Optimal control circuitry design for the digital p53 dynamics in cancer cell and apoptosis

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Abbreviations

IR	Ionizing Radiation
$[\dots]_{uncontr}$	Digitally uncontrolled proteins network
$[\dots]_{contr}$	Digitally controlled proteins network

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Abstract

Experimental work and theoretical models deduce a "digital" response of the p53 transcription factor when genomic integrity is damaged. The mutual influence of p53 and its antagonist, the Mdm2 oncogene, is closed in feedback. This paper proposes an aerospace architecture for translating the p53/Mdm2/DNA damage network into a digital circuitry in which the optimal control theory is applied for obtaining the requested dynamic evolutions of some considered cell species for repairing a DNA

damage. The purpose of this paper is not to improve the analysis of the actual mathematical models but to demonstrate the usefulness of such digital circuitry design capable to predict and detect the cell species dynamics for finding more information regarding the inner mechanism of the cell components.

The cell fate is newly conceived by the modified pulsing mechanism of p53 and other apoptotic species when the digital optimal control is applied to an apoptosis wiring diagram.

Introduction

In recent years there has been tremendous progress in efforts to identify the components of a human cell and their interactions. This gives hope of a useful strategy for attacking cancer in the near future. Nevertheless, hopes for cancer therapy have often vanished in a puff of smoke.

Nowadays, there is remarkable and increasing cooperation between biology and mathematics due in part to the decoding of the human genome. Dimensionally speaking, the dynamics of cancer cells seem to be described from a single-cell structure up to the macro-scale. A very interesting literature is dedicated to mathematical models and simulations regarding tumours as elasto-viscoplastic growing bodies.[1-10] These pointwise mathematical frameworks are useful for further models to describe adhesion mechanisms and angiogenesis. Since its discovery, the oncosuppressor p53 protein seems to play a prominent role in the evolution of a cancer cell. Activation and high concentration of p53 are the response to aberrant oncogene signals and this protein is capable of inducing the transcription of genes in charge of the cell-cycle arrest, DNA repair and apoptosis.[11-15] Browsing the state of the art, Reich et al.[16] have addressed the idea that increased expression of p53 in damaged cells may be explained by more than one mechanism. Clinical validation of developed anticancer drugs able to inhibit the function of oncoproteins leads to tumour regression depending on the cancer type with the main result of p53 restoration.[17] Bates et al.[18] propose a model in which E2F-1 (a

protein inherently activated by cell-cycle progression) in conjunction with another protein ($p14^{ARF}$), protects cells against oncogenic changes and then against aberrant proliferation.

At this point, an engineering frame of mind was adopted by the present author (whose disciplines lie inside aerospace technologies) when they faced the acknowledgement of the p53 protein structure and its role in the human single-cell scheme.

An outstanding recent work[19] not only confirms that the above mentioned p53 is inhibitory *in vivo* but also demonstrates that its activation is required for the pro-apoptotic target gene binding protein called IGFBP3. These conclusions seem to be related to a previous study of Bell et al.[20] in which their analysis showed that, at physiological temperatures, wild-type p53 was more than 50% unfolded with a 75% loss in DNA-binding activity.

Although these works can be considered milestones from a purely biological point of view, they remain far from being used mathematically, for two reasons: first, their adopted procedures make use of chemicals, reagents and enzymes and, secondly, they become inappropriate if one decides to translate them into unsteady mathematical simulations and computational predictions. The guidelines of the present paper are those given by references 11-15 and 21-24 in which theoretical and experimental studies about oscillations of the p53/Mdm2 feedback loop support the chance to mathematically describe this human single-cell proteins activity and their inner forms dynamics.

Briefly, these studies come to intriguing and converging conclusions i.e., p53 can be expressed into a series of discrete pulses after DNA damage and the whole p53/Mdm2 network system is constrained by a feedback loop and theoretically expressed by a digital scheme. However, this work shows differences in the analysis of results depending on the sign of the claimed feedback loops.

As a consequence, although these proposed mathematical models and experiments capture the oscillating characteristics of p53 and Mdm2, they show different and almost

conflicting points of view when linked to the interpretation of results in terms of output amplitudes, frequencies, interpulses and other oscillation parameters of the p53/Mdm2 dynamic responses. In short, a negative feedback loop generates damped oscillations, while schemes such as positive feedback loops of p53 may enhance undamped dynamics.

An important contribution for understanding the dynamics and variability of p53/Mdm2 system is given by Geva-Zatorsky et al.[12] They evaluate the amplitude and width of each peak of nuclear Mdm2-YFP (yellow-fluorescent-protein) and calculate the average of these properties. Ultimately, those authors obtained prolonged undamped oscillations in the p53/Mdm2 system. The onset of oscillations was synchronized with the DNA damage signal and cells gradually lost synchrony with each other due to variations in oscillation frequencies. The characteristic oscillation frequency in each cell was found by Fourier analysis.

But, leaving out the structure of the proposed model and whether or not the "noise" in the oscillations reflects internal noise during protein production rates, one has to catch the reliability of the model when Fourier analysis is employed for frequency detection and their power spectral density. Briefly, if prolonged undamped oscillations are obtained for Mdm2 protein, one has to check the behaviour – in the same frequency bandwidth – of its antagonist (and their forms) because cross-correlation factors and aliasing frequency distortion can seriously affect the detected value of the characteristic frequency especially in those digital schemes in which noise parameters and effects cannot be neglected.

Batchelor et al.[24] draw the conclusion that the p53/Mdm2 negative feedback loop is composed of interaction of two different timescales: one, a slow positive transcriptional arm and a fast negative protein-protein interaction arm. Their results show that the p53/Mdm2 feedback loop does not by itself drive sustained p53 oscillations. Thus, they identify the wild type p53-induced phosphatase 1, Wip1, as the central element

mediating a second (negative) feedback loop for chaining p53 and the upstream signalling proteins.

In the light of the state of the art, it is unambiguous the digital response of the p53/Mdm2 network. The mathematical models – based on a set of differential equations – seem to be reliable to predict the mutual influence of that network components.

Now, which benefits could be foreseen by replacing a human cell throughout a digital control system?

Maybe, for an audience that is not used to these methods, it could be very difficult to make sense out of the writer ‘s explanations. Then, it is a crucial step to give a preview of the usefulness of such approach.

Even though the mathematical methods based on a set of ordinary differential equations (ODEs) are quite well capable to describe the cell proteins and species interaction – in time and amount scales – they are confined in the cell behaviour and evolution in “itself”. In other words, the mathematical methods describe “de facto” the actual cell dynamics if or not a DNA damage is present, i.e. the mathematical approach remain only witness of the considered cell proteins interactions.

If and when a (single) human cell is translated into a digital wiring platform, the optimal digital control theory is capable to modify the previous cell behaviour. In other words, through a digital scheme, it is possible to identify and/or detect more effective species and kinases, concentration and timescale evolutions in order to achieve faster and modified evolutions of DNA repairing. Besides, some biological conflicting points of view regarding p53/Mdm2 network feedback signs resolve their discrepancy when a unifying digital control theory could highlight a cell inner mechanism not completely understood.

The scientific disciplines of the writer lie inside aerospace technologies but an outcome reproducibility of the proposed methods by molecular biologists and genetic

engineering supervisions could be helpful to find (or focus on) guidelines to act in some kind for convenient mutant species and /or chemical enzymes and kinases.

Following this mind frame, also the apoptosis cell fate can be newly interpreted.

For the present aim of this paper, two recent articles[14, 15] will be useful for improving a cellular circuitry which is capable of digitally processing the p53/Mdm2 system dynamics and giving a subject for discussion about its influence on apoptosis. Both these papers deal with the mechanisms for triggering p53 pulses in response to a DNA damage.

These authors elegantly showed how to obtain sustained p53 oscillations when the p53/Mdm2 negative feedback can be supplemented and integrated by a positive loop. Although negative feedback is necessary for triggering oscillations in the p53/Mdm2 system, it is not yet sufficient. In fact, if one considers a negative feedback loop with only two elements, it cannot oscillate.

