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CHANGE DETECTION IN THE DYNAMICS OF AN INTRACELLULAR PROTEIN SYNTHESIS MODEL USING NONLINEAR KALMAN FILTERING

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Abstract. A method for early diagnosis of parametric changes in intracellular protein synthesis models (e.g. the p53 protein - mdm2 inhibitor model) is developed with the use of a nonlinear Kalman Filtering approach (Derivative-free nonlinear Kalman Filter) and of statistical change detection methods. The intracellular protein synthesis dynamic model is described by a set of coupled nonlinear differential equations. It is shown that such a dynamical system satisfies differential flatness properties and this allows to transform it, through a change of variables (diffeomorphism), to the so-called linear canonical form. For the linearized equivalent of the dynamical system, state estimation can be performed using the Kalman Filter recursion. Moreover, by applying an inverse transformation based on the previous diffeomorphism it becomes also possible to obtain estimates of the state variables of the initial nonlinear model. By comparing the output of the Kalman Filter (which is assumed to correspond to the undistorted dynamical model) with measurements obtained from the monitored protein synthesis system, a sequence of differences (residuals) is obtained. The statistical processing of the residuals with the use of χ^2 change detection tests, can provide indication within specific confidence intervals about parametric changes in the considered biological system and consequently indications about the appearance of specific diseases (e.g. malignancies)

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1. **Introduction.** The paper studies the problem of parametric change detection in intracellular protein synthesis models, such as the model describing the p53 protein - mdm2 inhibitor dynamics. The $P53$ protein is of major importance for preventing the development of tumors since it enhances cell-cycle arrest and apoptosis. The concentration of the $P53$ protein in the cytoplasm is primarily controlled by another protein, known as inhibitor protein $mdm2$ within a feedback loop. The raise of the concentration of the MDM2 protein, causes the drop of the concentration of the P53 protein (downregulation). The deactivation (dissociation) of P53 is due to the ubiquitin molecules which the mdm2 binds to P53. Inversely, whenever the concentration of $P53$ increases, a transcription (synthesis) procedure for $mdm2$ is activated and consequently the levels of the MDM2 protein start to rise. By enhancing the concentration of mdm2, the concentration of $P53$ drops. This balancing procedure takes the form of a feedback loop, while it can be shown that a fixed point (equilibrium) for the $p53$-$mdm2$ dynamical system exists [14],[21],[25],[26], [41],[46]. A recent approach to chemotherapy has been to use drugs (such as Nutlins) that work by annihilating the $MDM2$ protein and consequently by blocking the disintegration effects that the $MDM2$ protein has on the $P53$ protein (ubiquitination), [1],[7], [9],[44]. This enables in turn a raise in the levels of the $P53$ protein and finally results in restraining the proliferation of the cancer cells [11],[13], [15],[24].

It has been shown that control of the levels of the concentration of the P53 protein, and in general of biological oscillators, can be succeeded by nonlinear feedback control schemes such as the ones based on differential flatness theory [5], [33], [35], [36], [34]. The control input is taken to be the infusion rate of the chemotherapy drug. In particular, about the P53 protein, its pharmacokinetics-pharmacodynamics is described by a complicated set of nonlinear differential equations. By applying differential flatness theory it is possible to transform this complicated model into the canonical Brunovsky form [3],[8], [16], [17], [22], [40], [38], [37]. In this latter form a single-input single-output description between the output (P53 protein) and the input (drug's infusion rate) is obtained. This permits the design of a feedback control law that can make the P53 protein concentration converge to the desirable levels.

Moreover, through the application of nonlinear estimation (identification) methods it has become possible to obtain numerical values for the parameters of the p53 protein - mdm2 inhibitor system [6], [19], [20], [23], [27], [43], [45], [49]. However, the parameters of such a model are subjected to uncertainties and parametric changes. Actually, the deviation of the protein synthesis model parameters from their nominal values is associated with deregulation of the cells population and is likely to provoke the appearance of malignancies. To detect pathological symptoms in the p53 protein synthesis the system's dynamics is emulated with the use of nonlinear Kalman Filtering. This model is parameterized with the nominal values which are associated with the system's normal condition [28], [29], [30]. The considered filtering approach is the Derivative-free nonlinear Kalman Filter [29], [33]. This consists of the application of the standard Kalman Filter recursion on the linearized equivalent of the protein synthesis system which has been obtained after applying differential flatness theory. Moreover, the filter makes use of an inverse transformation based again on differential flatness theory, so as to obtain estimates of the state variables of the initial nonlinear system.

Next, two sequences of data are generated. The first sequence consists of real measurements of the $P53^*$ protein concentration with are obtained at specific sampling instances. The second sequence is the Kalman Filter's output, again sampled at the same time instances. By comparing the two signals, a residuals (estimation error) sequence is obtained. The processing of the residuals with the use of statistical decision making criteria provides an indication about the existence of parametric changes (damages) in the p53-mdm2 protein synthesis model, which otherwise could not have been detected [2],[32]. Thus, by applying fault detection tests based on the χ^2 distribution it can be concluded if the p53 protein-mdm2 inhibitor system remains healthy and if the nominal parameter values for its model still hold [4],[18],[42]. Otherwise, a failure can be detected.

The structure of the paper is as follows: in Section 2 the dynamic model of the p53 protein - mdm2 inhibitor is analyzed and the associated differential equations are formulated. In Section 3 nonlinear feedback control of the p53 protein synthesis model is developed with the use of differential flatness theory. In Section 4 it is explained how parametric change detection in the aforementioned protein synthesis model can be succeeded with the use of statistical criteria, such as the χ^2 test. In Section 5 simulation tests are performed to show how the proposed change detection test succeeds to diagnose the existence of parametric variations in the protein synthesis model. Finally, in Section 6 concluding remarks are stated.

2. Dynamic model of the p53 protein - mdm2 inhibitor system.

2.1. Feedback control loops in the $p53$ protein - $mdm2$ inhibitor system.

Feedback control loops are widely met in intracellular protein synthesis processes and govern cellular dynamics [39]. The associated models are described by nonlinear differential equations, in certain cases with the appearance of time delays [10]. As mentioned, the concentration of the P53 protein is mainly controlled by the levels of the mdm2 protein within a negative feedback loop. The synthesis of the P53 protein is also affected by the ATM, ARF and E2F1 proteins through secondary feedback loops. The dynamic model of the p53 protein - mdm2 inhibitor system is described by Fig. 1. The meaning of the variables that appear in the p53 protein - mdm2 inhibitor dynamical system is as follows [7], [14], [15], [25]:

$p53$: mRNA concentration of the p53 gene after transcription, $P53$: concentration of the P53 protein in the cytoplasm after translation, $P53^*$: active form of the P53 protein that is produced after phosphorylation of P53, $mdm2$: mRNA concentration of the inhibitor protein mdm2 after transcription, $MDM2$: concentration of the MDM2 protein in the cytoplasm after translation, N: concentration of the chemotherapeutic drug, ATM: a protein that identifies the transcription of p53 and contributes to the phosphorylation of the P53 protein, ATM^*: concentration of the active form of the ATM protein. It contributes both to the phosphorylation of protein $P53$ and of protein $MDM2$, $e2f1$: mRNA concentration of the gene $e2f1$ after transcription, $E2F1$: concentration of the protein $E2F1$ after translation, $E2F1^*$: active form of the $E2F1$ protein, arf: mRNA concentration of the gene arf after transcription, ARF: concentration of the ARF protein after translation.

