

Application of Sensitivity Analysis for Molecular Drug Targets Searching with Regard to the NF- κ B Signaling Pathway

Malgorzata Kardynska, Jaroslaw Smieja, and Krzysztof Puszyński
Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland
Email: {malgorzata.kardynska, jaroslaw.smieja, krzysztof.puszyński}@polsl.pl

Abstract—Sensitivity methods have been originally developed for analysis of technical systems. Recently these methods have gained increasing importance in the analysis of biological systems. Among others, they allow to rank parameters of mathematical model describing complex biological system, according to their influence on system behavior. These parameters represent biochemical processes crucial for a given biological system and their modification (e.g. by using pharmacological agents) may prove beneficial in the treatment of many diseases. In this work, we propose a novel method of sensitivity analysis based on the frequency distribution of a model transient response. The method may be used to find potential molecular targets for new drugs and takes into account the heterogeneity of cell population with respect to their responses to a drug agent.

Index Terms—sensitivity analysis, frequency distribution, signaling pathways, NF- κ B, molecular drug targets

I. INTRODUCTION

The term signaling pathways (or regulatory pathways) refers to the cascades of biochemical processes involving creation, degradation and modification of various molecules, specific for a given pathway, as well as their transport between cellular compartments (such as cytoplasm, nucleus, mitochondria) 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. These cascades of biochemical processes are activated by events taking place inside a cell (e.g., DNA damage), changes in extracellular environment (e.g. in its chemical content or temperature), direct interactions with other cells (following their binding) or physical stresses (e.g., radiation, mechanical stress).

Modeling of signaling pathways has become an area of extensive research in recent years. It followed development of experimental techniques facilitating observation of intracellular processes at one hand and increasing awareness that experimental work must be

supported by mathematical modeling and analysis of models and data. One of the standard tools in analysis of signaling pathway models is Sensitivity Analysis (SA). Various methods of SA have been proposed, developed for either local or global sensitivity of systems under investigation. SA methods can serve a number of useful purposes, e.g. uncover technical errors in the model, identify critical regions in the parameter space or establish priorities for research [1]. Choosing the appropriate SA method should not only take into account the specificity of the analyzed model but also the objective of the research [2]. One of such objectives may be searching for the crucial processes in a given signaling pathway. Altering these processes, e.g. by using pharmacological agents, may result in drastic changes in cell responses and therefore indicate a potential target for new drugs. In this work we present a novel method of SA tailored precisely to search for components of cell-signaling network that could possibly become molecular targets for new drugs. The method can be used to support the design and development of new drugs and ultimately lead to the creation of drugs with a strong therapeutic effect at low doses.

II. METHODS

In the work we present a novel method of sensitivity analysis based on the frequency distribution of a model transient response. The method can be used to examine the sensitivity of the model to changes in parameters in the range that may represent the effect of drug administration (such as blocking/suppression of selected chemical processes). In this work, for the purposes of presenting our algorithm, the method was applied to examine a deterministic model described by ordinary differential equations. However, the method can be used to analyze any parametric model whose result is presented as a time course of a selected model variable.

In the proposed method, frequency spectra of system responses to a given stimuli are investigated, according to the following algorithm:

1. Run the simulation for nominal parameter values (denoted by $\mathbf{p}_n$), obtaining the model reference time courses $\mathbf{X}_n(\mathbf{p}_n, t)$.

Manuscript received November 17, 2020; revised January 22, 2021.

2. Choose the system output - for example, if the goal of treatment is to lower the level of a selected protein, the natural candidate for the system output is a variable describing the level of that protein (denoted by $x_{in}(p_n, t)$).
3. Calculate Discrete Fourier Transform (DFT) of $x_{in}(p_n, t)$ (denoted by $F_{in}(\omega, p_n)$).
4. For each parameter $p_j \in p_n$ generate a large set of its changed values $[\alpha \cdot p_j]$ where α is a random number drawn from a chosen distribution. The distribution should reflect experimental data for individual cells. The mean and range of parameter changes should be adjusted to result of drug administration and reflect heterogeneity of cells population, in which each cell may have different sensitivity to the drug. When there is no access to appropriate experimental data, it seems reasonable to use a normal or lognormal distribution.
 - 4.1. For each randomly drawn value of the parameter p_j run a simulation (with remaining parameters at their nominal values), obtaining $x_i(p_j, t)$.
 - 4.2. Calculate DFT of $x_i(p_j, t)$ (denoted by $F_i(\omega, p_j)$).
 - 4.3. Calculate the difference between DFT of the nominal response and the new response, defined as:

