Sloppy/Stiff Parameters Rankings in Sensitivity Analysis of Signaling Pathways

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Abstract: Sensitivity analysis methods have been developed for over half a century. However, their application to systems biology is a relatively new concept and has not been fully investigated. In this paperwe focus on creating parameter rankings based on sloppy/stiff parameter sensitivity analysis, that can be used to find the most important parameters and processes (that have the greatest impact on the system output) and subsequently can be used to reduce the number of experiments needed to precisely estimate parameters values or to indicate molecular targets for new drugs. In order to test the proposed procedure we performed sensitivity analysis of the HSF/NF-κB pathway model - a model combining two signaling pathways essential for cell survival.

1 INTRODUCTION

A biomathematical model is a description of a biological system using mathematical language. Such models are created to describe processes taking place at different levels: from a single cell to the entire population. In addition to many tiers of biological system, there are also many methods that can be used to describe them with mathematical language. In this paper we focused on deterministic models of so called signaling pathways, described by ordinary differential equations. Such models are powerful tools that allow us to develop and test several hypotheses about complex biological systems (Locke *et al*., 2005, Voit *et al*., 2006). In the literature there is a growing number of high dimensional models with a large number of parameters. As an example, we used a model combining two signaling pathways: HSF and NF-κB. However, methods for measuring biochemical parameters are limited and may introduces substantial inaccuracies (Maerkl and Quake, 2007). Therefore, each model should be checked with respect to its sensitivity to parameter changes.

The sensitivity analysis is an important tool used to determine how the change of parameters influence the system behavior. It provides information about the most important parameters that have the greatest impact on the system output (and as a consequence should be determined with the highest accuracy). Moreover it gives us information about robustness of the systems (Rand, 2008), which helps us validate the model. Most of the pathways should be robust with respect to changes in parameters in a relatively wide range which may represent the differences between individual cells, e.g. in the rate of biochemical reactions (characterized by different parameter values). Sensitivity analysis provides also a valuable insight into the importance of particular processes.

Sensitivity analysis methods are used to test mathematical models for over half a century. However, the methods used e.g. in automatic control cannot always be directly used in systems biology, and may lead to false conclusions. For this reason it is necessary to develop methods which take into account the specificity of biological systems and experimental data. In this paper we propose a new measure of parameter sensitivity. We use one of the most common methods used currently in sensitivity analysis of signaling pathways, known as sloppy/stiff parameter sensitivity analysis (Gutenkunst *et al*., 2007), however our work is

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focused on creating parameter rankings, that can be subsequently used either to reduce the model complexity (Kim *et al*., 2011) or indicate prospective molecular targets for new drugs (Marin-Sanguino *et al*., 2011). We compared our method of creating parameter rankings with the ranking based on the areas under curve of sensitivity function.

2 SLOPPY/STIFF PARAMETER SENSITIVITY ANALYSIS

Let the model be described by the state equation:

$$\frac{dy_{s,c}}{dt} = f\left(y_{s,c}, u, \theta\right), \tag{1}$$

where $y_{s,c}$ denoting number or concentration of molecules of species s in condition c, u is an input variable and θ are model parameters.

The change in model behavior as parameters θ varied from their nominal values θ^*can be quantified by the average squared change in molecular species time course (Gutenkunst *et al*., 2007):

$$C(\theta) \equiv \frac{1}{2N_sN_c}\sum_{s,c}\frac{1}{T_c}\cdot \cdot \int_0^{T_c}\left[\frac{y_{s,c}(\theta,t)-y_{s,c}(\theta^*,t)}{\sigma_s}\right]^2 dt, \tag{2}$$

where N_s and N_c are the total number of species and conditions, respectively, T_c is the sampling time and σ_s is the maximum value of species s across the conditions considered.

