SENSITIVITY ANALYSIS FOR DRUG EFFECT STUDY: AN $NF − \kappa B$ PATHWAY EXAMPLE

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Abstract—The complexity of biological signaling networks, especially the uncertainties associated with the model parameters, present challenges for understanding the behavior of such networks and hence hamper the translation of the modeling study into drug development process. Sensitivity analysis can help to determine which parameters are the “key drivers” of the model’s output. How to tailor the sensitivity study under drug perturbation based on the knowledge of available existing or potential drugs is considered in this paper. The goal is to evaluate the drug effect on the signaling pathway modeled by kinetic rate changes. Through an example simulation study of the response of $NF−\kappa B$ pathway to two drugs, it is observed that new or modified sensitivity analysis methods may be necessary for the purpose of drug effect study. In addition, the new method may also help us determine whether combination therapy can yield significant synergism when compared to their individual drug effect.

Index Terms—sensitivity analysis, signaling pathway, drug effect

I. INTRODUCTION

Many systems biology strategies rely on mathematical modeling and computer simulation to reveal and understand complex problems associated with biological systems. However, the difficulty associated with parameter estimation is a major challenge for mathematical models. Since direct measurements of biological parameters are rare, many of the parameters are estimated to lie within a large range characterized by a high degree of uncertainty [1]. Hence, examining the sensitivity of the model behaviors to parameter variations is necessary. Furthermore, understanding how cellular signaling will change under external input is important for drug development. However, the analysis of cellular signaling interactions is expected to create an enormous informatics challenge, maybe even greater than that of analyzing the genome. A key step towards a more quantitative understanding of signaling is to specify explicitly the kinetics of all chemical reaction steps in a pathway. Sensitivity analysis (SA) can help us find the most significant kinetic reactions which control the dynamic patterns of the response to external inputs such as drugs. This allows us to identify the key variables in the system and design experiments that measure changes only for those variables.

Sensitivity analysis methods have gained wide application in the study of biological systems, including signaling pathways, metabolic networks and genetic circuits. They provide valuable insights about the robustness of biological responses with respect to changes in biological parameters and pinpoint the model inputs which mostly contribute to the variation in model outputs [2]. Subsequently, the most sensitive parameters and their corresponding biological processes become the potential targets for further experimental analysis. Generally, SA can be used in two key areas of systems biology: 1) quantifying the variation in model outputs to parameter uncertainty (uncertainty analysis); and 2) identifying the parameters that mostly contribute to variation in the model outputs which drive the system behavior [3].

Local SA and Global SA techniques are the main techniques used to investigate the effects of variations in parameters. The sensitivity of a model based on the partial derivatives of the outcome with respect to its parameters is called local SA as the derivative is taken at a fixed point in the state space of model parameters. These methods belong to the class of one-factor-at-a-time (OAT) methods, because the net effect of a single varied parameter on the property of the outcome is taken while keeping the rest of the parameters at their fixed nominal values. These techniques have been applied to a number of signal transduction and metabolic pathway models [4]. However, since most biochemical reactions networks yield models of a nonlinear nature, local SA method can be of limited use when the analysis aims to assess the relative importance of uncertain factors [5]. On the other hand, Global SA investigates the sensitivity over the entire parameter space by simultaneously examining a whole range of parameters values. This technique is more appropriate when models are nonlinear or parameter values have large uncertainties [3]. In recent years, Global SA has attracted a lot of interest. Examples are the application of Multi-parametric sensitivity analysis (MPSA) and Morris method to $NF−\kappa B$ signaling pathway [6], [7], and MPSA, partial rank correlation coefficients (PRCC), weighted average of local sensitivities (WALS), Fourier amplitude sensitivity test (FAST) and Sobol method to Erk $−$ MAPK signaling pathway [1].

