Sensitivity Analysis-Based Approach for Identifying Key Steps in Cell Signaling for Hepatocytes Stimulated by Il-6

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Abstract

Signal transduction pathways generally consist of dozens of individual components and have an even greater number of parameters describing their reaction kinetics. While the general structure of some signaling pathways can be found in the literature, it is usually required to adapt the model and re-estimate some of the parameters from experimental data. However, it is not feasible to re-estimate hundreds of parameters due to the complexity and cost of the experiments associated with the generation of the data. Parameter sensitivity analysis is a useful tool for addressing this situation as it identifies the most important parameters, which also are the parameters which are easiest to estimate, for a signal transduction pathway. This paper presents a detailed parameter sensitivity analysis of the JAK/STAT and MAPK signal transduction pathway for stimulation with IL-6. Based upon the sensitivity analysis of the parameters, the most important step is the recruitment of the transcription factor to the dimer of the phosphorylated receptor complex. Sensitivity analysis also reveals that several other mechanism play an important role: desphosphorylation of the nuclear STAT3 dimer by PP2 as well as feedback inhibition by SOCS3.

Introduction

Understanding the regulatory mechanism of cell signaling can help in designing therapies for many diseases and injuries. However, the large number of components involved in the cell signaling pathways and the interaction between different signaling pathways (cross-talk) return results that are difficult to interpret. To address this issue a number of mathematical models (Asthagiri *et al*., 2001; Aksan *et al*., 2003; Huang *et al*., 1996; Schoeberl *et al*., 2002; Singh *et al*., 2006; Yamada *et al*., 2003) have been developed to improve the understanding of regulatory mechanism in the signaling pathways. Sensitivity analysis provides a powerful tool to analyze these mathematical models. The analysis can help improve the understanding of the signaling networks as it can be used to identify the contribution of individual parts of the model to the signaling pathway.

A variety of approaches to sensitivity analysis have been developed (Frey *et al*., 2002; Saltelli *et al*., 2005) and two methods, which are highly suitable for analyzing signaling

pathways, are investigated in this paper: (i) differential analysis (Frank, 1978; Hwang *et al*., 1978; Tomovic *et al*., 1972), which approximates the model by a first-order Taylor series; (ii) Fourier amplitude sensitivity test (FAST) (Cukier *et al*., 1973; McRae *et al*., 1982), which are based on the contributions of individual variables to the variance of the model output. The two techniques have been applied to identify the key steps in a mathematical model of an IL-6 signaling pathway (Singh *et al*., 2006) which contains the JAK (Janus-associated kinases)/STAT (signal transducers and transcription factors) pathway and the Ras/MAPK (mitogen-activated protein kinases) pathway. Based upon application to the IL-6 signaling pathway the advantages and disadvantages of the two sensitivity analysis techniques are investigated.

Differential analysis is widely used in the analysis of chemical reactions and also for analyzing signaling pathways (Gadkar *et al*., 2005; Hu *et al*., 2006; Liu *et al*., 2005). This technique entails the least computational burden but it returns only local information about a system. Additionally, it is a single parameter analysis technique which studies the effect of each parameter individually while fixing the remaining parameters at their nominal values. The Fourier amplitude sensitivity test (Cukier *et al*., 1973; McRae *et al*., 1982) is a global technique which is able to assess the behavior of the outputs over the entire domain of uncertainty of the parameters and to take parameter interactions into account.

Materials and Methods

Model descriptions

The IL-6 signaling pathway model analyzed in this work was developed in a recent paper by Singh *et al*. (2006), which describes signal transduction in hepatocytes induced by IL-6. This model contains two pathways: Janus-associated kinases (JAK) & signal transducers and transcription factors 3 (STAT3) are activated in one pathway while the other pathway involves the activation of mitogen-activated protein kinases (MAPK). This model is made up of 68 nonlinear ordinary differential equations which include 118 parameters and can be represented by:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}, \mathbf{p}, u) \,. \tag{1}$$

The equations are derived according to the law of mass reaction or Michaelis-Menten kinetics and the parameters, **p**, are the kinetic rate constants. The states, **x**, are the concentrations of the molecules in the pathway and the input, *u*, is the concentration of IL-6 that stimulates the pathway. The output variables are the concentration of $(STAT3N^*)_2$ (dimer of activated STAT3 in the nucleus). Due to the complexity of the system it is not possible to predict *a priori* which parts of the model are the main contributors to the dynamic behavior of the signaling pathway. Sensitivity analysis is used in this work to determine which parameters have the largest contribution to the signaling pathway for the chosen output variables.