$$\Delta F_i(\omega, p_j) = F_{in}(\omega, p_n) - F_i(\omega, p_j) \quad (1)$$

- 4.4. Calculate the index of deviation (denoted by A), based on the power spectrum of the calculated DFT [3]:

$$A_j = \frac{\sum_{k=0}^{M+1} |\Delta F_i(\omega_k, p_j)|}{\sum_{k=0}^{M+1} |F_{in}(\omega_k, p_n)|} \quad (2)$$

where $\omega_k = k/T$ ($k = 0, 1, \dots, 2M$), $M = \log_2 N - 1$, T is the final time of simulation, and N is the number of discrete time points in the simulation.

5. Calculate the mean μ and variance σ^2 of the index A for each parameter p_j .

The results of the algorithm can be presented in the form of parameter rankings based on average or variance of the index A for each parameter. While the significance of both rankings is high, their interpretation is quite different. Ranking based on the average will represent the potency of the drug - the higher the value, the greater the change in cell response at the population level. On the other hand, high values in the ranking based on variance will correspond to the high heterogeneity of cell responses, which is an adverse phenomenon. Therefore, the information contained in both rankings complement each other and thus help find promising molecular targets for drugs.

The presented method can be classified as a one-at-a-time SA. While these methods do not allow to fully examine the sensitivity of the model to parameter changes [4], their use is justified to search for molecular targets for new drugs when the drug has one target. For complex drugs, the use of the method is also possible, but requires the simultaneous change of two or more parameters.

III. RESULTS

A. Systems under Investigation

The applicability of proposed method is illustrated with an example of NF- κ B signaling pathway. Nuclear Factor κ B (NF- κ B) is a transcription factor that regulates expression of various genes and is widely involved in stress responses and the control of cell fate [5]. The NF- κ B -dependent pathway regulates cell responses to different types of stimuli, yet its primary function is the regulation of inflammatory and immune responses (e.g. via regulation of cytokine production) but is also involved in many other cellular processes (e.g.: apoptosis, cell cycle progression, angiogenesis, and metastasis) by controlling transcription of hundreds of different genes [6] and therefore it has been the subject of research for many years now. The great interest in NF- κ B signaling pathway also results from the fact that NF- κ B plays a key role in disease and in particular cancer progression. Up-regulation of the NF- κ B pathway is frequently observed in cancer cells, which may contribute to their resistance to treatment [7]. The frequency and amplitude of NF- κ B oscillations was shown to control target gene expression [8]-[10] and may have both proapoptotic or antiapoptotic functions [5]. In breast cancer, the NF- κ B activity stimulates tumour growth, metastasis and chemoresistance, therefore therapeutic inhibition of its activity is considered beneficial [11]. In particular, various ways of inhibition of NF- κ B pathway have been the focus of attention in many cancer studies [12]-[14]. In this work, we used a novel SA method to find key elements of the NF- κ B signaling pathway whose modification (e.g. using pharmacological agents) will inhibit the NF- κ B response.

We chose for analysis a two-feedback model of the NF- κ B regulatory module proposed by Lipniacki *et al.* [15] that includes regulation of IKK and A20. The model structure was subsequently extended to include additional interactions and various stimuli activating the NF- κ B response [8], [16]-[19], however, the core of the model remained unchanged. For the purpose of the demonstration, we decided to choose a less complex model, whose equations and parameters can be found in [15]. Table I presents model parameters and related biochemical processes, which we analyzed using proposed method.

It should be noted that we have excluded from the list (and also from the analysis) parameters that describe the physical properties of the cell (e.g. ratio of cytoplasmic to nuclear volume), because they cannot be pharmacologically modified and thus their analysis is pointless in the context of the purpose of the study.

B. Numerical Results

Activation of the NF- κ B signaling pathway (e.g. by binding a cytokine to a receptor on the surface of a cell membrane) leads to translocation of NF- κ B proteins from the cytoplasm to the nucleus, where they serve as a transcription factor for hundreds of genes. Considering this, the NF- κ B protein seems to be a natural candidate for

monitoring the activation and dynamics of the system. Therefore, we chose the concentration of nuclear NF- κ B as the system output in the proposed SA algorithm.

