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Sensitivity analysis of deterministic signaling pathways models

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Abstract. The paper is focused on application of sensitivity methods to analysis of signaling pathway models. Two basic methods are compared: local, based on standard sensitivity functions, and global, based on Sobol indices. Firstly, a general outline of modeling of signaling pathways by means of ordinary differential equations is briefly described. Afterwards, the methods of sensitivity analysis, known from literature, are introduced and illustrated with a simple example of a dynamical system of the second order. Subsequently, the analysis of the p53/Mdm2 regulatory module, which is a key element of any pathway involving p53 protein, is presented. The results of this analysis suggest that no single method should be chosen for investigation of any signaling pathway model but several of them should be applied to answer important questions about sources of heterogeneity in cells behavior, robustness of signaling pathways and possible molecular drug targets.

Key words: signaling pathways, sensitivity analysis.

1. Introduction

The terms *signaling pathways* or *regulatory pathways* relate to the cascade of processes, initiated by an external event (e.g., ligand binding to its specific receptor on a cell surface), or by an internal event (e.g., DNA damage). These processes involve creation or degradation of protein complexes, activation of enzymes and usually lead to activation or repression of transcription of genes specific for a given pathway. This results in production of new proteins (or their disappearance, if the genes are repressed) which may affect earlier stages of the cascade, thus creating positive or negative feedback loops. From a control theory perspective, the cell can be presented as a closed-loop system, as in Fig. 1.

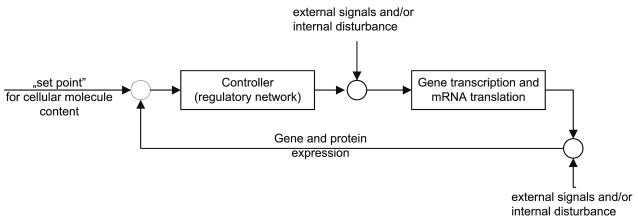

Fig. 1. A general block diagram representation of a signaling pathway. The diagram is simplified, since it does not include posttranslational modification of proteins, siRNA, etc.

Following rapid developments in new experimental techniques, mathematical modeling of regulatory pathways that control intracellular biological and chemical processes is gaining increasing interest in the biomedical research [1–3]. Analysis of biological data has led to much better understanding of the nature of intracellular processes. Though our knowledge of these processes has been rapidly expanded, still much more remains to be uncovered. Research efforts are hampered by at least several factors, high costs of experiments being not the least of them. So far, much more knowledge has been gained concerning the pathways structure than their dynamics. Despite a lot of efforts, relatively small number of models has been hitherto tested against experimental data, and, therefore, a lot of their parameters remain unknown. Moreover, due to their complexity, intertwining and lack of detailed knowledge of the mechanisms regulating each step of the signaling cascades, it is impossible to build precise mathematical description of entire pathways and take into account all factors playing a role in a realistic system. Therefore, analysis is always constrained to several most important processes. Nevertheless, the resulting models provide valuable insights into complex behavior on the cellular level [4], into kinetics of the involved proteins and their complexes and gives the predictions of the possible responses of whole system to the change in the level of a given activator or inhibitor. Thus, even simplified models can significantly contribute to the biological field.

There are many different methods that can be used to describe signaling pathways and their choice is subject to a particular question that the analysis should answer. In this paper deterministic models are considered, given by ordinary differential equations that describe concentration of molecules involved in the pathway are used. This gives a rise to a high dimensional model with a large number of parameters, that are unknown and difficult to estimate. Therefore, each model should be checked with respect to its sensitivity to parameter changes. Most of the pathways should exhibit robustness to parameter changes in a relatively wide range, as this corresponds to proper pathway dynamics in various cells that are not homogeneous and, as a result, characterized by different parameter values. Moreover, sensitivity analysis, as described in the following sections, provides a valuable insight into the importance of particular processes.

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In this paper, the sensitivity analysis is focused on creating parameter rankings, that can be subsequently used either to reduce the model complexity or indicate prospective molecular targets for new drugs. While the methods to obtain such rankings have been known for a long time, their implications, when applied to signaling pathway models, are not very well understood. This work is meant to explain the differences of these methods and their applicability in analysis of signaling pathways.

2. Models of signaling pathways

The models under consideration are based on two crucial assumptions:

- The concentrations are constant with respect to space (the system described by ODEs is an ideal (well-stirred) chemical reactor). This condition is usually satisfied unless molecular crowding [5, 6] takes place, as the average time to cross a cell by diffusion is approximately 1 minute for macromolecules and much less for small molecules; the time scale for analyzing signaling pathways is hours or even days [7].
- The number of molecules taking part in chemical reactions is large enough to apply the law of mass action to describe dynamics of these reactions. In most cases, this is satisfied, as in most reactions the number of molecules of any type reach tens of thousands or even hundred of thousands [8].

The basic processes taken into account in the models include production of new molecules (through gene transcription, mRNA translation and dimerization of molecules) and their degradation, dissociation of complexes and conformational changes in the form of molecules (most often caused by their phosphorylation or dephosphorylation) leading to their activation or inactivation. In addition, nuclear shuttling (i.e. transport of molecules between cytoplasm and nucleus) is incorporated into the model. However, this is done not by means of transport equations. Instead, a compartmental approach is applied, with cytoplasm and nucleus constituting separate compartments and flow between these compartments proportional to respective concentrations.

