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APPLICATION OF THE PIECE-WISE LINEAR MODELS FOR DESCRIPTION OF NONLINEAR BIOLOGICAL SYSTEMS BASED ON P53 REGULATORY UNIT

Magdalena Ochab[1], **Krzysztof Puszynski**[2], **Andrzej Swierniak**[3]

Institute of Automatic Control, Silesian University of Technology
ul. Akademicka 16, 44-100 Gliwice
[1]magdalena.ochab@polsl.pl, [2]krzysztof.puszynski@polsl.pl
[3]andrzej.swierniak@polsl.pl

ABSTRACT

We consider the application of the piece-wise linear system methodology for modelling biological processes. One of the most important cellular process is apoptosis - programmed cell death, which is regulated by protein p53. Due to high level of complexity the regulatory core of the protein p53 usually is modelled using nonlinear models, which are difficult in further analysis. Presented approach enables obtaining the biologically justified results, similar to those published elsewhere and in the same time creates opportunities for finding analytical solutions and performing further analysis.

INTRODUCTION

Biological processes, due to high complexity, are usually described by nonlinear models. However finding analytical solutions of such models is difficult and in most cases near to impossible. Thus nonlinear models can be solved only by numerical simulation. Additionally stability or sensitivity analysis of such models are very difficult. Taking into account all this disadvantages, we propose to model biological process by linear models with switchings. The theoretical basis of this method was presented in 1995 by Mestl et al. [2]. This method based on assumption that the interaction between variables can be represented by an operation on threshold functions. Nonlinearities in biological models often can be modelled by sigmoid functions, because the processes are activated after exceeding the threshold value of concentration of some proteins. In this method such nonlinearities are replaced by step functions $X_{ij} = X(x_i, \theta_{ij})$, where $X_{ij} = 0$ when $x_i < \theta_{ij}$, $X_{ij} = 1$ when $x_i \geq \theta_{ij}$. In the case of nonlinearities such as square nonlinearities, introduction of two or more threshold is necessary to get the proper dynamic of the system. The phase space is divided into rectangular, open and not necessarily closed boxes by thresholds for given variables. Within each box, the model is described by the system of linear differential equations.

Protein p53 due to his function in activation cell response after stress is described as 'the guardian of the genome'. After cellular stress such as DNA damage, p53 activates processes of cell cycle arrest, DNA repair and in case of irreparable DNA, it initiate apoptosis - programmed cell death [3]. The regulatory domain of p53 consists of two feedback loops. The negative feedback loop includes protein MDM2. Transcription of MDM2 is initiated by protein p53, and degradation of p53 is induced by MDM2[4]. The positive feedback loop includes protein PTEN, production

of which is activated by protein p53. PTEN regulate MDM2 transport from cytoplasm to nucleus, and in high concentration PTEN prevents p53 degradation by retaining MDM2 in cytoplasm [5].

In this work we investigate the possibility to transform/approximate the strongly nonlinear biological system to their simpler piece-wise linear models with switchings. Both models present only the main core of regulatory p53 domain so they consist of 4 variables: p53 (P), cytoplasmic MDM2 (C), nuclear MDM2 (N) and PTEN (T) and include positive and negative feedback loops. The symbol R stands for ionizing radiation which is input to the system. The structure of the models is presented in Fig. 1.

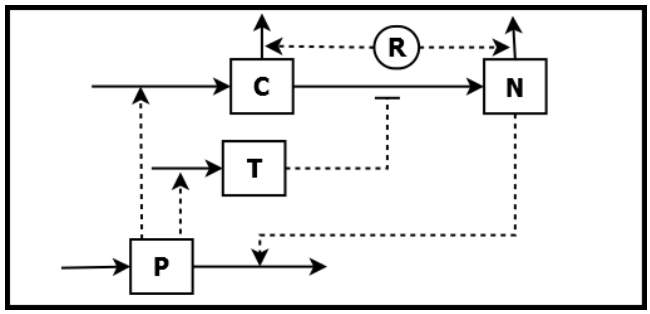

Figure 1. The p53 regulatory core: negative feedback loop works as P induces production of C which after modification to N degrades P; positive feedback loop works by negation of negative feedback loop - P induces production of T, which blockades conversion C to N and thus degradation of P.

