylate SFK and Zap70 interfering with their association and activation. This early signal propagates through a number of membrane bound/recruited scaffold proteins including LAT and SLP76. Eventually, the small G protein Ras is activated by a number of factors, initiating the MAPK cascade, ending in the phosphorylation of Erk. The activation of Erk is necessary to activate transcription factors critical for T cell responses.

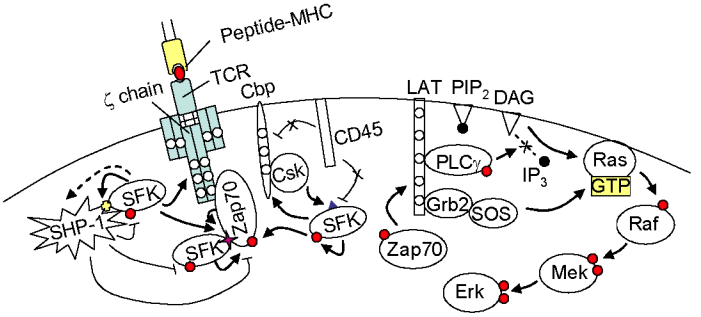

FIGURE 2. Schematic view of the TCR-activated Erk pathway. Arrows indicate protein catalyzed/facilitated phosphorylation or activation reactions while connectors with blunt ends indicate protein tyrosine phosphatase catalyzed dephosphorylation events. (Adapted from [11]).

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In addition to the Erk MAPK pathway, other signaling cascades that serve major roles in transmitting extracellular signals to the nucleus but not described herein include the PLC-γ (phospholipase C) pathway, the Ca^{2+} activated pathway(s), and the PI3K (phosphoinositide 3-kinase) pathway (reviewed in [12-15]). The end result of successful signaling through these cascades is the regulation of nuclear transcription factors (e.g., AP-1, NF-AT, NF-κB, GATA-3) leading to gene expression (or in some cases repression). These pathways bifurcate and interconnect in complex patterns which mathematical models help to resolve and understand.

3. APPROACHES TO MODELING AND ANALYSIS OF T CELLS

Currently the field employs many different approaches to mathematically model cellular processes and phenomena: ordinary differential equations (ODEs), compartmental models, partial differential equations (PDEs), population balance (PB) models, stochastic models, Bayesian graphical models, Boolean/logical models, and hybrid systems. It has been noted that there is no obviously appropriate modeling framework for representing all cellular processes [16-18]; it is intimately linked with the application and the nature of the available experimental data. Despite attempts to base the models on the underlying biochemical and biophysical mechanisms, most of the models generated are phenomenological partially due to experimental limitations to simultaneously measure concentrations, rates, diffusion, etc. within a living intact cell [18-20]. A formal algorithmic method or process for generating families of models, or evolving models, that are compatible with prior knowledge and experimental data would benefit the community [18]. The modeling community agrees that utilizing black box models to fit the data serves little utility for cellular systems, as they do not provide insight into the underlying mechanisms and dynamics.

For a life scientist, the cell context is defined by biochemical and genetic pathways that map interactions between participating elements (similar to that in Fig. 2). Regardless of the modeling framework, these signaling diagrams constrain the mathematical model structure. The underlying biochemical and genetic signaling maps are continually revised as more interactions are discovered; many of these can be found at http://www.kegg.org, http://stke.sciencemag.org/, and http://doqcs.ncbs.res.in. These pathway diagrams have been painstakingly assembled primarily through protein interaction and knock-out studies. Engineering methods have been applied to help construct maps for unknown signaling pathways by utilizing network inference techniques to generate Bayesian graphical models where the nodes represent the measured signaling elements or events and the connections between the notes indicate the relationship between the measured elements [20, 21]. The drawback associated with this method is the quantity of data necessary to extract significant relationships, the computational expense, and its inability to identify feedback loops which are numerous and play significant regulatory roles in the signaling pathways.

ODEs and PDEs are widely used in modeling because they describe system time course dynamics and are easy to numerically simulate using differential equation solvers, such as Matlab, Mathematica, and Sundials. Although ODEs are preferred by the engineering community for systems analysis, optimization, and control studies, PDEs may more accurately reflect the signaling dynamics as they simulate the system

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as a function of time and space. Regardless of the approach, differential equations can over-simplify reactions and only approximate kinetic reactions. Stochastic models more realistically portray cellular processes by simulating random, discrete events. However, they are more computationally expensive to simulate and difficult to systematically analyze. Discrete spatial models are also stochastic, but add in equations that control the position of every element. These models require detailed biochemical information to be accurate and are the most complicated and computationally expensive. Logic networks do not use kinetic information, but instead calculate probable steady-state results based upon a predetermined tendency towards predefined events. These logical models require little computation but require significant a priori biochemical data to accurately determine probabilities of transitions. This chapter describes a method to derive ODE based models that are limited in complexity, retain fidelity to the underlying biochemical processes, and are fully compatible with the available experimental data.