Moreover, in the paper of Zhang et al.[15], observations aimed at the employment of the p53 negative feedback loop and its observed oscillations address some important questions about the roles of positive feedback loops in generating and stabilizing oscillations and how apoptosis may be triggered by repeated pulses of p53.

It is my own firm belief that understanding the prominent role of the p53/Mdm2 loop and its sub-system dynamics offers a more than promising avenue for effective cancer therapy.

Now making reference to the previous studies of Ardito Marretta et al.[25, 26] regarding digital aerospace well-suited active control models and computer active control systems, *ad hoc* digital cellular circuitry is built up to pointwise recognize the cell damage checkpoint, the time-dependent p53 levels and the triggering of pulses. Moreover, a more complex digital cell scheme can determine different number of pulses when related to the expression of downstream genes and their evolution to give the chance –

in spin-off – to assign gradients of considered species with desired levels of p53 and/or leading to cell death.

Then, an integration of the proposed models – whatever type of feedback loop is employed – can be achieved. The digital control system theory, mathematically expressed in terms of state-space theory, could unify the disparate observations and offer the possibility of investigating apoptosis once the dynamics of inner protein forms is considered.

Adopted models and assumptions

What has aerospace science to do with cell biology? How, when and in what do these disciplines find a common subject? The human cell is a paramount design in which biochemistry, atomic theory, thermodynamics, biology and micro- and macro-scale structural laws – although not completely understood – can be translated into mathematical equations. Sometimes and not only for cell biology, some equations cannot be written and resolved without suitable assumptions and hypotheses. Let one example stand for all: although the fluid dynamic equations of Navier-Stokes should be (the only ones) applied for any fluid problems, they are, in fact, not soluble in closed form. Nevertheless, theories and models, simplified in such a way as to respect the physical phenomenon, like those of perfect gas or potential flows are currently employed for modern aerodynamic design! Let us now apply this (mathematical) scheme of working to a biological problem and its variables. To design suitable digital circuitry for a human single-cell, we start by taking into account the protein forms of p53 (p53 mono-ubiquitinated, p53 poly-ubiquitinated and p53 total, i.e., $p53_U$, $p53_{UU}$ and $p53_{tot}$, respectively) and Mdm2 (nuclear, cytoplasmic and phosphorilated, i.e., $Mdm2_{nuc}$, $Mdm2_{cyt}$ and $Mdm2P_{cyt}$, respectively) with their time-dependency from a set of equations[14]. A powerful tool for resolving the problem consists of processing the set of equations in a computational space, i.e., the *state-space* which mathematically represents all the possible conditions and combinations among the variables of the

problem. Once the state-space contains the necessary equations, the numerical solution will be the task of matrix calculus. In this case, through a matrix representation in the state-space of the mutual p53 and Mdm2 dynamics, a control matrix acting in positive feedback can be obtained and processed.

Control theory is widely employed in many engineering designs and several digital control strategies are currently applied to suppress or amplify instabilities and/or margins of safety. For digitally design of such control computer-aided systems, devoted algorithms must be developed and processed in appropriate circuitries. Similarly to typical digital control system design, a convenient set of mathematical equations may be available for processing the biological system parameters and their mutual influences.

For the single cell circuitry design, one can digitally transform the mathematical model proposed by Ciliberto et al.[14] (see Figs 1, 2) and the model #1 among those employed by Zhang et al.[15] (see Fig. 3).

Also, we digitally connect the control circuitries obtained from those models with a wiring diagram of apoptosis[15].

Making use of *Simulink*© software the mathematical models of Ciliberto et al.[14] and the model #1 employed by Zhang et al.[15] have been translated into assembled wiring digital platform and the single-cell digital circuitries of the two models (see tables 1 and 3 of, respectively) have been designed.

Cell digital wirings and connections were designed through a symbolic mathematical solution of the implemented ODEs of the employed models.[14, 15]

Cell species wiring diagrams and networks have been settled out with respect their biological interactions.

The final cell printed digital scheme triggers through a *Matlab*© computer recursive scheme based on Linear Quadratic Regulator given by the control theory of digital systems. And, more in detail, the digital circuits obtained in this paper have been obtained once the employed mathematical models[14, 15] have been translated into the

state-space domain according to the following mathematical scheme applied for a continuous time linear system, $\dot{x}$:

$$\dot{x} = Ax + Bu$$

with a quadratic cost function defined as

$$J = \int_0^\infty \left(x^T Q x + u^T R u \right) dt$$

The feedback control law that minimizes the value of the cost is

$$u = -Kx;\ K = R^{-1} B^T P$$

P is found by solving the continuous time algebraic Riccati equation

$$A^T P + PA - PBR^{-1} B^T P + Q = 0$$

Once the digital cell wiring platform, some observations are needed about the "digital control matrix" presence in the adopted cell circuitries.

For biology audience could be not immediate this presence and more than a question are unavoidable, i.e. what does the "digital control matrix" correspond to in an ODEs model? What does "switching on-off" the control matrix mean in term of biology (is it some kind of mutant, or another way to rewire the diagram)?

The employed control matrix derives for the control theory rules regarding the system dynamics. Even though it could be considered quite similar to a computer CPU, it governs not only all the mathematical processes in which the considered cell species are involved but also it is built up to numerically (and digitally) accomplish the feedback control law that minimizes the (dynamic) values of the chosen protein evolution (see its close-up in Fig. 2). The structure of the control matrix – once it is translated into digital scheme – has a pure mathematical frame and its presence is needed to simulate both the cell evolution in standard conditions ("off") as described by a set of equations concerning proteins evolution in "desired" condition if the cell genome is under attack and the modified and requested proteins signaling for accelerating the DNA repairing action ("on").

The most important features to underline are the obtained and "requested" dynamics of proteins network signaling to repair an imposed DNA damage. In terms of biology, it can be translated into a wild type form of one or more than one of the present transcription factor(s), or a different rate of phosphorilation in terms of protein terminals or kinases. The writer admits, at this step, his superficial knowledge of these biological subjects. The biological reproducibility will be desirable in the next future.

Further, one must note that the control matrix is only applicable to the model of Ciliberto et al.[14] and not to the employed model of Zhang et al.[15] because in the former the p53 influence on the DNA damage is mathematically connected, while, in the latter, this does not occur.

The reliability of the digital circuitry design is ensured in two different ways: in the first model – switching "off" the digital control matrix block (see Fig. 1) – we coherently obtain the same results as Ciliberto et al.[14] for the p53/Mdm2 dynamics when the same amount of DNA damage is imposed (see Fig. 4); moreover, the other digital circuitry faithfully repeats the p53/Mdm2 evolution of the model #1 of Zhang et al.[15], as shown in Fig. 5.

The implemented computational scheme and the mathematical procedures for matching and tuning the matrix cell optimal control are also outlined in a dedicated section of the paper.

Now, it should be noted that, in state-space theory, an applied digital wiring control scheme – based on the extended form of ODEs (ordinary differential equations) employed by Ciliberto et al.[14] and Zhang et al.[15] – should be not applicable in the state-space itself. This is due to the presence in the state-vector – as components – of both the p53 forms and its derivatives. To overcome this inconvenience, one must consider in the proposed mathematical models the absence of relationships among the first-derivatives on the right-hand side of the equations and those put on the left-hand side. Let us now describe in detail the procedures employed.

Model of Ciliberto et al. – Taking into account the system of non-linear/first order differential equations (ODEs) of Ciliberto et al.[14], one can describe both p53 and Mdm2 dynamics as well as the evolution of their forms. This mathematical approach is capable of deducing the mutual influence among these concentrations. The digital circuitry shall be able to reproduce the experimental basis of this model, i.e.:

a) Mdm2 and p53 are mainly degraded in the cell nucleus; b) Mdm2 is the activator of a reaction for degrading p53 in a ubiquitin-manner; c) Mdm2 attaches only two ubiquitins of p53 ($p53_U$ and $p53_{UU}$); d) three forms of p53 ($p53_U$, $p53_{UU}$, and $p53_{tot}$) induce transcription of Mdm2 in nonphosphorilated and cytoplasmic forms; e) for translocating into the nucleus, $Mdm2_{cyt}$ needs to be phosphorilated ($Mdm2_{cyt} \rightarrow Mdm2P_{cyt}$); f) the phosphorilated cytoplasmic $Mdm2P_{cyt}$ moves freely into and out of the nucleus; g) phosphorilation of $Mdm2_{cyt}$ is inhibited by $p53_{tot}$ in looping.