The basic feedback loop is that of the synthesis of the P53 protein under the inhibitor protein MDM2. When the concentration of the MDM2 protein increases, the concentration of the P53 protein is reduced (downregulation). This process is also known as proteolytic degradation. The MDM2 protein binds ubiquitin molecules to P53 which result to the dissociation of the $P53$ protein. On the other side, the

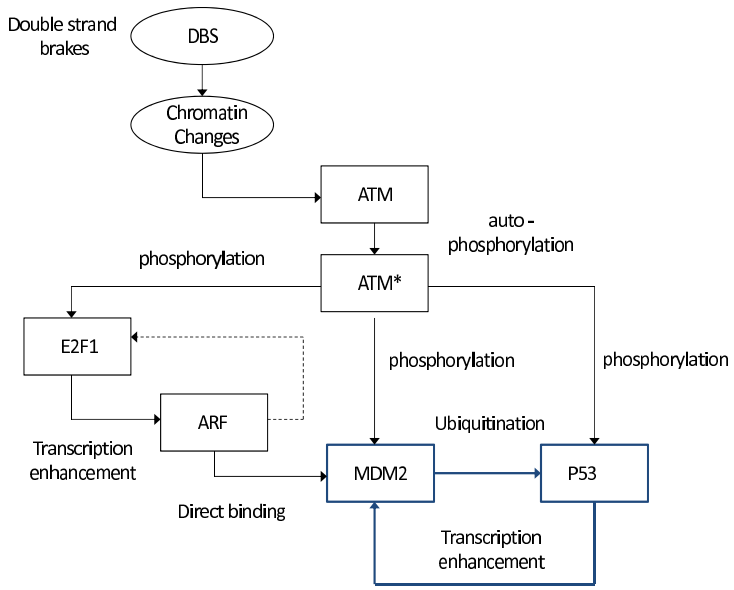


FIGURE 1. Feedback control loop of the p53 protein - mdm2 inhibitor system

increase of $P53$ enhances the transcription procedure of $mdm2$ and consequently the produced MDM2 protein will downregulate $P53$. In this manner the $p53$-$mdm2$ feedback loop converges to an equilibrium.

The role of the ATM protein is explained as follows: ATM is a protein that plays a sensor-detector role in the p53 network. ATM undergoes auto-phosphorylation which leads to its transformation to the active form ATM^*. This process can be accelerated by the exposure of the cell to radiation. In its turn ATM^*, through phosphorylation, contributes to the synthesis of the proteins $E2F1$, $MDM2$ and $P53$. The transformation of ATM^* through phosphorylation into $MDM2$ and $P53$ changes the equilibrium points of the $p53$-$mdm2$ loop. In particular, it enhances the levels of the $P53$ protein and attenuates the effects of $MDM2$ in the dissociation of the $P53$ protein. With the raise of the concentration of $P53$ cell cycle, arrest is also enhanced while the apoptosis rate is also increased.

Another loop, one can distinguish in the $p53$ network is between proteins $E2F1$ and ARF. As mentioned above, the ATM^* protein through its phosphorylation contributes to the synthesis of $E2F1$. In turn, the $E2F1$ protein contributes to the transcription into mRNA of the arf gene and consequently to the synthesis (translation) of the ARF protein. The increased concentration of the ARF protein results into downregulation of $E2F1$ and in this manner the $E2F1 - ARF$ loop closes and an equilibrium is reached. Moreover, ARF results into downregulation of the $MDM2$ and causes the rise of the levels of the $p53$ protein concentration. This also results to improved treatment against cancer cells. It has been confirmed that the removal of the ARF protein from human tissues is responsible for the appearance of breast, mind and lung tumors.

There are chemotherapy drugs that work by binding the $MDM2$ protein and consequently by preventing the $MDM2$ protein from deactivating the $P53$ protein

(ubiquitination). This is based on the infusion of $MDM2$ antagonists (Nutlins). By deactivating $MDM2$ these drugs restore the levels of concentration of the $P53$ protein and consequently contribute to the fighting against cancer cells. The effect of Nutlins on the $MDM2$ protein ($P53$-inhibitor) is defined by the following dynamics:

$$\dot{N} = \lambda_N - \mu_N N - k_6 \cdot N \cdot MDM2 \tag{1}$$

where N is the drug's concentration in the cytoplasm, λ_N is the drug's infusion rate, μ_N is the drug's degradation rate and $-k_6 \cdot N \cdot MDM2$ is the binding of the drug by the $MDM2$ protein. Usually, the infusion rate λ_N is taken to be constant. In the sections that follow it will be shown that the infusion rate can be controlled in such a manner that the levels of the concentration of the $P53^*$ protein are made to converge to desirable setpoints. A variable infusion rate can improve the efficiency of chemotherapy.

2.2. **State-space model of the $p53$ protein - $mdm2$ inhibitor system.** The following state variables are defined for the dynamic model of the $p53$ protein - $mdm2$ inhibitor system

$$\begin{array}{lllll} x_1 = p53 & x_2 = P53 & x_3 = P53^* & x_4 = mdm2 & x_5 = MDM2 \quad x_6 = N \\ x_7 = e2f1 & x_8 = E2F1 & x_9 = E2F1^* & x_{10} = arf & x_{11} = ARF \end{array} \tag{2}$$

The system can be described using the following state-space equations [14]

$$\begin{aligned} \dot{x}_1 &= \lambda_{p53} - \mu_{p53} x_1 \\ \dot{x}_2 &= a_{p53} x_1 - \mu_{53} x_2 - v_{p53} x_3 - \tfrac{K_1 ATM^* x_2}{K_{M_1} + x_2} - \tfrac{K_{cat} x_5 x_2}{a K_{13} + x_2} \\ \dot{x}_3 &= \tfrac{K_1 ATM^* x_2}{K_{M_1} + x_2} - v_{p53} x_3 - \tfrac{K_c at^* x_5 x_3}{a K_{13} + x_3} \\ \dot{x}_4 &= \lambda_{mdm2} - \mu_{mdm2} x_4 + \phi_{mdm2} \tfrac{x_3 (t - r_1)^{n_1}}{x_2(0)^{n_1} + x_3 (t - r_1)^{n_1}} \\ \dot{x}_5 &= a_{MDM2} x_4 - \mu_{MDM2} x_5 - \tfrac{K_2 ATM^* x_5}{K_{M_2} + x_5} - K_4 x_{11} x_5 - K_6 x_6 x_5 \\ \dot{x}_6 &= \lambda_N - \mu_N x_6 - K_6 x_6 x_5 \\ \dot{x}_7 &= \lambda_{e2f1} - \mu_{e2f1} x_7 \\ \dot{x}_8 &= a_{E2F1} x_7 - \mu_{E2F1} x_8 + v_{E2F1} x_9 - \tfrac{K_2 ATM^* x_8}{K_{M_3} + x_8} \\ \dot{x}_9 &= \tfrac{K_3 ATM^* x_8}{K_{M_3} + x_8} - v_{E2F1} x_9 - K_5 x_{11} x_9 \\ \dot{x}_{10} &= \lambda_{arf} - \mu_{arf} x_{10} + \phi_{arf} \tfrac{x_9 (t - r_2)^{n_2}}{x_8(0)^{n_2} + x_9 (t - r_2)^{n_2}} \\ \dot{x}_{11} &= a_{ARF} x_{10} - \mu_{ARF} x_{11} - K_4 x_{11} x_5 - K_5 x_{11} x_9 \end{aligned} \tag{3}$$