While there are some works on sensitivity analysis on signaling transduction pathways [4], [7], such as using Local SA and Global SA techniques discussed above, there are few study on sensitivity analysis under drug perturbation contexts. In this paper, considerations for sensitivity analysis under drug perturbation will be discussed. For example, it is observed that a drug may affect several parameters in a pathway simultaneously. In many cases, the drug may drive the parameters change in the same direction. For instance, it is shown that Bortezomib [8] will decrease the value of $NF−\kappa B$ pathway.
several parameters in $NF-\kappa B$ pathway simultaneously. We present some preliminary results by using two important inhibitor drugs, namely 1) proteasome inhibitor: Bortezomib and 2) drug A that competitively inhibit IKK in $NF-\kappa B$ pathway [9] as an example study. Abundant experimental data of the $NF-\kappa B$ pathway has been accumulated due to comprehensive research [10] allowing the construction of the $NF-\kappa B$ computational pathway model. With $NF-\kappa B$ model in hand, we examine three methods for drug effect study: the local SA, a simple global SA using orthogonal random sampling, and sequential parameters change.

II. SENSITIVITY ANALYSIS FOR DRUG EFFECT STUDY

In this section, we highlight the importance and need to find new sensitivity analysis methods that can better evaluate drug effects on signaling pathways. The interaction of a drug with signaling pathway is important to model and analyze because often the anticipated effect of the drug may be altered by unforeseen influences.

Drugs can generally be classified as either stimulants or inhibitors, which respectively increase or decrease reaction rates. Drug efficacy is the therapeutic response that it could produce. A direct drug modeling approach is to add the drug’s chemical species and reactions to the original pathway model. While this may be straightforward, it adds significant complexity to the pathway model. A practical method is to model the behavior of a drug as an inhibitor or stimulant and avoid increasing the number of chemical reactions or chemical species considered. Because stimulants and inhibitors alter the reaction rates of certain reactions, modeling the effect of a drug on a given chemical reaction can be accomplished by altering the kinetic coefficients. The amount of change of the kinetic coefficients is determined by the efficacy (pharmacodynamic or PD model) and metabolism rate (pharmacokinetic or PK model) of the drug.

Local SA investigates effect of a single varied parameter on the pathway outcome while keeping the rest of the parameters at their fixed nominal values. Because in many cases a drug may affect several parameters simultaneously, local SA cannot directly be applied to study drug effects. While global SA methods may consider the entire space of parameter changes, they do not consider the trend of parameters change due to drug perturbation. As a result, they may have low efficiency due to evaluation of unnecessary sample space. More importantly, they may provide misleading results due to the random sampling rather than systematic sampling.

In order to better understand the above analysis, an illustrative example with 2 parameters $p_1$ and $p_2$ is shown in Fig. 1. Assuming that the nominal values of $p_1$ and $p_2$ are $p_1^{nom}$ and $p_2^{nom}$ respectively, the entire sample space due to parameter changes is the big rectangle $AGHJ$. While local SA only examine the two lines $BF$ and $DE$, the global SA will scan the entire parameter space $AGHJ$ by random sampling. However, assuming the drug functions as an inhibitor and will cause both parameters to decrease, the parameter space for drug effect study will be $ABCD$. In general, this drug effect SA parameter space is much smaller than the original parameter space. In fact, assuming symmetry of the parameter space centered at the nominal values, the size of the drug effect SA parameter space is only $\frac{1}{2^N}$ of the size of the original parameter space, where $N$ is the number of parameters of interest. Furthermore, the direction of parameter change due to drug effect should be also taken into account to avoid misleading results.

