Apoptotic Regulatory Module as Switched Control System
Analysis of Asymptotic Properties

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Abstract: Switching control systems are getting increased interests due to their capability to exhibit simultaneously several kinds of dynamic behaviour in different parts of the system. Such hybrid systems can be applied in many different fields. We present the application of the switched control systems in modelling a biological system, precisely a p53-dependent apoptotic intercellular pathway. Biological experiments show that cells exhibit variety of different behaviours for the same external stimuli. Differences in cell responses lead to population split into fractions. We present the analysis of asymptotic properties of the apoptotic regulatory module with respect to a parameter which describes an effect of an external stress. Results show that the system can exhibit two types of behaviour: stabilization or oscillation near the equilibrium point.

1 INTRODUCTION

Switched control systems are a class of systems that consist of several linear subsystems and set of switching rules among them. Such systems are characterized by different models in dependence on the state of the system. As a result even simple model can have various types of dynamic behaviour for specified states of the system, which can result in chaos or multiple limit cycles. On the other hand, switched systems are relatively easy to analyse due to its partially linear properties (Klamka et al., 2013).

Among many other applications of switched systems, they can be applied to modelling the biological processes (Swierniak and Klamka, 2014). Biological and biochemical reactions usually are not spontaneous but are regulated by variety of regulatory factors, and consequently the process rates are described by the step-like function. To retain the switching behaviour majority of biological models are highly nonlinear, which implies difficulties in their analysis. The piece-wise linear models are easier to create, because values of the parameters correspond directly to the observed processes rates. Moreover the switched systems enable analysis of the properties, which are compatible with biological observations. Comparison of the nonlinear model results and the ones from piecewise linear model shows that the basic dynamics is the same (Ochab et al., 2016). Switched systems can be efficiently applied to modelling biological gene-protein networks, systems with complex dynamics.

Apoptosis is intercellular process of programmed cell death. It occurs in every multicellular organism in damaged, defective or no longer required cells. One of the key players in the apoptotic response to the DNA damage is the protein p53, which activates production of proteins responsible for apoptosis. The proper activity of the apoptotic pathway is crucial for the whole organism, because it enables elimination of the damaged cells and prevents carcinogenesis (Vousden and Lu, 2002; Schmitt and Lowe, 1999). Huge interest of the protein p53 and its regulatory network among the researchers is a result of high contribution of the cell with its p53 abnormalities in the cancer cells.

The main activity of the p53 is regulation protein production by acting as transcription factor. In normal healthy cell the low p53 level is maintained by the negative coupling with MDM2. The p53 activates MDM2 production, which in turn induces p53 degradation. External stress, such as DNA damage, induces MDM2 degradation. Decrease of the MDM2 results in stabilization of p53 and activation of the proteins production which are responsible for cell cycle blockade, damaged DNA repair and apoptosis. Results of biological experiments show that, depending on the stress level, the p53 can be maintained on different levels (Kracikova et al., 2013). In case of low stress, the normal cell division is blocked by the medium level of the p53 and processes of DNA repair are ini-

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tiated. However in this state the p53 level is not high enough to induce the process of apoptosis. Top level of external stress activates the positive feedback loop, which works through protein PTEN. Protein PTEN is induced by p53, and after accumulation in cytoplasm is able to block the negative feedback by blocking the MDM2 transport to nucleus. As a result the p53 increases to high level and apoptosis is activated (Jonak et al., 2016). Due to the great significance of the p53-dependent apoptotic pathway a wide range of experiments, both biological and mathematical, are performed to acquire fully knowledge about this process.

In our previous research we have examined the system behaviour for three different stress levels: 0, 4 and 9 a.u. (Ochab et al., 2017a). In present paper we study possible system responses and thus we focus our attention on the asymptotic properties of the control system for the whole range of parameter R, which stands for a level of stress.