The analysis was carried out for two hypothetical situations: (i) inhibition of selected biological processes (see Fig. 1) and (ii) induction of selected biological processes (see Fig. 2). In the first case, the parameter values in the model were reduced by the scaling factor α drawn from the normal distribution with $\mu = 0.1$ and $\sigma = 0.025$. As a result, the parameter values have been reduced by an average of 90%. This may correspond to the situation when we use inhibitors that selectively bind to enzymes which in turn leads to blocking of the affected reaction with a blocking efficiency of 90% at the population level. In the second case, the scaling factor α was also drawn from the normal distribution, but with parameters $\mu = 100$ and $\sigma = 25$. As a consequence, the parameter values have been increased, and thus selected reactions occur faster. An example of such a situation might be administration of enzyme activators, that bind to enzymes and increase their activity. In both cases, parameter rankings based on average (top panels) and variance (bottom panels) of the index A were presented. The symbols on the x-axis correspond to the parameters of the model and are explained in Table I.

TABLE I. THE LIST OF PARAMETERS OF THE NF- κ B MODEL

Parameter	Description
k_1	IKK activation caused by TNF
k_2	IKK inactivation caused by A20
k_3	IKK spontaneous inactivation
a_1	(IkB α NF- κ B) association
a_2	(IKK IkB α) association
a_3	(IKK IkB α NF- κ B) association
t_1	degradation of (IKK IkB α)
t_2	degradation of (IKK IkB α NF- κ B)
k_{prod}	IKKn production rate
k_{deg}	degradation of IKKa, IKKn and IKKi
c_1	inducible A20 mRNA synthesis
c_2	constitutive A20 mRNA synthesis
c_3	A20 mRNA degradation
c_4	A20 translation
c_5	A20 degradation
c_{1a}	inducible IkB α mRNA synthesis
c_{2a}	constitutive IkB α mRNA synthesis
c_{3a}	IkB α mRNA degradation
c_{4a}	IkB α translation rate
c_{5a}	IkB α degradation rate
c_{6a}	(IkB α NF- κ B) degradation
i_1	NF- κ B nuclear import
i_{1a}	IkB α nuclear import
e_{1a}	(IkB α NF- κ B) nuclear export
e_{2a}	IkB α nuclear export

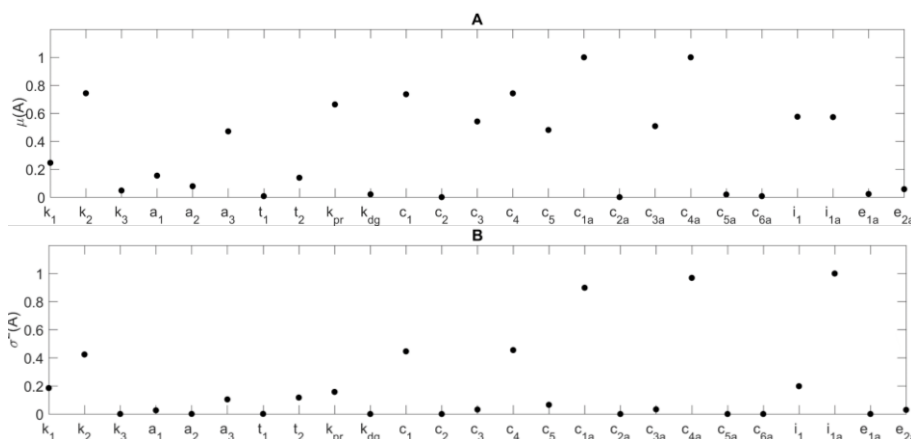


Figure 1. Parameter rankings showing the impact of reducing model parameters by the scaling factor α drawn from the normal distribution with $\mu = 0.1$ and $\sigma^2 = 0.025$ based on (A) average and (B) variance of the index A for each parameter.