Differential sensitivity analysis

Differential analysis is based on a Taylor series approximation. The output *y* can be expressed by

$$y(t,\mathbf{p}+\Delta\mathbf{p}) = y(t,\mathbf{p}) + \sum_{i=1}^{m} \frac{\partial y(t,\mathbf{p})}{\partial p_i}\Delta p_i + \frac{1}{2}\sum_{i=1}^{m}\sum_{j=1}^{m} \frac{\partial^2 y(t,\mathbf{p})}{\partial p_i \partial p_j}\Delta p_i \Delta p_j + \cdots . \tag{2}$$

for a perturbation $\Delta\mathbf{p}$ of the parameter vector $\mathbf{p} = [p_1, p_2, \cdots, p_m]^{\mathrm{T}}$. The first-order partial derivatives in the Taylor series are regarded as the sensitivity measures, $s_i(t,y)$, describing how the variations of the parameters affect the output variable:

$$s_i(t,y) = \frac{\partial y(t,p_i)}{\partial p_i}, \qquad i = 1, \cdots, m . \tag{3}$$

The dependence of the output on parameter variations is expressed by the linear approximation resulting in differential analysis being a linear method.

The system functions (Eq. 1) of the IL-6 signaling pathway consist of polynomial functions (mass reaction) and rational fraction functions (Michaelis-Menten kinetics) whose partial derivatives with respect to parameters can be calculated analytically. Differentiation of Eq. 1 with respect to p_i gives the following system of sensitivity differential equations:

$$\frac{d\mathbf{s}_i(t,\mathbf{x})}{dt} = \frac{d(\partial\mathbf{x}/\partial\, p_i)}{dt} = \frac{\partial(d\,\mathbf{x}/dt)}{\partial p_i} = \frac{\partial\mathbf{f}}{\partial\mathbf{x}}\frac{\partial\mathbf{x}}{\partial p_i} + \frac{\partial\mathbf{f}}{\partial p_i}, \tag{4}$$

or in the matrix form

$$\dot{\mathbf{s}}_i = \mathbf{J}\mathbf{s}_i + \mathbf{q}_i . \tag{5}$$

where $\mathbf{J} = \{\partial f_i/\partial x_j\}$ is the state Jacobian matrix and $\mathbf{q}_i = \{\partial f_i/\partial p_j\}$ is the derivative vector of the system functions with respect to the parameter. The sensitivity measures are calculated by solving the system equations (Eq. 1) and sensitivity equations (Eq. 5) simultaneously.

To eliminate the effects by different units of the parameters the sensitivity measures are often normalized by their nominal values

$$S_i(t) = \frac{\overline{p}_i}{\overline{y}} s_i(t) . \tag{6}$$

To simplify the interpretation of the results, the cumulative effect of the sensitivity over a time interval is usually considered. This is done by taking the root of the sum of the squares of the elements at different time points resulting in a sensitivity measure of a parameter given by

$$S_i = \sqrt{\sum_{t=0}^{N_T} S_i(t)^2} , \tag{7}$$

where N_T is the number of the time points.

Fourier amplitude sensitivity test (FAST)

As the output of the systems is affected by changes in the parameters, it is possible to use the variance of the changes in the output as an indicator of importance of the parameters. Fourier amplitude sensitivity test (FAST) uses the square root of the partial variance

$$S_i(t)=\sqrt{\mathrm{Var}_{p_j}(\mathrm{E}(y(t)\mid p_j))} \tag{8}$$

as a sensitivity measure for the parameter p_i. $\mathrm{Var}_{p_i}(\mathrm{E}(y\mid p_i))$, the variance of the conditional expectation $\mathrm{E}(y\mid p_i)$, denotes the contribution of a variation of the parameter p_i to the total variance Var(y). Efficiently estimating the variance is the key procedure of FAST as it is rarely the case in practice that the variance can be calculated analytically.