In matrix form, the state-space description of the system becomes

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \\ \dot{x}_4 \\ \dot{x}_5 \\ \dot{x}_6 \\ \dot{x}_7 \\ \dot{x}_8 \\ \dot{x}_9 \\ \dot{x}_{10} \\ \dot{x}_{11} \end{pmatrix} = \begin{pmatrix} \lambda_{p53} - \mu_{p53} x_1 \\ a_{p53} x_1 - \mu_{53} x_2 - v_{p53} x_3 - \tfrac{K_1 ATM^* x_2}{K_{M_1} + x_2} - \tfrac{K_c at x_5 x_2}{a K_{13} + x_2} \\ \tfrac{K_1 ATM^* x_2}{K_{M_1} + x_2} - v_{p53} x_3 - \tfrac{K^*_{cat} x_5 x_3}{a K_{13} + x_3} \\ \lambda_{mdm2} - \mu_{mdm2} x_4 + \phi_{mdm2} \tfrac{x_3 (t - r_1)^{n_1}}{x_2(0)^{n_1} + x_3 (t - r_1)^{n_1}} \\ a_{MDM2} x_4 - \mu_{MDM2} x_5 - \tfrac{K_2 ATM^* x_5}{K_{M_2} + x_5} - K_4 x_{11} x_5 - K_6 x_6 x_5 \\ -\mu_N x_6 - K_6 x_6 x_5 \\ \lambda_{e2f1} - \mu_{e2f1} x_7 \\ a_{E2F1} x_7 - \mu_{E2F1} x_8 + v_{E2F1} x_9 - \tfrac{K_2 ATM^* x_8}{K_{M_3} + x_8} \\ \tfrac{K_3 ATM^* x_8}{K_{M_3} + x_8} - v_{E2F1} x_9 - K_5 x_{11} x_9 \\ \lambda_{arf} - \mu_{arf} x_{10} + \phi_{arf} \tfrac{x_9 (t - r_2)^{n_2}}{x_8(0)^{n_2} + x_9 (t - r_2)^{n_2}} \\ a_{ARF} x_{10} - \mu_{ARF} x_{11} - K_4 x_{11} x_5 - K_5 x_{11} x_9 \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \lambda_N \tag{4}$$

which also written in the form

$$\dot{x} = f(x) + g(x)u \tag{5}$$

where $u = \lambda_N$ is the control input, and $f(x) \in R^{11\times 1}$, $g(x) \in R^{11\times 1}$ are vector fields. It will be shown that the considered model of the $p53$ protein - $mdm2$ inhibitor system is a differentially flat one. The flat output is defined as $y = [P^*_{53}, N, E2F1^*, ARF]$ or $y = [x_3, x_6, x_9, x_{11}]$. Thus one has $y = [y_1, y_2, y_3, y_4]^T$.

3. Nonlinear feedback control of the p53 protein system using differential flatness theory.

3.1. Definition of differentially flat systems.

Differential flatness theory will be used for implementing feedback control of the p53 protein-mdm2 inhibitor system. The main principles of differential flatness theory are as follows [8],[37]: A finite dimensional system is considered. This can be written in the form of an ordinary differential equation (ODE), i.e. $S_i(w, \dot{w}, \ddot{w}, \cdots, w^{(i)}),\ \ i = 1, 2, \cdots, q$. The term w denotes the system variables (these variables are for instance the elements of the system's state vector and the control input) while $w^{(i)},\ i = 1, 2, \cdots, q$ are the associated derivatives. Such a system is said to be differentially flat if there is a collection of m functions $y = (y_1, \cdots, y_m)$ of the system variables and of their time-derivatives, i.e. $y_i = \phi(w, \dot{w}, \ddot{w}, \cdots, w^{(\alpha_i)}),\ i = 1, \cdots, m$ satisfying the following two conditions [8],[29],[37]: 1) There does not exist any differential relation of the form $R(y, \dot{y}, \cdots, y^{(\beta)}) = 0$ which implies that the derivatives of the flat output are not coupled in the sense of an ODE, or equivalently it can be said that the flat output is differentially independent, 2) All system variables (i.e. the elements of the system's state vector w and the control input) can be expressed using only the flat output y and its time derivatives $w_i = \psi_i(y, \dot{y}, \cdots, y^{(\gamma_i)}),\ i = 1, \cdots, s$.

3.2. Differential flatness of the $p53$ protein - $mdm2$ inhibitor dynamical system.

From the sixth row of Eq. (3) and by solving with respect to x_5 one obtains

$$\begin{aligned} x_5 = \tfrac{\dot{x}_6 + \mu_N x_6}{-K_6 x_6} \Rightarrow x_5 = \tfrac{\dot{y}_2 + \mu_N y_2}{-K_6 y_2} \Rightarrow \\ x_5 = \tfrac{[0\ 1\ 0\ 0]\dot{y} + \mu_N [0\ 1\ 0\ 0] y}{-K_6 [0\ 1\ 0\ 0] y} \Rightarrow x_5 = f_5(y, \dot{y}) \end{aligned} \tag{6}$$

From the third row of Eq. (3) and by solving with respect to x_2 one obtains

$$\begin{aligned} & K_{M_1}\dot{x}_3 + \dot{x}_3 x_2 = K_1 ATM^* x_2 - v_{p53} K_{M_1} x_3 - v_{p53} x_2 x_3 - \\ & - K_{M_1} \tfrac{K^*_{cat} x_5 x_3}{aK_{13} + x_3} - \tfrac{K^*_{cat} x_5 x_3}{aK_{13} + x_3} x_2 \Rightarrow \end{aligned}$$

$$x_2 = \frac{K_{M_1}\dot{x}_3 - v_{p53} K_{M_1} x_3 + K_{M_1} \frac{K^*_{cat} x_5 x_3}{aK_{13} + x_3}}{K_1 ATM^* + v_{p53} x_3 + \frac{K^*_{cat} x_5 x_3}{aK_{13} + x_3} - \dot{x}_3} \Rightarrow \tag{7}$$

$$\begin{aligned} x_2 = \frac{K_{M_1}\dot{y}_1 - v_{p53} K_{M_1} y_1 + K_{M_1} \frac{K^*_{cat} f_5(y,\dot{y}) y_1}{aK_{13} + y_1}}{K_1 ATM^* + v_{p53} y_1 + \frac{K^*_{cat} f_5(y,\dot{y}) y_1}{aK_{13} + y_1} - \dot{y}_1} \Rightarrow \\ x_2 = f_2(y, \dot{y}) \end{aligned}$$

Equivalently, the second row of Eq. (3) is solved with respect to x_1. This gives

$$\begin{aligned} x_1 = \dot{x}_2 + \mu_{p53} x_2 + v_{p53} x_3 + \tfrac{K_1 ATM^* x_2}{K_{M_1} + x_2} - \tfrac{K_{cat} x_2 x_5}{aK_{13} + x_2} \Rightarrow \\ x_1 = f_1(y, \dot{y}) \end{aligned} \tag{8}$$

The fifth row of Eq. (3) is solved with respect to x_4. Thus, one obtains

$$\begin{aligned} x_4 &= \frac{\dot{x}_5+\mu_{MDM2}x_5+\frac{K_2ATM^*x_5}{K_{M_2}+X_5}+K_4x_{11}x_5+K_6x_6x_5}{a_{MDM2}} \Rightarrow \\ x_4 &= f_4(y,\dot{y}) \end{aligned} \tag{9}$$

