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On Parametric Sensitivity and Structural Robustness of Cellular Functions – the Oscillatory Metabolism of Activated Neutrophils

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Abstract— **Robustness of cellular functions is a key property of living organisms. Modelling and analysis of the genetic and biochemical networks underlying specific functions will enable quantification of the robustness as well as identification of the specific mechanisms providing robustness. Studies on cellular robustness has so far largely focused on parametric sensitivities, i.e., robustness of functions (behavior) with respect to changes in model parameters. In this paper we argue that robustness analysis of cellular models also should encompass structural robustness, i.e., robustness with respect to perturbations in the model structure. This is important not only to quantify the robustness of the cell functions themselves, but equally important, to gain knowledge about the quality of the model as such. In particular, if the model displays poor robustness against structural perturbations this serves as an indication of a potentially highly uncertain model and hence care must be exercised when interpreting the obtained parametric sensitivities. We here propose a simple method for analysing structural robustness of functions related to bistability and periodic oscillations in intracellular networks. The method is applied to a model of the oscillatory metabolism of activated neutrophils (white blood cells) recently proposed in Olsen et al., *Biophys J.*, 84:69-81, 2003. The model is found to be highly robust against parametric uncertainties, but is shown to display poor structural robustness. Indeed, attempting to divide the model into compartments, with the aim of emulating spatial distributions that exist *in vivo*, results in a qualitatively different model prediction.**

I. INTRODUCTION

Living organisms often display a striking robustness in the face of external and internal changes of the cellular environment. The robustness can, in principle, either be due to a redundancy of functionality, or be a result of feedback mechanisms within the biochemical networks generating the functionality. Feedback is also one of the main principles on which many cellular functions are founded. For instance, periodic controls, e.g., of the cell cycle and the circadian rhythm, as well as on-off controls, e.g., genetic switches, are based on nonlinear phenomena relying on feedback mechanisms, e.g., [1], [2], [3].

While robustness in technical feedback control systems typically is analyzed based on adding perturbations to the feedback structure, e.g., [4], analysis of robustness in biochemical networks has so far mainly focused on parametric sensitivities [5], [6]. Using parametric sensitivity as a measure of robustness is strictly based on the assumption that the underlying model structure is exactly known and that the model parameters represent physical properties of the network, such as kinetic rate constants for specific reactions. However, like in most other feedback systems, it is reasonable to assume that models for cellular processes in general have an uncertain structure, e.g., due to unmodelled phenomena such as intermediate reactions. In addition, cells are occasionally subject to perturbations that affect the structure of the biochemical network, e.g., due to gene silencing. Thus, robustness against perturbations to the model structure should be considered both to reveal potentially highly uncertain models as well as to gain insight into possible fragilities of cell functions with respect to structural changes.

In general, robustness against parametric uncertainty does not imply robustness against perturbations that affect the model structure. In this paper, we illustrate this point by analyzing a model of the oscillatory metabolism in neutrophils proposed in [7]. The choice of this specific model is motivated by the fact that it has been found to be relatively robust against parametric perturbations [8], but at the same time it has proven difficult to introduce spatial phenomena observed *in vivo*, such as travelling waves, while retaining the metabolic oscillations [9], [10]. The latter results indicate that relatively small changes in the model structure can completely alter the model predictions.

We first analyze the model with respect to parametric uncertainties and find, similar to what was found in [8], that the model is relatively robust against uncertainty in all parameters. Some parameters are found to display a significantly larger sensitivity than the others, but we here propose a modification that reduces also these sensitivities. In order to analyze the model robustness against unstructured perturbations, we employ a method based on a network representation proposed in [11] combined with linearization and analysis using the structured singular value μ [4]. While the structured singular value provides an efficient measure of structural robustness, it does not provide any information on what part of the network that is most fragile towards perturbations. To obtain such information we employ two analysis tools proposed in [11]. The model is shown to have very poor structural robustness, and the proposed modification that improves the parametric sensitivity is found to have essentially no effect on the structural robustness.