FAST uses the distribution functions to express the uncertainties of the parameters, assuming their joint probability density function is $f(\mathbf{p})$. From the joint probability density function, the variance of the output variable can be calculated to be

$$\mathrm{Var}\left(y\right)=\mathrm{E}\left(y^2\right)-\mathrm{E}\left(y\right)^2, \tag{9}$$

where the expectation is express by

$$\mathrm{E}\left(y\right)=\int\cdots\int y(\mathbf{p})f(\mathbf{p})dp_1dp_2\cdots dp_m. \tag{10}$$

The core feature of FAST is to attribute each parameter p_i to a transform function of a scalar s. When s varies, all the parameters are perturbed simultaneously. The series of transformations define a search curve in the parameter space. If the transformations are selected properly (Cukier *et al*., 1973; McRae *et al*., 1982; Saltelli *et al*., 1999), the search curve can scan every point in the parameter space. Then the multi integration over the parameter domain (Eq. 10) can be converted to a single integration over the s domain according to the ergodic theorem.

In a next step, FAST uses a Fourier transformation to calculate the single integration over the s domain. According to Parseval's theorem the integration along the real axis is equal to the sum of the Fourier coefficients in the frequency domain. The contribution of perturbations of a parameter to the total output variance is also determined by the Fourier coefficients.

For this work the squares of the sensitivity values at different time points are added to evaluate the cumulative effect over the time interval and the sensitivity measure of a parameter is given by

$$S_i=\sqrt{\sum_{t=0}^{N_T}S_i(t)^2}, \tag{11}$$

where N_T is the number of the time points.

Analysis of the Signaling Pathways and Comparison of Results

Results by the local sensitivity analysis and the global sensitivity analysis

The two sensitivity analysis methods are applied to the described IL-6 signaling pathway model (Singh *et al*., 2006). The number of required simulations for evaluation by FAST is approximately (Cukier *et al*., 1975)

$$N_s\approx 2.6N_p^{2.5} \tag{12}$$

where N_s is the number of simulations and N_p is the number of parameters. Since the model

contains 118 parameters, it would require nearly 400,000 simulations to determine the parameter sensitivities. However, to perform such a large number of simulations is not possible with the current computational resources. Instead, local parameter sensitivity analysis is performed on the model with 118 parameters and the 50 most important parameters from this local analysis are chosen as the candidates for global sensitivity analysis where other parameters are fixed on their nominal values.

In the analysis the concentration of IL-6 serves as the input to the model and is changed from 0 to 0.5 nM at time 0. The simulations are carried out for a 24 hr time period as the dynamic response of the system is captured within this time interval. The number of simulations for global analysis is chosen to be 25001 as this number satisfies the Nyquist theorem (Cukier *et al*., 1975).

The sensitivity profile of the activated transcription factor $(STAT3N^*)_2$ in the nucleus with respect to the parameter *kf7* by the differential analysis is shown in Fig. 1. The sensitivity values are all positive, i.e. an increase in the value of *kf7* will increase the concentration of $(STAT3N^*)_2$. The time-dependent sensitivity calculated by FAST is also shown in Fig. 2 for two different ranges of perturbations. One observation is that the sensitivity profile of FAST with small perturbations (Fig. 2a) is only different by a factor of a constant to the profile by the differential analysis (Fig. 1). This property will be explained later in the paper.

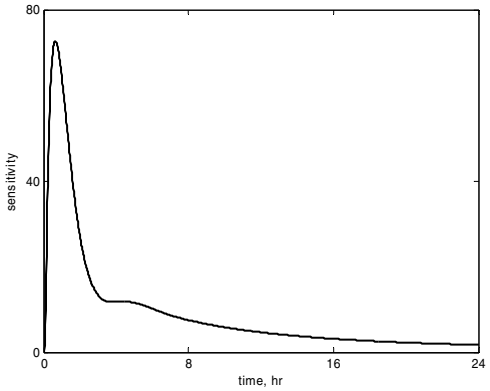
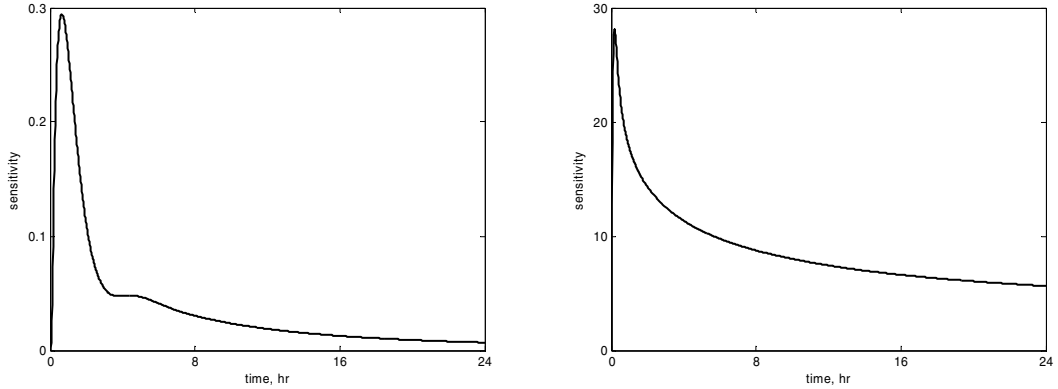


