

Qualitative and Quantitative Optimal Experimental Design for Parameter Identification of a MAP Kinase Model

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Abstract Mathematical models ensuring a highly predictive power are of inestimable value in systems biology. Their application ranges from investigations of basic processes in living organisms up to model based drug design in the field of pharmacology. For this purpose simulation results have to be consistent with the real process, i.e. suitable model parameters have to be identified minimizing the difference between the model outcome and measurement data. In this work graph based methods are used to figure out if conditions of parameter identifiability are fulfilled. In combination with network centralities, the structural representation of the underlying mathematical model provides a first guess of informative output configurations. As at least the most influential parameters should be identifiable and to reduce the complexity of the parameter identification process further a parameter ranking is done by Sobol' indices. The calculation of these indices goes along with a highly computational effort, hence monomial cubature rules are used as an efficient approach of numerical integration. All methods are demonstrated for a well known motif in signaling pathways, the MAP kinase cascade.

1. INTRODUCTION

Essentials in cell biology can be described and analyzed using deterministic models. For this purpose expert knowledge has to be converted into a suitable model structure M determining which and how model components interact. Even with a correct model structure, the identification of related model parameters $\theta \in \mathbb{R}^p$ can be a challenging task. Especially in the field of biology one has to accomplish the feat to determine a highly dimensional vector of unknown model parameters θ from sparse data, i.e., only a small subset of model states can be measured directly at a limited number of time points t_k .

Frequently, not all elements of the parameter vector θ can be identified uniquely for the considered model by a given measurement set due to a lack of structural identifiability. Qualitative optimal experimental design aims at finding an optimal input/output configuration of the system that guarantees structural identifiability without the knowledge of actual parameter values and real measurement data. To figure out which parameters are theoretically identifiable the identification problem may be reformulated as an observability problem (Fey and Bullinger [2009]). Now efficient methods of structural observability analysis can be applied. A new idea proposed in this work is to combine the structural representation of the model with network centralities (Koschützki and Schreiber [2008]). It turns out that this approach provides a first guess about highly informative measurement sets.

In the next step the most sensitive parameters have to be determined. A well known fact in systems biology is the sloppiness of biochemical systems (Gutenkunst et al. [2007]), i.e., only a small subset of parameters has a strong

impact on model behavior. Global sensitivities based on Sobol' indices may give information about these relevant parameters. Traditionally, Sobol' indices are computed by costly Monte Carlo simulations. In contrast, efficient methods of monomial cubature rules are applied in this work. This reduces the computational effort considerably.

Another important factor to be taken into account is the measurement noise as it results in uncertainties of the identified parameters $\hat{\theta}$. To derive meaningful models these uncertainties should be as small as possible. Consequently, a reliable determination and if possible a reduction of parameter uncertainties is fundamental in modeling. The task of quantitative optimal experimental design is to find experimental conditions that minimize the uncertainties in the identified parameters. Also this objective requires that structural identifiability can be ensured demonstrating the relevance of an a priori identifiability analysis.

In the following, challenges of parameter identification are discussed for the example of a MAP kinase model from literature (Behar et al. [2007]).

2. BACKGROUNDS

2.1 Structural Identifiability

The objective of parameter identification is to find parameter values θ that achieve an acceptable consistency between simulation results $y_s(t_K) \in \mathbb{R}^m$ and measurement data $y_d(t_K) \in \mathbb{R}^m$. For a given model structure M one has to ensure that all parameters θ are in principle identifiable by ideal input-output data, i.e., that the data are free of noise and continuous in time.

For linear ordinary differential equation systems (ode's)

the identifiability test can be done by an integral transformation, e.g., Laplace transformation (Walter and Lecourtier [1982]). However, the majority of processes in systems biology are described by nonlinear ode systems, often of the following form.

$$\dot{c}(t) = N \cdot r(c, \theta) \quad (1)$$

$$y_s(t) = g(c, \theta) \quad (2)$$

The temporal evolution of species concentrations $c(t) \in \mathbb{R}^n$ is determined by the stoichiometric matrix $N \in \mathbb{R}^{n \times v}$ and reaction rates $r \in \mathbb{R}^v$. The reaction rates r are often polynomial or rational functions of the concentrations c , if mass action kinetics or Hill kinetics are used.