The major published models related to T cell signaling dynamics are presented in Table 1. This table identifies the simulated cellular process, indicates the genesis of original experimental data to either generate or validate the model, states the role of optimization used in the model genesis or analysis, and summarizes any engineering or model analysis performed. Optimization occurrences in the table were utilized for parameter identification during the model construction or model structure minimization or variation. The optimizations for parameter identification were both global and local searches over pre-specified ranges. Optimization of model structure was performed through either model reduction techniques, using algorithms such as principal component analysis, flux analysis, and minimum cut sets, or varying reaction structures for optimal data fitting. In the case of Bayesian networks, optimization of model structure is built into the algorithms. Most of the model analyses performed were stability analyses, sensitivity analyses, and steady state analyses. Sensitivity analyses ranged from manual parameter manipulation to applications of global algorithms such as multi-parametric sensitivity analysis (MPSA).

TABLE 1. Summary of Published Optimization and Systems Analysis of Mathematical Models of T cells and Relevant Signaling Pathways

Source	Model Type	Process	Experimental results	Optimization performed	Model analysis
[22]	ODE	Receptor dynamics	None	None	Stability analysis
[5]	ODE	Signal transduction	Ca^{2+} with film-thinning apparatus	Local parameter ID	Local parameter sensitivity
[23]	ODE	Signal transduction	None	None	Stability analysis
[24]	ODE	Signal transduction	Sensitivity to small ΔSHP-1	None	Local parameter sensitivity
[25]	ODE	Signal transduction	Zap70 phosphorylation	Local parameter ID	Stability analysis, nonlinear dynamics
[11, 26]	ODE	Signal transduction	Receptor expression	Local and global parameter ID	GSA, stability analysis
[27]	ODE and Logical	Signal transduction	None	None	Stability analysis

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[19]	ODE	Signal transduction	None	None	Stability analysis
[28]	ODE	Signal transduction	None	Hill coefficient estimation	Stability analysis
[29]	ODE	Signal transduction	None	None	Stability analysis
[30]	ODE	Cell differentiation	None	Parameter ID (method unpub)	Stability analysis
[31]	ODE	Signal transduction	Experimental validation of model	Parameter ID (method unpub)	Local parameter sensitivity analysis
[32]	ODE	Signal transduction	None	Model reduction and linearization	Steady state analysis
[33, 34]	ODE	Signal transduction	None	None	MPSA
[35]	ODE	Receptor kinetics	None	Local parameter ID, model structure variation	Local parameter sensitivity
[36]	ODE	Signal transduction	None	Model reduction	Global, time-varying parameter sensitivity analysis, principal component analysis, flux analysis
[37]	PDE	Receptor dynamics	None	None	Local parameter sensitivity
[38]	PDE	Receptor dynamics	Experimental validation of model	None	Local parameter sensitivity
[39]	PDE	Signal transduction	None	None	Control coefficients
[40]	Stochastic	Signal transduction	None	None	Local parameter sensitivity
[41]	Stochastic	Receptor dynamics	None	None	Local parameter sensitivity
[42]	Stochastic	Receptor dynamics	TCRp and pZap in knockout mice	None	Local parameter sensitivity
[43]	Logical	Signal transduction	None	None	Logical analysis
[44]	Logical	Signal transduction	None	Model structure variation	Logical analysis
[45]	Logical	Signal transduction	None	Minimal cut sets	Logical steady-state analysis, dependency matrix
[46]	Algebraic	Signal transduction	None	None	Local parameter sensitivity
[47]	Algebraic	Receptor dynamics	None	None	Local parameter sensitivity
[48]	Algebraic	Receptor dynamics	None	None	Local parameter sensitivity
[20]	Graphical	Signal transduction	Phosphorylation data	Bayesian network formation	None
[49]	Graphical	Signal transduction	None	None	Stability analysis
[50]	Graphical	Gene regulation	Their published cDNA data	Bayesian network formation	None
[21]	Graphical	Cell differen-tiation	Proliferation and phosphorylation data	Bayesian network formation	None

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4. OPTIMIZATION AND SENSITIVITY ANALYSIS FUNDAMENTALS

As demonstrated in the previous section, mathematical model development has relied upon optimization techniques primarily for parameter identification and systems analysis predominantly for sensitivity analyses or stability analyses. This section generally describes constrained optimization and sensitivity analyses in an effort to illustrate how they direct and support model development in a systematic manner.