2 METHODS

2.1 PLDE Model Analysis

Piece-wise linear differential equation models (PLDE models) consist of set of linear differential functions and set of rules defining the subset of functions, which is used for a specified state. For biological systems the general equation is defined as:

$$\frac{dx_i}{dt} = p_i(X) - d_i(X)x_i, \quad i = 1,2,...,n \qquad (1)$$

where x_i is a protein, p_i is a production rate, d_i is a degradation rate and X is a state of the system described by the set of boolean functions. The phase space is divided by thresholds into regulatory domains. In each domain the system is described by a linear (affine) model. The boundaries of the domains are defined by threshold values denoted by θ_{ij}, where i is the variable (protein) and j is a number of the threshold for the given variable. On the boundaries, values of the parameters can switch and consequently the system is not continuous. Additionally the system contains switching variables, which define if the specific variable is above or below its threshold. The switching variables are boolean and are denoted by Z_i, where i indicates a number of the variable. The boolean variables are applied to define the regulatory domains. Each domain is defined by a boolean vector B which specifies relation of all the variables to its threshold. Moreover relation between variables and thresholds defines the values of the parameters in the model.

In the piece-wise linear models two types of stationary points are distinguished: regular stationary points and singular stationary points.

2.1.1 RSP

The regular stationary points, shortly RSPs, exist inside the regulatory domain. The RSPs are localized by application of steady state condition for each domain. If the calculated point lies inside boundaries of the considered domain, it is asymptotically stable and is called RSP. A method determining RSP can be described as follows:
for each subsystem described by linear equation model

- calculate steady state $dx_i/dt = 0$
- check localization of the point, if the point lies inside the boundaries, it is the RSP.

2.1.2 SSP

Localization of the singular stationary points, shortly SSPs, is much more challenging, because they exist when at least one of the variables lies on its threshold. In our analysis we use the method proposed for gene regulatory networks (Plahte et al., 1994; Mestl et al., 1995; Plahte et al., 1998). In the simplest case only one variable is equal to its threshold which means that the SSP is located on the boundary.

The SSPs can be described by a number of the variables which lie on the threshold. In the simplest case only one variable is located on the boundary and the other variable values are not restricted. It is worthy to notice that such case is possible only if the variable is directly dependent on its own threshold value. To be precise, the SSP lies on the threshold θ_{ij} for variable $x_i = \theta_{ij}$, if the derivative $\partial F_i/\partial Z_{ij}$ is greater than 0, where F_i is the time derivative dx_i/dt and Z_{ij} is switching variable.

The switched system is not continuous on the thresholds, so to calculate the derivatives, we replace the step function by the continuous sigmoid function with the limit [0,1]. Thus the switching function is a monotonic mollifier defined as $Z_{ij} = Z(x_i, \theta_{ij}, \delta)$:

$$Z_{ij} = \begin{cases} 0 & \text{for } x_i \leq \theta_{ij} - \delta, \\ \text{increases from 0 to 1} & \text{for } x_i \in \langle \theta_{ij} - \delta, \theta_{ij} + \delta \rangle \\ 1 & \text{for } x_i \geq \theta_{ij} + \delta \end{cases}$$

Parameter δ denotes the distance from the threshold, so with δ tending to zero, the monotonic mollifier approaches the step function.

In order to determine existence of the SSP in the case when two or more variables are equal to

their thresholds, we apply the procedure described by Mestl et al. (Mestl et al., 1995). For all sets of the threshold variables:

- replace values of the variables by corresponding thresholds
- determine values of the switching variables, which are not related to the analysed thresholds, as 0 or 1
- set time derivatives to 0
- solve the set of equations to calculate values of switching variables related to the analysed thresholds
- check assumptions: values of switching variables must be included in the range $[0,1]$ and values of remaining variables must be in the specified range, if yes, there is a SSP

In the system with SSP localized on the crossing of boundaries oscillations can be observed, because there exist a limit cycle around the SSP.

2.2 p53-Dependent Apoptotic Model

A piece-wise linear model of the p53 regulatory module was presented in our previous paper, where we compared its results with the results obtained by the nonlinear model (Ochab et al., 2016). The model consists of 4 variables, which correspond to different types of proteins: P - p53, C - cytoplasmic MDM2, N - nuclear MDM2, T - PTEN. Production of p53 (P) is constant and its degradation is increased by nuclear MDM2 (N). Production of cytoplasmic MDM2 (C) and PTEN (T) is induced by p53 (P). Nuclear MDM2 (N)results from nuclear import of the cytoplasmic form (C), which is regulated by T. Consequently in this system two feedback loops exist. The first one is negative and it exists between P, C and N, because p53 (P) induces production of its own inhibitor. The second one is positive, due to blockade of the negative feedback by PTEN (T), which is induced by p53 (P). All the dependencies between proteins are presented on the diagram (Fig. 1).