To analyze model sensitivity to parameter variation we considered the Hessian matrix corresponding to cost function $C(\theta)$. Since biochemical parameters very often have different units and widely varying scale to eliminate the impact of relative changes in parameter values the derivatives with respect to $log\theta$ are taken:

$$H^C_{j,k} = \frac{d^2C}{dlog\theta_j dlog\theta_k}, \tag{3}$$

where j and k denotes j−th and k-th parameter, respectively. The Hessian describes the quadratic behavior of the cost function C near the point θ^*. H^C can be calculated as (Gutenkunst *et al*., 2007):

$$H^C_{j,k} = \frac{1}{N_sN_c}\sum_{s,c}\frac{1}{T_c\sigma_s^2}\cdot \cdot \int_0^{T_c}\frac{dy_{s,c}(\theta^*,t)}{dlog\theta_j}\frac{dy_{s,c}(\theta^*,t)}{dlog\theta_k}dt. \tag{4}$$

Based on Eq. (4) the sensitivity of the entire model (for all species s across all considered conditions c) to parameter variation can be calculated. However, the sensitivity of individual species or sensitivity of the model in specific condition could be also examined by taking into account only one species or condition.

The Hessian matrix is positive, definite and symmetric, so it has real eigenvalues λ and eigenvectors v. Analyzing H^C corresponds to approximating the surfaces illustrating deviations from nominal system response. The surface is N_p-dimensional ellipsoids, where N_p is the number of parameters in the model. The principal axes of the ellipsoids are the eigenvectors of H^C, and the width d_i of the ellipsoids along each principal axis is proportional to one over the square root of the corresponding eigenvalue λ_i (Gutenkunst *et al*., 2007):

$$d_i = \frac{1}{\sqrt{\lambda_i}}. \tag{5}$$

Figure 1: An example of ellipse illustrating deviations from nominal system response for a simple model with two parameters θ_1 and θ_2. d_1 and d_2 denotes the width of the ellipse along each principal axis, corresponding to eigenvalues λ_1 and λ_2 respectively, while v_1 and v_2 denotes the eigenvectors defining the position of the ellipse.

The narrowest axes are called “stiff”, and the broadest axes “sloppy”. The meaning of eigenvalues and eigenvectors of H^C is illustrated on a simple example, where Hessian describes an ellipse in the θ_1/θ_2 parameter space (Figure 1).

The relative widths d_1 and d_2, shown in the Figure 1, allow us to identify “sloppy” and “stiff” principal axis of the ellipse. However, the degree to which the principal axes of the ellipsoids are aligned to the bare parameter axes is also important. It can be estimated by comparing the ellipsoids

intersections I_i with each bare parameter axis i, calculated as:

$$I_i = \sqrt{\frac{1}{H^C_{i,i}}}, \quad (6)$$

and projections P_i onto each bare parameter axis i, calculated as:

$$P_i = \sqrt{\left(inv\, H^{x^2}\right)_{i,i}}. \quad (7)$$

If I_i / P_i = 1, then one of the principal axes of the ellipsoids lies along bare parameter direction i, however in biological systems this occurs very rarely. More often the ellipses are skewed from single parameter directions (Gutenkunst *et al*., 2007).

Although I_i / P_i ratio provides some useful information, it does not link the skewing rate with the width of particular principal axes of the ellipsoids, which is also very important because it would help us to identify the most significant parameters in the model. To relate these width (corresponding to "sloppy" and "stiff" principal axes) with specified parameter changes we propose another index, which is used to create the parameters ranking. The index is defined for the j-th parameter as:

$$r_j = \sum_i \left|\frac{v_{j,i}}{d_i}\right| \quad (8)$$

where the sum is calculated over all principal axes, d_i is the width of the ellipsoid along i-th principal axis, and $v_{j,i}$ is the element of the i-th eigenvector corresponding to the j-th parameter.

3 SENSITIVITY ANALYSIS OF THE HSF/NF-ΚB PATHWAYS MODEL

In order to test the applicability of the procedure described above we performed sensitivity analysis of the HSF/NF-κB pathway model, which described in (Smieja *et al*., 2015). The model combines two signaling pathways essential for cell survival.