The mathematical models employed in the study of cellular processes, including the T-cell signaling and activation process studied in this chapter, can be generally categorized as dynamical systems. In such a system, a finite dimensional variable $x \in \Re^n$, called the state variable, is identified as a minimal set of variables needed to represent the entire state of the system at any given time; its time-course trajectory will thus summarize the dynamics of the underlying biological process. In addition to the state variable, other types of variables can also be identified. For example, input variable $i \in R^l$ consists of those variables that one can control to influence the evolution of the state variable, such as those modeling external stimuli and, in the case of the example studied in this chapter, the exogenous input introduced to account for the incompleteness of the model structure. The output variable $y \in \Re^q$ is a set of variables whose values can be measured through experiments. The progress of the state x in time can be described by an ODE called the dynamics equation:

$$\frac{dx}{dt} = f(p, i, x, t), \tag{1}$$

and the output can be determined from the state through a transformation:

$$y = g(p, x, t). \tag{2}$$

Here, $p \in \Re^m$ is a set of parameters that can affect both the state dynamics and the way the output y is determined. In stochastic models, random perturbations may also be introduced to the above two equations to accommodate the various uncertainty inherent in the system, resulting in stochastic differential equations (SDEs).

In this framework, after the state, the input and the output variables are identified, the goal of developing dynamical system models for a cellular process can be typically decomposed into two stages. In the first stage, the model structure, namely, the specific forms of the functions f and g, is determined by taking into consideration the nature of the underlying biochemical reaction processes. Some of the commonly used forms of f for this purpose are linear, bilinear, and rational functions [51]; thus the model structure is specified by the way that these forms are combined in the dynamics equation. Once a model structure has been settled, in the second stage, the value of the parameter p is then adjusted so that the system output y produced under this particular model structure and parameter value agrees with the experimentally measured data $\hat{y}$. It is possible that these two stages may be repeated multiple times before a satisfactory model can be reached. Optimization and sensitivity analysis can play a large role in directing this model generation as described in this section and further illustrated in the following example.

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4.1. Sensitivity analysis

The mathematical models of cellular signaling processes typically have largely unknown parameter values as the models are not mechanistic and the parameters often account for the effects of multiple events. For example, many ODE model parameters account for diffusion without explicit representation of the process. Revisions, extensions, or adaptations of previously published models may require entirely new parameter values as the explicit addition or removal of a process or element may alter the interpretation of parameters. Hence, there is considerable uncertainty in all model parameter values. Given this uncertainty, it is important to understand how sensitive the model output is to variations in parameter values.

Conducting a sensitivity analysis not only quantifies the sensitivity of the output with regards to the model parameter values but also provides information for directing optimization (as described in the next section), model development and experiment design [26, 52]. The output of a sensitivity analysis helps to identify dominant processes or elements, recognizes events/elements that can be considered negligible, and suggests areas where future model modifications or additional experimental data would be valuable. Typically sensitivity analyses have been used for model reduction [34, 36]; we investigate their use in model genesis and parameter identification.

For large highly nonlinear models, the sensitivity of a model output to variations in a single parameter value is not apparent. This problem becomes more complex when considering that large models typically have numerous parameters, many of which may be uncertain. While a local sensitivity analysis determines the model sensitivity to parameter variations over a localized region around the nominal parameter values, a global sensitivity analysis (GSA) investigates the sensitivity over the entire parameter space. For a more complete review of sensitivity analysis of complex kinetic systems applicable towards chemical systems, see [53].

The effects from small changes in parameter values can be expressed by a Taylor series expansion about those changes with first order, second order, and higher order terms. For simplicity consider an algebraic expression $h(p)$ where $p \in \Re^m$, the expected value of the function for a small change in p is given by:

$$h(p+\Delta p) = h(p) + \sum_{j=1}^{m} \frac{\partial h}{\partial p_j}\Delta p_j + \frac{1}{2}\sum_{j=1}^{m}\sum_{i=1}^{m} \frac{\partial^2 h}{\partial p_j \partial p_i}\Delta p_i \Delta p_j + \cdots \qquad (3)$$

This expansion is only valid in a small region of the parameter space about the nominal values. The local sensitivity can be computed exactly using deterministic methods that differentiate the model (reviewed in [53, 54]). The decoupled direct method, often applied to chemical systems, differentiates the mathematical model of the system with respect to each parameter and then solves the fully differentiated system [55]. The first order local sensitivities, namely the slope of the model output for small variations in one parameter about the nominal parameter values, can be estimated using a finite difference approximation. This "brute force" method computes the model output at least twice: once at the nominal parameter values and again with a small perturbation at one parameter value. The sensitivity is estimated as the change in the model output divided by the change in the parameter value:

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$$\frac{\partial h}{\partial p_i} = \frac{h(p + \Delta p_i) - h(p)}{\Delta p_i} \tag{4}$$

This estimate can be inaccurate if the perturbation chosen is too large and so violates the local linearity assumption.