As mentioned earlier in the text, the variables in the models represent (molar) concentrations of molecules of a given type. They can be either concentrations in a whole cell (usually, when modeling prokaryotic cells or in simplified models built for eukaryotic cells) or separate nuclear and cytoplasmic concentrations (for eukaryotic cells).

The model represents a reaction network, described by a nonlinear state equation

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

where $\mathbf{X} = [x_1 \quad x_2 \quad \dots \quad x_n]^T$ is a state vector with x_i denoting molar concentration of molecules of type i (in case of two-compartmental model, molecules in nucleus and cytoplasm are separate species) and $\mathbf{u}$ is an input variable and $\mathbf{p}$ are model parameters. An important property of these systems is that for any time t all variables are nonnegative.

Usually, the models take into account only a single (scalar) input, which corresponds to extracellular ligand concentration (or, e.g., the dose of irradiation). Moreover, in most cases, it is assumed that the intracellular response is either proportional to the input, or it is a simple on/off (or binary) switch.

3. Sensitivity analysis

The sensitivity analysis is an important tool used to determine how the change of parameters influence system behavior. It helps to identify those parameters that have the greatest impact on the system output both in steady and transient states, at the same time providing information about robustness of these systems [9], a property that should characterize most of the signaling pathways. Though it was developed over half a century ago, its application into system biology is a relatively new concept (though it is a required step in development of the models).

Interpretation of the results of application of sensitivity methods to signaling pathway models goes far beyond standard sensitivity/robustness conclusions. Among others, they also provide means to simplify high dimensional models that arise in systems biology [10] and can be used to indicate prospective "hit points", or molecular targets for the drugs against diseases associated with particular signaling pathways, if the ultimate research goal is to affect the pathway dynamics in a most effective way [11]. A good overview of the methods and their applicability can be found in [12].

Two main categories of sensitivity analysis methods can be distinguished: local and global. The local sensitivity analysis provides information on the effect of a small deviation a single parameter from its nominal value on the system output. Global sensitivities, in turn, describe how the system output changes when multiple parameters change within a relatively wide range.

3.1. Local sensitivity analysis. Let us first discuss the concept of local sensitivities. The approach described in this section is used to help in the analysis of the dynamic behavior of the whole system and is not related to the experimental measurements. Therefore, the state is considered to be the output.

Let the model be described by the state Eq. (1), whose solution is

$$\mathbf{X}(\mathbf{p_n}, t), \tag{2}$$

where $\mathbf{p_n}$ denotes the nominal parameter vector. The first-order sensitivity coefficients s_{ij}, describing the influence of the i-th parameter on the j-th state variable are defined as [13, 14]:

$$s_{ij} = \frac{\partial x_i}{\partial p_j} \tag{3}$$

and the absolute sensitivity matrix as

$$\mathbf{S} = \frac{\partial \mathbf{X}}{\partial \mathbf{p}} = \begin{bmatrix} s_{11} & s_{12} & \cdots & s_{1m} \\ s_{21} & s_{22} & \cdots & s_{2n} \\ \vdots & \vdots & \vdots & \ddots \\ s_{n1} & s_{n2} & \cdots & s_{nm} \end{bmatrix}. \tag{4}$$

More precisely, s_{ij} are sensitivity functions, as they change in time.

Since an analytical solution of (2) is usually not available, sensitivity coefficients must be calculated in some other way. One of the approaches that can be applied is the direct differential method [15]. Calculating partial derivative of (1) with respect to p_j yields

$$\frac{d}{dt}\frac{\partial \mathbf{X}}{\partial p_j} = \frac{\partial f}{\partial \mathbf{X}}\frac{\partial \mathbf{X}}{\partial p_j} + \frac{\partial f}{\partial p_j} = J \cdot S_j + F_j, \tag{5}$$

where

$$J = \frac{\partial f}{\partial \mathbf{X}} = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} & \cdots & \frac{\partial f_2}{\partial x_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial f_n}{\partial x_1} & \frac{\partial f_n}{\partial x_2} & \cdots & \frac{\partial f_n}{\partial x_n} \end{bmatrix} \tag{6}$$

is the Jacobi matrix,

$$F_j = \frac{\partial f}{\partial p_j} = \begin{bmatrix} \frac{\partial f_1}{\partial p_j} \\ \frac{\partial f_2}{\partial p_j} \\ \vdots \\ \frac{\partial f_n}{\partial p_j} \end{bmatrix} \tag{7}$$

is the parametric Jacobi matrix, and

$$S_j = \frac{\partial \mathbf{X}}{\partial p_j} = \begin{bmatrix} s_{1j} \\ s_{2j} \\ \vdots \\ s_{nj} \end{bmatrix} \tag{8}$$

is the column sensitivity vector with respect to the j-th parameter.