Fig 1. The local sensitivity profile of $(STAT3N^*)_2$ with respect to the parameter *kf7*.



a b

Fig 2. The sensitivity profiles of $(STAT3N^*)_2$ with respect to the parameter *kf7* by FAST with perturbations (a) from 99% to 101% nominal value and (b) from 10% to 1000% nominal value.

The results obtained by the two analysis techniques for small perturbations (99%-101% nominal values) and large perturbations (10%-1000% nominal values) are summarized in Table 1. The parameters are ranked by their cumulative effect over the time interval (Eq. 7 and Eq. 11 respectively). To compare the two procedures the rank values are normalized by the largest rank value.

Table 1. Summary of the results by the two methods

No	Differential Analysis		FAST			
			ΔP: 99-101%		ΔP: 10-1000%	
	Symbol	Value	Symbol	Value	Symbol	Value
1	kf7	1	kf7	1	kf7	1
2	kf32	0.748	kf32	0.748	kf21	0.9782
3	kf21	0.7129	kf21	0.7128	Vm24	0.7639
4	kf8	0.7061	kf8	0.706	kf8	0.7593
5	kb7	0.6667	kb7	0.6667	kf26	0.7532
6	kf20	0.5628	kf20	0.5627	kf27	0.7389
7	kb20	0.5492	kb20	0.5491	kf28	0.6619
8	kf42	0.4772	kf42	0.4773	kb7	0.6252
9	Vm24	0.4503	Vm24	0.4503	kf31	0.6019
10	kf26	0.4503	kf26	0.4503	ka26	0.5989
11	kf27	0.4472	kf27	0.4472	kf29	0.5936
12	kf45	0.4191	kf45	0.419	kf70	0.592
13	Km24	0.4131	Km24	0.4132	kf48	0.5865
14	ka26	0.4077	ka26	0.4077	Km24	0.569
15	kf70	0.4071	kf70	0.4071	kf20	0.5657
16	kf31	0.4056	kf31	0.4055	kb28	0.563
17	kb27	0.3922	kb27	0.3921	kb27	0.554
18	kf28	0.388	kf28	0.388	kb48	0.5274
19	kb28	0.3872	kb28	0.3871	kf71	0.4793
20	kf29	0.3654	kf29	0.3654	kb29	0.4765
21	kb29	0.3591	kb29	0.3591	kf42	0.4625
22	kf71	0.3301	kf71	0.3301	kf32	0.4577
23	kb45	0.3027	kb45	0.3028	kb20	0.4196
24	kf19	0.2603	kf19	0.2603	kf19	0.4077
25	kf18	0.2195	kf18	0.2196	kb18	0.3138

Comparison of results from local analysis and global analysis techniques

It can be concluded from Table 1 that the results of the global analysis with small perturbations are very similar to those of the local analysis. The results by FAST when the parameters are perturbed from 99 percent to 101 percent of their nominal values are identical to the ones computed for local analysis. In fact the two analysis techniques are equivalent when the system is linear in the parameters and the parameters are independently uniform distributed. Suppose the output is

$$y(t) = a_1(t)p_1 + a_2(t)p_2 + \cdots + a_m(t)p_m, \tag{13}$$

then the partial variance of *y* with respect to p_i in the FAST method is

$$\mathrm{Var}_{p_i}(\mathrm{E}(y(t) \mid p_i)) = a_i^{\,2}(t) \cdot \mathrm{Var}(p_i) = a_i^{\,2}(t) \cdot \frac{(p_{ui} - p_{li})^2}{12} \overline{p}_i^{\,2}, \tag{14}$$

where $\overline{p}_i$ is the nominal value of parameter p_i, $p_{ui} \cdot \overline{p}_i$ and $p_{li} \cdot \overline{p}_i$ are the upper bound and the lower bound, respectively. The normalized partial differential is given by

$$\frac{\overline{p}_i}{\overline{y}} \frac{\partial y(t)}{\partial p_i} = a_i(t) \frac{\overline{p}_i}{\overline{y}}. \tag{15}$$