We introduce 4 threshold to divide phase space into regulatory domains. There is one threshold value for p53 in order to model the activation of MDM2 and PTEN production (parameters p_2^* and p_3^* respectively) by accumulated p53. One threshold for PTEN is used to model the blockade of the MDM2 transport to nucleus. PTEN level exceeding the threshold value signifies decrease of the MDM2 transport rate (k_1^*). Additionally there are two threshold values for nuclear MDM2, which separates the three levels of p53 degradation: low, medium and high. There are no threshold

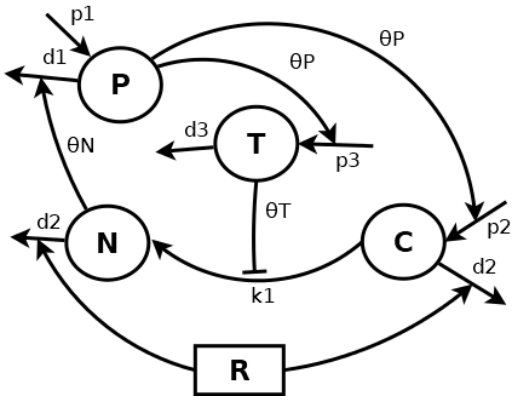

Figure 1: Model of the p53 regulatory core. Symbols of the variables, switching variables and model parameters are taken from the model (2).

for cytoplasmic MDM2, because none of the analysed processes are dependent on its level. Due to existence of four thresholds, the model contains four switching variables Z_P, Z_T, Z_{N1}, Z_{N2}, which define if the specific variable is above or below its threshold. Moreover each domain is defined by a vector $B = [P,N,T]$, where P, N and T are the boolean-like states denoting if the corresponding variable is below (0) or above (1 in case of P and T and 1 or 2 in case of N) its threshold. For example domain [110] denotes the subspace, where $P > \theta_P$, $\theta_{N1} < N < \theta_{N2}$ and $T < \theta_T$. Please, note that parameters which are dependent on the system state are marked with *.

The control system is linear, precisely affine, with a constant input. The general linear differential state equation can be written in the following form:

$$\dot{\mathbf{x}}(\mathbf{t}) = \mathbf{A}\mathbf{x}(t) + \mathbf{b}, \quad t \in [0, +\infty) \tag{2}$$

where the $\mathbf{x}(\mathbf{t}) \in R^n$ is a state vector, $\mathbf{A}$ is a given $n \times n$ - dimensional control matrix and $\mathbf{b} \in R^n$ is a constant input. They are defined below:

$$\mathbf{x}(t) = \begin{bmatrix} P(t) \\ C(t) \\ N(t) \\ T(t) \end{bmatrix} \qquad \mathbf{b} = \begin{bmatrix} p_1 \\ p_2^* \\ 0 \\ p_3^* \end{bmatrix}.$$

Moreover,

$$\mathbf{A} = \begin{bmatrix} -d_1^* & 0 & 0 & 0 \\ 0 & -(k_1^* + d_2(1+R)) & 0 & 0 \\ 0 & k_1^* & -d_2(1+R) & 0 \\ 0 & 0 & 0 & -d_3 \end{bmatrix}$$

where

$$\begin{aligned} p_2^* &= p_{20} + p_{21} Z_P \\ p_3^* &= p_{30} + p_{31} Z_P \\ d_1^* &= d_{10} + d_{11} Z_{N1} + d_{12} Z_{N2} \\ k_1^* &= k_{10} - k_{11} Z_T \end{aligned}$$

$$Z_P = \begin{cases} 0 & \text{if } P < \theta_P \\ 1 & \text{if } P > \theta_P \end{cases}$$

$$Z_{N1} = \begin{cases} 0 & \text{if } N < \theta_{N1} \\ 1 & \text{if } N > \theta_{N1} \end{cases}$$

$$Z_{N2} = \begin{cases} 0 & \text{if } N < \theta_{N2} \\ 1 & \text{if } N > \theta_{N2} \end{cases}$$

$$Z_T = \begin{cases} 0 & \text{if } T < \theta_T \\ 1 & \text{if } T > \theta_T \end{cases}$$

The degradation of the MDM2, both cytoplasmic and nuclear, is increased by stress factor denoted by R. The values of the parameters given above are presented in Table 1 and the values of threshold are presented in Table 2 Values of the parameters base on our previous research (Ochab et al., 2016) and the biological results (Jonak et al., 2016).