The ninth row of Eq. (3) is solved with respect to x_8. Thus one obtains

$$\begin{aligned} &K_{M_3}\dot{x}_9+\dot{x}_9x_8 = K_3ATM^*x_8 - v_{E2F1}K_{M_3}x_9 - \\ &-v_{E2F1}x_8x_9 - K_5K_{M_3}x_{11}x_9 - K_5x_{11}x_9x_8 \Rightarrow \\ &x_8 = \frac{K_{M_3}\dot{x}_9+v_{E2F1}K_{M_3}x_3+K_5K_{M_3}x_{11}x_9}{K_3ATM^*-\dot{x}_9-v_{E2F1}x_9-K_9x_{11}x_9} \Rightarrow \\ &x_8 = f_8(y,\dot{y}) \end{aligned} \tag{10}$$

The eighth row of Eq. (3) is solved with respect to x_7. Thus one obtains

$$\begin{aligned} x_7 &= \frac{\dot{x}_8+\mu_{E2F1}x_8-v_{E2F1}x_9+\frac{K_2ATM^*x_8}{K_{M_3}+x_8}}{a_{E2F1}} \Rightarrow \\ x_7 &= f_7(y,\dot{y}) \end{aligned} \tag{11}$$

The eleventh row of Eq. (3) is solved with respect to x_{10}. It holds

$$\begin{aligned} x_{10} &= \frac{\dot{x}_{11}+\mu_{ARF}x_{11}+K_4x_{11}x_5+K_5x_{11}x_9}{a_{ARF}} \Rightarrow \\ x_{10} &= f_{10}(y,\dot{y}) \end{aligned} \tag{12}$$

Moreover, from the sixth row of Eq. (3) and using that $x_5 = f_5(y,\dot{y})$ and $x_6 = y_2$ one obtains about the control input $u = \lambda_N$

$$\begin{aligned} u = \lambda_N &= \dot{x}_6 + \mu_N x_6 + K_6x_6x_5 \Rightarrow \\ \lambda_N &= f_u(y,\dot{y}) \end{aligned} \tag{13}$$

Thus one has that all state variables and the control input of the $p53$ protein - $mdm2$ inhibitor system are functions of the flat output y and of its derivatives. Consequently, the dynamical system of $P53$ is a differentially flat one.

3.3. **Flatness-based control of the $p53$ protein - $mdm2$ inhibitor system.** It will be shown that using the differentially flat description of the $p53$ protein - $mdm2$ inhibitor system it is possible to transform it to the canonical Brunovsky form. It holds that $y_1 = x_3$ therefore

$$\dot{y}_1 = \dot{x}_3 \Rightarrow \dot{y}_1 = \frac{K_1ATM^*x_2}{K_{M_1}+x_2} - v_{P53}x_3 - \frac{K^*_{cat}x_5x_3}{aK_{13}+x_3} \tag{14}$$

Consequently, the second derivative of y_1 is found to be

$$\ddot{y}_1 = \frac{(K_1ATM^*\dot{x}_2)(K_{M_1}+x_2)-(K_1ATM^*x_2)\dot{x}_2}{(K_{M_1}+x_2)^2} - v_{p53}\dot{x}_3 - \frac{-K^*_{cat}(\dot{x}_5x_3+x_5\dot{x}_3)(aK_{13}+x_3)-(K^*_{cat}x_5x_3)\dot{x}_3}{(aK_{13}+x_3)^2} \tag{15}$$

After intermediate operations one obtains

$$\begin{aligned} \ddot{y}_1 &= \frac{K_1ATM^*K_{M_1}}{(K_{M_1}+x_2)^2}\dot{x}_2 - v_{p53}\dot{x}_3 \\ &-\frac{K^*_{cat}aK_{13}x_5\dot{x}_3}{(aK_{13}+x_3)^2} - \frac{K^*_{cat}x_3}{(aK_{13}+x_3)}\dot{x}_5 \end{aligned} \tag{16}$$

and after substituting the derivatives of x_3 and x_5 one gets

$$\begin{aligned} \ddot{y}_1 = &\frac{K_1ATM^*K_{M_1}}{(K_{M_1}+x_2)^2}[a_{p53}x_1 - \mu_{p53}x_2 - v_{p53}x_3 - \frac{K_1ATM^*x_2}{K_{M_1}+x_2} - \frac{K_{cat}x_5x_2}{(aK_{13}+x_2)^2}] - \\ &-[v_{p53} + \frac{K^*_{cat}aK_{13}x_5}{(aK_{13}+x_3)^2}]\cdot[\frac{K_1ATM^*x_2}{K_{M_1}+x_2} - v_{p53}x_3 - \frac{K^*_{cat}x_5x_3}{(aK_{13}+x_3)}] - \\ &-\frac{K^*_{cat}x_3}{(aK_{13}+x_3)}[a_{MDM2}x_4 - \mu_{MDM2}x_5 - \frac{K_2ATM^*x_5}{K_{M_2}+x_5} - K_4x_{11}x_5 - K_6x_6x_5] \end{aligned} \tag{17}$$

By differentiating once more with respect to time one obtains

$$y_1^{(3)} = f(y, \dot{y}) + g(y, \dot{y})u \tag{18}$$

where the control input $u = \lambda_N$ is the input rate of the chemotherapy drug, while functions $f(y, \dot{y})$ and $g(y, \dot{y})$ are defined as follows:

(i) function $f(y, \dot{y})$

$$\begin{aligned} f(y,\dot{y}) = & -\frac{2(K_{M_1}+x_2)\dot{x}_2 K_1 ATMK_{M_1}}{(K_{M_1}+x_2)^4}[a_{p53}\dot{x}_1 - \mu_{p53}\dot{x}_2 - v_{p53}\dot{x}_3 - \frac{K_1 ATM^* x_2}{K_{M_1}+x_2} - \\ & - \frac{K_{cat}x_5 x_2}{aK_{13}+x_2}] + \frac{K_1 ATM^* K_{M_1}}{(K_{M_1}+x_2)^2}[a_{p53}\dot{x}_1 - \mu_{p53}\dot{x}_2 - v_{p53}\dot{x}_3 - \\ & - \frac{K_1 ATM\dot{x}_2(K_{M_1}+x_2) - K_1 ATM^*\dot{x}_2}{(K_{M_1}+x_2)^2} - \frac{K_{cat}(\dot{x}_5 x_2 + x_5\dot{x}_2)(aK_{13}+x_2) - K_{cat}x_5 x_2\dot{x}_2}{(aK_{13}+x_2)^2}] - \\ & \frac{-K^*_{cat}aK_{13}\dot{x}_5(aK_{13}+x_3)^2 - K^*_{cat}aK_{13}x_5 2(aK_{13}+x_3)\dot{x}_3}{(aK_{13}+x_3)^4}\cdot[\frac{K_1 ATM^* x_2}{K_{M_1}+x_2} - v_{p53}x_3 - \frac{K_c at^* x_5 x_3}{aK_{13}+x_3}] - \\ & - [v_{p53} + \frac{K^*_{cat}aK_{13}x_5}{(aK_{13}+x_3)^2}]\cdot[\frac{K_1 ATM^*\dot{x}_2(K_{M_1}+x_2) - K_1 ATM^* x_2\dot{x}_2}{K_{M_1}+{x_2}^2} - \\ & - v_{p53}\dot{x}_3 - \frac{K^*_{cat}(\dot{x}_5 x_3 + x_5\dot{x}_3)(aK_{13}+x_3) - K^*_{cat}x_5 x_3(aK_{13}+x_3)}{(aK_{13}+x_3)^2}] - \frac{K^*_{cat}\dot{x}_3(aK_{13}+x_3) - K_c at^* x_3\dot{x}_3}{(aK_{13}+x_3)^2}\cdot \\ & [a_{MDM2}x_4 - \mu_{MDM2}x_5 - \frac{K_2 ATM^* x_5}{K_{M_2}+x_5} - K_4 x_{11}x_5 - K_6 x_6 x_5] \\ & - \frac{K^*_{cat}x_3}{(aK_{13}+x_3)}\cdot[a_{MDM2}\dot{x}_4] - \mu_{MDM2}\dot{x}_5 - \frac{K_2 ATM^* x_5 - K_{M_2}ATM^* x_5\dot{x}_5}{K_{M_2}+{x_5}^2} - \\ & - K_4(\dot{x}_{11}x_5 + x_{11}\dot{x}_5) - K_6 x_6\dot{x}_5] - \frac{K^*_{cat}x_3}{(aK_{13}+x_3)}[-\mu_N x_6 - K_6 x_6 x_5](-K_6 x_5) \end{aligned} \tag{19}$$