Finally, Eqs. (1) and (5) can be combined and solved simultaneously to find sensitivity coefficients:

$$\begin{aligned} \dot{\mathbf{X}} &= f(\mathbf{X}, \mathbf{p}, u, t) \\ \dot{S}_j &= J \cdot S_j + F_j \end{aligned} \tag{9}$$

The initial conditions for sensitivity functions are given by

$$S_j(0) = \frac{\partial x(0)}{\partial p_j}. \tag{10}$$

These initial conditions are often put to be equal to 0 (e.g. [16]), which is true in all cases when arbitrary initial conditions $x_i(0)$ are assumed. It is also the case in this paper, as both examples considered here represent only forced component of dynamical models responses (initial conditions for all variables are assumed to be zero). However, if the task was to analyze a response of a system whose initial state is its equilibrium, reached for another input value, initial conditions would depend on model parameters and (10) would have to be applied to find initial values of sensitivity functions.

In many systems different parameters may take values that are distributed over several orders of magnitude. The same holds true also for model state variables. Therefore, instead of the absolute sensitivities, the relative sensitivities are defined:

$$\overline{s_{ij}} = \frac{\partial x_i}{\partial p_j} \cdot \frac{p_j}{x_i}. \tag{11}$$

Such normalization makes it possible to compare relative influence of any parameter change on system behavior, regardless of the scale of either the parameter or the state variable.

Having calculated relative sensitivities, the analysis can be focused on either of the following goals:

- checking which parameters are relevant for steady state and which for transient dynamics – the whole time course of sensitivity coefficients are taken into account then;
- creating the ranking of parameters that subsequently indicates which processes are the most important for the signaling pathway; consequently, this provides valuable information for experimental research about possible molecular targets in the pathway under consideration;
- finding correlation among parameters, important if the experiments are designed to estimate model parameters.

As far as ranking of parameters is concerned, it is based on cumulative indices. They can be calculated either for each state variable separately, or for the whole system. The importance of the j-th parameter for the i-th state variable can be measured as

$$S^*_{ij} = \frac{1}{T}\int_0^T |s_{ij}(\tau)| d\tau, \tag{12}$$

where T denotes the time horizon of the simulation, or [17]:

$$S^*_{ij} = \frac{1}{N}\sqrt{\sum_{k=1}^{N} |s_{ij}(k)|^2}, \tag{13}$$

where N denotes the number of integration steps in the simulation and the sum is calculated taking consecutive values of s_{ij} computed in simulation. Similarly, the overall effect of a j-th parameter change on the whole system can be measured as

$$S^{tot}_j = \sum_{i=1}^{n} S^*_{ij}. \tag{14}$$

4. Global sensitivity analysis

It should be noted that the local sensitivity analysis, while useful, has a significant drawback in the sense that it does not allow to capture general sensitivity of the system. In fact, multiple parameters can be changed in a given system. Depending on system structure, some of these changes can increase and some decrease the effect comparing to the change of a single parameter. Therefore, as mentioned at the beginning of this section, methods for global sensitivity analysis have been

developed. Most of them consist in simulation of the model for a large number of parameter sets and subsequent transformations of the results. What is important, their applicability is not constrained to deterministic models; stochastic models can also be analyzed this way. The key issue in successful application of these methods is in appropriate sampling of parameter space. Many works devoted to this subject can be found in the literature, dealing with both theoretical aspects of Latin hypercube sampling or factorial sampling plans (e.g. [18–20]) and their application to signaling pathways analysis (e.g. [17, 21]).

Usually, for signaling pathways models parameter values are randomly generated from one of two possible distributions:

- normal or Gamma distribution, if the nominal value of the parameter has already been determined and known from literature;
- uniform distribution, defined on a wide range of biologically acceptable parameters, if the parameters are not known.

Afterwards, one of the two families of methods is applied:

- calculating local sensitivities for each simulation and subsequently averaging them over all simulations [10, 22];
- variance-based sensitivity methods, where
  - the ratio of variance of a model output to the average value is calculated, serving as a sensitivity index [23], or
  - variance of a model output is decomposed into partial variances contributed by changes in individual parameters, the sensitivity indices are subsequently derived from the ratio of the partial variance to the total variance of model output. [10].

In the latter case, the most popular approach seems to be the so called Sobol's method [24]. Two kinds of sensitivity indices are calculated there. One is a first-order sensitivity that measures the fractional contribution of a change of a single parameter to the variance of output. The other is the total effect sensitivity, or the sum of all the sensitivities involving the model input of interest over the full range of parameters values explored. The general idea of this method is rewritten below, basing on [24].

Once again, let us suppose that the model be described by the state equation (1), whose solution is (2). Let us also divide the set of all M parameters arbitrarily into two subsets y and z such that:

$$y = (p_{k_1}, \ldots, p_{k_m}), \tag{15}$$

where $1 \leq m \leq M-1$, $1 \leq k_1 \leq \ldots \leq k_m \leq M$, and z contains the remaining $M-m$ parameters. Then, two sensitivity indices for each subset y can be defined:

$$\begin{aligned} S_y &= \frac{D_y}{D}, \\ S_y^{tot} &= \frac{D_y^{tot}}{D}, \end{aligned} \tag{16}$$

where D is a total variance caused by any feasible changes in parameter values, D_y is the variance of the model response in the case when only parameters from the subset y change. D_y^{tot} is the variance in the case when at least one of the changed parameters belongs to y. Actually, the total variance D is obtained by summing all possible D_y.