NONLINEAR MODEL OF P53 REGULATORY UNIT

The nonlinear model of p53 regulatory was presented by Kozlowska and Puszynski ([1] - to appear). This model includes very strong nonlinearities to present oscillatory behaviour and bistability. The model equations are presented below. The parameters and their values are presented in the Table 1.

$$\frac{dP}{dt} = p_1 - d_1 \ N^2 \ P, \tag{1}$$

$$\frac{dC}{dt} = p_2 \ \frac{P^4}{P^4 + k_2^4} - k_1 \ \frac{k_3^2}{k_3^2 + T^2} \ C - d_2 \ (1+R) \ C, \tag{2}$$

$$\frac{dN}{dt} = k_1 \ \frac{k_3^2}{k_3^2 + T^2} \ C - d_2 \ (1+R) \ N, \tag{3}$$

$$\frac{dT}{dt} = p_3 \ \frac{P^4}{P^4 + k_2^4} - d_3 \ T. \tag{4}$$

PIECE-WISE LINEAR MODEL OF P53 REGULATORY UNIT

We created model of regulatory p53 module using piece-wise linear system. The preliminary version of this model was presented in the conference material [6]. We have improved it by fitting parameter values that allows to achieve more accurate behaviour of the model. The state space is divided by threshold values of variables into domains. Each domain includes a different version of our linear model, described by various parameters and the system is "switching" from one model to other. The differential equations of the linear models are as follows:

Table 1. Nonlinear model parameters

Parameter	Description	Value
Parameters for both models		
p_1	p53 production rate	8.8
d_2	MDM2 degradation rate	1.375×10^{-5}
d_3	PTEN degradation rate	3×10^{-5}
R	Ionising radiation	4 or 9
Nonlinear model parameters		
d_1	p53 degradation rate	1.375×10^{-14}
p_2	MDM2 production rate	440
p_3	PTEN production rate	100
k_1	MDM2 transport rate	1.925×10^{-4}
k_2	p53-dependent genes activation coefficient	1×10^5
k_3	PTEN-dependent MDM2 transport coefficient	1.5×10^5

$$\frac{dP}{dt} = p_1 - d_1^K(N)\ P, \tag{5}$$

$$\frac{dC}{dt} = p_2^K(P) - k_1^K(T)\ C - d_2\ (1+R)\ C, \tag{6}$$

$$\frac{dN}{dt} = k_1^K(T)\ C - d_2\ (1+R)\ N, \tag{7}$$

$$\frac{dT}{dt} = p_3^K(P) - d_3\ T. \tag{8}$$

Using the assumptions presented by Mestl [2], we determine the parameters which should change in step-like way according to the variables values. The MDM2 and PTEN production rate is directly dependent on the protein p53 concentration which activate its transcription. After crossing the threshold value for p53, the MDM2 and PTEN production rates increase. Similarly we introduce the threshold value for the protein PTEN. After crossing the threshold, the rate of MDM2 transport from cytoplasm to nucleus is dramatically decreased due to PTEN activity as a controller of MDM2 transport. Taking into account unsaturated character of p53 degradation induced by protein MDM2, we decided to determine two threshold values for MDM2-dependent p53 degradation. In normal cell state, nuclear MDM2 level is high so the p53 degradation is rapid. After stress MDM2 level is decreased so the degradation of p53 slows down. For very low MDM2 level degradation of p53 is even slower. The threshold variable values divide state space into domains (Fig.2). The Tab. 2. contains the values of the parameters of the linear models, the rules of the parameter changes and the thresholds for the variables.

RESULT

Using created models we simulate the cell response after ionizing radiations. The ionizing radiation induce DNA damage and the rapid degradation of the MDM2 so it is modelled by increasing the MDM2 degradation rate. The radiation is added in two different dose: 4 Gy and 9 Gy.

In the nonlinear model, after irradiation with 4 Gy dose, we observe decrease of MDM2, which induces increase of protein p53 level. The system after 50 hours of oscillations stabilises on the medium increased p53 level (Fig. 3A). The higher radiation dose induces bigger and more rapid MDM2 degradation. In the early stage of the response the protein levels oscillate. After approximately 15 hours the high increase of PTEN is observed. PTEN in high concentration