(ii) function $g(y, \dot{y})$

$$g(y, \dot{y}) = -\frac{K^*_{cat}x_3}{aK_{13}+x_3}(-K_6 x_5) \tag{20}$$

By defining the new control input $v = f(y, \dot{y}) + g(y, \dot{y})u$, the dynamics of the active $P53$ protein can be written in the form

$$y^{(3)} = f(y, \dot{y}) + g(y, \dot{y})u \Rightarrow y^{(3)} = v \tag{21}$$

A suitable feedback control law for the system of Eq. (21) is

$$v = y_d^{(3)} - k_1(\ddot{y} - \ddot{y}_d) - k_2(\dot{y} - \dot{y}_d) - k_3(y - y_d) \tag{22}$$

where the gains k_1, k_2 and k_3 are chosen such that the characteristic polynomial of the closed-loop system is a Hurwitz-stable one. The dynamics of the tracking error is $e = y - y_d = P53^* - P53^*_d$ is given by

$$e^{(3)} + k_1\ddot{e} + k_2\dot{e} + k_3 e = 0 \tag{23}$$

finally results into $lim_{t\to\infty}e(t) = 0$. The control input that actually applied to the $p53$ protein - $mdm2$ inhibitor system is given by

$$u = g(y, \dot{y})^{-1}[v - f(y, \dot{y})] \tag{24}$$

It is noted that the p53 protein - mdm2 inhibitor system exhibits the so-called zero dynamics [12]. This means that the model contains internal state variables which do not appear as outputs in the linearized equivalent of the p53-mdm2 model given in Eq. (18). Since the internal state variables describe also proteins concentration they are expected to vary within specific intervals. The boundedness of the internal state variables implies also boundedness of the control input, thus finally enabling state variable $P53^*$ to converge to the desirable setpoints.

Remark 1. First, about delays in the p53 protein - mdm2 inhibitor dynamics, denoted as $x_3(t - r_1)$ and $x_9(t - r_2)$ respectively, it is pointed out that: (i) none of these terms appears in the input-output linearized model for which control is developed and which is finally described by Eq. (19) and Eq. (20), (ii) even if delay

terms were present in the model of Eq. (19) and Eq. (20) these could be substituted by their Taylor series expansions and thus the effects of time delays could be handled as disturbances and could be easily compensated by the robustness of the control loop [47],[48]. Second, it is noted that, the considered dynamical model is practically decoupled and one arrives to control output y_1 by one single control input which is the drug infusion rate. Finally, it is noted that the appearance of zero dynamics in the p53-mdm2 system does not affect the stability of the control loop, because the state variables which constitute the zero dynamics remain bounded.

Remark 2. To identify dynamically the parameters of the p53-mdm2 state-space model of Eq. (3), one can perform again Kalman Filtering, or can apply nonlinear least squares methods such as the Levenberg-Marquardt method [28]. In the Kalman Filter approach for unknown parameters identification, the state vector to be estimated by the Kalman Filter is taken to be the unknown parameters vector θ, which is assumed to be updated in time by $\theta(k+1) = \theta(k) + w(k)$, where $w(k)$ is a noise vector of known covariance matrix. The measured variable to be used by the Kalman Filter is the flat output $y = P_{53}^{*}$, which appears as output in the linearized dynamics of the p53-mdm2 system given in Eq. (21).

4. Detection of parametric changes with the use of statistical criteria.

4.1. State estimation using the Derivative-free nonlinear Kalman Filter.

To apply the feedback control law of Eq. (24) and Eq. (22) to the system of the p53 protein synthesis it is possible to use measurements of the concentration of the active $P53^{*}$ protein at the cytoplasm, however the derivatives of $P53^{*}$ with respect to time are missing. These have to be estimated with the use of a filtering method. To this end, the Kalman Filter recursion is used on the linearized equivalent of the p53 protein - mdm2 inhibitor that is described by Eq. (21).

Using the transformation of the protein synthesis model given in Eq. (18) to Eq. (21), the dynamics of the $p53$ protein - $mdm2$ inhibitor system is written in the following canonical Brunovsky form

$$\begin{array}{c} \dot{z} = Az + Bv \\ z_m = Cz \end{array} \tag{25}$$

or equivalently,

$$\begin{pmatrix} \dot{z}_1 \\ \dot{z}_2 \\ \dot{z}_3 \end{pmatrix} = \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} z_1 \\ z_2 \\ z_3 \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} v \tag{26}$$

with measurement equation given by

$$z_m = \begin{pmatrix} 1 & 0 & 0 \end{pmatrix} z \tag{27}$$

For the dynamics of the $p53$ protein - $mdm2$ inhibitor system that is described in Eq. (26) and Eq. (27) it is possible to perform state estimation using the Kalman Filter recursion. The application of Kalman Filtering on the linearized equivalent of the system and the use of an inverse transformation based on the expression of the initial state variables as functions of the flat output (see Eq. (6) to Eq. (10)) enables also to obtain estimates for the state variables of the initial nonlinear dynamical system of Eq. (4). This recursive estimation and inverse transformation procedure constitutes the *Derivative-free nonlinear Kalman Filter*. The state estimator is

$$\begin{array}{c} \dot{\hat{z}} = A_o \hat{z} + B_o v + K(z_m - \hat{z}_m) \\ \hat{z}_m = C_o \hat{z} \end{array} \tag{28}$$

where $A_o = A$, $B_o = B$ and $C_o = C$. In the design of the associated disturbances' estimator one has the dynamics defined in Eq. (28), where $K \in R^{3\times 1}$ is the state estimator's gain and matrices A_o, B_o and C_o have been defined in Eq. (26) to Eq. (27). The discrete-time equivalents of matrices A_o, B_o and C_o are denoted as $\tilde{A}_d$, $\tilde{B}_d$ and $\tilde{C}_d$ respectively, and are computed with the use of common discretization methods [29],[32]. Next, a Derivative-free nonlinear Kalman Filter can be designed for the aforementioned representation of the system dynamics [29],[30]. The associated Kalman Filter-based disturbance estimator is given by [28],[32].

measurement update:

$$\begin{aligned} K(k) &= P^{-}(k)\tilde{C}_d^T[\tilde{C}_d \cdot P^{-}(k)\tilde{C}_d^T + R]^{-1} \\ \hat{z}(k) &= \hat{z}^{-}(k) + K(k)[\tilde{C}_d z(k) - \tilde{C}_d \hat{z}^{-}(k)] \\ P(k) &= P^{-}(k) - K(k)\tilde{C}_d P^{-}(k) \end{aligned} \tag{29}$$

time update:

$$\begin{aligned} P^{-}(k+1) &= \tilde{A}_d(k)P(k)\tilde{A}_d^T(k) + Q(k) \\ \hat{z}^{-}(k+1) &= \tilde{A}_d(k)\hat{z}(k) + \tilde{B}_d(k)\tilde{v}(k) \end{aligned} \tag{30}$$

The Derivative-free nonlinear Kalman Filter is parameterized using the nominal model of the p53 protein - mdm2 inhibitor system, that is the model that describes the normal (healthy) condition. Next, two sequences of data are processed (see Fig. 2(a)). The first sequence consists of real measurements of the p53 protein concentration which are obtained at specific sampling instances. The second sequence is the Kalman Filter's output, sampled again at the same time instances. By comparing the two signals, the residuals (estimation error) sequence is generated. The processing of the residuals with the use of statistical decision making criteria provides an indication about the existence of parametric changes (faults) in the protein synthesis model, which otherwise could not have been detected [2],[32]. Thus, by applying fault detection tests based on the χ^2 distribution it can be concluded if the p53 protein-mdm2 inhibitor system remains healthy and if the nominal parameter values for its model still hold (see Fig. 2(b)). Otherwise, a failure can be detected.