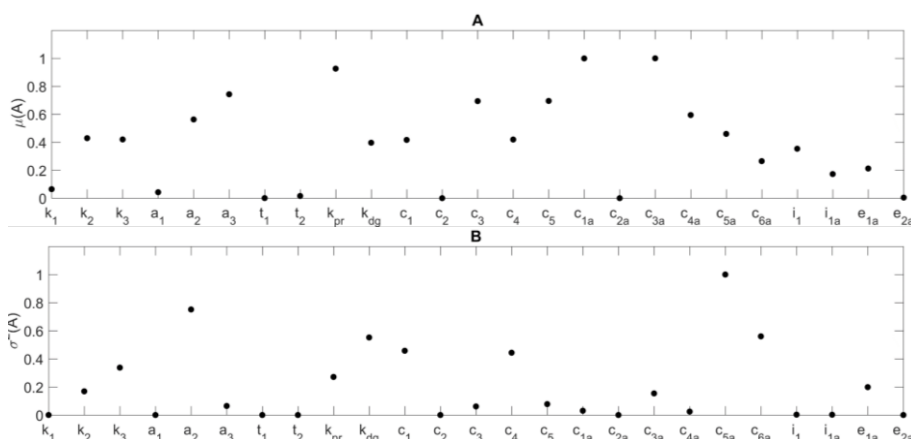


Figure 2. Parameter rankings showing the impact of increasing model parameters by the scaling factor α drawn from the normal distribution with $\mu = 100$ and $\sigma^2 = 25$ based on (A) average and (B) variance of the index A for each parameter.

To validate parameters rankings, we performed a simulation analysis. Based on the rankings, we chose four parameters (two parameters for each ranking set) that should change the model response most significantly and subsequently we simulated the model response with modified parameter values. According to the parameter rankings based on average of the index A, the parameters c_{1a} and c_{3a} have been chosen in the case of inhibition, while c_{1a} and c_{4a} in the case of induction. Parameter values were modified in a range that corresponded to parameter rankings, namely $\alpha=0.05$, $\alpha=0.1$ or $\alpha=0.15$ when parameter values were reduced and $\alpha=50$, $\alpha=100$ or $\alpha=150$ when parameter values were increased.

The time courses obtained for the parameters modified by the scaling factor α are presented in Fig. 3 and are consistent with parameter rankings. Increasing or decreasing selected parameters resulted in significant upregulation or downregulation of the NF- κ B response. To predict whether a change in a given parameter will result in upregulation or downregulation, a thorough knowledge of the system and its parameters is necessary. Furthermore, it can be expected that opposite changes in certain parameters may result in a similar effect on the system, e.g. reducing the transcription rate for a particular mRNA may have a similar effect as increasing the degradation rate for this mRNA.