NF-κB is a family of transcription factors that regulate the transcription of hundreds of genes, including genes that determine cell fate. It has been proved that NF-κB can play an antiapoptotic role in cancer cells, e.g. *via* activation of anti-apoptotic genes (Cataldi *et al*., 2003). Upregulation of the NF-κB pathway is frequently observed in cancer cells, which contributes to their resistance to the anticancer treatment (Hayden and Ghosh, 2012; Perkins, 2012). Therefore inhibition of NF-κB pathway may constitute one of the goals in anticancer therapies. Experimental results show that heat shock induces such inhibition in cancer cells (Janus *et al*., 2011). However, the precise mechanisms of interactions between HSF and NF-κB pathways are not fully understood yet. Development of a combined mathematical model of these pathways and its subsequent computational analysis should help to develop the most efficient anticancer therapy protocols.

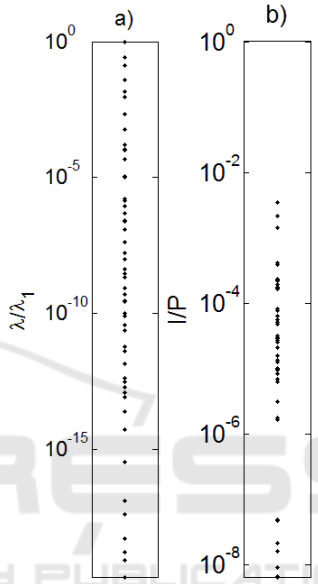

Figure 2: The eigenvalues (a) and *I/P* (b) spectrum of the HSF/NF-κB pathways model.

So far, numerous models of NF-κB pathway have been developed, whereas much fewer models of HSF pathway have been published. The model proposed in our work was based on the previously published ones, which described either NF-κB (Lipniacki *et al*., 2004) or HSF (Szymanska and Zylicz, 2009) pathways separately. In order to incorporate crosstalk between HSF and NF-κB pathways, they had to be modified: nuclear and cytoplasmic levels of proteins and complexes had to be separated and constitutive and inducible HSPs were described by separate variables. The interactions between the HSF and NF-κB pathways take into account creation HSP:IKK complexes, temperature-dependent inactivation of proteins located upstream of IKK activation and inhibition of NF-κB import to the nucleus under heat shock condition. The reactions taken into account are summarized in the Table 1.

We checked the sensitivity of the system

following the procedure described in the previous chapter. We have chosen nuclear NF-κB as one of the state variables to illustrate applicability of the method. The eigenvalues and *I/P* spectrum is plotted on Figure 2, while parameter ranking based on index r_j (Eq. (8)) is shown on Figure 3. To compare the results with other commonly used procedure, a parameter ranking based on area under curve of sensitivity functions is show on Figure 4. Due to high number of parameters the horizontal axes contains only numbers corresponding to the parameters listed in Table 2.

In both presented rankings the position of most parameters is comparable. However there are some significant differences, e.g. in parameters 37 and 45, corresponding to the ratio of cytoplasmic to nuclear volume (k_v) and IkBa mRNA degradation rate (c_{3a}), respectively. The parameter k_v is indicated as the most important by the sloppy/stiff method, while in the ranking based on sensitivity function parameter c_{3a} seems to be more important. To check which of these two parameters has greater influence on the system response, we performed three simulations: 1) for nominal parameter values, 2) for parameter k_v increased by 30% and 3) for parameter c_{3a} increased by 30%. The results of these three simulations are shown in Figure 5. By comparing these three time courses, we can see that parameter k_v significantly increases the maximum concentration of free nuclear NF-κB and in this term the ranking based on sloppy/stiff method seems to be more reliable. However, changing the parameter c_{3a} results in phase shift in system response, what in biological systems can also be very important.