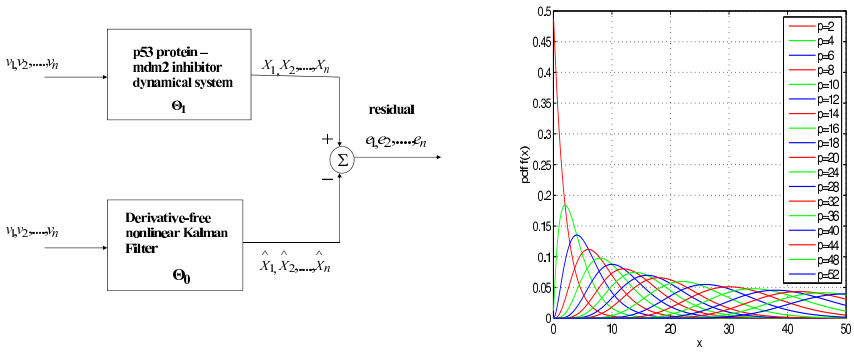
4.2. **Fault detection.** The residuals' sequence (differences between the real output of the monitored protein synthesis model and the one estimated by the Kalman Filter) is a discrete error process e_k with dimension $m\times 1$. Actually, it is a zero-mean Gaussian white-noise process with covariance given by E_k. A conclusion can be stated based on a measure of certainty that the parameters of the dynamic model of the protein synthesis model remain unchanged. To this end, the following *normalized error square* (NES) is defined [31]:

$$\epsilon_k = e_k^T E_k^{-1} e_k \tag{31}$$

The normalized error square follows a χ^2 distribution. An appropriate test for the normalized error sum is to numerically show that the following condition is met within a level of confidence (according to the properties of the χ^2 distribution)

$$E\{\epsilon_k\} = m \tag{32}$$

This can be succeeded using statistical hypothesis testing, which are associated with confidence intervals. A 95% confidence interval is frequently applied, which is specified using $100(1-a)$ with $a = 0.05$. Actually, a two-sided probability region is



(a) **(b)**

FIGURE 2. (a) Statistical change detection test based on the processing of residuals, (b) Probability density function of the χ^2 distribution, for various degrees of freedom p

considered cutting-off two end tails of 2.5% each. For M runs the normalized error square that is obtained is given by

$$\bar{\epsilon}_k = \frac{1}{M}\sum_{i=1}^{M}\epsilon_k(i) = \frac{1}{M}\sum_{i=1}^{M}e_k^T(i)E_k^{-1}(i)e_k(i) \tag{33}$$

where ϵ_i stands for the i-th run at time t_k. Then $M\bar{\epsilon}_k$ will follow a χ^2 density with Mm degrees of freedom. This condition can be checked using a χ^2 test. The hypothesis holds, if the following condition is satisfied

$$\bar{\epsilon}_k \in [\zeta_1, \zeta_2] \tag{34}$$

where ζ_1 and ζ_2 are derived from the tail probabilities of the χ^2 density. For example, for $m = 20$ and $M = 100$ one has $\chi^2_{Mm}(0.025) = 1878$ and $\chi^2_{Mm}(0.975) = 2126$. Using that $M = 100$ one obtains $\zeta_1 = \chi^2_{Mm}(0.025)/M = 18.78$ and $\zeta_2 = \chi^2_{Mm}(0.975)/M = 21.26$.

4.3. **Fault isolation.** By applying the statistical test into n subsystems (local protein synthesis loops) of the aggregate protein synthesis model, it is also possible to find out the subsystem that has deviated from normal functioning. In the case of a single failure one has to carry out n χ^2 statistical change detection tests. Actually, out of the n χ^2 statistical change detection tests, the one that exhibits the highest score (or equivalently indicates the largest parameter deviation from the nominal value) are those that identify the local protein synthesis loop that has been subjected to disease (the damaged components for this local loop are the changed parameters in its state-space description).

In the case of multiple failures one can identify the subset of local protein synthesis loops that have been subjected to parametric change by applying the χ^2 statistical change detection test according to a combinatorial sequence. This means that

$$\binom{n}{k} = \frac{n!}{k!(n-k)!} \tag{35}$$

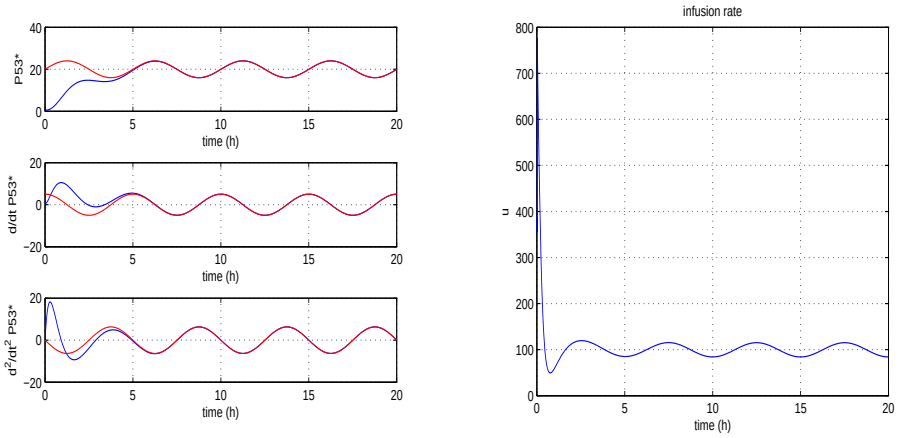
tests have to take place, for all sets in the protein synthesis model that comprise n, $n-1$, $n-2$, $\cdots$, 2, 1 local loops. Again the χ^2 tests that give the highest scores indicate the local loops which are most likely to have been subjected to parametric change. This approach enables to assess the magnitude of deviation from nominal values that the protein synthesis model has undergone and to focus on the part that exhibits the most significant parametric change.

Remark 3. It has been shown that by applying differential flatness theory, the input-output linearized model of Eq. (21) is obtained having as output the concentration of the P_{53}^* protein and as input the infusion rate of the chemotherapy drug. The effects to this model of parametric uncertainties, external perturbations as well as of time delays can be represented as an additive disturbance input $\tilde{d}$. The redesign of the Kalman Filter as a disturbance observer enables the estimation of the non-measurable state variables of the model, as well as the estimation and compensation of the aforementioned additive disturbance input. The stability features of flatness-based control of the p53 protein - mdm2 inhibitor model, after the inclusion of the disturbance observer in the control loop, are similar to those of LQG control. About, zero dynamics and due to the boundedness of the state variables which do not appear as outputs in the linearized p53-mdm2 model, it can be assured that in Eq. (24) functions $f(y, \dot{y})$ and $g(y, \dot{y})$ will be also bounded. This also implies that the control signal that is computed from the feedback law of Eq. (24) is bounded. Consequently the proposed control scheme is a feasible one and can be implemented in practice.