Statistical methods are generally used to estimate global sensitivities for models of cellular processes by varying numerous model parameters simultaneously to help understand how the model output varies over the entire parameter space and elucidate parametric interactions. These methods can be categorized as either regression or variance based [56]. Regression based methods are most applicable when each parameter is independent of the others; a rank-transformed regression method can accommodate nonlinear models but is only valid for monotonic models. Variance based methods do not make assumptions about the model type. However, these variance based models are computationally expensive. The variance methods can estimate the main-effect index, the contribution from a single parameter, and the total effects sensitivity index to account for interactions between parameters. A comparison of the relative parameter sensitivity rankings for the T-cell Erk MAPK pathway model [26] indicates that the regression based method, partial rank correlation coefficient (PRCC) [57-59], and variance based methods such as the Sobol's method [60] and extended Fourier amplitude sensitivity test (FAST) [61] methods give similar results. Not surprisingly, the extended FAST method was the most computationally efficient of the variance-based methods.

The extended FAST computes the main-effect and total-effect indices from numerous model outputs evaluated over the *m*-dimensional parameter space sampled along curves that vary in angular frequency for each parameter. This is defined by the following transformation function:

$$p_i = \frac{1}{2} + \frac{1}{\pi}\arcsin\left(\sin\left(\omega_i s + \varphi_i\right)\right) \tag{5}$$

where each parameter varies periodically at an angular frequency ω_i and random phase shift φ_i, with a scalar variable $s \in (-\pi, \pi)$. (The angular frequencies are chosen to be linearly independent from one another.) With this transformation, the model output function can be expressed as a Fourier series with respect to s. The FAST sensitivity indices are computed from the Fourier series coefficients [61]. The extended FAST sensitivity analysis is applied in the following example to quantify the parameter sensitivities and direct model development.

4.2. Optimization

For a given model structure, the problem of parameter identification can be solved by a method outlined in the following, which makes use of the sensitivity analysis results described in the previous section. The parameter identification problem is often formulated as an optimization problem, namely, finding the parameter values p for which the simulated model output y fits the experimental data $\hat{y}$ the "best". For example, in one possible model of the T cell signaling diagram, the system output consists of $q = 4$ components, corresponding to the four relevant signaling elements: percentages of TCR receptors expressed on the surface and internalized, and

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percentages of Zap70 and Erk phosphorylated. Let j be an index of these signals, and assume that measurements of the j-th output have been taken at n_j different times $t_{j,1}, t_{j,2}, ..., t_{j,n_j}$ to yield the data $\hat{y}_{j,1}, \hat{y}_{j,2}, ..., \hat{y}_{j,n_j}$. Then an objective function to measure how well the simulated outputs of the model fit the experimental data can be chosen as

$$J(p) = \sum_{j=1}^{q} \sum_{i=1}^{n_j} w_{j,i} [y(p, t_{j,i}) - \hat{y}_{j,i}]^2, \tag{6}$$

which is the weighted least squares error (WLSE) between the simulated outputs and the experimentally measured time course data with properly chosen weights $w_{j,i}$. The notation $y(p, t_{j,i})$ is adopted to highlight the dependency of the simulated output on the choice of the parameter value p: the output y depends on the state x, whose evolution is in turn affected by p. With the objective function defined above, the parameter identification problem can be formulated as

$$\begin{aligned} &\min && J(p) \\ &s.t. && \frac{dx}{dt} = f(p, i, x, t), y = g(p, x, t) \end{aligned} \tag{7}$$

which is a constrained optimization problem. The constraints are specified by state dynamics and the output function. Additional constraints may exist that bound the range of the parameter variations.

Many existing methods can be used to solve the above constrained optimization problem. However, most of them suffer from local minimum trap and poor convergence rate [62]. Since the generic algorithm (GA) excels at finding (rough) global minimum and gradient based methods can provide fast convergence rate, the combination of these two are adopted in this chapter: first a good initial guess is obtained using GA and then the solution is further improved using a gradient descent algorithm. To expedite the search in the second steps, the true gradient vector can be computed using the sensitivities of the states with respect to the parameters. Define $s_{jk}(p,t) = \partial x_j(p,t) / \partial p_k$ as the sensitivity of the state x_j with respect to parameter p_k at time t under parameter p. Taking the derivative of the equation $\dot{x}_j = f_j(p, x, t)$ with respect to p_k, leads to the following ODE:

$$\frac{ds_{jk}}{dt}(p,t) = \frac{\partial f_j}{\partial p_k}(p,x,t) + \sum_{i=1}^{n} \frac{\partial f_j}{\partial x_i}(p,x,t) \cdot s_{ik}(p,t). \tag{8}$$