3 RESULTS

As we showed in our previous paper (Ochab et al., 2017a), for different size of stress - reflected by different values of the parameter R, the system response can be significantly different. In order to check all possible realizations of the system for whole range of stress, we examine existence of the different RSPs and SSPs for $R \in [0, +\infty\rangle$. The system behaviour for specified values of R can be visualized by the transition diagram. The domains are marked by the rectangles with vectors determining their states. The arrows show the directions of the transitions between domains. Transition diagrams show in which domain the RSPs exist and are useful in localizing the closed sequences between domains, which can contain the SSPs around which the systems response can oscillate. The simplest way to create transition diagram is calculation of the RSP in each domain and determine whether the solution stay in the analysed domain or move to another. Exemplary, for stress $R = 5$, there is one RSP in the system in the domain [101] and one possible limit cycle between domains [010], [110], [120] and [020] (Fig. 2).

3.1 Regular Stationary Point - RSP

In this section we determine ranges of the R in which the RSP exists in any domain. For each domain we write the model equations and equal them to 0. Then we solve the system of equation to find values of R, which assures that the stationary point lays inside analysed domain. In the apoptotic switched model the regular stationary points can exist only in 3 domains. The RSPs exist:

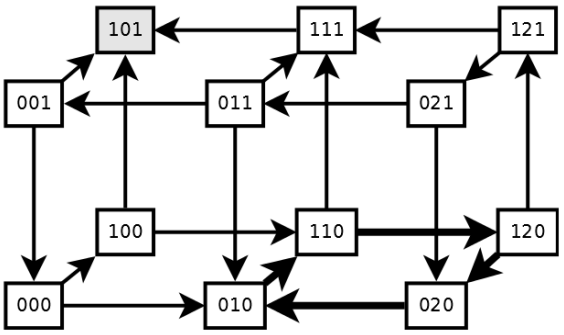

Figure 2: State transition diagram for the apoptotic model for $R = 5$. Gray domain contains RSP, bold arrows emphasize the closed sequence around the SSP.

- in domain [020] for $R \in [0, 0.8635\rangle$
- in domain [111] for $R \in \langle 1.0322, 1.9154\rangle$
- in domain [101] for $R \in \langle 1.9154, +\infty\rangle$.

The values of the variable P in the steady states in each domain are presented in table 3. For $R \in [0.8635, 1.0322\rangle$ there is no RSP in the system, so the system response does not stabilize on one value but oscillates in a limit cycle.

The regular stationary points correspond to the asymptotically stable cell response in different cases. For low stress the RSP exists in domain [020] and corresponds to the low p53 level and the high nuclear MDM2 level, which agrees with biological observation of normal cells, where low p53 level is maintained by high degradation rate and, in turn by high MDM2 level. An increase of the parameter R results in disappearing of the RSP in domain [020] and arising in domain [111] and afterwards in [101]. The increase of the R corresponds to higher external stress level, which induces cell damages, the MDM2 degradation and consequently accumulation of the p53 (see Table 3 with increasing values of p53 in stationary points).

In biological experiments after high stress level the increased p53 level is observed. The p53 level in cell determines the cell response, the medium average p53 level can be assigned to the cells with repairable damages and excluded proliferation, whereas the high p53 level indicates cells with unrepairable damages and apoptosis activation.

3.2 Singular Stationary Point - SSP

In the apoptotic model thresholds exist for 3 variables: P, N and T, and consequently, the SSP can exist in 3 types of subspaces: on the plane where one

Table 1: The values of the model parameters.

Parameter	Description	Value	Unit
p_1	spontaneous P production rate	8.8	1/sec
p_{20}	spontaneous C production rate	2.4	1/sec
p_{21}	P-induced C production rate	21.6	1/sec
p_{30}	spontaneous T production rate	0.5172	1/sec
p_{31}	P-induced T production rate	3.6204	1/sec
d_{10}	spontaneous P degradation rate	$9.8395 * 10^{-5}$	1/sec
d_{11}	N-induced P degradation rate	$6.5435 * 10^{-5}$	1/sec
d_{12}	N-induced P degradation rate	$1.6283 * 10^{-4}$	1/sec
d_2	spontaneous C and N degradation rate	$1.375 * 10^{-5}$	1/sec
d_3	spontaneous T degradation rate	$3 * 10^{-5}$	1/sec
k_{10}	spontaneous N transport rate	$1.5 * 10^{-4}$	1/sec
k_{11}	T-inhibited N transport rate	$1.4713 * 10^{-4}$	1/sec

Table 2: The values of the thresholds for variables.