II. THE ACTIVATED NEUTROPHIL

The model we have chosen for this study was first presented in [7], and will in the following simply be referred to as *the Olsen model*. The Olsen model describes the metabolic activity of activated neutrophils. Neutrophils are

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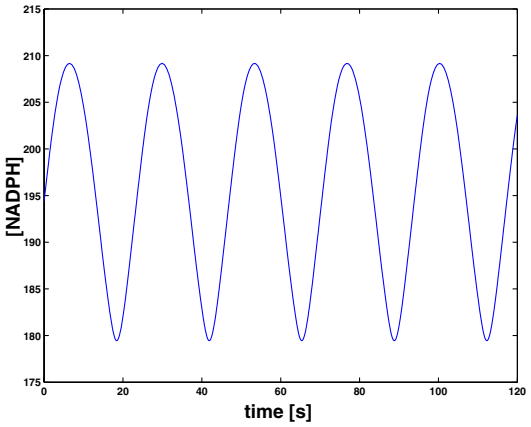

Fig. 1. Temporal oscillations in the concentration of NADPH in cytoplasm of an activated neutrophil.

a type of white blood cells that constitute a central part of the immune defensive system, and they become active when they sense a bacteria, or another invader, which it is their task to kill. As part of the activation, neutrophils increase their production of NADPH, and the concentrations of the involved components display temporal oscillations and spatial waves. The temporal oscillations are illustrated in Figure 1. The oscillations and waves have been recorded experimentally through a fluorescence technique measuring the NADPH concentration. The Olsen model proposes a metabolic mechanism explaining these oscillations.

The Olsen model consists of 16 states and 25 parameters, but due to conservation relationships the number of independent states is only 14. All states correspond to concentrations of various compounds which are divided between two different sub-cellular compartments; the cytosol and the phagosome. NADPH resides in the cytoplasm, and it is here that the oscillations and waves have been observed. In the phagosome reside the poisonous superoxides, and this is where the bacteria is engulfed and killed. The mechanisms in the Olsen model involve reactions in the cytoplasm and in the phagosome, as well as linear and nonlinear transport (diffusion and facilitated transport) between the two compartments. In [7] it is claimed that all ingoing components are necessary to explain the observed oscillations, and the necessity of several of the mechanisms in the model was also demonstrated experimentally. The results presented here will provide a more in-depth analysis of this statement.

A. Spatial Extension of the Olsen Model

The original Olsen model was not spatially extended and could therefore not explain the experimentally observed waves running along the cell. A natural suggestion, however, is to use the Olsen model as a base, and divide the cytosol into spatial sub-compartments. This approach has been attempted in [9] and [10] and it was shown that a straightforward subdivision of the Olsen model did not result in an oscillating solution with travelling waves. It was assumed that the chemicals in the cytosol were transported within the cytosol by means of ordinary diffusion, and several different values of the chemical diffusion constants were tried. As expected, with sufficiently high diffusion constants the spatially extended model behaved like the undivided, i.e. oscillated with the same frequency and amplitude, and with all parts of the cytosol synchronized. As the diffusion constants were lowered a phase shift, between the oscillations in the different compartments, appeared. However, this phase shift did not lead to a wave-phenomenon since it also resulted in the temporal oscillations disappearing altogether. Since the oscillations disappeared even for relatively large values of the diffusion constants, it serves as an indication that the predictions of the Olsen model are sensitive to relatively small changes in the model structure.

B. A more detailed HMS model

In the neutrophil, the major source of NADPH production is the hexose monophosphate shunt (HMS). In the original Olsen model the production is simply modeled as a constant inflow, described by a constant parameter k_{12}. The parameter sensitivity analysis performed below reveals that the model is relatively sensitive with respect to perturbations in this parameter. To analyze whether this is an artifact due to the simplicity of the HMS description, we also studied a modified Olsen model with a more elaborate HMS description. In the extended model a constant flow through glycolysis leads to a production of glucose-6-phosphate which is broken down in three enzymatic reactions to ribulose-5-phosphate, and in this process two $NADP^+$ are converted into two NADPH. The modified model with the new HMS description is based on a similar model developed by Ursula Kummer [12], and is available upon request.

III. Parametric Sensitivity

In order to quantify the robustness of the Olsen model with respect to parametric variations, we consider first a nonlinear and then a linear sensitivity analysis.