Moderately complex nonlinear ode systems can be handled by methods of differential algebra (Saccomani [2004]) but large scale systems still remain intractable. As an alternative for larger systems, a local method has been proposed that only assigns a probability of non-identifiability (Sedoglavic [2002]). To overcome the limitations in system size the original ode system (Eq. 1) is restated in the following way (Fey and Bullinger [2009]). The state vector $c(t)$ is extended by the rates $r(t)$ and if existing by denominators $m(t)$ of Michaelis Menten or Hill kinetics, respectively.

$$\dot{c}_e(t) = f(c_e) \quad (3)$$

$$y_s(t) = g(c_e) \quad (4)$$

Now model parameters θ are implicitly given by the knowledge of initial conditions of the extended ode system (Eq. 3), i.e., if $c_e(0) = [c(0)^T, r(0)^T, m(0)^T]^T \in \mathbb{R}^{n_e}$ is observable all parameters are in principle identifiable.

The proof of observability of nonlinear systems is usually as complex as the proof of identifiability. But in the latter case methods of structural observability analysis become applicable, i.e., conditions that are necessary for observability are checked. The structural analysis can be applied on the basis of a directed graph (digraph) $D(v, e)$ with n different nodes v_i representing the states of the extended ode system (Eq. 3). The existence of an edge $e_{i,j}$ from node v_i to v_j is determined by non-zero elements $a_{j,i}$ of the adjacency matrix A^* . The $a_{i,j}$ element of A^* is set to 1 if the derivative $\frac{\partial f_i(c_e)}{\partial c_{e_j}}$ exists and to 0 if this is not the case. In a similar way, an adjacency matrix C^* of the output function y_s (Eq. 4) can be derived.

The extended system (Eq. 3) is called structural observable (Reinschke [1988]) if the following two conditions are fulfilled:

- All nodes indicating elements of the extended state vector $c_e(t)$ are directly or indirectly connected to nodes related to $y_s(t)$.
- $s - \text{rank}[A^*; C^*] = n_e$.

Here $s - \text{rank}$ represents the structural rank of a matrix. Both conditions can be checked by highly efficient methods of graph analysis, e.g., the algorithm of Shortest Path (Dijkstra [1959]) determines the output connectivity of every node v_i to the output y_s . Calculating the structural rank of a matrix can be easily done by the Ford-Fulkerson algorithm (Ford and Fulkerson [1956]).

2.2 Qualitative Optimal Design

If a system turns out to lack structural observability and hence structural identifiability, there are two possible ways to proceed. One possibility is to check how sensitive the system is against the unidentifiable parameters. Very often the behavior of a biological system is dominated by a tiny subset of all model parameters. Then it may be sufficient, if this subset of parameters can be identified, even if some of the insensitive parameters cannot be determined uniquely. The question of computing parameter sensitivities is treated in Section 2.3.

The second possibility is to look for other measured quantities y_d that ensure structural observability and identifiability. In many cases several sets of measurements fulfilling this condition will exist. Then the question comes up, which set of measurements should actually be chosen. A simple heuristic criterion that may help to answer this question is presented in what follows.

Up to this point, the structural representation of the ode system is only used for the discrete decision if a model is identifiable or non-identifiable. In addition, the representation of the internal information flow via the digraph $G(v, e)$ may provide a first guess about highly informative measurement sets, as is discussed in the following. First the situation is treated, that no real measurement data nor guesses about parameter values are available. The idea proposed here is to use network centralities to assess the importance of every node in $G(v, e)$. The "Status Index of Katz" (Katz [1953]) is calculated (Eq. 5) for this purpose.

$$c_K(i) = \sum_{k=1}^{\infty} \sum_{j=1}^{n_e} \alpha^k (A^{*k})_{ji} \quad (5)$$

In (Eq. 5), j is an index over all n_e nodes in the graph, k stands for paths of length k . $((A^*)^k)_{ji}$ is nonzero if there is a path of length k from node v_j to v_i . Hence, the status index $c_K(i)$ assumes large values for a node v_i that is linked directly or indirectly to many other nodes. To guarantee convergence of the infinite sum in (Eq. 5), a damping factor α is introduced. The inverse of α has to be bigger than the largest eigenvalue of A^* .