In the present study, to achieve the optimal control scheme – based on an *LQR*-type (*Linear Quadratic Regulator*) kernel – a suitable manipulation of the previous set of ODEs has been done for writing and using them from the extended form into the state-space representation.

Following this procedure, a dynamic matrix representation of the p53/Mdm2 system is obtained in which the time-dependent and mutual influence of these proteins can be fully described. From now on, the inner mechanisms of degradation of forms of cell proteins and/or, phosphorilation, are marginally mentioned, the purpose of the present work being a global mathematical procedure to design a cellular circuitry and the post-processing of the output.

In agreement with to but differently from Ciliberto et al.[14], in the present procedure, a non linear ODE has been employed to write the set of equations with respect to the p53 protein and not to the total p53. In more detail, the state-vector for p53 and its concentrations shall be

$$\underline{x}(t) \square \left[[p53][p53_U][p53_{UU}][\dot{p}53][\dot{p}53_U][\dot{p}53_{UU}] \right]^T$$

where the overdot represents the time-derivative and the subscripts *U*, *UU* identify the first-, the poly-ubiquitin protein forms and *T* (upper the right-hand square bracket) the transpose of the vector, respectively; while the vector

$$\underline{b} = \begin{bmatrix} k_{s53} & 0 & 0 & 0 & 0 & 0 \end{bmatrix}^T$$

contains the coefficient of Ciliberto et al.[14].

We consider the level of [$Mdm2_{nuc}$(t)] as a time variable and a matrix $\underline{\underline{P}}$ for the dynamic activity of p53 (time-variant). Mdm2 dynamics can be expressed by a vector in the state-space.

Once the proposed state-space representation gives the same results as the model of Ciliberto et al.[14], the digital optimal control law has been implemented and based on the assumption that the matrix is such that [$Mdm2_{nuc}$] is equal to a constant. Then, the compact expression

$$\mathbf{P} = \left\{ \underline{\underline{P}}_{ij}; i, j = 1,2,3 \; so \; that \; p_{ij} \in \Re \right\}$$

(in which the symbols $\in$ and $\Re$ mean "belonging to" and range of real numbers, respectively) represents the time-invariant dynamic super-matrix. Using the digital scheme, several simulations have been performed and they allowed the identification of the constant value of [$Mdm2_{nuc}$] equal to 0.1 in such a way as to obtain a rate of DNA repair much more quickly than was obtained by Ciliberto et al.[14]

Model #1 of Zhang et al. – At first, the question formulated in this work seems to be appropriate, i.e., is a negative feedback loop (p53 upregulates Mdm2, which deactivates p53) sufficient to explain the observed oscillation? Conversely, we put another question: if the negative feedback loop between p53 via Wip1 (see Batchelor et al.[24]) is essential to maintain the uniform shape of p53 pulse, is it possible to find a unification

of the "opposite" points of view shown in Zhang et al.[15] and Batchelor et al.[24]? Maybe this opportunity is more than possible.

In fact, from a purely mathematical point of view, the feedback becomes positive if it is considered as a *part* of a complete typical digital system. Instead, if one looks at the single state-vector quantities (in feedback) containing all the forms of p53, then the feedback loop becomes "hybrid" (positive - negative) in itself, because any element of the control matrix has opposite sign with each other. In a schematic representation, one has the control matrix as follows

$$\underline{\underline{K}} = \begin{bmatrix} +k_{11} & -k_{12} & +k_{13} \\ \vdots & +k_{22} & -k_{23} \\ sym & \cdots & +k_{33} \end{bmatrix}; k_{ij} > 0$$

The optimal control matrix is derived from the stabilizing solution of the Riccati equation as a function of the dynamic matrix of the protein p53 whose elements are the reaction coefficients of Ciliberto et al.[14]. The above k_{ij} matrix elements are, in turn, functions of those coefficients.

In the model #1 of Zhang et al.[15], Mdm2 activates p53. Combined with p53-induced Mdm2 transcription, Mdm2 thereby enhances its own synthesis. The values of stable steady state concentrations are obtained once the level DNA damage is set equal to zero. When this level is different from zero, the degradation of nuclear Mdm2 increases and its concentration begins to fall. The interesting result of this model is that if the damage level is quickly repaired, the p53/Mdm2 control system develops a single-pulse response to repair the DNA damage itself. The second pulse occurs if the level of the DNA damage is relatively high. Also in this case, the designed digital circuitry faithfully reproduces the results as shown in Fig. 5.

Apoptosis – Zhang et al.[15] show a wiring diagram of apoptosis and apply it to their model #1 (see Fig. 2 of Zhang et al.[15]). Although the above mentioned models[14, 15] give different evolutions of the DNA damage repairing, digital control theory in state-space

can unify these models on the basis of the developed control matrix. Indeed, a well-suited optimal control law allows any DNA damage evolution to be assigned to the realized human single-cell circuitry. Then, we take into account the DNA damage level and shape proposed by Zhang et al.[15] applied to the model of Ciliberto et al.[14] once it is connected to a wiring diagram of apoptosis (see Figs 1, 3).

Results and discussions

The first "generation" of mathematical models for p53/Mdm2 interaction takes into account a simplified description of the negative effect of the oncogene Mdm2 on its antagonist p53, i.e., the inhibition of p53 transcriptional activity and the p53 degradation through the binding of Mdm2 to the p53 itself. Successively, Lahav et al.[22] confirm the p53/Mdm2 negative feedback loop oscillations once functional p53-CFP (cyan fluorescent protein) and Mdm2-YFP (yellow fluorescent protein) fusion proteins are observed through time-lapse fluorescent microscopy. After γ-irradiation at a 20min resolution during 16h of growth, these authors measured the total fluorescence in the nuclei of over 200 different cells through a movie technique. Their conclusions regarding the p53 digital behaviour consist of different fractions of cells showing zero, one, two or more pulses as a function of γ-irradiation dose; the width of each pulse was 350±160min, the timing of the first pulse maximum being rather variable (360±240min) after damage; the time between the maxima of two consecutive pulses is more precise, i.e., 340±100min. Thus, they found that in the p53/Mdm2 feedback loop system, the number of pulses, but not the size or shape of each pulse, depends on the level of the input signal.

Here, we consider the running digital control process for the previous selected models of Ciliberto et al.[14] and Zhang et al.[15] and evaluate the p53 dynamics in interaction with the other protein parameters and the DNA damage levels.

Model of Ciliberto et al. – Looking at the simulation output when the digital control matrix is switched "on", it has been roughly noted that the $p53_{tot}$ and $Mdm2_{nuc}$ levels increase throughout the same timescale (see Fig. 6) when compared to the results having the digital control matrix switched "off"; since high levels of $p53_{tot}$ involve a decrease in the DNA damage, we now switch the [$Mdm2_{nuc}$] level to its lowest value in such a way as to obtain a positive feedback loop according to the digital control theory rules.

Surprisingly, the results change deeply. Digital optimal control is then able to realize remarkable effects: first of all, a DNA damage repair speed faster than 50% (see Fig. 7) and – in cascade – a relevant variation of p53/$Mdm2_{nuc}$ dynamics. From the comparison between Figs 4 and 6, one may deduce that the oscillation parameters of p53/Mdm2 have been modified in terms of amplitudes (higher concentrations) and interpulse (time-shift in the second pulse of about 40min). The initial conditions of Ciliberto et al.[14] being equal, the faster rate of DNA damage repair can strongly affect the response of the whole p53/$Mdm2_{nuc}$ dynamics. Moreover, the optimal control matrix is able to output sustained amplitudes of both p53 and $Mdm2_{nuc}$ values.