The nonlinear analysis is based on performing one-parameter continuation of the model so as to determine the distance between the two Hopf bifurcation points at which the metabolic oscillations emanate for variations in single parameters. The actual distances were obtained using continuation methods available in the Janet software (`www.fys.dtu.dk/~Janet`), and only local bifurcations were considered. The results are given in Table I. As can be seen from the table, all parameters can be varied significantly without the metabolic oscillations disappearing. The largest sensitivity, with this measure, is found for the parameters k_{12} and k_{13} which both can be varied about $\pm 20\%$ without removing the metabolic oscillations.

For the linear parametric sensitivity analysis, we employ the standard metabolic control analysis formula

$$\frac{p}{X}\frac{dX}{dp}$$

where p is the single parameter that is varied and X is the entity which is considered [13]. We here choose to study

parameter	nominal value	lower limit	upper limit	range %
k_1	5.0×10^{7}	1.2×10^{7}	1.5×10^{9}	76.00
k_{-1}	58	0	7.2×10^{3}	100
k_2	1.0×10^{7}	6.3×10^{4}	4.9×10^{19}	99.37
k_3	4.0×10^{3}	2.3×10^{3}	1.9×10^{4}	42.50
k_4	2.0×10^{7}	2.5×10^{6}	8.5×10^{7}	87.50
k_5	1.0×10^{7}	0	1.3×10^{10}	100
k_6	1.0×10^{5}	1.6×10^{4}	6.7×10^{5}	84.00
k_7	1.0	0	9.6×10^{2}	100
k_8	5.0×10^{7}	5.4×10^{6}	1.6×10^{8}	89.20
k_9	5.0×10^{8}	1.2×10^{4}	-	100
k_{10}	1.0×10^{7}	5.4×10^{3}	1×10^{19}	99.95
k_{11}	6.0×10^{7}	5.2×10^{6}	4.4×10^{9}	91.33
k_{12}	3.0×10^{-5}	2.1×10^{-5}	3.5×10^{-5}	16.67
k_{13}	1.25×10^{-5}	1.0×10^{-5}	1.8×10^{-5}	20.00
k_{-13}	4.5×10^{-2}	0	0.19	100
k_{14}	30	1.4	3.1×10^{15}	95.33
k_{15}	30	9.1	7.2×10^{14}	69.67
k_{16}	10	1.8	1.8×10^{15}	82.00
k_{17}	10	0.7	2.2×10^{15}	93.00
k_{18}	0.009	0	3.2×10^{15}	100
V	288×10^{-6}	6.9×10^{-6}	0.08	97.60
L	550	1.1	3.4×10^{8}	99.80
K_O	1.5×10^{-6}	0	2×10^{-4}	100
K_{NADPH}	60×10^{-6}	1.0×10^{-6}	0.054	98.33

TABLE I

PARAMETER REGIONS WITH SUSTAINED OSCILLATIONS WHEN PERTURBING ONE PARAMETER AT A TIME. THE NOMINAL STATE CORRESPOND TO THE OLSEN MODEL WITH NADPH INFLUX (K12) EQUAL TO 3.0E-5M/S, AND THE SUPEROXIDE DIFFUSION CONSTANT (K18) EQUAL TO 0.009.

the frequency and amplitude of the oscillations. The results for the amplitude are shown in Figure 2. In the figure the parameters have been sorted according to the median value of all amplitude sensitivities (since each state gives rise to an individual sensitivity). The median is the middle line in each box and the other two lines display the maximum and minimum sensitivity (except for the effect on the amplitude of melatonin in the cytosol by parameters k_{16} and k_{17} which has been discarded for clarity reasons). The corresponding analysis of the period gives similar results, and we hence conclude that neither the linear nor the nonlinear parametric sensitivity analysis reveals any particularily sensitive parts of the model, but that k_{12} and k_{13} are the most critical parameters.

The two parameters which lead to the highest model sensitivity, k_{12} and k_{13}, are also the two parameters corresponding to the simplest reaction descriptions. Since k_{12} is employed to describe an in reality relatively complex set of HMS reactions, we consider whether a more detailed description of the HMS reactions can reduce the sensitivity. For this purpose we study a model with a more elaborate HMS description, as described above, and the result for the amplitude sensitivity is displayed in Figure 3. In the new model, k_{12} and the parameters with indexes starting with 0 are parameters affecting the NADPH production. As can be seen, none of these parameters are ranked among the most important parameters, and we thus conclude that the high sensitivity for k_{12} in the Olsen model was caused by a model simplification. Whether the proposed model modification also has an impact on the structural robustness of the model is considered below.