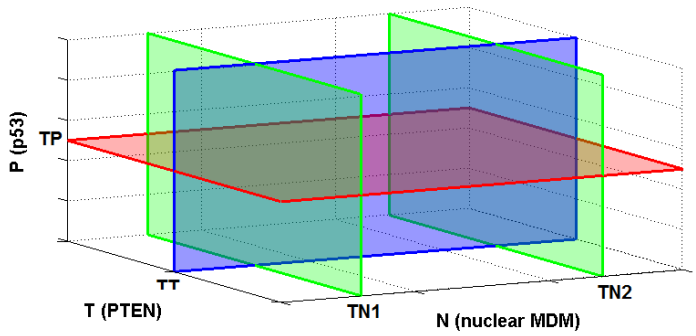

Figure 2. The division of state space into domains

Table 2. Linear model parameters and threshold values

Threshold values			
Parameter	Description	Value	
TN_1	$1st$ nuclear MDM2 threshold	4×10^4	
TN_2	$2nd$ nuclear MDM2 threshold	8×10^4	
TP	p53 threshold	4.5×10^4	
TT	PTEN threshold	1×10^5	
linear model parameters			
Parameter	Description	Condition	Value
d_1^K	cumulative p53 degradation rate	$N(t) < TN_1$ $TN_1 \leq N(t) < TN_2$ $TN_2 \leq N(t)$	9.8295×10^{-5} 1.6383×10^{-4} 3.2765×10^{-4}
p_2^K	cumulative MDM2 production rate	$P(t) < TP$ $TP \leq P(t)$	2.4 24
p_3^K	cumulative PTEN production rate	$P(t) < TP$ $TP \leq P(t)$	0.5172 4.1376
k_1^K	cumulative MDM2 transport rate	$T(t) < TT$ $TT \leq T(t)$	1.5×10^{-4} 2.8667×10^{-6}

block transport of MDM2 to nucleus so the degradation of protein p53 is decreased. High p53 level indicates the activation of cells apoptosis in such case (Fig. 3.B).

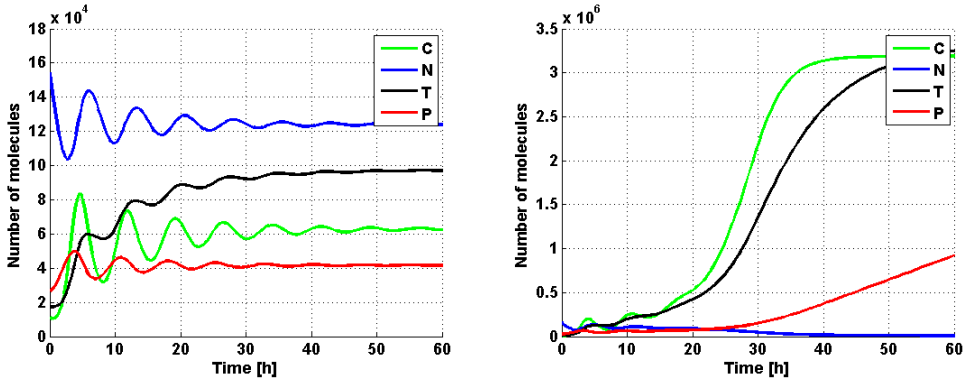

Figure 3. Proteins level in nonlinear model after irradiation. A. Irradiation 4 Gy. B. Irradiation 9 Gy.

In the piece-wise linear model the 4 Gy irradiation induces decrease of nuclear MDM2 under the first threshold, which results in decreased p53 degradation. Increase of protein p53 level under assumed threshold induces increase production of MDM2 and PTEN. The increase of protein MDM2 over threshold results in return to high p53 degradation rate followed by lower Mdm2 and PTEN production which closes the cycle. The step switches of parameter values are presented in Fig. 4C. The regular parameter switches result in oscillation in proteins levels (Figure 4A). After 9 Gy dose of the irradiation in the cells very high MDM2 decrease occurs. Increase of p53 protein level induces production of MDM2 and PTEN, and, similarly to response for 4 Gy, in the system recurrent changes of parameters values can be observed. Due to long time of high p53 level, concentration of PTEN increases over the threshold for MDM2 transport blockade. 20 hours after irradiation, the MDM2 transport to nucleus is significantly diminished so the protein MDM2 in nucleus decreases under the second threshold (Figure 4D). The protein p53 degradation is very low and its level grows to high value and stabilizes. In such cells the apoptotic decision is activated (Figure 4B).