Solutions to the above equations for all j and k yield the state sensitivity functions at the given parameter value p. The gradient of the objective function $J(p)$ with respective to the k-th parameter p_k can then be obtained as:

$$\frac{\partial J}{\partial p_k} = 2 \sum_{\alpha=1}^{q} \sum_{i=1}^{n_\alpha} w_{\alpha,i} [y(p, t_{\alpha,i}) - \hat{y}_{\alpha,i}] \cdot \left[\frac{\partial g(p, x, t_{\alpha,i})}{\partial p_k} + \sum_{j=1}^{n} \frac{\partial g(p, x, t_{\alpha,i})}{\partial x_j} \cdot s_{jk}(p,t) \right] \tag{9}$$

In the gradient descent algorithm, the sensitivity variables are first obtained from the initial guess p^{old}. The gradient of the objective function with respect to p is then computed using the above expression. The parameter is then updated as:

$$p^{new} = p^{old} + \delta \cdot \frac{\partial J}{\partial p}, \tag{10}$$

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where δ is the adjustable step size. This process is repeated until certain convergence criterion is met (e.g., no significant improvement in the objective function).

Note that this approach of sensitivity analysis is a special case of the much more powerful technique called the adjoint method. The adjoint method, introduced by Lions in [63] and further developed by Jameson [64] in the context of aerodynamic design, computes the gradient of an objective function whose variables are subject to PDE constraints. One of its advantages is the flexibility with which optimization problems can be formulated. In the context of parameter identification of the mathematical modeling of cellular processes, several generalized optimization problems can be formulated and solved. For example, the system dynamics equation can admit time-varying parameter $p(t)$; thus for any given model structure, a much better fitting of the experimental data may be achieved by the optimized time-varying parameter than that achievable by a constant one. Another generalization is that the system dynamics may allow mode switching, resulting in hybrid system models [65]. For instance, as the concentration of certain signaling element varies, some reaction rates may change abruptly. We are currently exploring these directions.

5. MODEL EVOLUTION FOR T CELL SIGNALING EVENTS

This section provides an example of the generation of experimental data and the application of constrained optimization and sensitivity analyses to direct the evolution of an ODE model of T cell activation.

5.1. Data generation

All mathematical models of cellular processes require experimental data, whether knock-out experiments or detailed proteomic data. When feasible, previously published data is utilized to help determine interactions, concentrations, and kinetic parameters. More often than not, the necessary data to support model development is not available. A benefit of generating new data is self-consistency: each data point can be produced under the same conditions and with similar degrees of reliability.

To generate a model of T cell activation, detailed time-course data was required. Time-course data determines the degree of activation of an element over time. Ideally, data would be generated for each element of the model. However, investigators are limited by instrumentation, reagents, and indicators. For instance, the phosphorylation state of a protein can be determined using either western blots (which average the results over thousands of cells) or flow cytometry (which produces single-cell data);but both methods require reliable, phospho-specific antibodies.

For this example, the phosphorylation of two kinases, Zap70 and Erk, and TCR location were measured. Western blots were used to determine the percent phosphorylation of Zap70 and Erk over time, while flow cytometry was used to monitor the surface expression of TCR and the accumulation of a fluorescent antibody controlled by TCR internalization. Time points were chosen so that the majority of data was of early times but still covered 90 minutes.

Specifically, the human leukemia Jurkat E6.1 T cell line was utilized. Prior to stimulation, cells were resuspended in serum free media at 1×10^8 cells/ml and

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incubated for 30 minutes at 37°C. Cells were then stimulated through the TCR by incubation with 10 μg/ml anti-CD3ε (UTCH1, BD Bioscience) on ice for 5 minutes. Cells were transferred to 37°C and incubated for different time durations up to 90 minutes. For quantitative SDS-PAGE and immunoblotting, cell lysates were analyzed on 12% SDS-PAGE gels followed by transfer to a nitrocellulose membrane. The membrane was stained with antibodies against human phospho-Zap70 Y319 (rabbit polyclonal, Cell Signaling Technology), phospho-Erk1/2 pT202/pY204 (rabbit polyclonal, Cell Signaling Technology) and α-tubulin (mouse monoclonal, Sigma), followed by incubation with Odessey® anti-mouse Alexa Fluor 680 and anti-rabbit IRDye 800 fluorescent secondary antibodies (LI-COR Biosciences). Treated membranes were scanned and fluorescent signals were quantified using an Odessey® infrared imager. For flow cytometry, following stimulation as above, cells were washed and incubated for 1hr at 4°C with FITC conjugated goat anti-mouse IgG (Santa Cruz Biotechnology) in staining buffer. Stained cells were washed once and resuspended in staining buffer at 2×10^6 cells/ml for flow cytometry analysis using a Beckman-Coulter Cytomics FC500 flow cytometer (Purdue Cancer Center Analytical Cytology facility). For intracellular TCR experiments, cells were stimulated as above with 10 ug/mL Alexa Fluor 700 conjugated anti-CD3 antibody (Biologic). At each time point, cells were then washed in stripping buffer, fixed, and analyzed on the FC500. Total surface expression levels were obtained by stimulating with conjugated antibody on ice and washing away excess label. The fluorescent antibody is assumed to neither leave the cell nor be degraded; therefore, the intracellular MFI represents the cumulative internalization of TCR.