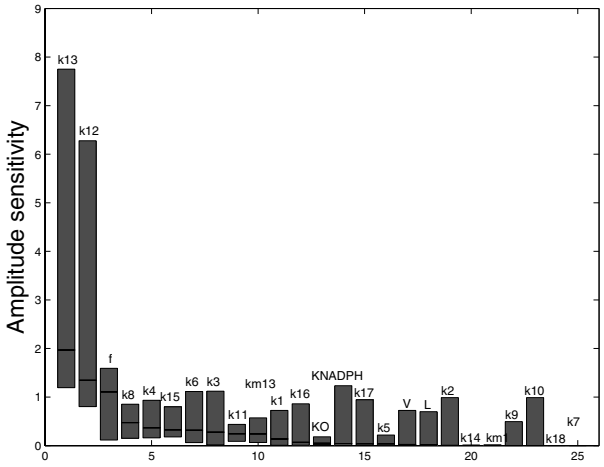

Fig. 2. Parametric sensitivity for the amplitudes in the original Olsen model. The most important parameters are sorted to the left.

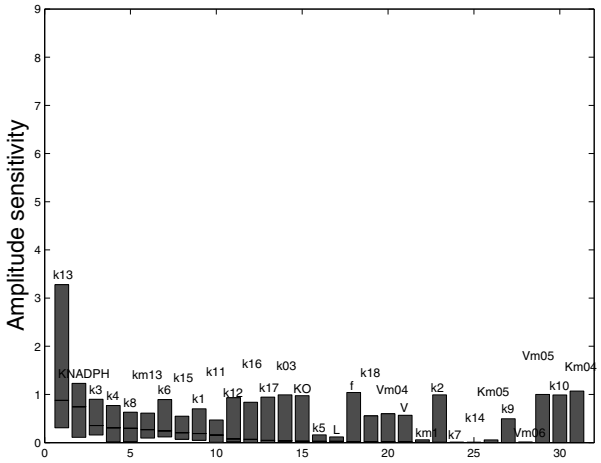

Fig. 3. Parametric sensitivity for the amplitudes in the Olsen model with a more detailed HMS reaction. Now no parameters corresponding to the old k_{12} parameter, in Figure 2, are present among the important ones.

IV. STRUCTURAL ROBUSTNESS

In this section we consider the structural robustness of the Olsen model. This is based on considering the system as a biochemical network with nodes corresponding to pools of biochemical compounds, e.g., metabolites in the cytoplasm and phagosome, and the links between these corresponding to interactions imposed by chemical reactions and transport mechanisms [11]. An illustration of the network corresponding to the Olsen model is shown in Figure 4. Only 14 components are included, corresponding to the number of independent concentrations in the model. As can be seen from the figure, there is a relatively high degree of connectivity in the model, i.e., each node is directly connected to a relatively large number of other nodes. With the network

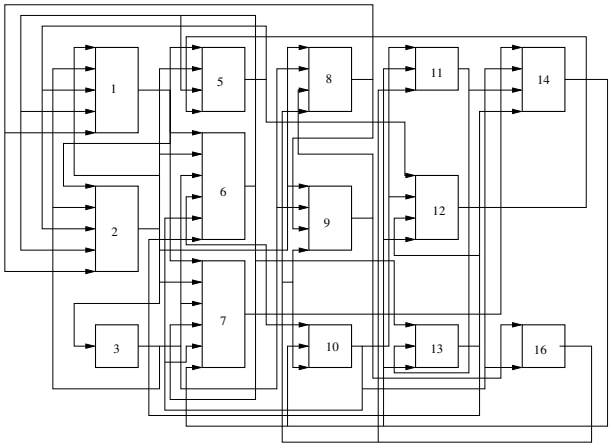

Fig. 4. Network representation of Olsen Model. The nodes correspond to the concentrations of individual biochemical components, while the connections stem from chemical reactions and transport mechanisms.