5. **Simulation tests.** The protein concentration state variables of the p53 model were measured in micro-Mol (μM). Indicative nominal values for the parameters of the p53 protein synthesis model are: $\lambda_{p53} = 2.1(\mu M{\cdot}h^{-1})$, $\mu_{p53} = 0.2(h^{-1})$, $a_{p53} = 5.3(h^{-1})$, $v_{p53} = 0.2(h^{-1})$, $K_1 = 2.1(h^{-1})$, $K_2 = 0.2(h^{-1})$, $K_3 = 2.3(h^{-1})$, $K_4 = 0.2(\mu M^{-1}h^{-1})$, $K_5 = 0.1(\mu M^{-1}h^{-1})$, $K_6 = 0.001(\mu M^{-1}h^{-1})$, $K_{13} = 3.2(\mu M)$, $ATM_s = 0.005(\mu M)$, $a = 0.001$, $K_{M_1} = 0.1\mu M$, $K_{M_2} = 0.2\mu M$, $K_{M_3} = 0.3\mu M$, $K_{cat} = 0.31h^{-1}$, $K_{cat}^* = 2.10(h^{-1})$, $\lambda_{mdmd2} = 0.4(\mu M{\cdot}h^{-1})$, $\mu_{mdm2} = 0.6(h^{-1})$, $\phi_{mdm2} = 0.7(\mu{\cdot}Mh^{-1})$, $a_{MDM2} = 0.8(h^{-1})$, $\mu_{MDM2} = 0.9(h^{-1})$, $\mu_N = 0.05(h^{-1})$, $\lambda_{e2f1} = 0.3(\mu{\cdot}Mh^{-1})$, $\mu_{e2f1} = 0.4(h^{-1})$, $a_{E2F1} = 0.5(h^{-1})$, $\mu_{E2F1} = 0.6(h^{-1})$, $v_{E2F1} = 0.7(h^{-1})$, $\lambda_{arf} = 0.4(\mu M{\cdot}h^{-1})$, $\mu_{arf} = 0.5(h^{-1})$, $\phi_{arf} = 10.6(\mu M{\cdot}h^{-1})$, $a_{ARF} = 0.7(h^{-1})$, $\mu_{ARF} = 0.8(h^{-1})$.

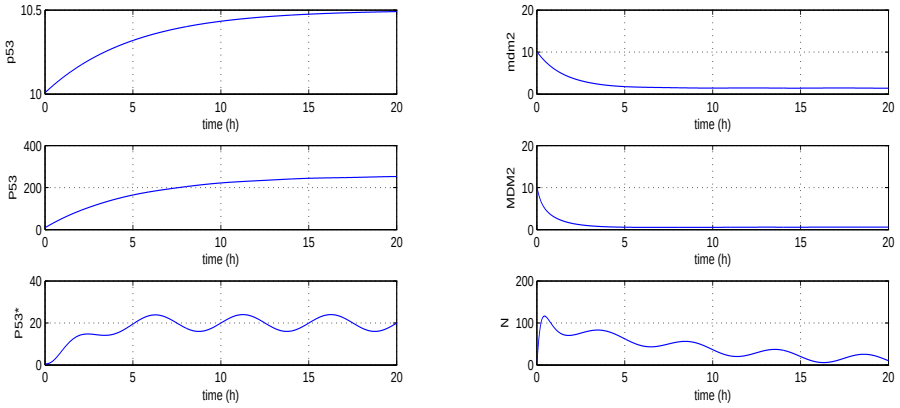
First, an exact and fault-free dynamical model of the p53 protein - mdm2 system was considered. The response of the p53-mdm2 protein synthesis model to nonlinear feedback control is depicted in Fig. 3 to Fig. 5. It can be noticed that under the proposed feedback control the concentration of the target state variable, that is the active $P53^*$ proteins converges to the desirable levels. Moreover, the rest of the model's state variables which are implicitly affected by the control input (zero dynamics of the system) remain also bounded.

Next, the χ^2 statistical change detection criterion was used for finding parametric changes in the protein synthesis model. The proposed fault diagnosis method was capable of detecting the existence of parametric changes in the p53-mdm2 protein synthesis model. The obtained results are depicted in Fig. 6 to Fig. 9. The fault thresholds are determined by the confidence intervals of the χ^2 distribution. The χ^2 distribution has $d = 3$ degrees of freedom. The number of iterations was $M = 2000$. Thus, for an 98% confidence interval the associated upper and lower fault thresholds are $U = 2.8886$ and $L = 3.1136$. For parameter K_{cat}^* the nominal



(a) **(b)**

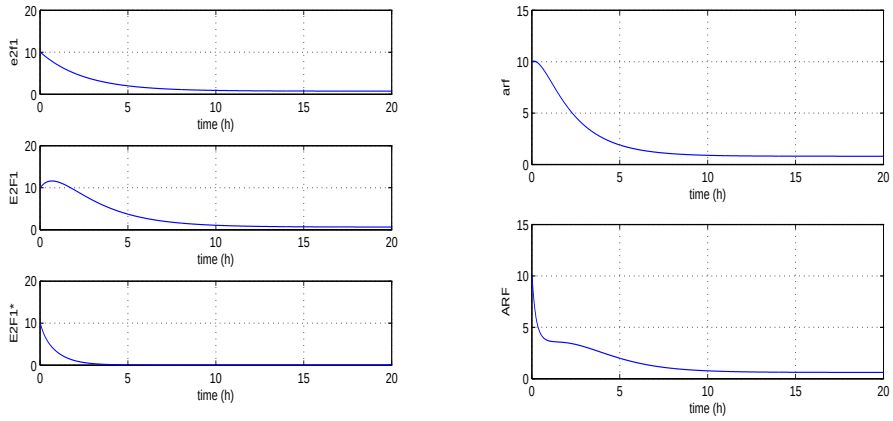
FIGURE 3. Dynamical model without faults: (a) nonlinear feedback control of the $P53^*$ protein concentration (blue line) and convergence to the associated setpoints (red lines), (b) infusion rate as control input



(a) **(b)**

FIGURE 4. Dynamical model without faults: (a) variation of the $p53$ mRNA concentration, $P53$ concentration in the cytoplasm and active $P53^*$ concentration, (b) variation of the $mdm2$ mRNA concentration, $MDM2$ concentration in the cytoplasm and active $MDM2^*$ concentration

value was $K^*_{cat} = 2.10$ while after change the value became $K^*_{cat} = 3.30$. For parameter K_1 the nominal value was $K_1 = 2.1$ while after change the value became $K_1 = 8.1$. Finally, for parameter K_{13} the nominal value was $K_{13} = 3.2$ while after