Evidently, nodes with high values $c_K(i)$, i.e., nodes that are strongly connected, are good candidates for measurement generation. If during the process of model refinement real data y_d and consequently first guesses of θ become available these information can be easily included by weighted edges. The weight w_{ij} of an edge e_{ij} is correlated to the sensitivities $\frac{\partial f_j(c_e)}{\partial c_{e_i}}$ and $\frac{\partial g_j(c_e)}{\partial c_{e_i}}$, respectively. In this way, the qualitative optimal experimental design is closely linked to the quantitative optimal design, i.e., searching for operation conditions that minimize parameter uncertainties.

2.3 Global Sensitivity Analysis

Especially for models in systems biology, the influence of different model parameters θ on the model output varies strongly. On the one hand there are parameters $\theta_l \subset \theta$ that can be changed by magnitudes without influence on the dynamic behavior and on the other hand a slight change of certain parameters $\theta_h \subset \theta$ leads to a strong output

variation. Evidently the focus of parameter identification should lie on the latter subset θ_h .

To detect the different influence of model parameters θ on y_s related parameter sensitivities have to be determined. If the variation of θ is quite small and their values are almost certainly known then the sensitivities can be determined by a local method using the Fisher Information Matrix (FIM) (Eq. 6).

$$FIM = \left(\frac{\partial y_s}{\partial \theta} \Big|_{\theta} \right)^T \frac{\partial y_s}{\partial \theta} \Big|_{\theta} \quad (6)$$

Usually this is not the case and global methods taking parameter uncertainties explicitly into account have to be applied. These requirements are automatically fulfilled by variance based approaches. Treating parameters θ and the output y_s as random variables one is interested to quantify the amount of variance that each parameter θ_i contributes to the variance of the output $\sigma^2(y_s)$.

The ranking of a parameter θ_i is done by the amount of output variance that would vanish, if this parameter θ_i is assumed to be known. Formally, for every assumed known parameter θ_i a conditional variance $\sigma_{-i}^2(y_s|\theta_i)$ can be determined. The subscript $-i$ indicates that the variance is taken over all parameters other than θ_i . As the hypothesis of a known parameter θ_i is misleading the expected value of the conditional variance $E_i \left[\sigma_{-i}^2(y_s|\theta_i) \right]$

has to be determined, where the subscript E_i illustrates that the expected value is only taken over the parameter θ_i . Now, the output variance $\sigma^2(y_s)$ can be separated (Saltelli et al. [2005]) into the following two additive terms.

$$\sigma^2(y_s) = \sigma_{-i}^2(E_i[y_s|\theta_i]) + E_i[\sigma_{-i}^2(y_s|\theta_i)] \quad (7)$$

The variance of the conditional expectation $\sigma_{-i}^2(E_i[y_s|\theta_i])$ represents the contribution of parameter θ_i to the variance $\sigma^2(y_s)$ indicating the importance of this parameter. The normalized expression (Eq. 8) is known as the first order sensitivity index (Sobol' [1993]) and shall be used in the following for parameter sensitivity analysis.

$$S_i = \frac{\sigma_{-i}^2(E_i[y_s|\theta_i])}{\sigma^2(y_s)} \quad (8)$$

Usually, the multidimensional integrals, i.e., determining $\sigma^2(y_s)$ or $\sigma^2(y_s|\theta_i)$, are evaluated by Monte Carlo methods. This is correlated with a highly computational effort. To reduce computation cost and to avoid a random exploration of the parameter space \mathbb{R}^p using Monte Carlo methods (Sobol' [2001]) monomial cubature rules are applied in this work. Samples θ^j of \mathbb{R}^p and related weights w^j are chosen deterministically to represent the parameter uncertainties (Eq. 9,10).

$$E[\theta] = \bar{\theta} \approx \sum_j^{SP} w^j \theta^j \quad (9)$$

$$\sigma^2(\theta) \approx \sum_j^{SP} w^j (\theta^j - \bar{\theta})(\theta^j - \bar{\theta})^T \quad (10)$$