Table 1: Reaction list for the HSF/NF-κB pathways model (Smieja *et al*., 2015).

NF-κB subsystem	HSF subsystem
$IKKn \xrightarrow{k_{deg}} Ø$	$Prot \xrightarrow{T,\, k_5} mfProt$
$IKKn \xrightarrow{TNF,\, TRAF2,\, n_1} IKKa$	$mfProt + HSP_{cons} \xrightarrow{k_1} HSP_{cons}{:}mfProt$
$IKKa \xrightarrow{A20,\, TNF,\, n_2} IKKi$	$mfProt + HSP_{ind} \xrightarrow{k_1} HSP_{ind}{:}mfProt$
$IKKa \xrightarrow{n_3} IKKi$	$HSP_{cons}{:}mfProt \xrightarrow{a,\, k_{-1}} HSP_{cons} + Prot$
$IKKa \xrightarrow{k_{deg}} Ø$	$HSP_{ind}{:}mfProt \xrightarrow{a,\, k_{-1}} HSP_{ind} + Prot$
$IKKi \xrightarrow{k_{deg}} Ø$	$HSP_{cons} + HSF \xrightarrow{k_3} HSP_{cons}{:}HSF$
$NF\text{-}κB_{nuc} \xrightarrow{c_1} NF\text{-}κB_{nuc} + A20_t$	$HSP_{ind} + HSF \xrightarrow{k_2} HSP_{ind}{:}HSF$
$A20_t \xrightarrow{c4} A20_t + A20$	$HSP_{ind}{:}HSF \xrightarrow{k_{-2}} HSP_{ind} + HSF$
$A20 \xrightarrow{c_5} Ø$	$HSP_{cons}{:}HSF + mfProt \xrightarrow{k_{-3}} HSP_{cons}{:}mfProt + HSF$
$A20_t \xrightarrow{c3} Ø$	$3HSF \xrightarrow{k_4} HSF_3$
$IKKa + IκBα \xrightarrow{a_2} IKKa{:}IκBα$	$HSF3 + HSP_{ind} \xrightarrow{k_{-4}} HSP_{ind}{:}HSF + 2\ HSF$
$IKKa{:}IκBα \xrightarrow{k_{d3}} IKKa$	$HSP_{ind} \xrightarrow{k_{d2}} Ø$
$IκBα \xrightarrow{c_{5a}} Ø$	$HSPmRNA \xrightarrow{k_{d1}} Ø$
$IκBα_t \xrightarrow{c_{3a}} Ø$	$HSF_3 \xrightarrow{k_{tr}} HSF_3 + mRNA$
$NF\text{-}κB + IκBα \xrightarrow{a_1} NF\text{-}κB{:}IκBα$	$HSPmRNA \xrightarrow{k_{tl}} HSPmRNA + HSP_{ind}$
$NF\text{-}κB{:}IκBα \xrightarrow{c_{6a}} Ø$	$HSF_{cons} + IKKa \xrightarrow{k_6} HSP_{cons}{:}IKK$
$IKKa + NF\text{-}κB{:}IκBα \xrightarrow{a3} IKKa{:}NF\text{-}κB{:}IκBα$	$HSF_{ind} + IKKa \xrightarrow{k_6} HSP_{ind}{:}IKK$
$IKKa{:}NF\text{-}κB{:}IκBα \xrightarrow{k_{d4}} IKKa + NF\text{-}κB$	$HSP_{cons}{:}IKK \xrightarrow{k_{-6}} HSP_{cons} + IKKn$
$NF\text{-}κB \xrightarrow{k_v,\, i_1} NF\text{-}κB_{nuc}$	$HSP_{ind}{:}IKK \xrightarrow{k_{-6}} HSP_{ind} + IKKn$
$NF\text{-}κB_{nuc} + IκBα_{nuc} \xrightarrow{a_1} NF\text{-}κB_{nuc}{:}IκBα_{nuc}$	$TRAF \xrightarrow{T,\, k_{5TRAF}} mf\ TRAF$
$NF\text{-}κB_{nuc}{:}IκBα_{nuc} \xrightarrow{e_{2a}} NF\text{-}κB{:}IκBα$	$mf\ TRAF2 + HSP_{cons} \xrightarrow{k_{1TRAF}} HSP_{cons}{:}mf\ TRAF2$
$NF\text{-}κB_{nuc} \xrightarrow{c_{1a}} NF\text{-}κB_{nuc} + IκBα_t$	$mf\ TRAF2 + HSP_{ind} \xrightarrow{k_{1TRAF}} HSP_{ind}{:}mf\ TRAF2$
$IκBα_t \xrightarrow{c_{4a}} IκBα_t + IκBα$	$HSP_{cons}{:}mf\ TRAF2 \xrightarrow{a,\, k_{-1TRAF}} HSP_{cons} + TRAF2$
$IκBα \xrightarrow{k_v,\, i_1} IκBα_{nuc}$	$HSP_{ind}{:}mf\ TRAF2 \xrightarrow{a,\, k_{-1TRAF}} HSP_{ind} + TRAF2$
$IκBα_{nuc} \xrightarrow{e_{1a}} IκBα$	$HSF_{3\ cyt} \xrightarrow{k_v,\, t_{1i}} HSF_{3\ nuc}$
	$HSF_{3\ nuc} \xrightarrow{t_{1e}} HSF_{3\ cyt}$
	$HSP_{cons,cyt} \xrightarrow{k_v,\, t_{2i}} HSP_{cons,nuc}$
	$HSP_{cons,nuc} \xrightarrow{t_{2e}} HSP_{cons,cyt}$
	$HSP_{ind,cyt} \xrightarrow{k_v,\, t_{3i}} HSP_{ind,nuc}$
	$HSP_{ind,nuc} \xrightarrow{t_{3e}} HSP_{ind,cyt}$