The dual action of the control matrix has different damping effects: these are more evident for the second pulse of p53 and almost irrelevant for Mdm2. This different behaviour in the second pulse is linked to the local gradient of the DNA damage repair pattern; while in the first pulse, the difference – between the uncontrolled and controlled models – of the DNA damage levels is very high, the local amount and then the gradients are quite superimposable up to 220min. When the second pulse triggers, the DNA local damage levels are totally different in shape (gradient) and amplitudes. In the uncontrolled digital system, the gradient is confined inside a range of 80min, while in the controlled one, the range becomes wider up to 120min. In any case, as regards the p53/$Mdm2_{nuc}$ system, the action of the control matrix implies an overall effect, i.e., an amplifying of concentrations scattered along the same timescale. Also, the global interpulse of the p53/Mdm2 network is shifted; from a comparison between Figs 4 and 6, one can deduce that its first pulse triggers at the same time interval (200÷240min); while, in the digital control system, the second pulse occurs after a delay of 600+48min. Both the uncontrolled and controlled digital circuitries show in-phase oscillations of the single protein forms, the frequency of the $[p53_{tot}/Mdm2_{nuc}]_{uncontr}$ system being equal to 3.78 10^{-5}Hz and 3.3 10^{-5}Hz for $[p53_{tot}/Mdm2_{nuc}]_{contr}$ network.

The use of appropriate cell digital machinery reveals interesting perspectives for future biology spin-off. Some of them seem to be relevant in the next future: mathematical prediction of cell behaviour in absence of *in vivo* tests; future investigation for pharmacological and/or drug therapies to realize the optimal dynamics of cell proteins; proteins dynamics to inhibit or amplify some species against others; the influence of some cell species in presence of a DNA damage. Strictly speaking, accuracy of results, freedom in choosing any other cell proteins forms, genotypes and/or target genes, circuitry components manipulations and low computational resources yield reason enough for these perspectives. Let us now exploit the wide range of regulations of a digitally-aided cell scheme. For example, is the proposed digital machinery able to replicate *in toto* the cell protein dynamics when genomic damage remains constant? And moreover, what is the influence of the dynamic control matrix on the DNA repair pattern in the absence of an external input from one of the proteins considered? In other words, the sense and the goal of the challenge are not only the acceleration of DNA repair through a digital control matrix nor to simulate cell protein evolution of a human cell under cancer attack but to assign the task of cell defence to a mechanism governed by an integrated bio-digital system. Ultimately, the digital control circuitry shall disregard the protein evolution which "normally" occurs in a cell with genomic damage but, instead, it becomes a new supervisor to *ex novo* assign the required laws of protein behaviour. Thus, starting (for example) from the p53 oscillations as components of a state-vector in the state-space and whatever the constant DNA damage level, this *must* be repaired. The deduction from these assertions is that the cancer cell could not yet become aware of having the strength to play the mortal match. Now, or in the near future, an external digital control has or will have the task of dictating the rules of the game.

Having this in mind, we start to adjust the cell digital platform of Fig. 1 to obtain a modified circuitry like that of Fig. 8 in which the digital control matrix is directly linked

to the p53/Mdm2/DNA damage system. To check the capability of the digital control matrix to externally govern the p53/$Mdm2_{nuc}$ network – independently from the natural and/or aberrant protein evolution after DNA damage – we introduce an ionizing amount 5 times greater than the dose of Ciliberto et al.[14]

$$IR = ampl \cdot heav\left(0 \le t \le 10\right); ampl = 5\,unit$$

The simulation results shown in Figs 9-10 demonstrate the attendance of the digital control matrix - in the absence of a direct p53 signal arising from a damaged cell and feedback the p53 state-vector components only – modulates the p53/Mdm2 oscillations until the damage is totally repaired. The number of pulses and amplitudes of the p53/Mdm2 system are now quite different from those obtained with the previous simulations. Two effects are more evident, i.e., the phase-shift between the p53/Mdm2 network (see after the second pulse in Fig. 10) and the delay time after the fifth pulse. According to the local gradients of the DNA repair pattern (see Fig. 9), the p53/Mdm2 system undergoes forward steady-stable conditions.

Model #1 of Zhang et al. – As mentioned before, this model has been converted into a digital platform circuitry for two reasons: one, to check the reliability of the cell digital machinery and, second, for the proposed apoptosis wiring diagram shown and linked by those authors to this model. As mentioned before – in the light of the interchangeability of the previous digital control matrix – we adopt this model and its protein forms to analyse the response of the system when an apoptosis wiring diagram is considered. In this model, Mdm2 activates p53 and, in positive feedback, p53 and $Mdm2_{cyt}$ abruptly increase. At this point, an increasing quantity of Mdm2 migrates into the nucleus so giving degradation of p53 and a decrease in its level. As a consequence, the Mdm2 rate decreases and, accordingly, the $Mdm2_{nuc}$ level drops (see Fig. 5). In this model, the initial DNA damage is repaired at a constant rate. When a wiring diagram of apoptosis is connected to this model, those authors define three forms of p53 (*helper*, *killer* and *lurker*) and follow the evolution of these forms to elegantly deduce and propose a

digital apoptosis mechanism by numbering the p53 pulses. In agreement with to but differently from those authors, we link their apoptosis wiring diagram – digitally converted – to cell digital circuitry having a digital control matrix (see Fig. 1).

Apoptosis – the decision on cell fate is now conceived and bound through the modified pulsing mechanism of p53 and other considered species when the cellular digital optimal control system is applied to an apoptosis wiring diagram (see Figs 1, 3, 8). Following Zhang et al.[15], we take into account their apoptotic model (see table 5 of their mentioned work). In sequence, switching "on" and "off" the cell digital control matrix, the controlled and uncontrolled feedback networks give results concerning the time evolution of the apoptotic species (cyclin-dependent kinase inhibitor, p21; p53-regulated apoptosis-inducing protein 1, p53AIP1; apoptotic protease activating factor 1, APAF1; and target gene "cytoc" as a functional of APAF1 in apoptosome expression) , the value of apoptosome parameter (expressed by the Heaviside function) being equal to 1 for matching (or not) the cell death. A comparison of Figs 11 and 12 show remarkable information. First of all, instabilities of apoptotic species vanish in the digital control system and, in sequence, variations of cell death parameter in terms of timescale and triggering occur. In the uncontrolled system, the cell fate triggers at 124.61min and goes on for 32.3min, while in the digital controlled network cell fate triggers at 115.3min and remains for 27.7min. Moreover, interesting topics of discussion can be derived from the previous results when they are sketched all at once on the same timescale (see Figs 13 and 14).

Looking at the results shown in Figs 13-14, one could venture a comment about a "suspicious" behaviour of p21 protein In fact, both the uncontrolled and controlled systems (although with different triggering and time range) show apoptotic phase just in correspondence with the inflexion point of the considered species amount evolution. A possible and common (for both the networks) unification could be mathematically expressed (the writer steps aside about the possible biological implications): cell death

triggers when the local gradients of the species (p21 being equal to zero or being inactive) reach their maximum values at the same instant; apoptotic phase remains until all the considered species match their numerical maximum values; then p21 triggers (sic!). Once the local gradient of p21 reaches its transient maximum value, the cell death phase ends.

Following the above mentioned procedure in which the digital control matrix is directly linked to the p53/Mdm2/DNA damage system, we come back to turn on the modified circuitry of Fig. 8 to externally govern the p53/$Mdm2_{nuc}$ network – independently from the natural and/or aberrant protein evolution after DNA damage – and we introduce an ionizing amount 5 times greater than the dose of Ciliberto et al.[14]

The DNA damage level being the same as in Fig. 9, the modified circuitry scheme of Fig. 8, having the digital matrix directly linked to the p53/Mdm2 network, faithfully replicates the apoptotic species of a cell in which a constant DNA damage level is considered (see Fig. 15).