These indices represent the influence of change of the parameters on the defined model response. A higher index value for a specific parameters set means that simulation results are more dependent on the changes of the parameters that belong to this set. In particular, if Sobol indices for a single parameter are equal to 1, then the response depends only on this parameter. When Sobol indices are equal 0 for single parameter changes, then reactions associated with these parameters can be omitted without any consequences on models response.

Analytical determination of the D_y, D_y^{tot} and D values is in most cases impossible. Therefore, a numerical approach based on Monte Carlo simulations is required. The Monte Carlo based algorithm developed to determine their values [24] requires that we should divide the set of the parameters into two subsets (y, z), where y is the subset of the parameters for which we want to calculate Sobol indices and z is the subset containing the remaining parameters. Next step is to sample two points from the parameters space from the uniform distribution of the range $< 0, 1 >$ (this assumption, needed for convergence of the method, requires appropriate rescaling of the parameters in the analyzed models) to receive $P = (y, z)$ and $P' = (y', z')$. Then three simulations of the model should be made, for parameters sets $P = (y, z)$, $P_1 = (y, z')$ and $P_2 = (y', z)$. If we assume that $x(P)$ is model simulation results for parameters set P (e.g. signal level in time T), then after N simulations we receive:

$$\frac{1}{N}\sum_{i=1}^{N} x(P) \to x_0, \tag{17}$$

$$\frac{1}{N}\sum_{i=1}^{N} x^2(P) \to D + x_0^2, \tag{18}$$

$$\frac{1}{N}\sum_{i=1}^{N} (x(P) * x(P_1)) \to D_y + x_0^2, \tag{19}$$

$$\frac{1}{N}\sum_{i=1}^{N} (x(P) * x(P_2)) \to D_z + x_0^2, \tag{20}$$

which allows for determination od D, D_y and D_z. After that by using the formula:

$$D_y^{tot} = D - D_z \tag{21}$$

and Eqs. (16) we can calculate Sobol indices for a chosen subset y of the parameters set.

It should be noted that because of the convergence properties in the formulas (17)–(20) the value of N should be large enough.

The Sobol indices calculated as described above can be used for determining the parameter ranking. As mentioned above the Sobol indices gives us the influence of the selected

subset of the parameters on the simulation results. However, any single parameter can be chosen for different subsets of parameters. Therefore, to estimate the total influence of the specific parameter on the model response, we have to consider all the subsets in which this parameter can be included. Similarly, if we want to construct the ranking of all parameters, we have to calculate Sobol indices for all possible subsets y of the parameter set, except for two cases. The first one is an empty y subset, in which case Sobol indices are always equal to 0 and the second case is when all parameters are included in y and the set z is empty. Then, the Sobol indices are always equal to 1.

When applied to analysis of time responses of a dynamical system, the Sobols indices are not the single values but actually functions of time. As a result, a single value of the index cannot constitute a measure of the influence of parameter changes on the system response. Therefore, the average values of the indices in time are chosen to be the partial scores L_k for all parameters that belong to a given subset y_k. Additionally we decide to distinguish the influence of the partial score for a given parameter depending on the size k of the subset y_k, for which the partial score was received. That way, smaller subsets have higher influence on the final score. According to this the final score for a single parameter r is calculated from the formula:

$$J_r = \sum_{k=1}^{M-1} \frac{1}{k} * L_k^{(r \in y)}, \tag{22}$$

where M is the size of the parameters set and $r \in y$ means that above sum is calculated for all L_ks received from y_k that contain the parameter r.

After calculation of the all J_r values, the ranking of the parameter is built. A higher J_r means that the model response is more dependent to the parameter r and this parameter receives higher position in the ranking.

5. A simple example – sensitivity analysis of an oscillating system

In order to illustrate differences between different parameter rankings introduced in the preceding sections, a simple second order system will by analyzed.

Let us consider a step response of a standard 2nd order oscillatory system, given by its transfer function

$$K(s) = \frac{X(s)}{U(s)} = \frac{k\omega_n^2}{s^2 + 2\xi\omega_n s + \omega_n^2}, \tag{23}$$

where $X(s)$ and $U(s)$ are Laplace transforms of the output and input signals, respectively. Its state variables model can be chosen in a standard way in a phase space, so that $\mathbf{X}^* = [x_1 \quad x_2]^T = [x \quad \dot{x}]$. Then

$$\begin{cases} \dot{x}_1 = & x_2 \\ \dot{x}_2 = & -2\xi\omega_n x_2 - \omega_n^2 x_1 + k\omega_n^2 u \end{cases} \tag{24}$$

Let us assume $k = 1$, $\xi = 0.5$ and $\omega_n = 1$ as the model nominal parameters and focus the sensitivity analysis on the output $y = x_1$ (Figs. 2a–c). The normalized sensitivity functions, calculated from (11) are shown in Fig. 2d. As expected, the influence of the parameter k is constant in time, and it is the only parameter whose change results in changing steady state value of the output. Sensitivity functions calculated with respect to parameters ω_n and ξ imply that changing this parameters affects oscillatory behavior. Having calculated these sensitivity functions, it is easy to determine parameter rankings.