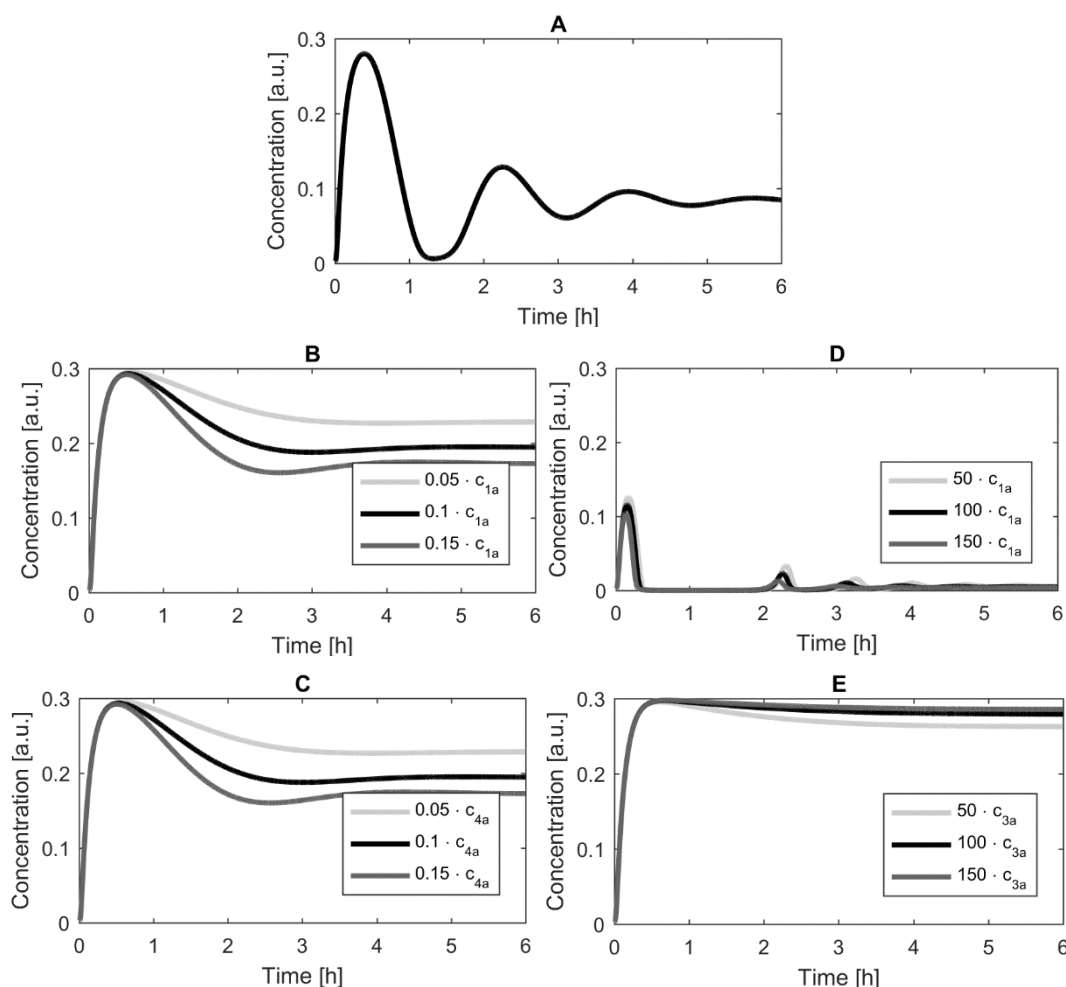


Figure 3. Time courses of nuclear NF- κ B simulated for (A) nominal parameter values or (B-E) selected parameter values modified by the scaling factor α . In the simulations, only one parameter was changed at a time, the rest of the parameters were taken from the nominal values.

In addition, it was found that reducing the parameters c_{1a} and c_{3a} resulted in a large variance of the NF- κ B response (Fig. 3 B and C), which is also in line with the results of the sensitivity analysis (see Fig. 1B). In contrast, the variance of the NF- κ B response after increasing the parameters c_{1a} and c_{4a} (Fig. 3DE) is significantly lower, despite the fact that these parameters were randomly drawn from the normal distribution with a large standard deviation ($\sigma = 25$).

C. Discussion

In both considered situations (inhibition and induction of selected biological processes) the analysis showed a significant impact of parameters related to transcription and translation of the I κ B α protein, a primary inhibitor of NF- κ B that keeps NF- κ B sequestered in an inactive state in the cytoplasm. Considering that either I κ B α protein or its transcript can be predicted as potential molecular target for a new drug.

Based on simulation analysis, it was found that modifying parameters related to $\text{I}\kappa\text{B}\alpha$ transcription and translation can lead to upregulation or downregulation of the NF- κB response. In the first case, we observe a constant translocation of nuclear NF- κB and the disappearance of its oscillation, which will probably result in the upregulation of hundreds of NF- κB dependent genes. In the second case, we observe the suppression of the NF- κB response, visible as the reduction of its oscillation's amplitude and frequency as well as the reduction of the time for which NF- κB is observed in the cell nucleus.

Considering the role of NF- κB in cancer progression, the suppression of the NF- κB signaling pathway may have a therapeutic application. The analysis showed that this effect can be achieved by modification (namely upregulation) of $\text{I}\kappa\text{B}\alpha$ mRNA synthesis. In addition, the results of the analysis confirmed the low variance of the NF- κB response resulting from a random increase in the $\text{I}\kappa\text{B}\alpha$ mRNA synthesis rate (see Fig. 2B), which may lead to less heterogeneous response in the cell population after drug administration. Therefore, $\text{I}\kappa\text{B}\alpha$ mRNA was selected as the best molecular target for new drugs aimed at suppressing the NF- κB response. Regulation of mRNA can be achieved by using specific microRNA (miRNA) molecules that can be used for both upregulation and downregulation of mRNA. This conclusion agrees with the latest research in the field of cancer therapies that is considering the use of miRNA for therapeutic purposes [20]

IV. CONCLUSION

We presented a new method of sensitivity analysis of signaling pathway models, aimed at finding potential drug targets. The method is based on system responses to a given input function analyzed in the frequency domain and allows to rank parameters according to their influence on system behavior.

Applicability of the method has been illustrated with an example of chosen regulatory module. Using the method, we thoroughly analyzed the NF- κB signaling pathway, which is a signaling pathway of great importance in the development of cancer and its therapeutic inhibition is considered beneficial.