It can be concluded that the square root of the partial variance is proportional to the normalized partial differential. This also illustrates that linearization of the model is a good approximation in a small range around the nominal point.

If global analysis techniques use small perturbations, then the parameter interactions become negligible since the system under study is approximately linear in the parameters. However, if large perturbations are applied then the nonlinear properties of the system become dominant and the parameter interactions will have a significant effect on the results. For example, as the perturbations increase the importance of the parameter *kf*32, as noted by its position on the list, decreases from 2 to 22 as computed by FAST. This change can serve as an indicator that local analysis may not always be appropriate when dealing with systems where parameter values are within a large uncertainty interval.

Local analysis techniques have been extensively explored in the past while global methods have become more popular over the last 20-30 years. The reason for this is that, while global analysis techniques provide more information and can even reduce to the results derived from local analysis, they are computational significantly more expensive than local techniques. For example, for a computer with a P4 3.4G CPU, 2G Memory, and a Windows operating system, the time for the differential method to obtain the sensitivity measures of all 118 parameters is roughly 25 minutes while the time for the FAST method is more than 12 hours even though only 50 parameters are used in this latter case. Due to the computational effort, local analysis methodologies cannot be completely replaced by global analysis techniques. Local analysis can act as a valuable screening method to identify the important parameters for further investigation by global techniques.

Discussions and Conclusions

Significance of recruitment of the transcription factor STAT3 to the dimer of the phosphorylated receptor complex (IL6-gp80-gp130-JAK)$_2$.*

It is seen from Table 1 that the parameter *kf*7 is computed to be the most important parameter by all two sensitivity analysis techniques. *kf*7 is the forward rate constant of the reaction where the transcription factor STAT3 in the cytoplasm is recruited to the dimer of the

phosphorylated receptor complex (IL-6-gp80-gp130-JAK*)$_2$. Subsequently, the activated STAT3 dimers translocate to the nucleus. This reaction is the initial step for signal transduction through the JAK/STAT pathway.

The dimer of the phosphorylated receptor complex (IL-6-gp80-gp130-JAK*)$_2$ also activates the Ras/MAPK pathway by recruiting the Src homology domain 2 (SH2)-containing protein tyrosine phosphatase SHP2. As a result, the recruitment of STAT3 competes with the recruitment of SHP2. When *kf7* is large then the receptor complex (IL-6-gp80-gp130-JAK*)$_2$ is more likely to participate in signaling through the JAK/STAT pathway instead of the MAPK pathway. Thus *kf7* has a significant effect on the activation of STAT3.

Varying *kf7* also drastically changes the importance of other parameters. The results calculated by the FAST method when *kf7* is fixed at different values are shown in Table 2. In the case of large *kf7* the affinity of STAT3 binding to (IL-6-gp80-gp130-JAK*)$_2$ is high and the JAK/STAT pathway is highly activated. Inhibitors in the JAK/STAT pathway will have a significant effect on inactivating the transcription factor. The parameters *kf*20, *kf*21 (the nuclear phosphatase PP2 deactivates the phosphorylated STAT3 dimer in the nuclear), *Vm*24, *kf*26, *kf*27 (the feedback-inhibition by the suppressor of cytokine signaling 3, SOCS3) are ranked at the top positions. On the other hand, in the case of small *kf7* the JAK/STAT pathway is partially activated. The effects of the inhibitors in the pathway decline and the positions of their parameters decrease. However, the cross-talk with the Ras/MAPK pathway becomes more important. The parameters *kf*48 (recruitment of Ras-GTP* to (IL6-gp80-gp130-JAK*)$_2$-SHP2*-Grb$_2$-Sos) and *kf*32 (inhibition by recruitment of SHP2 to (IL6-gp80-gp130-JAK*)$_2$) are ranked at the top positions. Other parameters in the Ras/MAPK pathway such as *kf*71, *kb*48, *kf*42 also become more important when *kf7* is small. The significant changes with different values of *kf7* prove the key role that recruitment of the transcription factor to the dimer of the phosphorylated receptor complex in the JAK/STAT pathway plays.