Thresholds	Description	Value	Unit
θ_P	P threshold value	$4.5 * 10^4$	molecules
θ_{N1}	1^{st} N threshold value	$4 * 10^4$	molecules
θ_{N2}	2^{nd} N threshold value	$8 * 10^4$	molecules
θ_T	T threshold value	$1 * 10^5$	molecules

Table 3: Values of p53 (P) in regular stationary point for different values of stress R.

R	Domain	P_s
0 - 0.8635	[020]	$3.3559 \cdot 10^4$
1.0322 - 1.9154	[111]	$5.3714 \cdot 10^4$
1.9154 - $+\infty$	[101]	$8.9435 \cdot 10^4$

variable lies on its threshold, on the crossing of the planes, where two variables lie on thresholds, and on the point, where all three variables lie on their thresholds.

In order to check if the model can contain the SSP on the plane, we calculate the derivatives of all the variables with respect to its switching variables $\partial F_i/\partial Z_{ij}$. In this model partial derivatives for all the variables P, C, N and T do not directly depend on their switching variables Z_{ij} so all $\partial F_i/\partial Z_{ij}$ are equal to zero. Consequently in this system there is no SSP on the surface of the single boundary.

To determine an existence of the SSP on the crossing of two boundaries we analyse all the existed cases. For all combinations of two thresholds from θ_P, θ_T and θ_{N1} or θ_{N2} the procedure described in section 2.1.2 was applied with attitude to determine values of the stress R, for which the assumptions are satisfied. For the whole range of the parameter R, the SSP can exist only in two subspaces.

3.2.1 SSP 1: [θ_P, θ_{N2}, $T < \theta_T$]

The SSP exists on the crossing of the threshold of the p53 and the MDM2, precisely for P equal to θ_P, N equal to θ_{N2} and T smaller than θ_T. The model equations describing the steady state in this subspace are as follows:

$$\begin{aligned} 0 &= p_1 - (d_{10} + d_{12}Z_{N2})\theta_P, \\ 0 &= p_{20} + p_{21}Z_P - (k_{10} + d_2(1+R))C, \\ 0 &= k_{10}C - d_2(1+R)\theta_{N2}, \\ 0 &= p_{30} + p_{31}Z_P - d_3\ T. \end{aligned} \tag{3}$$

The switching parameters Z_P and Z_{N2} are in the range $[0,1]$ and the value of the T variable is smaller than θ_T for $R \in \langle 0.8635, 7.7038 \rangle$.

3.2.2 SSP 2: [θ_P, θ_{N2}, $T > \theta_T$]

The second SSP in the system exists for P equal to θ_P, N equal to θ_{N2} and T greater than θ_T. The model equations describing the steady state are presented below:

$$\begin{aligned} 0 &= p_1 - (d_{10} + d_{12}Z_{N2})\theta_P, \\ 0 &= p_{20} + p_{21}Z_P - (k_{10} - k_{11} + d_2(1+R))C, \\ 0 &= (k_{10} - k_{11})C - d_2(1+R)\theta_{N2}, \\ 0 &= p_{30} + p_{31}Z_P - d_3\ T. \end{aligned} \tag{4}$$

The switching parameters Z_P and Z_{N2} are in the range $[0,1]$ and the value of the T variable is greater than θ_T for $R \in \langle 0.7059, 1.0322 \rangle$.

The last possible case, is localization of the SSP in the crossing of three boundaries. In this model two such points exist and should be analyzed: [θ_P, θ_{N1}, θ_T] and [θ_P, θ_{N2}, θ_T]. In both cases the values of the switching variables Z_P, Z_{N1} (or Z_{N2}) and Z_T are not included in the range $[0,1]$ so independently of the parameter R, the SSP cannot exist there.