To sum up, we have reason to believe that the evidence of designing a cell digital platform could be useful and representative in studying p53/Mdm2 evolution in a cancer cell and its fate. This is a first step. Limitations of the current digital design can be found in its application to a single-cell and the hypothesis of the considered number of apoptotic species (one has to remember that only some cases of Zhang et al.[15] match cell death).

Nevertheless, those limitations can become future qualities when the proposed digital cell circuitry is enhanced for multi-cellular systems and for suitable feedback of the ionizing radiation signal, which will be studied in future work.

Outcome reproducibility

Once the initial conditions of stable steady-state are considered (see table 1), a digital control circuitry design can be performed through the protein [p53] state-space representation (or the equivalent [Mdm2] in feedback), i.e.:

$$\underline{\dot{x}}(t) = \underline{\underline{P}}\left(\left[Mdm2_{nuc}(t)\right]\right)\underline{x}(t) + \underline{b} \tag{1}$$

$$\underline{z}(t) = \left[\left[Mdm2_{nuc}\right]\left[Mdm2_{cyt}\right]\left[Mdm2P_{cyt}\right]\left[Mdm2_{nuc}\right]\left[Mdm2_{cyt}\right]\left[Mdm2P_{cyt}\right]\right]^T \tag{2}$$

$$\underline{\dot{z}}(t) = \underline{\underline{M}}\left(\left[p53_{tot}(t)\right], k_{d2}(t)\right) + \underline{c}\left(\left[p53_{tot}(t)\right]\right) \tag{3}$$

$$\underline{c} = \left[0\ f\left(\left[p53_{tot}(t)\right]\right)0000\right]^T \tag{4}$$

where the subscripts *nuc*, *cyt* and *Pcyt* identify nuclear, cytoplasmic and phosphorilated cytoplasmic protein forms, respectively; while the vector (4) contains the known function f of Ciliberto et al.[14]

More precisely, some auxiliary matrices have been employed as follows:

$$\underline{\underline{K}} = LQR\left(\underline{\underline{P}}, \underline{\underline{BB}}, \underline{\underline{Q}}, \underline{\underline{R}},\right) \tag{5}$$

where $\underline{\underline{K}}$ is the digital optimal control law matrix, $\underline{\underline{BB}}$ the input-state transition matrix, $\underline{\underline{Q}}$ and $\underline{\underline{R}}$ are here positive definite matrices. The pre-multiplying factors of the matrices $\underline{\underline{Q}}$ and $\underline{\underline{R}}$ have been imposed in such a way that the pattern of the components of the state-vectors $\underline{x}(t)$, $\underline{z}(t)$ and the DNA damage are quite similar to those of Ciliberto et al.[14], while the optimal control matrix has to accomplish the task to accelerate the DNA repair process according to the equation:

$$\frac{d\left[DNA_{dam}\right]}{dt} = kDNA[IR] - k_d DNA\left[p53_{tot}\right]\frac{\left[DNA_{dam}\right]}{J_{DNA} + \left[DNA_{dam}\right]} \tag{6}$$

in which *IR* represents the functional of the imposed radiation dose.

The system of differential equations obtained must be processed and resolved, i.e.:

$$\begin{cases} \underline{\underline{P}}^T \underline{\underline{S}} + \underline{\underline{S}}\,\underline{\underline{P}} - \left(\underline{\underline{S}}\,\underline{\underline{\underline{\underline{BB}}}}\right)\underline{\underline{R}}^{-1}\left(\underline{\underline{\underline{\underline{BB}}}}^T\right) + \underline{\underline{Q}} = 0 \\ \underline{\underline{K}} \equiv \textit{optimal control law} = \underline{\underline{R}}^{-1}\left(\underline{\underline{\underline{\underline{BB}}}}^T\,\underline{\underline{S}}\right) \end{cases} \tag{7}$$

from the first equation of the above system, one obtains the Riccati stabilizing solution $\underline{\underline{S}}$, and the second equation is then solved.

The optimal control matrix terms being a function of the following parameters:

$$\begin{cases} \underline{\underline{K}} = \left\{ k_{ij} \in \Re \,/\, k_{ij} = f\left(q_{ij}, r_{ij}, [Mdm2_{nuc}], k_f, k'_{d53}, k_{d53}, k_r\right) \right. \\ [Mdm2_{nuc}] = const \\ q_{ij} \in \underline{\underline{Q}} \\ r_{ij} \in \underline{\underline{R}} \end{cases} \tag{8}$$

easy mathematical manipulation of the equation of p53 yields:

$$\frac{d}{dt}[p53_{tot}] = \frac{d}{dt}[p53] + \frac{d}{dt}[p53_U] + \frac{d}{dt}[p53_{UU}] \tag{9}$$

and then one obtains:

$$\begin{aligned} &\frac{d}{dt}[p53] = -k'_{d53}[p53] - \left(k'_{d53}[p53_U] + \frac{d}{dt}[p53_U]\right) + \\ &-\left(\left(k'_{d53} + k_{d53}\right)[p53_{UU}] + \frac{d}{dt}[p53_{UU}]\right) \\ &\frac{d}{dt}[p53_U] = k_f[Mdm2_{nuc}][p53] - \left(k'_{d53} + k_r + k_f[Mdm2_{nuc}]\right)[p53_U] + k_r[p53_{UU}] \\ &\frac{d}{dt}[p53_{UU}] = k_f[Mdm2_{nuc}][p53_U] - \left(k'_{d53} + k_{d53} + k_r\right)[p53_{UU}] \end{aligned} \tag{10}$$

Now, if the optimal control law is applied, further terms belonging to the matrix $\underline{\underline{K}}$ must be added, and then the extended form of the final system of equations is obtained. Rearranging the system (10), one comes to:

$$\frac{d}{dt}[p53] = -\left(k'_{d53} + k_f\,[Mdm2_{nuc}]\right)[p53] + k_r\,[p53_U] + k_{s53} + \sum_{n=1}^{3} k_{1,n} x_{n,1}$$
$$\frac{d}{dt}[p53_U] = k_f\,[Mdm2_{nuc}][p53] - \left(k'_{d53} + k_r + k_f\,[Mdm2_{nuc}]\right)[p53_U] +$$
$$+ k_r\,[p53_{UU}] + \sum_{n=1}^{3} k_{2,n} x_{n,1} \qquad (11)$$
$$\frac{d}{dt}[p53_{UU}] = k_f\,[Mdm2_{nuc}][p53_U] - \left(k'_{d53} + k_{d53} + k_r\right)[p53_{UU}] + \sum_{n=1}^{3} k_{3,n} x_{n,1}$$

For the adopted values of the matrix elements, k_{ij}, see table 2. Once the *LQR* has been performed, those elements were obtained by a linear combination of the rate constants shown in table 1.

Model simulation

All the recursive routines and circuitries were processed using *Matlab*/*Simulink* platforms at the Department of Mechanical Engineering – University of Bath (UK) – under the supervision of doctor Michael Carley.