5.2. ODE model development

A minimal signaling diagram was constructed to contain only the elements whose time course has been measured. This signaling diagram was a gross simplification that lumped many signaling processes (transport, multiple phosphorylation events, etc.) into individual events but still captured the information transfer between the signaling elements considered. The diagram was translated into a mathematical model with rates defined by the Law of Mass Action and simple chemical kinetics. This generated nonlinear ordinary differential equations with only a few rate parameters. The model could be represented as in Eqs. (1) and (2) without an exogenous input. The rate parameters associated with this minimal structure were largely unknown as they were not defined mechanistically. In the initial iteration, time course data existed to define the dynamics of each model variable so the dimension of the parameter space, m, was significantly less than the number of experimental data points, N. Thus, the parameter identification problem was well posed [66] and any inability to fit the data suggested the omission of important processes.

The discrepancies between the simulated and the experimental were addressed in the next model generation either by adding an omitted process or through the use of a piecewise linear exogenous input, $i \in R^l$. This exogenous input was temporarily utilized to mediate intermediate reactions and events to fit the available data. The variable time nodes of the exogenous input nodes were defined as (t_0, t_1,…$t_{l/2}$) with

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their corresponding magnitudes denoted by (i_0, i_1,…$i_{l/2}$) where $\frac{l}{2} \le \left\lfloor \frac{(N-1)/2 - m}{2} \right\rfloor$. Linear interpolation between time nodes computed the exogenous input value in the model. If the maximum allowable $l < 2$ and/or an acceptable fit was not obtainable with the model using the exogenous input, additional experimental data was obtained either from literature or the laboratory to further develop the model.

After fitting with an exogenous input, the function of the exogenous input was interpreted within the known signaling pathway and replaced by signaling element(s) or event(s) that serve a similar function. This process was iterated until the data was well fit and all possible exogenous inputs were interpreted as known signaling elements or processes. In some instances, the current state of knowledge was not capable of recreating the action of the exogenous input, thereby suggesting the presence of an unknown signaling event to investigate via future experiments. This approach encouraged the mathematical translation of the signaling pathways to be developed in a stepwise manner with well defined inputs and outputs, consistent with the approaches suggested in [7, 8]. The unknown model parameters and definition of the exogenous input in terms of node times and magnitudes were identified using local and global optimization techniques as described in the optimization section.

Model development evolved in two manners with the first guided by expert knowledge of the signaling pathway details and the second being directed through a sensitivity analysis. The sensitivity analysis identified the most sensitive portions of the model to address in order to remedy the inadequacies in the model fitness. The initial model was the same for both approaches.

5.3. Results

The process began with the creation of a basic diagram, Fig. 3(a), containing the measured elements: active Zap70 (Zap*), active Erk (Erk*), and three forms of TCR: internalized TCR, TCRi, bound TCR, TCRb, and phosphorylated TCR, TCRp. (TCRb and TCRp comprise the TCR surface expression data.) Translation of the signaling diagram created an initial model with unknown parameters. The fitted simulation results, shown in Fig. 4, matched the surface expression data well. However, after the initial time period, the simulation results deviated from the experimental data for the internalized receptors and the activated Zap70 and Erk dynamics. Receptor internalization, Zap70 and Erk activation were inappropriately sustained and required downregulation. Subsequently, two parallel approaches were taken and compared: evolution directed by expert knowledge and evolution directed by sensitivity analyses.

5.3.1. Evolution directed by expert knowledge

The evolution of the minimal models as directed by expert knowledge is illustrated in Fig. 3 (b) and (c). As receptor phosphorylation initiates and sustains receptor internalization and Zap70 activation, signaling mechanisms to mediate this process were reviewed. In the second iteration, an exogenous input, *i*, was incorporated to modify the TCRp dynamics and degradation of internalized receptors. (The degradation rate is affected by protein tyrosine kinase (PTK) activity during

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stimulation [67] while the recycling rate remains relatively constant.) A GA optimization problem was solved to define the exogenous input and determine the best fit model parameters. The resulting model dramatically improved the fitted dynamics (see Fig. 4). With the action of the exogenous input mediating the phosphorylation of the TCR, internalization ceased and Zap70 and Erk returned to basal activity levels following their initial activation.