Due to an existence of the SSPs in two subspaces, two types of oscillations can be observed. Significantly wider range of the parameter R have the SSP in the region [θ_P, θ_{N2}, $T < \theta_T$]. This SSP results in undamped oscillations between low and high p53 level and medium and high MDM2 level. Such oscillations are a consequence of the negative feedback loop, and are related to the delayed cell response in case of the repairable damages. Biological experiments show that for the low stress level a cell makes an attempt to repair its damages and comes back to the normal state. The oscillation of the p53 level prevents cell division but in the same time does not induce cell elimination (Geva-Zatorsky et al., 2006; Bar-Or et al., 2000). Consequently if the damages are not unrepairable, the cell has got time to come back to the normal state.

The second SSP indicates an existence of the cycle between the p53 and the MDM2 for high PTEN level. Such situation occurs only for a very narrow range of R parameters. This case is possible for low external stress, when the initial high PTEN level is maintained over the θ_T threshold by oscillation between low and high p53 level. However in biological cells high PTEN level is observed only after p53 activation, so such situation is not probable concluding from the biological results.

3.3 Bifurcation Diagram

Depending on the R parameter value, the stationary points exist in different domains or on the different boundaries. For small R the RSP exists in domain [020] and the P level is low. With increase of the R value, in the system the second stationary point, SSP, appears. Depending on the initial conditions the system can stabilize in the domain [020] or oscillate between domains with low and high P and medium and high N. With further increase of the R, in the system any RSP does not exist, so the only possible system response is oscillation. In quite narrow range of the R parameter values, in the system two singular stationary points exist, which results in two different cycles and different levels of proteins. With further increase of R, in the system the RSP in the domain [111] arises, which is characterized by the medium P level. For R greater than 1.9154 in the system the RSP arises in the domain [101] with the high P level. The SSP exists in the system for R smaller than 7.7038 which means, that depending on the initial conditions, in the system two types of response can be observed: stabilization or oscillations (see Fig. 3).

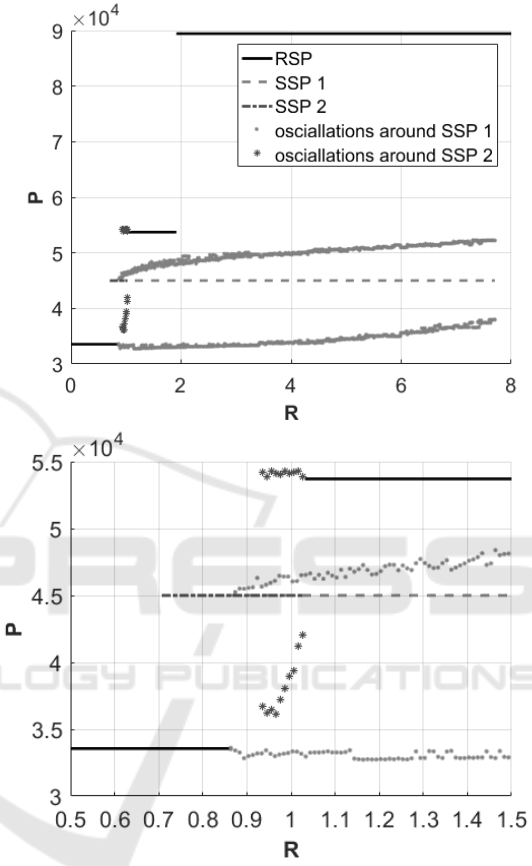

Figure 3: Level of the variable P in dependency on the parameter R. Top figure: results for the whole range of R. Bottom figure: magnification of the figure for $R = \langle 0.51.3 \rangle$. SSP 1 - values of P in the SSP with $T < \theta_T$, SSP 2 - values of P in the SSP with $T > \theta_T$. Please notice the lack of oscillations around SSP 2 for $R \in \langle 0.8635, 0.95 \rangle$ - see text for explanation.

The dependency between variables N and R is presented on the Fig. 4. With increase of R, the N level is decreased. For a wide range of the R parameter, in the system two types of result exists, stabilization in the steady state or oscillation of the protein levels, which are a consequence of the simultaneous existence of the two stationary points: SSP and RSP.