Appendix A

The coefficients used are shown in tables 1-2

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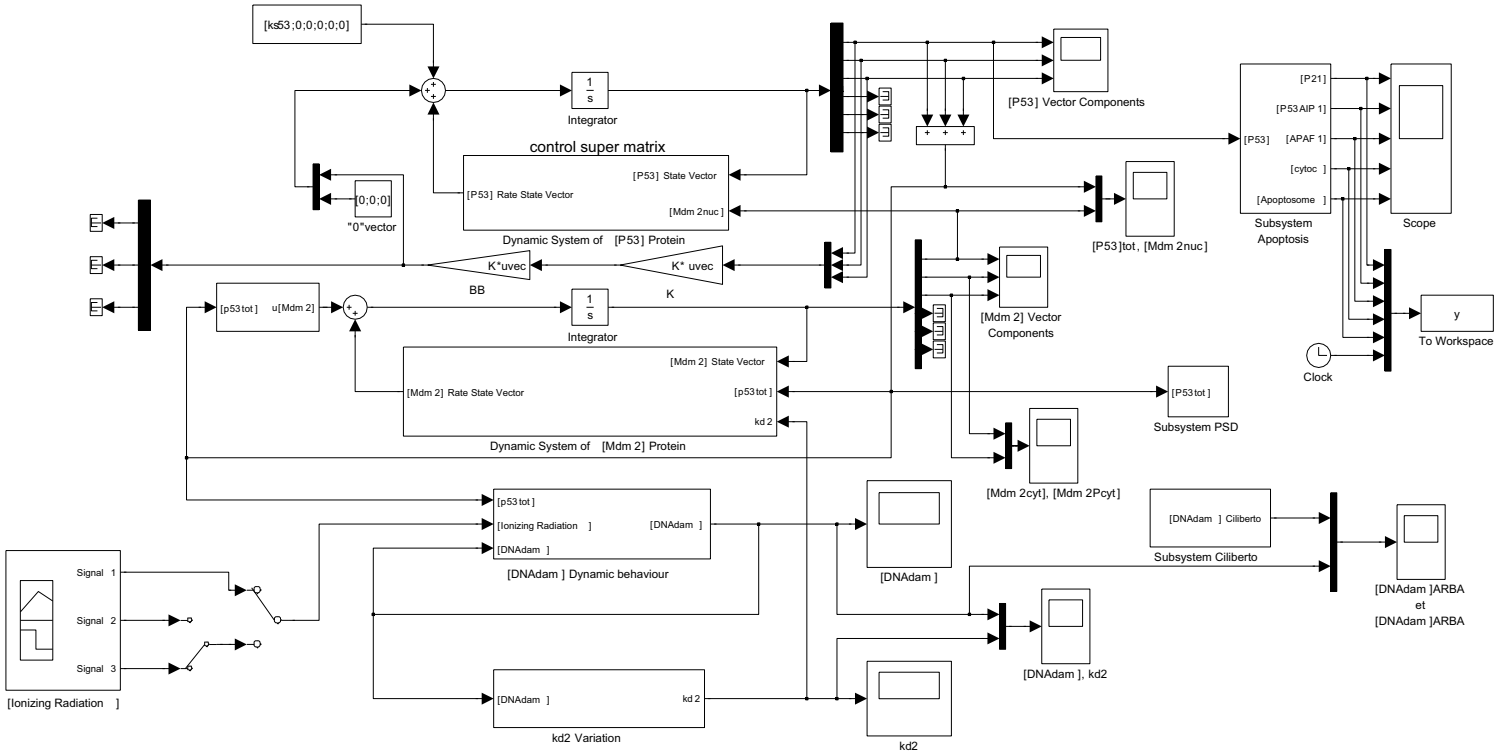
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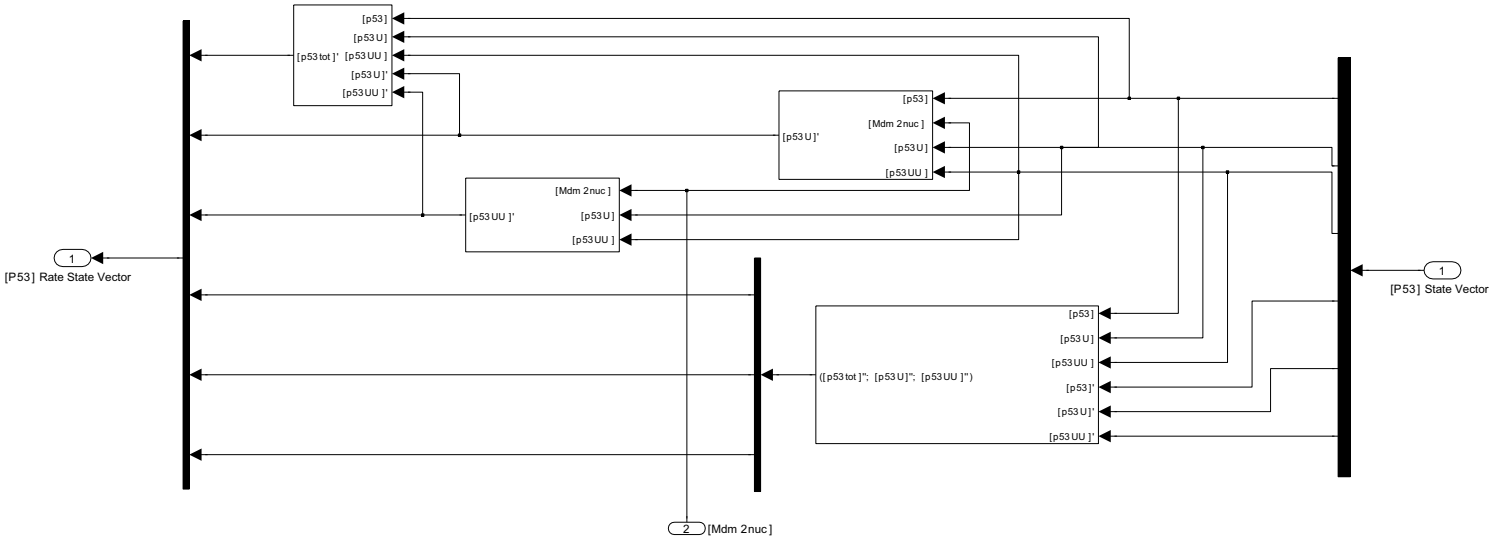