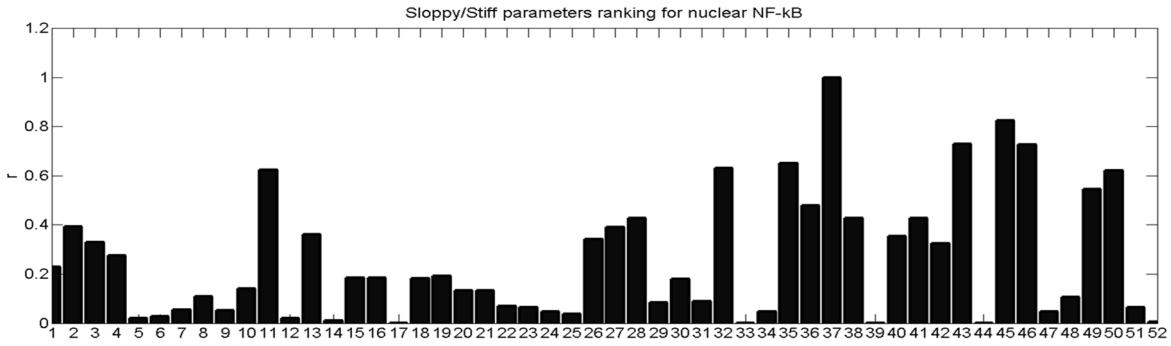

Figure 3: Parameter ranking based on sloppy/stiff method.