III. EXAMPLE STUDY

A. Pathway Model with Drug Input

Twenty years following the identification of $NF-\kappa B$, research in $NF-\kappa B$ signaling pathway is still intense, because numerous stimuli that activate $NF-\kappa B$ and the large number of genes that are related to cell survival, apoptosis, and cell mitigation are regulated by $NF-\kappa B$ [10]. $NF-\kappa B$ is typically expressed transiently to activate certain target genes. Under normal conditions, it is kept in the cytoplasm by the inhibitor proteins $I\kappa B$. When an upstream stimulus activates $I\kappa B$ kinases ($IKK$), it phosphorylates $I\kappa B$, which then be degraded in a ubiquitin-protesome pathway. The degradation of $I\kappa B$ results in the translocation of $NF-\kappa B$ from the cytoplasm to the nucleus where it activates the transcription of specific cellular genes as well as its own inhibitor, $I\kappa B\alpha$, giving rise to a negative feedback control [9]. Recently, $NF-\kappa B$ signalling pathway has become a focal point for intense drug discovery and development efforts [11]. In this paper, sensitivity analysis under drug perturbation on $NF-\kappa B$ pathway is studied.
**Fig. 3:** Dynamic features of nuclear \( NF - \kappa B \) using global sensitivity analysis with random orthogonal sampling.

**TABLE I: Parameter Values (Nominal Values)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reaction type</th>
<th>Biochemical reaction</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_4 )</td>
<td>complex formation</td>
<td>( NF - \kappa B + I\kappa Ba \rightarrow NF - \kappa B : I\kappa Ba )</td>
<td>30</td>
<td>( \mu M^{-1} min^{-1} )</td>
</tr>
<tr>
<td>( a_7 )</td>
<td>complex formation</td>
<td>( NF - \kappa B : I\kappa Ba + IKK \rightarrow NF - \kappa B : I\kappa Ba : IKK )</td>
<td>11.1</td>
<td>( \mu M^{-1} min^{-1} )</td>
</tr>
<tr>
<td>( d_1 )</td>
<td>dissociation</td>
<td>( NF - \kappa B : I\kappa Ba + IKK \rightarrow NF - \kappa B : I\kappa Ba )</td>
<td>0.03</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( d_1 )</td>
<td>dissociation</td>
<td>( NF - \kappa B : I\kappa Ba + IKK \rightarrow NF - \kappa B : I\kappa Ba : IKK )</td>
<td>0.075</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( t_{deg} )</td>
<td>degradation</td>
<td>( I\kappa Ba \rightarrow 0 )</td>
<td>0.006</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( t_{deg} )</td>
<td>degradation</td>
<td>( NF - \kappa B + IKK \rightarrow NF - \kappa B : I\kappa Ba : IKK )</td>
<td>0.0013</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( k_{t_1} )</td>
<td>transport</td>
<td>( NF - \kappa B \rightarrow NF - \kappa B )</td>
<td>0.0048</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( t_{p_2} )</td>
<td>transport</td>
<td>( I\kappa Ba \rightarrow I\kappa Ba )</td>
<td>0.025</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>transport</td>
<td>( NF - \kappa B : I\kappa Ba \rightarrow NF - \kappa B : I\kappa Ba )</td>
<td>0.84</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( t_{p_1} )</td>
<td>transport</td>
<td>( NF - \kappa B \rightarrow NF - \kappa B )</td>
<td>0.05</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( t )</td>
<td>synthesis (delay)</td>
<td>( NF - \kappa B \rightarrow NF - \kappa B + I\kappa Ba )</td>
<td>40</td>
<td>min</td>
</tr>
<tr>
<td>( k_{deg} )</td>
<td>inactivation</td>
<td>( IKK \rightarrow 0 )</td>
<td>0.002</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( r_4 + d_4 )</td>
<td>catalyzed degradation</td>
<td>( NF - \kappa B : I\kappa Ba + IKK \rightarrow NF - \kappa B + IKK )</td>
<td>11.1</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( r_1 )</td>
<td>catalyzed degradation</td>
<td>( I\kappa Ba + IKK \rightarrow IKK )</td>
<td>2.22</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>( \delta_{\text{synthesis}} )</td>
<td>synthesis</td>
<td>( NF - \kappa B \rightarrow NF - \kappa B + I\kappa Ba )</td>
<td>0.24</td>
<td>( min^{-1} )</td>
</tr>
</tbody>
</table>