Notice the lack of oscillations around SSP for $R \in \langle 0.8635, 0.95 \rangle$ on Fig. 3 (bottom panel). It is caused by the close proximity of the SSP to the θ_T boundary which causes that the trajectories escape from the

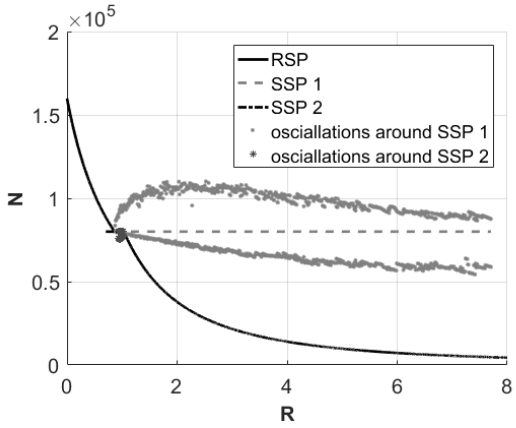

Figure 4: Level of the variable N in dependency on the parameter R. SSP 1 - values of N in the SSP with $T < \theta_T$, SSP 2 - values of N in the SSP with $T > \theta_T$.

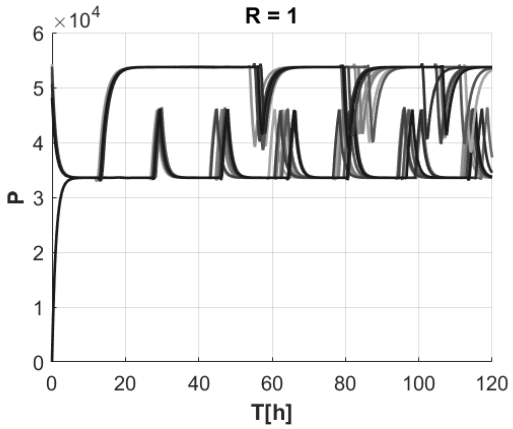

Figure 5: Time course of the variable P for different initial conditions for value of parameter R equals 1.

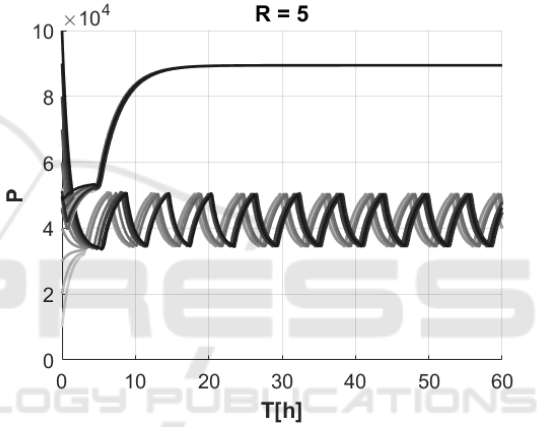

Figure 6: Time course of the variable P for different initial conditions for value of parameter R equals 5.

oscillatory mode. With the increasing R, SSP recedes from the θ_T boundary and with R greater than 0.95, trajectories do not cross the threshold θ_T thus oscillations appear. Nevertheless the presented method is unable to detect analytically this phenomenon which is its weakness.

More generally the weakness of the presented method is impossibility to analytically determine the attraction pools for the calculated stationary points. The numerical simulation for different initial conditions shows, that in the case of $[\theta_P, \theta_{N2}, T > \theta_T]$ the attraction pool is very small.

3.4 Numerical Results

To testify the achieved results we calculated the system response for two values of parameter R for different initial conditions. For R equals 1, in the system two SSPs exist and consequently on the time courses the two types of oscillations are observed. Interestingly both SSPs are on the same borders, precisely for subspaces with $P = \theta_P$ and $N = \theta_{N2}$, but one point is under θ_T threshold and the second one is above. Consequently, even if the values of the variable P in the stationary points are the same, the time courses are significantly different (Fig. 5). For the higher value of R, in the system one RSP and one SSP exist. Consequently, depending on the initial conditions, the system can approach the steady point in the domain [101] or oscillate in the limit cycle around the singular stationary point (Fig. 6). In the case of modelling cell population using stochastic approach, different types of responses are received. Consequently the cell population is divided into several fractions, which present different behaviours (Ochab et al., 2017b).

4 CONCLUSIONS

Analysis of the switched system demonstrate the properties of the apoptotic intercellular pathway. The localization and the types of the existing stationary points correspond with the biological results. The presented method can be efficiently applied to piecewise linear systems to examine properties of the protein regulatory networks, nevertheless the results show that this method is not free from imperfections. Lack of the analysis of the attraction pools can lead to false determination of the system behaviour based on existence of the singular stationary points without attraction pools. In the future we want to overcome this difficulty and improve the proposed methodology.

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