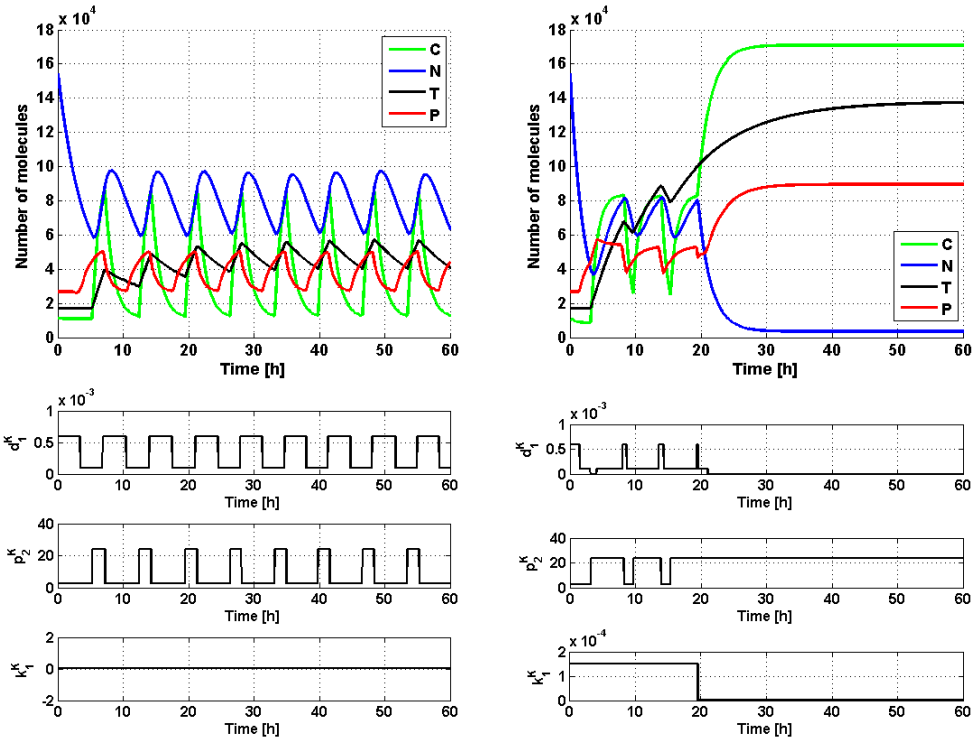

Figure 4. Proteins level in linear model with switchings after irradiation. A. Irradiation 4Gy. B. Irradiation 9 Gy. C. Switchings of parameters after irradiation 4Gy. D. Switchings of parameters after irradiation 4Gy

CONCLUSION

In this work we show that the piece-wise linear models can be used to simplify even strongly nonlinear biological systems. This allows us to take an advantage of linear systems features such as possibility to getting the analytical solutions and further analysis. Because the models are very simplified the presented time courses of the systems are only approximation of the presented in real cells. Nevertheless the main system dynamics is still maintained. We still have stable equilibrium points; first without any stimuli in which cells proliferate and second, in which cell

dies due to apoptosis. Between them we have third equilibrium around which cells oscillate trying to repair they damage. We have checked the cell responses for two different doses of irradiation: 4 Gy and 9 Gy. In both models 4 Gy irradiation dose induces the oscillations of protein levels. This oscillations are the results of existence of negative feedback loop between p53 and MDM2. In the early response after 9 Gy irradiation in the systems the oscillations of the proteins levels occur. After 9 Gy dose the decrease of protein MDM2 is bigger than decrease after 4 Gy, so the protein p53 level is higher and the production of protein PTEN ran with higher rate. High protein PTEN level activates the positive feedback loop: it induces blockade of MDM2 transport to the nucleus, so the degradation of p53 decrease to very low values. Therefore the high p53 level can be maintain by time long enough to active apoptosis.

The results of these two model simulations differ, however the general response is the same. Both models are very simple, so they can only approximately present the cellular behaviour. Anyway the main and the most important features of this system in both cases were presented properly: oscillations and the bistability of the model due to the negative feedback loop and the ability to maintain high p53 level due to the positive feedback loop. Both of the models have some restrictions. In the nonlinear model the proteins cytoplasmatic MDM2 and PTEN increase to very high concentrations, which cannot be obtained in the living cells (Figure 3B). In the linear model, the step parameter switch induces very rapid, not real changes in proteins concentrations (Figure 3C).

Both models are very useful for modelling p53 regulatory core. However the linear model with switchings has got a very significant advantage. We can found the analytical solution for the linear model, so we need not to solve it numerically. Additionally, we can easily analyse the property of such system, like stability, sensitivity or controllability.

The main problem of biological models are determination of the parameter values. In nonlinear models determination of the parameters of nonlinear functions is necessary, even if their biological meaning is difficult to define (such as parameters k_1 and k_2 in first model). On the other hand in the piece-wise systems we need to determine the threshold for variables. The biological experiment usually does not determine exact proteins numbers but justifies the use of the approximate description such as low/middle/high protein level. Additionally, the rate of many processes is described by the sigmoid function because after crossing the minimal number of proteins needed for activation, the process run with its characteristic rate. For many processes it is reasonable to create only one threshold - a boundary between low and high protein level and the activated and inactivated process. In some cases, such as square function of MDM2-induced p53 degradation, using bigger number of threshold is justified. Number of the introduced threshold must be a compromise between the accuracy of the model and the quantity of the parameters needed to be determined.

ACKNOWLEDGEMENTS

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