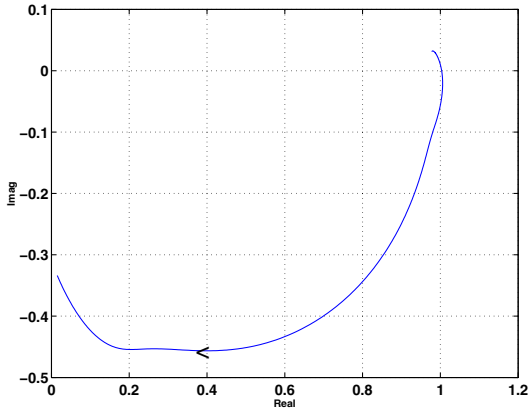

Fig. 5. Critical characteristic locus of network corresponding to Olsen model linearized around the nominal unstable steady-state underlying the limit cycle. The feedback structure corresponds to positive feedback such that the critical Nyquist point is $+1$.

perspective, the large number of feedback connections also become transparent.

To analyze the robustness of the limit cycle behavior, generated by the network, we consider the system at the unstable steady-state underlying the stable limit cycle for the nominal parameter values in [7]. Since each pool of biochemical compound are stable by themselves, i.e. there are no autocatalytic effects, it can trivially be concluded that the oscillating instability is created by feedback interactions between the compounds. This is also formally verified by disconnecting all feedback in the linearized network and considering the eigenvalue loci of the corresponding loop-gain. According to the generalized Nyquist criterion, the feedback will induce an oscillating instability if some eigenvalue locus encircles the point $+1$[1] in the complex plane and the crossing of the positive real axis to the right of $+1$ is for a non-zero frequency. The loop-gain of the network is obtained from the linearized state space model [11]

$$\Delta\dot{x} = \tilde{A}\Delta x(t) + (A - \tilde{A})\Delta u(t)$$

where A is the Jacobian obtained at the unstable steady-state and $\tilde{A}$ is a diagonal matrix containing the diagonal elements of A. The signal $\Delta u(t)$ is the feedback signal, and the closed-loop network is hence obtained by letting

$$\Delta u(t) = \Delta x(t)$$

In transfer-matrix form, the corresponding loop gain of the network is then

$$L(s) = (sI - \tilde{A})^{-1}(A - \tilde{A})$$

For the Olsen model we find only one eigenlocus that encircles the $+1$ point, and this eigenlocus is shown in Figure 5. The crossing of the real axis is for the frequency $\omega_0 = 0.323\ rad/s$, corresponding to a period of $T = 19.5s$ which is close to the observed period in Figure 1. The crossing of the real axis is at 1.003, and hence very close to the critical point $+1$, indicating that there exist some very small perturbation of the network that will stabilize the steady-state and thereby remove the limit cycle corresponding to the metabolic oscillations. Thus, we should expect the existence of a limit cycle in the model to be fragile with respect to some specific perturbation of the model structure.

To quantify the *instability margin* of the underlying steady-state, we employ the structured singular value μ [4]. Thus, we add a perturbation to the feedback signal $\Delta u(t)$ on the following form

$$\Delta u_p(t) = (I + \Delta_I)\Delta u(t)\ ; \quad \Delta_I = diag(\delta_i)$$

where Δ_I is a diagonal and complex valued perturbation matrix. The perturbation corresponds to adding a relative perturbation to the direct effect of each metabolite on the other metabolites. Using the structured singular value we can compute the smallest size $|\delta_i| = 1/\mu$ of the perturbation that moves one eigenlocus of the loop-gain L to the critical point $+1$ at a given frequency. Since the perturbation in principle may move some locus other than the critical one to $+1$, it is necessary to verify that the perturbation in fact moves the critical locus to $+1$. At the nominal steady-state we find that the structured singular value $\mu = 380$ (maximum over frequency) and we define the corresponding instability margin to be

$$\delta_i = 1/\mu = 0.0026$$

Thus, a relative perturbation of less than 0.3 % in the feedback signals will stabilize the network steady-state, and hence remove the limit cycle behavior (assuming there exist no other unstable solution of the nonlinear network for the given parameter values). To consider whether improved robustness can be obtained for other parameter values than the nominal ones used in [7], we compute the stability margin along the one-parameter continuation curve connecting the two Hopf points of the nonlinear model for each parameter. The result for the parameter k_{12} is shown in Figure 6, and

[1]We employ a positive feedback structure, hence the critical point is $+1$ rather than -1 as in negative feedback structures.