Table 2. Results by FAST for cases where *kf7* is fixed at different values (10%, 25%, 100%, 400%, 1000% nominal value) while other parameters are perturbed from 10% to 1000% of their nominal values

No.	10% kf7		25% kf7		100% kf7		400% kf7		1000% kf7	
1	kf48	22.88	kf48	45.277	kf21	127.3585	kf21	323.21	kf21	511.08
2	kf32	18.081	kf21	42.556	kf32	106.9433	kf8	243.69	kf8	390.52
3	kf71	16.949	kf32	39.696	kf8	99.75406	kf27	232.26	kf27	384.29
4	kf21	16.548	kf71	37.665	kf48	99.24594	kf26	228.37	kf26	378.72
5	kb7	15.759	kb7	35.332	kb7	95.06144	Vm24	225.41	Vm24	378.13
6	kf8	14.831	kf8	34.198	kf26	89.23968	kb7	218.43	kb7	328.7
7	kf20	14.648	kf20	32.326	kf20	87.89009	kf32	198.67	kf31	324.98
8	kb48	14.523	Vm24	31.753	kf27	87.20311	kf31	194.15	kf28	323.04
9	kf26	12.357	kf26	31.492	Vm24	85.29502	kf20	191.61	kf70	307.79
10	Vm24	12.307	kb48	30.322	kf71	83.55244	kf28	189.73	kf29	307.24
11	kf42	11.993	kf42	28.416	kb48	77.61289	kf29	183.4	ka26	306.14
12	kf19	11.922	kf27	27.999	kf31	74.35815	kf48	183.24	kf20	280.47

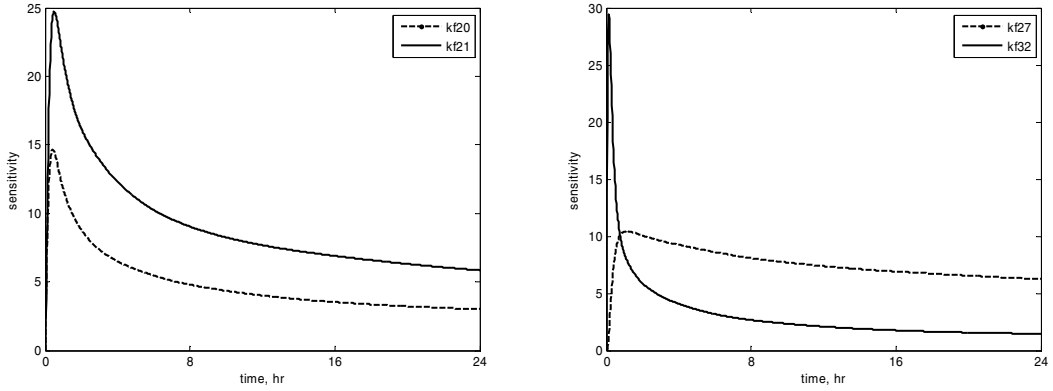
13	kb18	11.738	kf19	27.856	Km24	69.80567	kf70	180.86	Km24	276.32
14	kf18	11.498	kb20	27.677	kf29	69.50425	ka26	180.68	kb27	276.1
15	kb20	10.831	kf28	27.443	ka26	69.12723	Km24	171.16	kb28	274.2
16	kf45	9.9303	kf31	24.98	kf28	69.02862	kb48	165.23	kb29	257.07
17	kf27	9.9222	kf18	24.165	kf70	67.89437	kb28	162.67	kf32	242.28
18	ka26	9.8	kf29	23.371	kf42	67.3212	kb27	161.49	kf48	239.7
19	kf31	9.7821	kf70	23.362	kf19	63.41135	kb29	150.92	kb48	235.82
20	Km24	9.7425	Km24	22.964	kb20	62.75963	kf71	149.57	kb20	213.38

Deactivation of the JAK/STAT signaling transduction pathway by negative control mechanisms

There are several regulatory mechanisms in the IL-6 signaling pathway which have a major effect on signaling through the pathway. These include the cytoplasmic tyrosine phosphatase PP1, the nuclear tyrosine phosphatase PP2, the suppressor of cytokine signaling 3 SOCS3 and the Src homology domain 2 (SH2)-containing protein tyrosine phosphatase SHP2. Sensitivity analysis identified reaction parameters associated with these mechanisms as being of high importance to signal transduction.