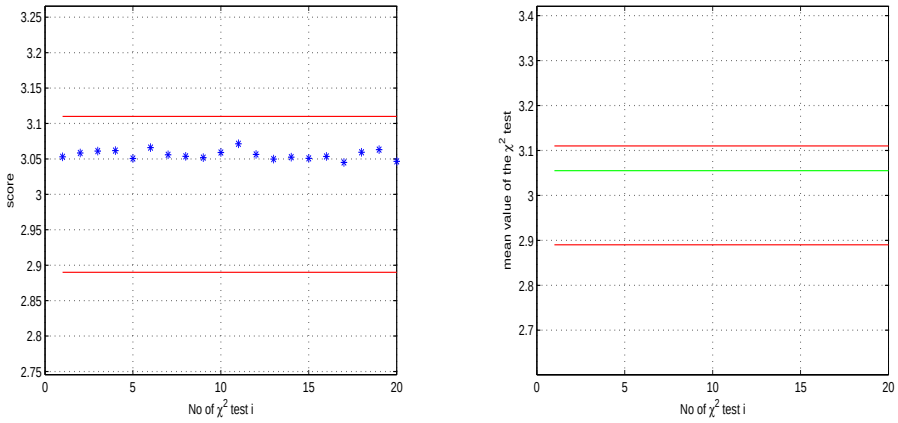
(a) **(b)**

FIGURE 5. Dynamical model without faults: (a) variation of the $e2f1$ mRNA concentration, $E2F1$ concentration in the cytoplasm and active $E2F1^*$ concentration, (b) variation of the arf mRNA concentration, ARF concentration in the cytoplasm

change it became $K_{13} = 5.2$. It can be noticed that when the parameters of the model of the p53 protein - mdm2 inhibitor system remained at their nominal values the statistical change detection test returned a value that was within the upper and lower fault thresholds. On the other hand, when deviation from the nominal parameters values took place the result of the χ^2 fault detection test exceeded clearly the fault boundaries.

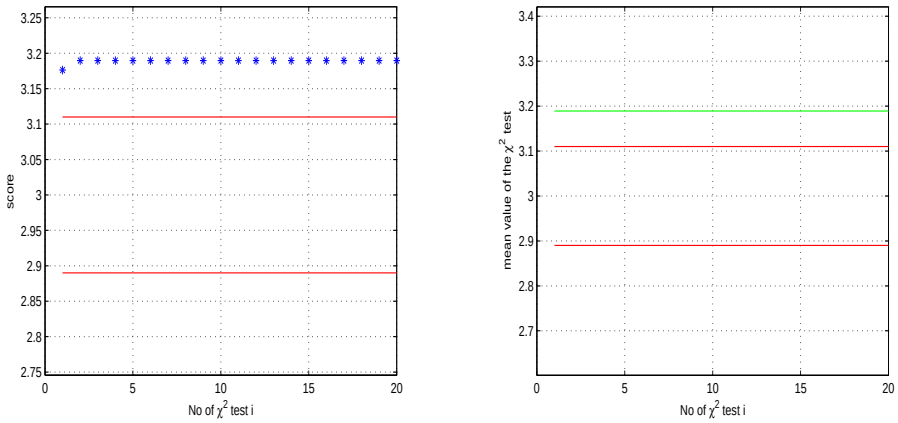
6. **Conclusions.** The paper has proposed a systematic method for detecting parametric changes in the model of the p53 protein - mdm2 inhibitor system. First, it has been shown that the considered protein synthesis loop is differentially flat, which means that all its state variables and the control inputs can be expressed as functions of certain state vector elements (that constitute the flat output) and of the associated flat output derivatives. The differential flatness property enables to transform the nonlinear protein synthesis model into a canonical linear form for which the design of state feedback controller becomes easier.

Next, the paper has analyzed the problem of detection of parametric changes in the protein synthesis model. The dynamic behavior of the p53 protein in normal condition has been described with the use of nonlinear Kalman Filtering. The considered filter, known as Derivative-free nonlinear Kalman Filter, consists of the standard Kalman Filter recursion applied on the linearized model of the system. It also makes use of an inverse transformation based on differential flatness theory which enables to obtain estimates for the state vector elements of the initial nonlinear model. By comparing the filter's output against the output of the real p53 protein - mdm2 system a sequence of error measurements (residuals) is obtained. Further processing of the residuals with the use of a statistical change detection criterion, that is the χ^2 test, enables to diagnose if parametric changes have taken place in the protein synthesis model.



(a) **(b)**

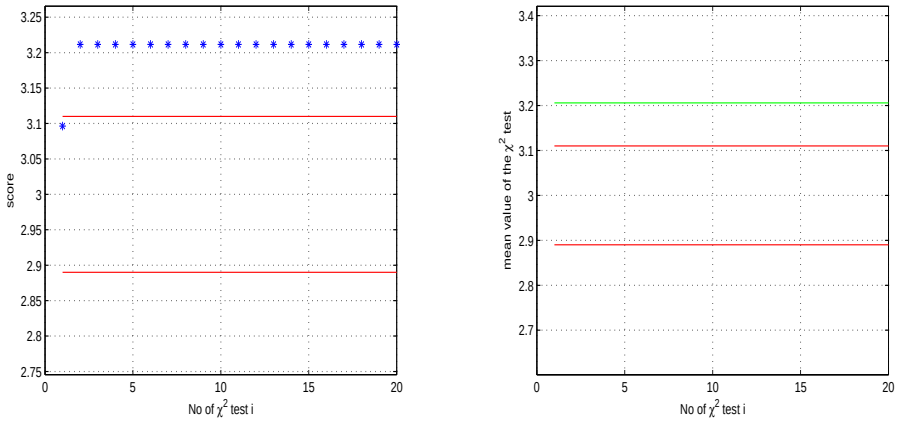
FIGURE 6. No parametric change (confidence interval 98% denoted with red lines): (a) Individual values of the χ^2 tests, (b) mean value of the χ^2 test denoted with green line



(a) **(b)**

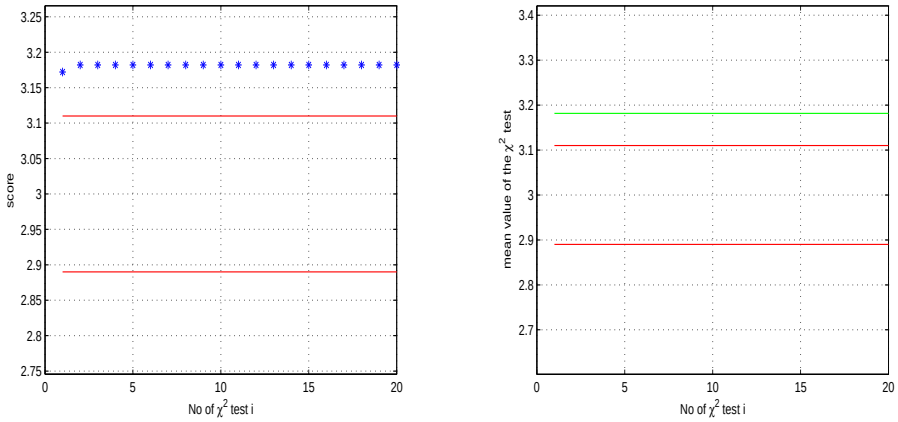
FIGURE 7. Change in parameter K^*_{cat} (confidence interval 98% denoted with red lines): (a) Individual values of the χ^2 tests, (b) mean value of the χ^2 test denoted with green line

Apart from protein synthesis models, the paper results can be generalized to other health monitoring problems of biological systems, such as hormone synthesis models and gene networks. Thus, the paper's method can contribute to diagnosing of deviation of the above mentioned systems from the normal condition and also in identification of specific parametric changes which are associated with certain diseases.



(a) **(b)**

FIGURE 8. Change in parameter K_1 (confidence interval 98% denoted with red lines): (a) Individual values of the χ^2 tests, (b) mean value of the χ^2 test denoted with green line



(a) **(b)**

FIGURE 9. Change in parameter K_{13} (confidence interval 98% denoted with red lines): (a) Individual values of the χ^2 tests, (b) mean value of the χ^2 test denoted with green line

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