Details of sample point selection can be found in (McNamee and Stenger [1967]) where a suite of exact monomial rules is presented. Every sample θ^j is propagated

via the ode system (Eq. 1) to the output function y_s^j at time point t_k . The resulting set of output functions $y_s^j(t_k)$ provides an approximation about the variance of $y_s(t_k)$ (Eq. 11,12).

$$E[y_s(t_k)] = \bar{y}_s(t_k) \approx \sum_j^{SP} w^j y_s^j(t_k) \quad (11)$$

$$\sigma^2(y_s(t_k)) \approx \sum_j^{SP} w^j (y_s^j(t_k) - \bar{y}_s(t_k))(y_s^j(t_k) - \bar{y}_s(t_k))^T \quad (12)$$

The overall number of samples SP is correlated to the precision of the used monomials. Here rules are applied using a number of $SP = 2p^2 + 1$ for determining $\sigma^2(y_s)$ and $SP = 2(p-1)^2 + 1$ for $\sigma^2(y_s|\theta_i)$, respectively. The computational effort is quadratically related to the dimension of the unknown parameters θ , if necessary the effort could be reduced further using monomials of lower degree, e.g., $SP = 2p + 1$ for the unconditional output variance.

3. CASE STUDY

The capability of living cells to react on external stimuli by an appropriate response is essential in changing environments. Hence, signaling pathways sensing external stimuli, converting them into an intracellular signal that generates a response are of high interest in systems biology. Especially, as a malfunction of these pathways can cause a number of diseases a better understanding of underlying processes can lead to novel treatment methods.

Mathematical modeling and model analysis can play a part in contributing improvements in this field of biology. Consequently, there is a strong need for highly predictive models, i.e., the model must be able to describe the real process quite well even under conditions that were not part of a former parameter identification process.

As shown in the previous section the parameter identifiability is a prerequisite to fulfill these requirements. The presented methods are demonstrated for a quite common signaling motif, namely the mitogene activated protein kinase (MAP kinase) (Behar et al. [2007]). In general, the MAP kinase pathways mediates diverse processes ranging from gene transcription right up to programmed cell death. The cascade consists of three enzymes that are activated sequentially allowing a variety of response patterns. Under the assumption that the pool of each individual enzyme is constant over the time, the related ode system consists of 3 ode's for the activated enzymes (Eq. 13-15) and comprises 14 unknown model parameters, i.e., $\theta \in \mathbb{R}^{14}$.

$$K\dot{K}K^* = \frac{k1 \cdot u \cdot (1 - KKK^*)}{k1m + (1 - KKK^*)} - \frac{v2 \cdot KKK^*}{k2m + KKK^*} - \frac{k5 \cdot K^* \cdot KKK^*}{k5m + KKK^*} \quad (13)$$

$$K\dot{K}^* = \frac{k3 \cdot KKK^* \cdot (1 - KKK^*)}{k3m + (1 - KKK^*)} - \frac{v4 \cdot KKK^*}{k4m + KKK^*} \quad (14)$$

$$\dot{K}^* = \frac{k6 \cdot KKK^* \cdot (1 - K^*)}{k6m + (1 - K^*)} - \frac{v7 \cdot K^*}{k7m + K^*} \quad (15)$$

Only rate expressions r_i of mass action and Michaelis Menten kinetics are applied. Consequently, a reformulation into a parameter free ode system by extending the state

vector is possible (Eq. 3). The resulting system provides the basis of the structural observability analysis.

In a first step the dedicated adjacency matrix A^* is determined leading to the digraph $G(v, e)$ (Fig. 1). As mentioned the digraph has to be sufficiently connected as a condition for structural identifiability. From every node v_i there must exist a path to nodes that are measurement candidates. Here, the three activated enzymes are assumed to be theoretically measurable. In Table 2 path lengths from every node v_i to nodes v_j related to one of the potential outputs y_s are written down. Only finite paths can be found in the first three lines of Table 2, i.e, the first condition of structural identifiability is fulfilled.

Now the second condition, the full structural rank, has to be proved. As already the adjacency matrix A^* provides a full structural rank every output configuration ensures structural identifiability.