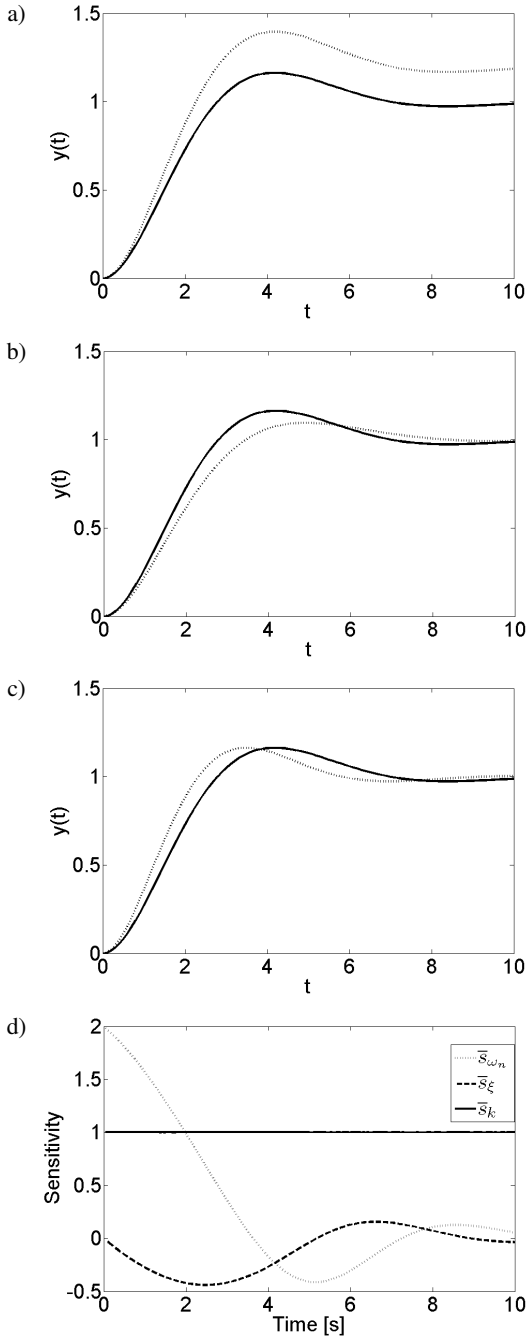

Fig. 2. a)–c) Output of the model (23) calculated for nominal parameter values (solid line) and (a) k, (b) ξ, (c) ω_n increased by 20%. d) Normalized sensitivity functions for this system

For global sensitivity analysis two numerical experiments were performed, one basing on sampling parameter values from uniform distribution (over the interval $[p_n - 0.5p_n, p_n + 0.5p_n]$ where p_n denote nominal values of parameters, $p_n \in k, \xi, \omega_n$) and another from normal distribution (with mean equal to the nominal value and standard deviation equal to $0.33 * 0.5p_n$ for each parameter).

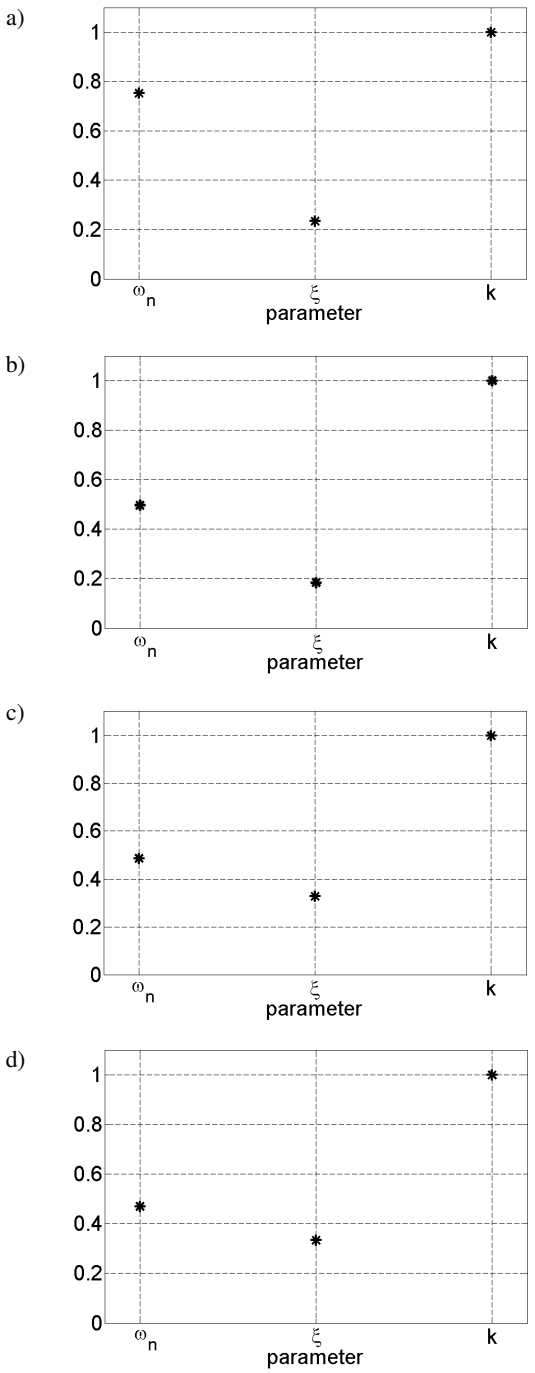

Fig. 3. Comparison of parameter rankings obtained with (a) local sensitivity analysis and the integral index (12); b) local sensitivity analysis and the L^2 index (13); c) Sobol method, parameters sampled from normal distribution; d) Sobol method, parameters sampled from uniform distribution

In each case 2000 samples were generated. If the sample value obtained from the normal distribution was not from the interval $[p_n - 0.5p_n, p_n + 0.5p_n]$, it was rejected as an outlier.