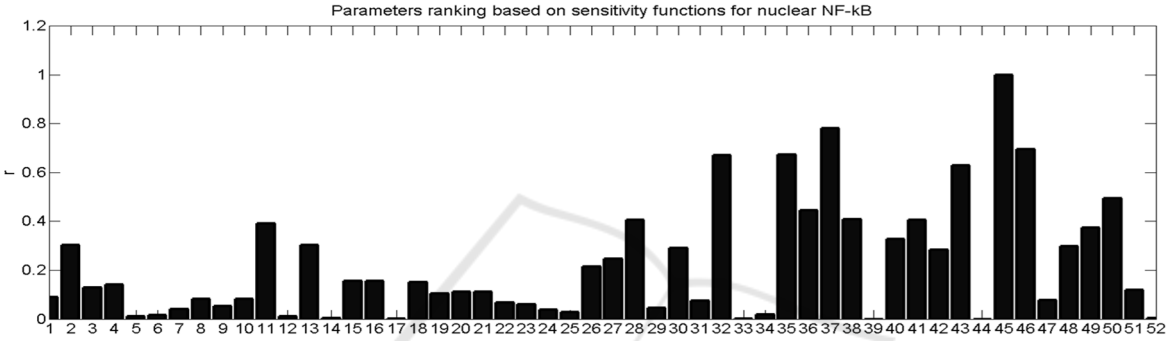

Figure 4: Parameter ranking based on sensitivity functions.

Table 2: List of parameters in the HSF/NF-κB pathways model (Smieja *et al*., 2015).

No.	Name	No.	Name	No.	Name
1	k_1	19	k_{d2}	36	k_{deg}
2	k_{-1}	20	t_{1i}	37	k_v
3	k_{1TRAF}	21	t_{1e}	38	c_1
4	k_{-1TRAF}	22	t_{2i}	39	c_2
5	k_2	23	t_{2e}	40	c_3
6	k_{-2}	24	t_{3i}	41	c_4
7	k_3	25	t_{3e}	42	c_5
8	k_{-3}	26	t_4	43	c_{1a}
9	k_4	27	n_1	44	c_{2a}
10	k_{-4}	28	n_2	45	c_{3a}
11	k_5	29	n_3	46	c_{4a}
12	k_{5TRAF}	30	a_1	47	c_{5a}
13	k_6	31	a_2	48	c_{6a}
14	k_{-6}	32	a_3	49	i_1
15	k_{tr}	33	k_{d3}	50	i_{1a}
16	k_{tl}	34	k_{d4}	51	e_{1a}
17	a	35	k_{prod}	52	e_{2a}
18	k_{d1}				

It should be noted that contrary to standard rankings based on sensitivity function, the proposed ranking reflects the influence of parameter changes on the system output, not only in the case when a single parameter is varied but also when it changes together with other ones. However, computational complexity is the same as for calculating sensitivity functions. The variance-based approaches (e.g. Sobol, 2001), would require much more computational power.

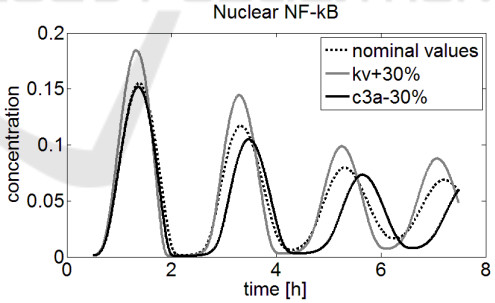

Figure 5: The comparison of three simulation runs:1) for nominal parameter values (dotted line), 2) for parameter k_v increased by 30% (gray line) and 3) for parameter c_{3a} increased by 30% (black line).

4 CONCLUSIONS

Parameters rankings are a useful tool that allows us to indicate parameters that are most important for the dynamics of a given pathway. In this paper we presented a new method for creating the parameters ranking based on the popular sloppy/stiff parameter

sensitivity analysis. Taking into account the example presented in this work we showed that the method can provide valuable information about the most important parameters that have the greatest impact on the system output.

Moreover, the work shows that the parameters rankings for the same model may vary depending on the applied methodologies. Various parameters rankings may be sensitive to various changes in response (e.g. quantitative or qualitative changes). For this reason the choice of sensitivity analysis method must be adapted to the purpose of research and the type of model we investigate. Furthermore it is a good practice to examine the sensitivity of the system using various methods and compare the results.

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