The nuclear tyrosine phosphatase PP2 phosphorylates the dimer of phosphorylated transcription factor STAT3 so that they can translocate from the nucleus. The inactivated transcription factors in the cytoplasm will be phosphorylated again by recruitment to the receptor complex. The cycling of STAT3 between the nucleus and the cytoplasm plays an important role in signal transduction (Haspel *et al*., 1996; Yamada *et al*. 2003), as it decreases the accumulation of the transcription factors in the nucleues and prolongs the signal duration. This observation is supported by the sensitivity analysis. The parameter *kf*21 in the decomposition of the recruitment of PP2 to the transcription dimer to deactivate STAT3 is ranked 2nd by the two global sensitivity analysis techniques and the rank values are only slightly less than those of *kf*7. The parameter *kf*20 in the formation of PP2 and the dimer of activated STAT3 also have a high rank. Based on the sensitivity profiles of *kf*21 and *kf*20 shown in Fig. 3a, both parameters have significant effects on the overall signal strength.

The deactivation mechanisms by SOCS3 and SHP2 have been extensively studied in the literature (Lehmann *et al*., 2003; Schmitz *et al*., 2000; Sommer *et al*., 2005). Fig. 3b shows the sensitivity profiles of *kf*32 (recruitment of SHP2 to gp130) and *kf*27 (recruitment of SOCS3 to gp130). The sensitivity of *kf*32 has a peak after a very short period of time as SHP2 has a significant effect on the signal amplitude and it is important for early signal modulation. As time passes, the sensitivity of *kf*32 becomes smaller than the sensitivity of *kf*27 indicating that reactions associated with the effect of SOCS3 have a strong effect on the signal duration of the system. Though both inhibitors bind to the same receptor gp130, their effects are distinct. The prediction by sensitivity analysis is consistent with the conclusions from experiment data (Lehmann *et al*., 2003), as SOCS3 has a major effect on the long-term system response, while at the same time it takes roughly 30 minutes before a significant amount of SOCS3 is formed in these experiments.



a b

Fig 3. The sensitivity profiles of (a) *kf*20 and *kf*21, and (b) *kf*32 and *kf*27 by FAST

The cytoplasmic tyrosine phosphatase PP1 attenuates the signal transduction by dephosphorylation of activated STAT3. However, from the sensitivity analysis results the parameters, such as *kf*10, *kf*11, in the reactions where PP1 binds to the activated STAT3 and its dimmer, have small rank values. This means that the inhibitor PP1 does not significantly affect the JAK/STAT signaling pathway.

Conclusion

Mathematical modeling and simulation of complex signaling pathways has received increasing attention in the area of quantitative cell biology over the least few years. As many of the underlying biological mechanisms are not fully understood, it is important to study the effect of uncertainties on a system and determine which parameters should be estimated from data to account for these uncertainties. Towards this end, sensitivity analysis is a powerful tool to analyze mathematical models containing uncertain parameters.

Two sensitivity analysis techniques were investigated in this work and their advantages and disadvantages were discussed based upon application to an IL6 signaling pathway. While the results returned by the different techniques were similar to local analysis, the Fourier amplitude sensitivity test (FAST) has some advantage as it is a global sensitivity method which can explore the whole parameter space and take parameter interactions into account.

From the sensitivity analysis results it is identified that binding of the transcription factor STAT3 to the dimer of the phosphorylated receptor complex (IL6-gp80-gp130-JAK*)$_2$ is the most important reaction governing these pathways. Among the regulatory mechanisms in the pathway, reactions involving PP2 were determined to be the most important ones for the JAK/STAT pathway. Parameter associated with reactions involving SHP2 have a large effect on the signal amplitude while parameters associated with reactions involving SOCS3 mainly affect the signal duration. Parameters associated with reactions related to PP1 had the least effect of the ones mentioned here.

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