In the presented example, the method proved to be able to capture the influence of parameter changes on system transient time responses and based on this, to select parameters that can potentially become molecular targets for new drugs.

The analysis emphasized the huge potential that may have regulation of transcription process using miRNA, which is consistent with the latest research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Malgorzata Kardynska and Jaroslaw Smieja develop the sensitivity analysis method; Malgorzata Kardynska implemented the algorithm and conducted numerical

simulations; Malgorzata Kardynska and Jaroslaw Smieja analyzed the results; Malgorzata Kardynska wrote the paper; Krzysztof Puszyński acquired funding and administered the work; all authors had approved the final version.

ACKNOWLEDGMENT

This work was financially supported by internal grants of Silesian University of Technology 02/010/BK_19/0164 (JS) and NCN grant UMO-2016/23/B/ST6/03455 (KP, MK). Calculations were performed on the Ziemowit computational cluster (<http://www.ziemowit.hpc.polsl.pl>) created in the POIG.02.01.00-00-166/08 project (BIO-FARMA) and expanded in the POIG.02.03.01-00-040/13 project (Syscancer).

REFERENCES

- [1] A. Saltelli, *Global Sensitivity Analysis: The Primer*, Chichester: John Wiley & Sons, 2008.
- [2] J. Smieja, M. Kardynska, and A. Jamroz, "The meaning of sensitivity functions in signaling pathways analysis," *Discrete Cont. Dyn-B*, vol. 19, no. 8, pp. 2697-2707, 2014.
- [3] A. Prosek, M. Leskovaar, and B. Mavko, "Quantitative assessment with improved fast Fourier transform based method by signal mirroring," *Nucl. Eng. Des.*, vol. 238, no. 10, pp. 2668-2677, 2008.
- [4] A. Saltelli, K. Aleksankina, W. Becker, P. Fennell, F. Ferretti, N. Holst, *et al.*, "Why so many published sensitivity analyses are false: A systematic review of sensitivity analysis practices," *Environ. Modell. Softw.*, vol. 114, pp. 29-39, 2019.
- [5] M. S. Hayden and S. Ghosh, "Shared principles in NF-kappa B signaling," *Cell*, vol. 132, no. 3, pp. 344-362, 2008.
- [6] S. Vallabhupurapu and M. Karin, "Regulation and function of NF-kappa B transcription factors in the immune system," *Annu. Rev. Immunol.*, vol. 27, pp. 693-733, 2009.
- [7] N. D. Perkins, "NF-kB: Tumour promoter or tumour suppressor?" *Trends in Cell Biology*, vol. 14, pp. 64-69, 2004.
- [8] L. Ashall, C. A. Horton, D. E. Nelson, P. Paszek, C. V. Harper, K. Sillitoe, *et al.*, "Pulsatile stimulation determines timing and specificity of NF-kappa B-dependent transcription," *Science*, vol. 324, no. 5924, pp. 242-246, 2009.
- [9] R. A. Kellogg and S. Tay, "Noise facilitates transcriptional control under dynamic inputs," *Cell*, vol. 160, no. 3, pp. 381-392, 2015.
- [10] S. Tay, J. J. Hughey, T. K. Lee, T. Lipniacki, S. R. Quake, and M. W. Covert, "Single-cell NF-kappa B dynamics reveal digital activation and analogue information processing," *Nature*, vol. 466, no. 7303, pp. 267-271, 2010.
- [11] M. E. Velazquez, P. O. Saloma, M. I. P. Arreola, K. E. N. Castro, J. I. Castro, and J. M. Montor, "The role of cytokines in breast cancer development and progression," *J. Interf. Cytok. Res.*, vol. 35, no. 1, pp. 1-16, 2015.
- [12] M. Kardynska, A. Paszek, J. Smieja, D. Spiller, W. Widlak, M. R. H. White, *et al.*, "Quantitative analysis reveals crosstalk mechanisms of heat shock-induced attenuation of NF-kappa B signaling at the single cell level," *Plos Comput. Biol.*, vol. 14, no. 4, 2018.
- [13] J. D. Domenech, R. Pippa, M. Tapia, P. Gascon, O. Bachs, and M. Bosch, "Inactivation of NF-kappa B by proteasome inhibition contributes to increased apoptosis induced by histone deacetylase inhibitors in human breast cancer cells," *Breast Cancer Res. Tr.*, vol. 112, no. 1, pp. 53-62, 2008.
- [14] A. Zanutto, E. Braganhol, R. Schroder, L. H. T. D. Souza, R. J. S. Dalmolin, M. A. B. Pasquali, *et al.*, "NF kappa B inhibitors induce cell death in glioblastomas," *Biochem. Pharmacol.*, vol. 81, no. 3, pp. 412-424, 2011.
- [15] T. Lipniacki, P. Paszek, A. R. Brasier, B. Luxon, and M. Kimmel, "Mathematical model of NF-kappa B regulatory module," *J. Theor. Biol.*, vol. 228, no. 2, pp. 195-215, 2004.
- [16] P. Paszek, S. Ryan, L. Ashall, K. Sillitoe, C. V. Harper, D. G. Spiller, *et al.*, "Population robustness arising from cellular