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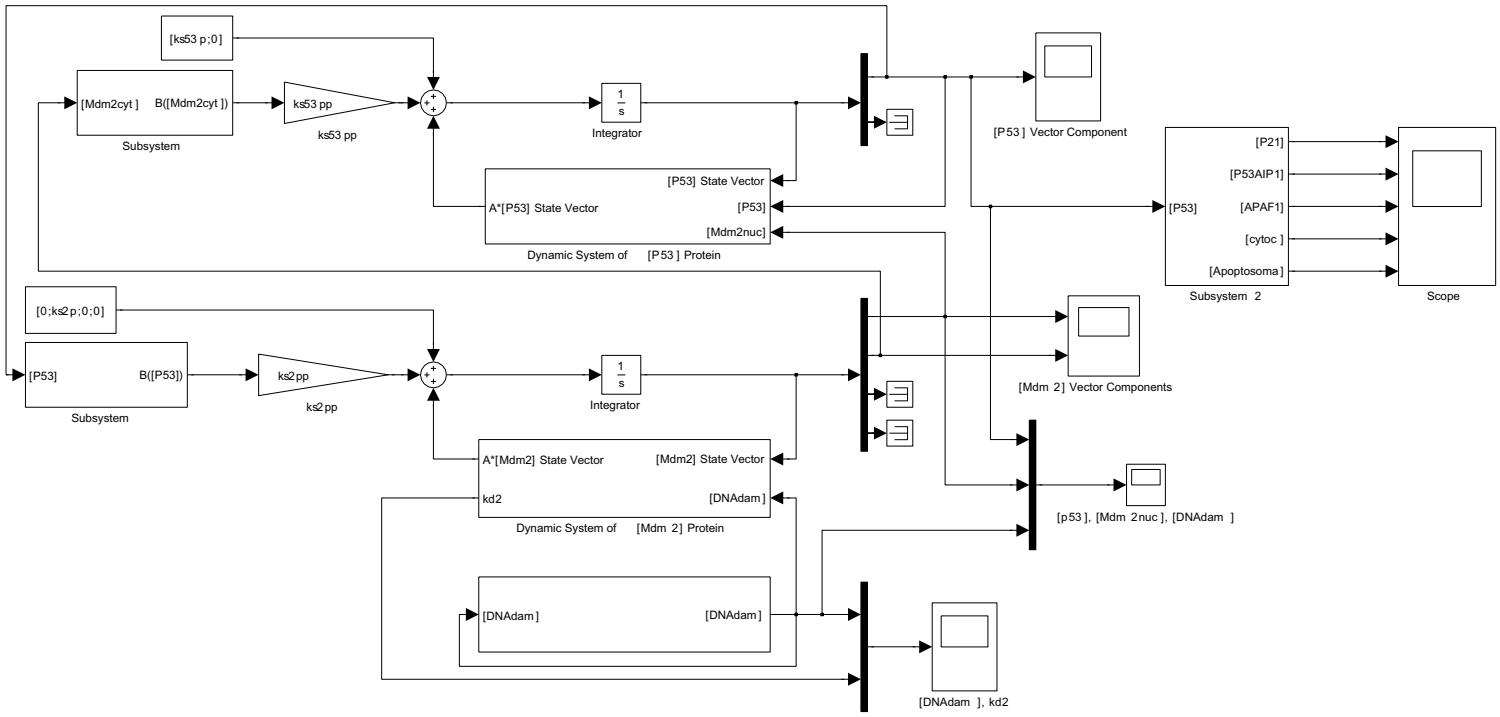
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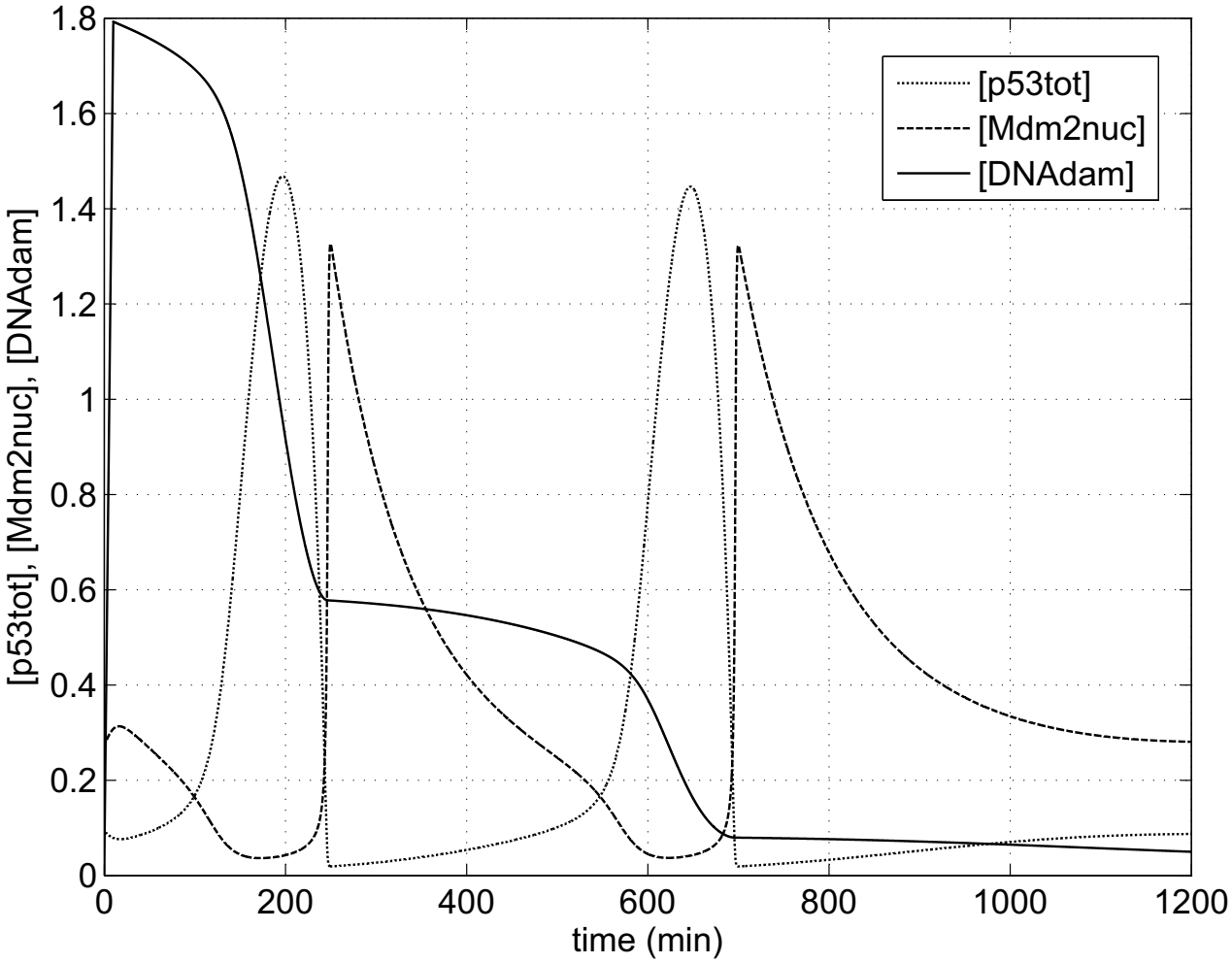
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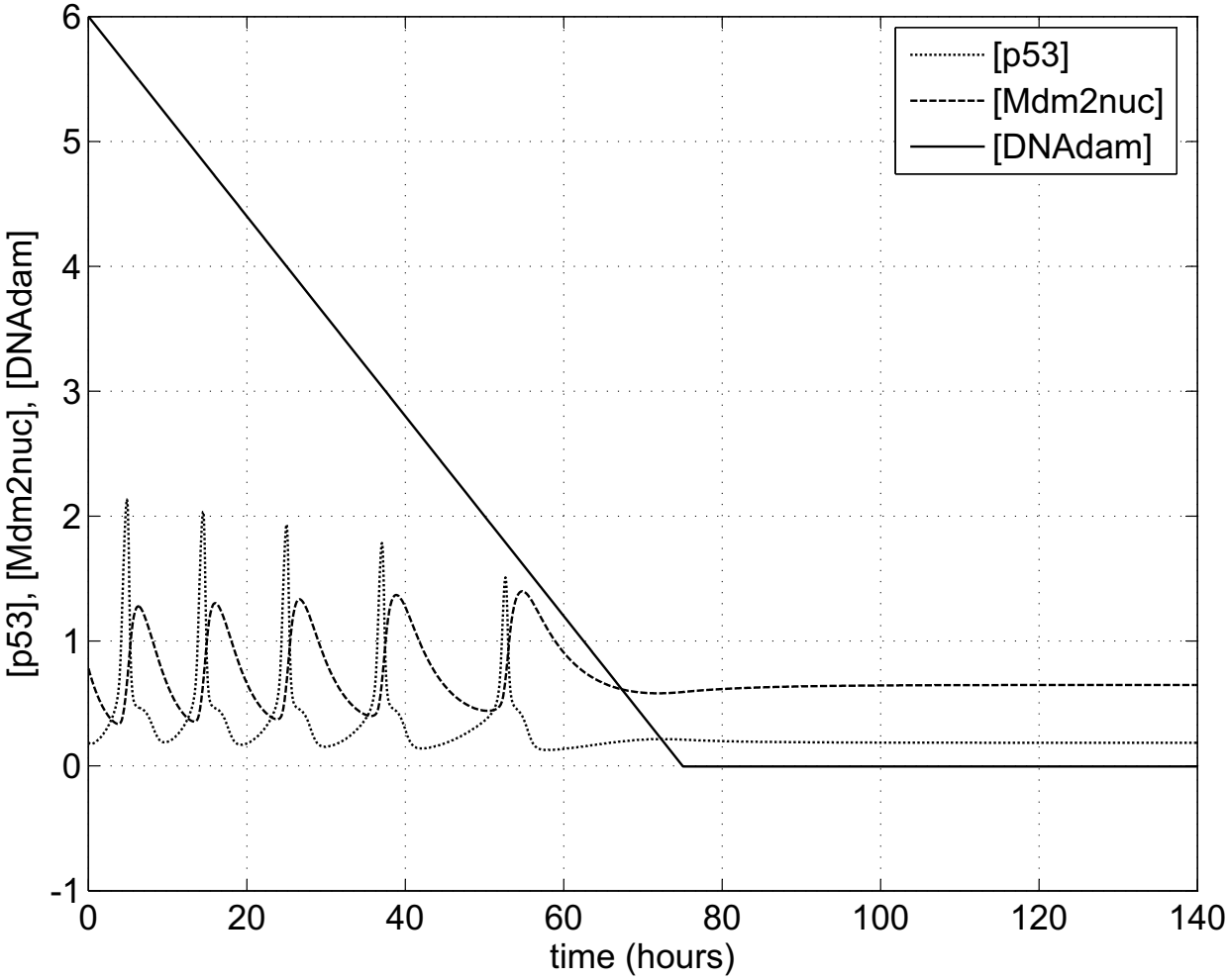