As practical identifiability requires structural identifiability the result can be verified using the FIM (Eq. 6). Model parameters θ are practically identifiable if the FIM has a full rank (Walter and Pronzato [1997]). The FIM is evaluated at parameter values given by literature (Behar et al. [2007]). Furthermore, almost perfect measurement data are assumed, i.e, very low measurement noise and a high number of measurement data points. All three measurement candidates provide an appropriate FIM, confirming the previous result that is only based on the model structure.

The reformulation of the original model (Eq. 1) to a system of an extended state vector (Eq. 3) enables not only the proof of structural identifiability it also provides the detection of further interesting measurement candidates. For the MAP kinase model the information about single rates r_i seems to be sufficient to guarantee parameter identifiability, e.g., the output connectivity of r_1 is shown in the 4th line of Table 2.

The feedback from K^* to KKK^* has a strong impact to parameter identification. A deactivation of this feedback changes the structural identifiability fundamentally. As indicated by the 5th and 6th line of Table 2 the loss of information coupling from K^* to KKK^* leads to infinite path lengths when measuring K^* or KKK^* . The states $c_{e, nO} = [K^*, m_5, m_6, r_8, r_9, r_{10}]$ are not output connected by KKK^* , i.e., the related parameters $\theta_{nI} = [k_6, k_6m, v_7, k_7m]^T$ are not identifiable. In a similar way the number of non identifiable parameters increases to $\theta_{nI} = [k_3, k_3m, k_4, v_4, k_6, k_6m, v_7, k_7m]^T$ when measuring only KKK^* . At least K^* has to be included into the measurement set to ensure structural identifiability.

As shown for the original MAP kinase model, measurement data of only one of the activated enzymes are sufficient for parameter identifiability. For the real parameter estimation process not only the identifiability is of interest but also the information content of measurements. To derive meaningful models with low parameter uncertainties highly informative measurement data are needed. Usually, the assessment of the information content is only available after running first experiments providing real measurement data.

Here, the three potential outputs, KKK^* , KK^* , and K^* , are ranked using the "Katz status index" (Tab. 1). Based

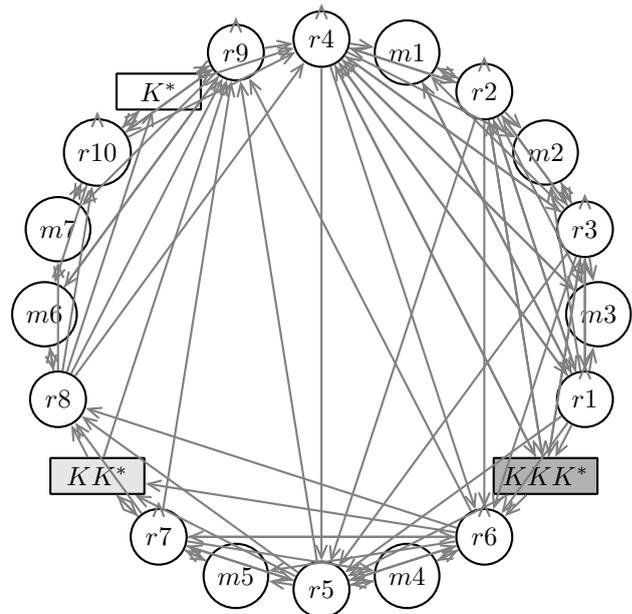


Figure 1. Digraph $G(v, e)$

y_s	c_K
KKK^*	1.00
KK^*	0.93
K^*	0.89

Table 1. Normalized Katz status index c_K

on graph analysis KKK^* is rated as the most informative one followed by KK^* and K^* , respectively. Conclusions about measurement candidates can be drawn before any experimental data y_d or estimates of model parameters θ are given.

As sensitivities, e.g. $\frac{\partial f_j(c_e)}{\partial c_{e_i}}$ and $\frac{\partial g_j(c_e)}{\partial c_{e_i}}$, are not considered at this stage, graph based result may provide only a first guess of highly informative outputs. Nevertheless, this approach is a good starting point for the parameter identification process and can be easily extended by qualitative information during the framework of model refinement.