heterogeneity," *P. Natl. Acad. Sci. USA*, vol. 107, no. 25, pp. 11644-11649, 2010.

- [17] Y. J. Wang, P. Paszek, C. A. Horton, D. B. Kell, M. R. H. White, D. S. Broomhead, *et al.*, "Interactions among oscillatory pathways in NF-kappa B signaling," *Bmc. Syst. Biol.*, vol. 5, 2011.
- [18] Y. J. Wang, P. Paszek, C. A. Horton, H. Yue, M. R. H. White, D. B. Kell, *et al.*, "A systematic survey of the response of a model NF-kappa B signalling pathway to TNF alpha stimulation," *J. Theor. Biol.*, vol. 297, pp. 137-147, 2012.
- [19] S. Zambrano, M. E. Bianchi, and A. Agresti, "A simple model of NF-kappa B dynamics reproduces experimental observations," *J. Theor. Biol.*, vol. 347, pp. 44-53, 2014.
- [20] R. Rupaimoole and F. J. Slack, "MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases," *Nat. Rev. Drug Discov.*, vol. 16, no. 3, pp. 203-221, 2017.

Copyright © 2021 by the authors. This is an open access article distributed under the Creative Commons Attribution License ([CC BY-NC-ND 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/)), which permits use, distribution and reproduction in any medium, provided that the article is properly cited, the use is non-commercial and no modifications or adaptations are made.



Malgorzata Kardynska received PhD degree in biocybernetics and biomedical engineering from the Silesian University of Technology, Gliwice, Poland in 2018.

She is currently employed at the Silesian University of Technology in Gliwice as a postdoctoral research assistant. She completed the scientific internship in the Systems Microscopy Centre, School of Biological Sciences within the Faculty of Biology, Medicine and Health at the University of

Manchester. Her research interests are focused on mathematical modeling and analysis of signaling pathways and regulatory networks, as well as application of sensitivity methods in analysis of biological systems.

Dr. Kardynska has co-authored more than 20 peer-reviewed publications in scientific journals and conference proceedings.



Jaroslaw Smieja received PhD and DSc (habilitation) degrees in automatic control from the Silesian University of Technology, Gliwice, Poland, in 2000 and 2012, respectively.

He is an associate professor at the Silesian University of Technology and teaches numerous courses, including systems dynamics, optimization methods, bioinformatics and mathematical modeling in biology and medicine. His research interests

include application of mathematical modeling and control theory methods to analysis of biological systems both at intracellular and cell population levels.

Dr. Smieja is an author and co-author of over 80 peer-reviewed publications and reviewer for many international scientific journals.



Krzysztof Puszyński received PhD and DSc (habilitation) degrees in biocybernetics and biomedical engineering respectively in 2009 and 2017 all from the Department of Automatic Control, Silesian University of Technology in Gliwice, Poland.

He is currently an assistant professor at the Silesian University of Technology. He has been on long-term scientific internships at Cross Cancer Institute University of Alberta in Edmonton, Canada and Istituto di Analisi dei Sistemi ed Informatica „Antonio Ruberti” in Rome, Italy. His research interests are in controlling intracellular processes, bioinformatics, biomedical modelling and control, therapy planning and mathematical epidemiology.

Dr. Puszyński is an author or co-author of around 82 papers in international and Polish journals and conferences proceedings as well as 1 monography entitled "system engineering approach to planning anticancer therapies" published by Springer. He is also a reviewer of 10 international journals from the JCR list.