An ODE model of the \( NF - \kappa B \) pathway developed by Hoffmann et al [12] is adopted in this study to model the simultaneous interactions of the multi-component \( NF - \kappa B \) signaling pathway. The FDA approved drug proteasome inhibitor bortezomib [8] and drug A [9] are the two drugs under consideration. The schematic of drug action could be found in Fig.2 of paper [9]. Drug A, a protein kinase inhibitor is chosen to achieve this because it competitively inhibit \( IKK \) with the same binding kinetics as that of the natural reaction involving \( NF - \kappa B : I\kappa Ba \) and \( IKK \). The effect of the proteasome inhibitor bortezomib which inhibit \( I\kappa Ba \) degradation is adjusted through the parameter setting related to individual terms for \( I\kappa Ba \) and \( NF - \kappa B : I\kappa Ba \) molecules rescued from inhibition of \( I\kappa Ba \) degradation [9]. Detailed parameter setting and ODE model could be found in appendix posted on webpage “http://nsf-rise.pvamu.edu/webpage/NSFRIA/1238918.htm”. The sensitivity of parameters that affected by each drug will be studied in next section. Furthermore, Bortezomib, in high dosage modulates a variety of cellular processes that may contribute to toxicity because the proteasome which is responsible for the \( I\kappa Ba \) degradation has additional protein degrading roles [8]. Sensitivity of the combination therapy with Bortezomib and drug A is studied to investigate the possibility of designing a therapy to induce a better effect and contain toxicity to a certain threshold.

**B. Simulation Results and Analysis**

Using the adopted ODE model of the \( NF - \kappa B \) pathway, we perform sensitivity analysis for different drug inputs, namely bortezomib, drug A and their combination therapy. The kinetic rate values are from [9] and listed in Table.I. Both drugs are inhibitors and they are designed to reduce the level of nuclear \( NF - \kappa B \). In terms of their effects on the pathway, it is shown in [9] that bortezomib will reduce the values of kinetic rate variables \( r_4, d_4, d_1 \) and \( r_1 \), while drug A will reduce the value of \( a_7 \).

The numerically simulated \( NF - \kappa Bn \) profiles are quantified with three dynamic feature score (Steady State, Peak and Slope) as shown in Fig.2. We tested two methods: 1) global SA with orthogonal sampling; 2) sequential variation of kinetic rate values. For sequential variation, all the drug-affected kinetic rate variables are assumed to reduce at the same rate with increase in dosage. Simulation is done as variables are equally reduced from the nominal values (100%) to 10% with a 10% step size. Whereas for global SA, each of the drug-affected kinetic rate variables are randomly sampled within a range of 5% to 100% since their value can only be suppressed due to drug input. 100 sets of the 5 drug-affected kinetic rate variables are sampled. The result of the three dynamic features scores observed for each sample point is then normalized with the nominal score. All simulations were run using MATLAB for t=100hrs to allow the system to reach...
Sensitivity of dosage on kinetic rate variables. Its sensitivity increases with the increase in dosage, and the effect of the combination therapy is larger compared to the individual effect of both drugs. In our example study, the sequential change of parameters indicates improved drug effect with increased dosage, while the random change results in complicated patterns.

3) Sensitivity analysis for combination of drugs could demonstrate whether such combination is effective: the sum effect of the combination is larger than the sum of the individual effect of both drugs.

IV. CONCLUSIONS

Sensitivity analysis (SA) can help us improve the predictive capacity of the signaling pathway model and refine parameter estimates by identifying the most influential parameters. However, many existing SA methods may not fit the needs for drug effect analysis. In this study, we have the following observations:

1) Usually a typical drug affect a set of parameters in the pathway models, as a result, we need to carry out sensitivity analysis for this set of parameters simultaneously rather than individually.

2) When we perform sensitivity analysis for this set of parameters simultaneously to study drug effect, the change in parameter values can be done sequentially (a drug may change different parameters in different scale, weighted quotients could be added for further study) or randomly; the trend will be different. In our example study, the sequential change of parameters indicate improved drug effect with increased dosage, while the random change results in complicated patterns.

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REFERENCES