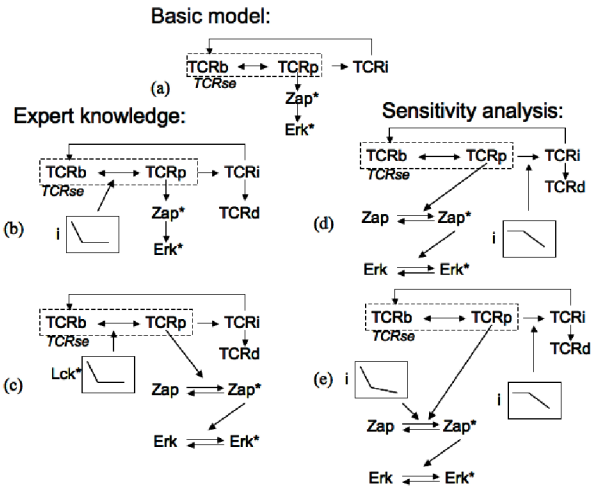

FIGURE 3. Evolution of system diagrams of minimal models. (a) Original, most basic model. (b),(c) Model evolution directed by expert knowledge only. (d), (e) Model evolution directed by sensitivity analysis only.

Not surprisingly, the fitted time course of the exogenous input qualitatively matched the time course of Lck, a known TCR kinase [68]. Therefore, in the next generation, Fig. 3 (c), the model was altered to more closely resemble the known signaling pathway elements and dynamics. Namely, the exogenous input was replaced by an Lck waveform with dynamics consistent with that reported in literature and the phosphorylation and dephosphorylation process of Zap70 and Erk activation and inactivation were incorporated. The resulting simulations after fitting by both the GA and the adjoint method are shown in Fig. 5. The parameters for this system were first obtained by GA, dashed lines, which have noticeable deviations from the experimentally measured data (points with error bars) (see Fig. 5). The bold lines are the simulated responses using the parameters obtained by the local gradient method. As can be seen, the latter represent a much better fit for the experimental data, demonstrating the utility of combining GA and gradient algorithms as described in the optimization section.

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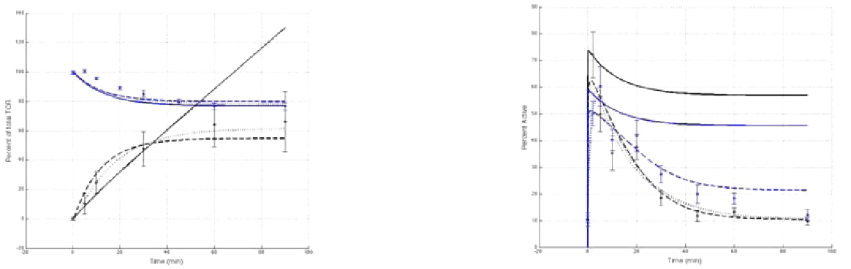
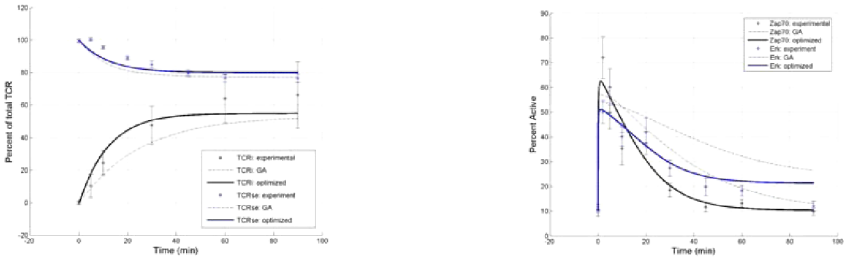


(a) (b)

FIGURE 4. Evolution directed by expert knowledge. Comparison of fitted minimal model A (continuous lines), B (dotted lines), and C (dashed lines) simulations with experimental data (points with error bars). (a) TCR dynamics. (b) Percent activation of Zap70 and Erk.



(a) (b)

FIGURE 5. Results from model C. Comparison of the simulated responses using the initial guess obtained by GA (dashed lines), using the parameters obtained by the local gradient descent algorithm (bold lines), and experimental data (points with error bars). (a) TCR kinetics, (b) Percent activation of Erk and Zap70.