To allow comparison of different ranking indices, all of them have been normalized to a maximum value and shown in Fig. 3. Even in such simplified model, the parameter rankings obtained from different methods are clearly different. Though the ranking order is the same for all methods (k is the most and ξ the least important), the relative strength of their influence varies with the method applied. The local sensitivity methods assign much higher importance to the parameter ω_n than to ξ, whereas in the global sensitivity indices they are closer to each other. This is understandable, as both parameters affect, among others, the frequency of the output oscillations and global methods, contrary to the local ones, allow to evaluate the impact of two or more parameter changing. Another interesting property exposed by the global rankings is that as long as the range within which parameters change is not to large, it makes no difference if their values are sampled from normal or uniform distribution. This implies that knowing precise nominal values is not necessary to describe system behavior. Of course, this latter conclusion holds only for a particular range of parameter changes. Nevertheless, such property is important in analysis of signaling pathways, in which nominal parameter values are virtually unknown.

As the next, biological example show, in more complex systems even the order of parameters, implied by the rankings can depend on the particular method.

6. Sensitivity analysis of the p53-Mdm2 regulatory module

In this section a simple regulatory module, that is at core of any signaling pathway involving p53 protein (Fig. 4), is analyzed.

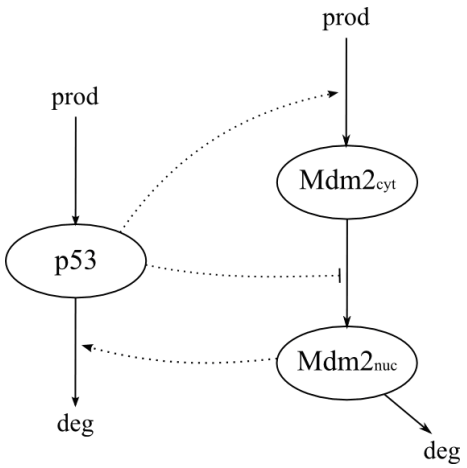

Fig. 4. The simplest p53-Mdm2 regulatory module

Its dynamics is described by the following equations [25]:

$$\frac{d(p53)}{dt} = ms_1 - k_{d1} \cdot (p53) \cdot (Mdm2_{nuc})^2, \qquad (25)$$

$$\frac{d(Mdm2_{cyt})}{dt} = n \cdot \left(s_2 + \frac{s_3 \cdot (p53)^3}{s_4^3 + (p53)^3} \right) - k_1 k_2 \cdot \frac{(Mdm2_{cyt})}{k_2 + (p53)}, \tag{26}$$

$$\frac{d(Mdm2_{nuc})}{dt} = \frac{k_1 k_2 \cdot (Mdm2_{cyt})}{k_2 + (p53)} - k_{d2} \cdot (Mdm2_{nuc}), \tag{27}$$

where the variables $(p53)$, $(Mdm2_{cyt})$, $(Mdm2_{nuc})$ denote concentrations of total p53 protein, cytoplasmic and nuclear Mdm2, respectively. The parameters m and n are the numbers of p53 and Mdm2 gene copies, respectively, s_1, s_2 and s_3 are the production rates per gene copy, k_{d1} and k_{d2} are p53 and Mdm2 degradation rates, respectively, and k_1 is Mdm2–mediated nuclear import rate. The model properties have been thoroughly discussed in [25] but its sensitivity analysis has not been performed yet.

Dynamics of this system is illustrated in Fig. 5. Nominal parameter values were taken from [25] and are given in Table 1.