The FIM in combination with the Cramer Rao inequality (Eq. 16) is used in the following to determine the covariance matrix of estimated parameters $\hat{\theta}$ (Kay [1993]).

$$\sigma^2(\hat{\theta}) \approx FIM^{-1} \quad (16)$$

Under the assumption of very low measurement noise and a high number of data samples, $\sigma^2(\hat{\theta})$ is calculated for all three potential outputs. Naturally, the stimulus u of the MAP kinase model, which enters (Eq. 13), has a strong impact on the dynamic behavior. The trace of the parameter covariance matrix is shown in (Fig. 2) for different values of u .

As indicated by the "Katz Status index" KKK^* provides the most informative measurement data, i.e., the trace of $\sigma^2(\hat{\theta})$ is the smallest one at $u=0.25$. Whereas, data generated by K^* leads to the most uncertain parameters.

In addition, the predictive power of MAP kinase model can be further improved focusing only on the most important parameters. Due to the sloppiness of biological systems [Gutenkunst et al. [2007]] the 14 unknown model parameters θ can be grouped into important θ_h and less important θ_l parameter subsets, respectively, using Sobol' indices, as described in Section 2.3. To determining the

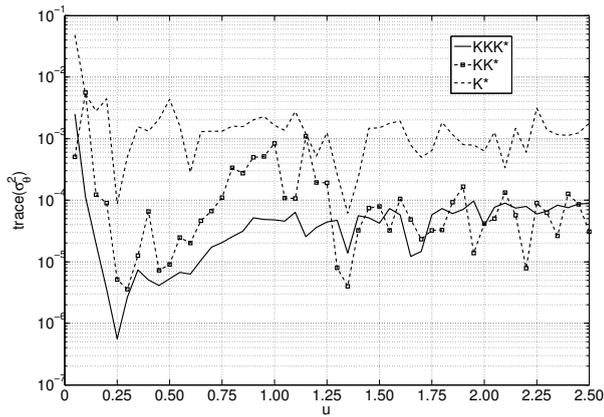


Figure 2. Trace of σ_θ^2 related to different stimuli u

output variance $\sigma^2(y_s)$ that is caused by the 14 parameters a overall number of $2 \cdot 14^2 + 1 = 393$ simulation runs have to be done. As shown, for every first order sensitivity index S_i the expected value of the related conditional variance (Eq. 12) has to be calculated. This increases the total number of function evaluations to 1017 for every index S_i , whereas it is still less computational cumbersome than other sample based methods. Usually, several thousand samples have to be evaluated by Monte Carlo methods.

A relative standard deviation of 10% of the literature parameter values is assumed leading to results shown in (Fig. 3(a) - 3(c)). Here, sensitivity indices S_i relative to every activated enzyme at different time points are given. Obviously, the most important parameters are $\theta_h = [k1, k12, k3, k6]^T$. These parameters have to be determined as precisely as possible whereas the left 10 parameters θ_l can be fixed to literature values reducing the complexity of the parameter identification process dramatically.

4. CONCLUSION

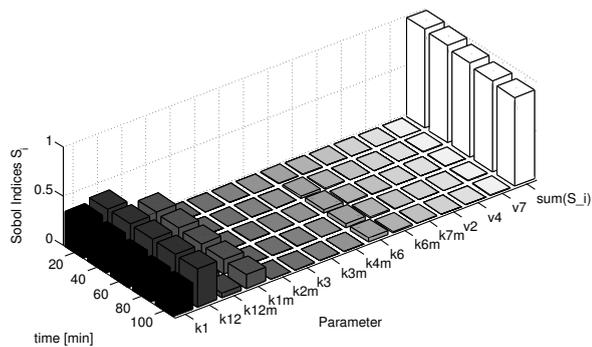
Here, a quite efficient framework is given to study necessary conditions of parameter identifiability. Based on an extended state vector, a digraph is generated making algorithms of graph analysis applicable. If the reformulated system provides a full structural rank and all states are output connected, all model parameters are structurally identifiable. In addition, network centralities provide a first guess of highly informative output candidates at a point where no measurement data or guesses about model parameters are available. If alternatively, no suitable output configuration, ensuring structural identifiability for all parameters, can be determined, a concentration of the most important model parameters is suggested.