5.3.2. Evolution directed by sensitivity analysis

A parallel approach utilized sensitivity analyses to direct model iterations. With this approach, illustrated in Fig. 3 (d) and (e), the next step was determined by the results of an extended FAST analysis as described in the sensitivity analysis section. Analysis of model A revealed it to be most sensitive to the receptor internalization rate parameter. The second generation model employed an exogenous input, *i*, to control internalization with physiological activation of Zap70 and Erk. GA optimization defined the exogenous input and found the model parameters. The exogenous input, i, achieved a closer fit to surface and cumulative internalized TCR without a significant improvement to the fitness of the Zap70 and Erk dynamics (Fig. 6, dotted lines). Ideally, the exogenous input would be replaced in the next generation model by a known signaling element or event, but this could not be achieved. An extended FAST sensitivity analysis of model D was performed to improve the Zap70 (and thereby Erk) fit. This analysis found Zap70 dynamics were slightly more sensitive to its phosphorylation parameter than all others. Therefore, a second exogenous input was

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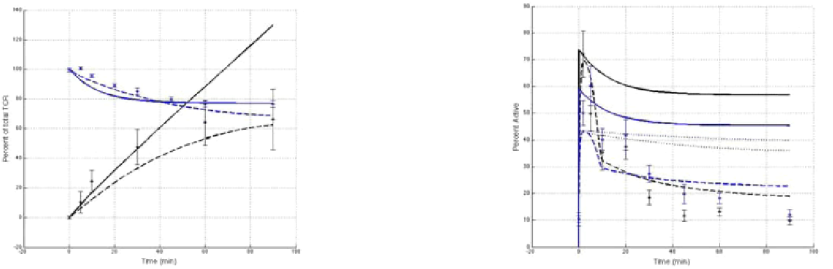
added to phosphorylate Zap70 in Fig. 3 (e) with results shown in Fig. 6, dashed lines. This iteration required a more complicated input than used previously, and also decreased the dependence of Zap70 on TCRp by an order of magnitude from model D.

5.3.3. Comparison of approaches

Although both approaches to guiding model modifications resulted in reasonable fits to experimental data, the model evolution pathway diverged immediately. Expert knowledge directed the placement of the first exogenous input to the TCR phosphorylation process, while a sensitivity analysis indicated that manipulating the internalization process would most significantly alter the output. The simulations from the two approaches exhibited similar surface and cumulative internalization kinetics; however, as shown in Fig. 7, the simulated TCR phosphorylation differed dramatically. The expert knowledge approach downregulated TCRp while the sensitivity analysis approach allowed TCRp to remain elevated. Additional experimental data for TCRp is necessary to definitively determine which simulation was correct for TCRp, if either.



(a) (b)

FIGURE 6. Evolution directed by sensitivity analysis. Comparison of fitted minimal model A (continuous lines), D (dotted lines), and E (dashed lines) simulations with experimental data (points with error bars). (a) TCR dynamics, which did not vary between D and E. (b) Percent activation of Zap70 and Erk.

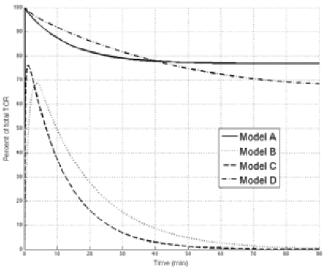


FIGURE 7. Comparison of evolution approaches. TCRp dynamics for each model.

The sensitivity analysis directed approach resulted in a more complicated model without better fits to experimental data. Furthermore, it was not possible to replace or interpret the identified exogenous inputs by known signaling elements or events as in

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the first approach. From this simple example, it is clear that sensitivity analyses should not be used in isolation to direct model genesis. Instead, expert knowledge supplemented by sensitivity analysis is more likely to succeed.

6. SUMMARY

The majority of published systems analysis and optimization methods conducted on mathematical models relating to T cell signaling dynamics were for parameter identification, model reduction, and stability, sensitivity and steady state analyses. Herein, a method to address model structural uncertainty through the extensive use of optimization techniques with exogenous inputs was proposed and evaluated. Under the guidance of expert knowledge, the resulting model captures the dominant biochemical events to explain the experimental observations. Although sensitivity analyses can identify dominant processes or elements, its direction of model modifications was not productive for these complex biological systems. Nevertheless, sensitivity analysis in support of expert knowledge-driven model evolution has significant potential. Thus, optimization and sensitivity analysis can help systematically evolve mathematical models that are appropriate for advancing biomedicine. The proposed process limits the model complexity, retains sufficient model accuracy, and utilizes expert knowledge to ensure the model structure is consistent with the known biochemical pathways. Although this methodology was developed for studying T cell signaling pathways, it is broadly applicable to other cell types and can accommodate a wide range of data types.

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