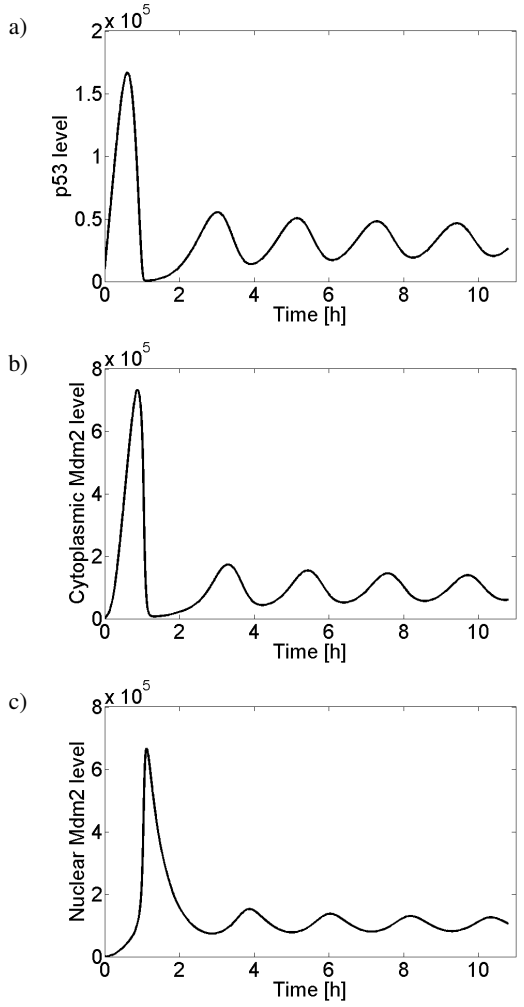

Fig. 5. Time responses for each state variable in p53/Mdm2 model: a) p53; b) Mdm2$_{cyt}$; c) Mdm2$_{nuc}$. Output is scaled in number of molecules, calculated from molar concentrations for an average cell volume

Table 1
Simple p53-Mdm2 model parameters

Parameter	Value
s_1	16 s^{-1}
s_2	8 s^{-1}
s_3	80 s^{-1}
s_4	105
k_{d1}	10213 s^{-1}
k_{d2}	$2.2 \cdot 10^{-4}$ s^{-1}
k_1	$3.5 \cdot 10^{-3}$ s^{-1}
k_2	2300
m	2
n	2

Figure 6 shows parameter rankings obtained with local sensitivity analysis. In this particular system, all of them are similar, which implies that any of the two commonly used sensitivity indices can be used. They show that the most important parameter in the system is k_{d2}, which is a degradation rate of Mdm2 protein. This implies that the work of the regulatory module can be most effectively affected by targeting the process of Mdm2 degradation. Such conclusion can be very important with respect to search for new molecular drug targets.

It seems reasonable to assume that if the goal is to analyze how change of parameters corresponds to different dynamics in a heterogenic cell population, the sensitivity index (12) is more appropriate. On the other hand, if the model is built mainly to reflect particular experimental results, (13) is preferable, especially if the sum is calculated not for all simulation points, but only for the time points in which measurements were made.

For global sensitivity analysis parameter values were sampled from uniform distribution (over the interval $[0.01p_n, 100p_n]$ where p_n denote nominal values for each parameter).

Not surprisingly, since the rankings calculated for each of the three state variables are similar, analysis of the effect of parameter changes on the whole system (see Fig. 7) leads to the same conclusion. However, the ranking provided by the Sobol indices is quite different and so are its implications. It should be noted that type of analysis allows to evaluate effects of changing not only a single parameter, but their various combinations as well.

Therefore, while the local sensitivity analysis may indicate how to change system behavior by targeting just one element of the pathway, analysis of possible different behavior of cells due to their heterogeneity (reflected by different parameters) should be performed with Sobol indices. Then, even more important than the particular order of parameters, implied by the ranking is the fact that their weights indicated by the index value fit into much narrower range, when compared with the results of local sensitivity analysis. This means that in this particular pathway, a deviation from what could be a nominal value of one parameter, can be compensated by an appropriate change (within a similar range) of another, thus allowing evolution of heterogenic, yet robust cell population.