The importance of parameters is calculated by a variance based sensitivity analysis. To reduce the computational burden of evaluating highly dimensional integrals numerically, monomial cubature rules of precision 5 are applied. In general, focusing on an important parameter subspace is quite beneficial to the identification process, i.e., the quality of the estimated parameters can be improved.

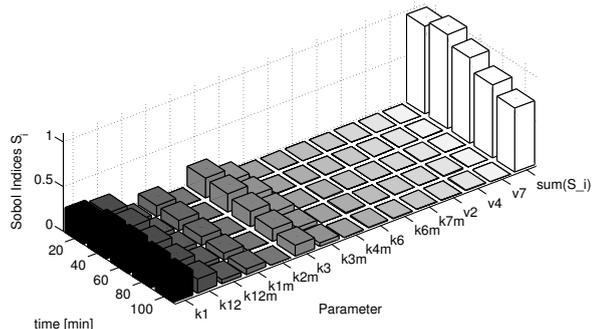
If the uncertainties of identified parameters are still to high, more informative measurement data have to be generated using methods of quantitative optimal design. The quantification of these parameter uncertainties is usually

	K	KK^*	KK^*	K^*	m_1	m_2	m_3	m_4	m_5	m_6	m_7	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}
$y_s = KKK^*$	0	3	2	2	2	2	2	5	5	3	3	1	1	1	1	4	4	4	2	2	2
$y_s = KK^*$	2	0	3	3	3	3	3	2	2	5	5	2	2	2	2	1	1	1	3	3	3
$y_s = K^*$	3	2	0	5	5	5	5	3	3	2	2	4	4	4	4	2	2	2	1	1	1
$y_s = r_1$	2	3	2	1	2	2	2	5	5	3	3	0	1	1	1	4	4	4	2	2	2
.....
$y_s = KKK^*$	0	∞	∞	∞	2	2	-	∞	∞	∞	∞	1	1	1	-	∞	∞	∞	∞	∞	∞
$y_s = KK^*$	2	0	∞	∞	3	3	-	2	3	∞	∞	2	2	2	-	1	1	1	∞	∞	∞
$y_s = K^*$	3	2	0	5	5	5	-	3	3	2	2	4	4	4	-	2	2	2	1	1	1

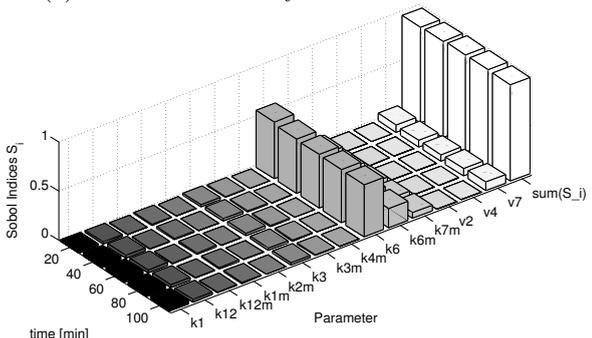
Table 2. Checking output connectivity - Using the algorithm of Dijkstra, the path lengths between all nodes v_i , representing states of the extended system, to nodes of the potential outputs y are shown. All finite entries indicate an output connectivity of corresponding nodes, whereas paths of infinity length stand for unconnected nodes. Entries above the dotted line belong to the original model, entries below are related to the model with the deactivated feedback.



(a) First order sensitivity indices related to KKK^*



(b) First order sensitivity indices related to KK^*



(c) First order sensitivity indices related to K^*

Figure 3. First order sensitivity indices of all model parameters for the three activated enzymes at different time points t_k are shown.

done by the Fisher information matrix (FIM). As this method implies a linear relation of measurement data and model parameters the FIM is of limited value for a broad class of mathematical models, as even the simplest of ordinary differential equations result in a non-linear problem for parameter identification. So in most cases, sample based approaches seem to be more reliable for uncertainty quantification. Promising candidates of this type, which rely on the Sigma Point method (Julier and Uhlmann [1996]), have been introduced recently (Heine et al. [2008], Schenkendorf et al. [2009]).

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