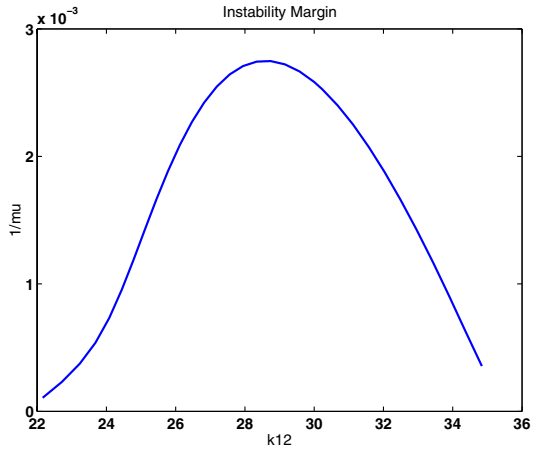

Fig. 6. Instability margin $1/\mu$ of steady-state underlying limit cycle as a function of the parameter k_{12} for which limit cycles exist in the Olsen model.

as can be seen the nominal value $k_{12} = 30$ provides a close to maximum robustness in terms of the μ-measure. Similar results are obtained for all other parameters in the model, i.e., the nominal parameter values used in [7] give close to maximum robustness for the given model structure. Thus, the model structure appears relatively unrobust regardless of the parameter values used. We realize that a definite conclusion would require simultaneous variations of all the parameters to optimize the robustness, a rather exhaustive task not included here.

We repeated the computations for the model extended with the HMS reactions, but find that this extension, while reducing the parametric sensitivity, has essentially no effect on the structural robustness as quantified by the structured singular value.

Because the μ-computations in principle are based on applying the same magnitude perturbation to all feedback channels, it does not provide any information on which part of the network which is most sensitive to the perturbation. To obtain such information we employ two measures proposed in [11]. The first measure is based on adding a real valued perturbation to one feedback signal at the time

$$\Delta u_{pi} = (1 + \epsilon_i)\Delta u_i \;\; ; \;\; \Delta u_{pj} = \Delta u_j, j \neq i$$

and determine the size of the perturbation ϵ_i needed to stabilize the network. The resulting ϵ_i values for the nominal parameter values are shown in Figure 6. As can be seen from the figure, the instability of the network is most sensitive with respect to perturbations in the feedback of components 5 (peroxide in phagosome) and 14 (free oxygen in cytoplasm). However, the instability displays a relative large sensitivity with respect to all components apart from components 8 (MLTH in phagosome) and 10 (NADPH in cytoplasm) (components 4 and 15 were considered dependent variables, according to the two algebraic constraints, in the analysis presented here). Indeed, we find that leaving out the dynamic effects of components 8 and 10 has a very

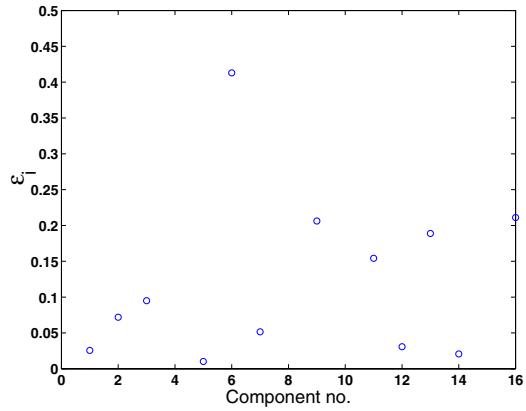

Fig. 7. Real relative perturbations required in the effect of individual components to stabilize the steady-state of the Olsen model with nominal parameter values.