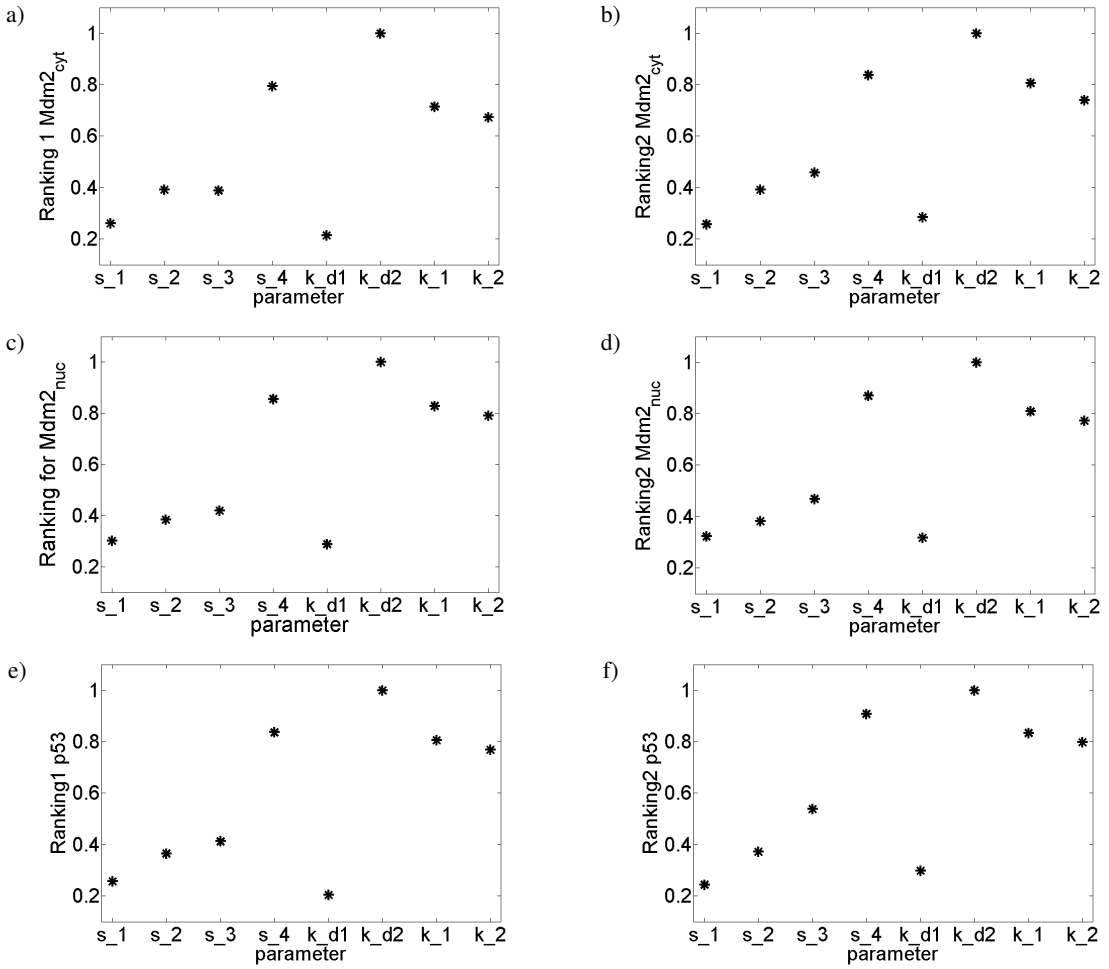

Fig. 6. Comparison of parameter rankings for single variables obtained with local sensitivity analysis and a), c), e) the integral index (12); b), d), f) the L^2 index (13). Parameters m and n were not changed, as they are integer numbers representing gene copies and they appear in the model in the product with another parameters

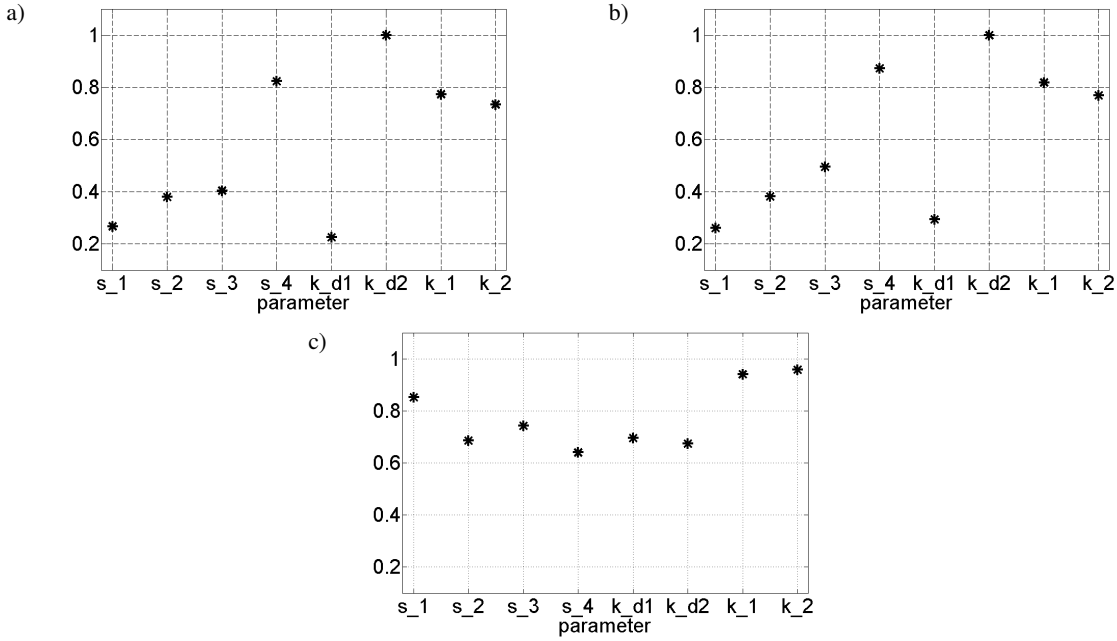

Fig. 7. Comparison of cumulative parameter rankings, describing influence of parameter changes on dynamics of the system as a whole: a) local sensitivity analysis and the integral index (12); b) local sensitivity analysis and the L^2 index (13); c) Sobol method

7. Conclusions

The sensitivity analysis is one of the necessary tools in *in silico* investigation of signaling pathways. However, a particular method that can be chosen should depend on which of two aims are reached for. At a preliminary stage of model building, local sensitivity analysis is more convenient as it is less demanding from computational point of view. Moreover, if model parameters are relatively well known, this method can be used to indicate prospective "hit points", or molecular targets, if the ultimate research goal is to affect the pathway dynamics in a most effective way.

On the other hand, if the research is focused on finding relation between changes of parameters (corresponding to heterogeneity of cells) and the dynamics of a complex system that is the signaling pathway, Sobol indices are more appropriate. If their absolute values are low, it implies a relative robustness of the pathway, which should be its important property in most cases.

It should be also noted that heterogeneity in cellular responses to external stimuli, often attributed to stochasticity of intracellular processes, can be reflected also in deterministic models through change of parameters. Then, though the models remain deterministic in their nature, their parameters can be treated as random variables and global sensitivity analysis is once again a convenient tool in their investigation.

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