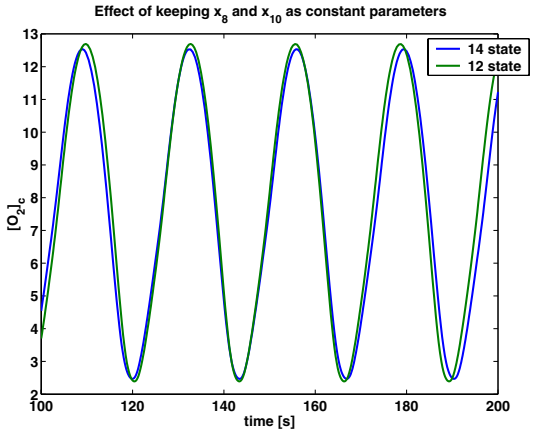

Fig. 8. Limit cycle for 14 and 12 state models, respectively, with $k_{12} = 30e-6$. For the 12 state model we keep $x_8 = [MLTH]_p = 248\mu M$ and $x_{10} = [NADPH]_c = 198\mu M$ which correspond to the underlying steady-state values of the full model.

small effect on the limit cycle of the model. Thus, the limit cycle can be predicted with a 12-state model. The limit cycle, in terms of O_2 concentration in the cytoplasm is shown in Figure 8.

The second structural robustness measure proposed in [11] is based on adding a complex valued perturbation to one element of the loop-gain L_{ij} at the time

$$L_{p,ij} = L_{ij}(1 + \delta_{ij}) \; ; \quad L_{p,kl} = L_{kl}, kl \neq ij$$

and determining the size of $|\delta_{ij}|$ needed to stabilize the network. If no perturbation of a particular L_{ij} can stabilize the network then we define the corresponding $\delta_{ij} = \infty$. This perturbation corresponds to perturbing the direct effect of one compound j on another compound i. As shown in [11], the δ_{ij}-measure is straightforward to compute from the state-space description of the network. The δ_{ij}-measure for the 15 most sensitive elements of the loop-gain L are shown in Figure 9. As can be seen from the figure, the most sensitive interactions involve components 1, 2 and 5 which are all in the phagosome. Indeed, it is easily verified

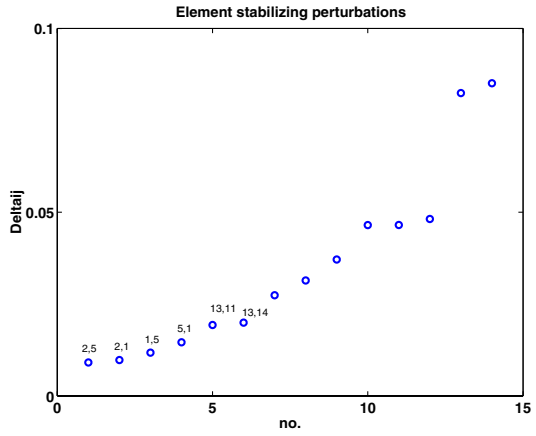

Fig. 9. Magnitude of relative perturbations $|\delta_{ij}|$ required in element i,j of the open-loop network $L(s)$ to stabilize the closed-loop network corresponding to the Olsen model with nominal parameter values.

that only small perturbations of the Jacobian corresponding to these components will bring the RHP eigenvalues at the nominal steady-state to the imaginary axis. For instance, a perturbation of A_{25} by 1.5% will bring all eigenvalues to the LHP thereby stabilizing the steady-state of the network. This perturbation can, however, not be achieved by changing single model parameters. Thus, if the model structure indeed is correct this sensitivity may not pose a real robustness problem. However, the sensitivity as such points to a possibly fragile model structure. This fragility is to some extent reflected in the fact that the oscillations cease to exist when attempting to include spatial phenomena, that exist *in vivo*, in the model [9], [10].

V. Discussion and Conclusions

The analysis of robustness of cellular functions is a key issue within the emerging area of Systems Biology. While most results so far have been based on analysing parametric sensitivities, we have in this paper argued that also structural robustness should be considered. This is important both to reveal potentially highly uncertain model structures, and to detect structural fragilities in cellular networks as such. We proposed to consider the effect of various types of perturbations to the network structure in order to quantify the structural robustness. As an example system we considered the metabolic oscillations in activated neutrophils, and showed that a previously proposed model, while relatively insensitive to parameter changes, displayed a poor robustness to perturbations in the model structure. Also, we proposed biologically motivated modifications to the model that reduced the parametric sensitivity, but that turned out to have little or no effect on the structural robustness. The results on structural robustness can serve to explain why previous authors have experienced problems in attempting to extend the model to involve spatial phenomena, as existing *in vivo*.

Acknowledgement

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