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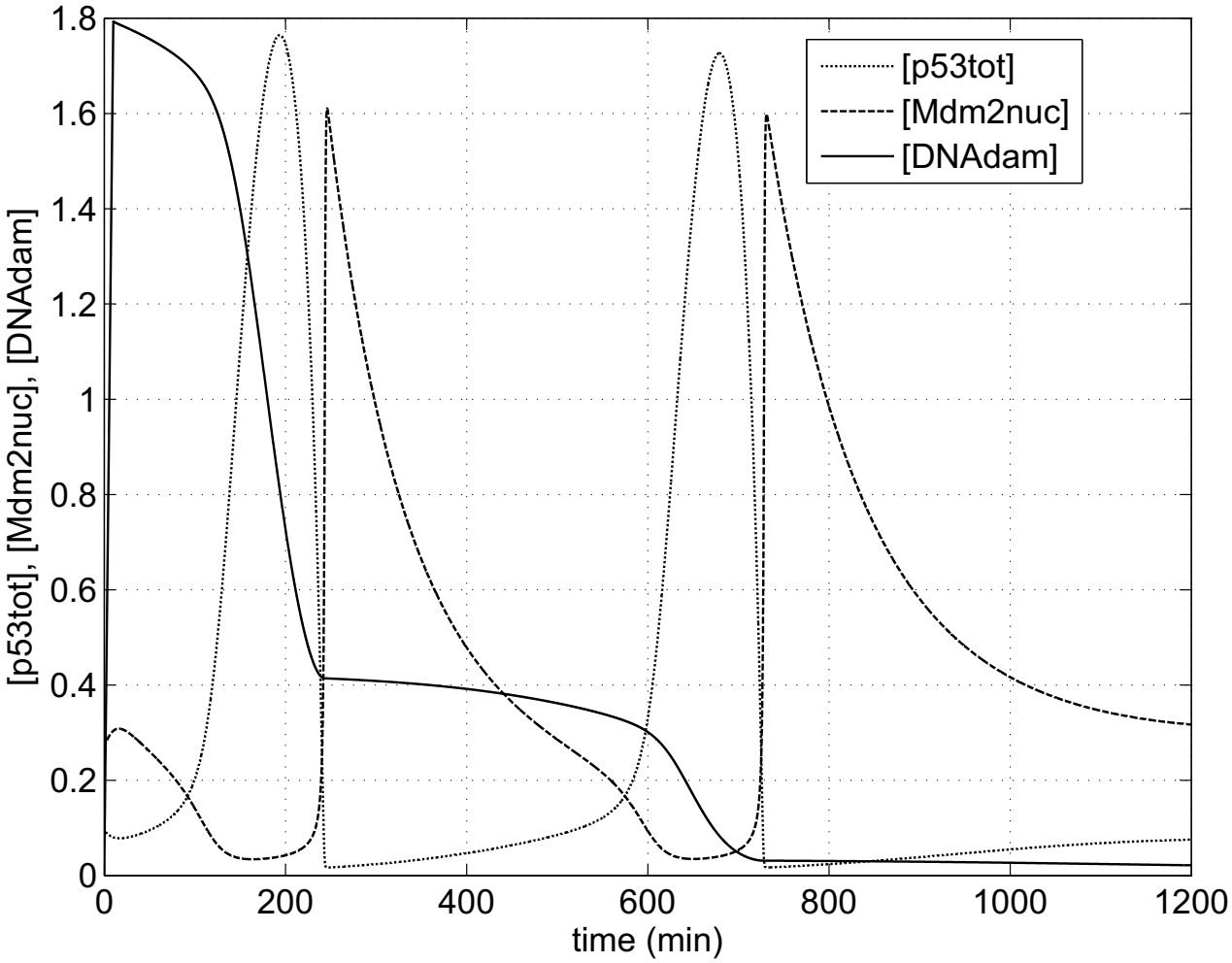
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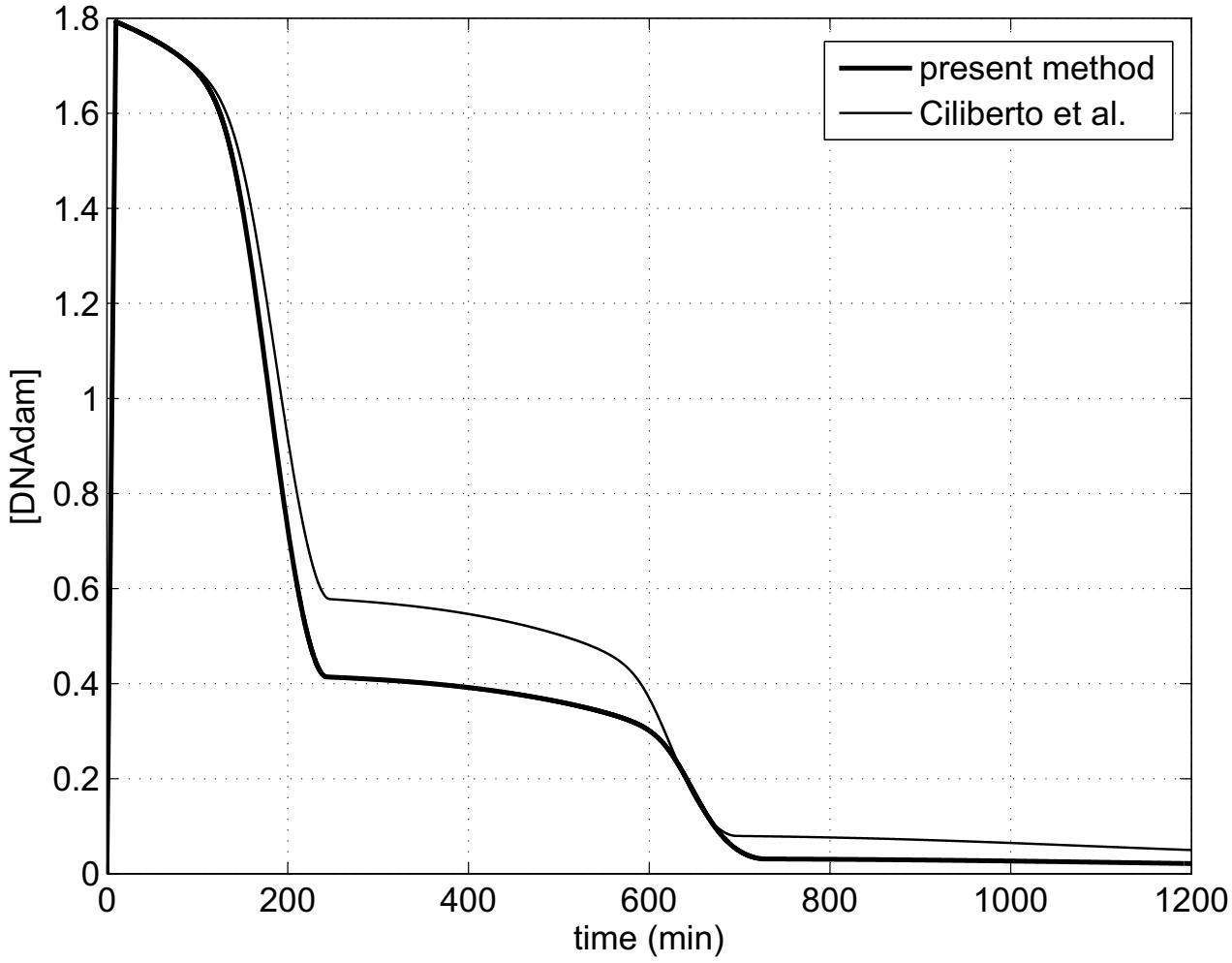
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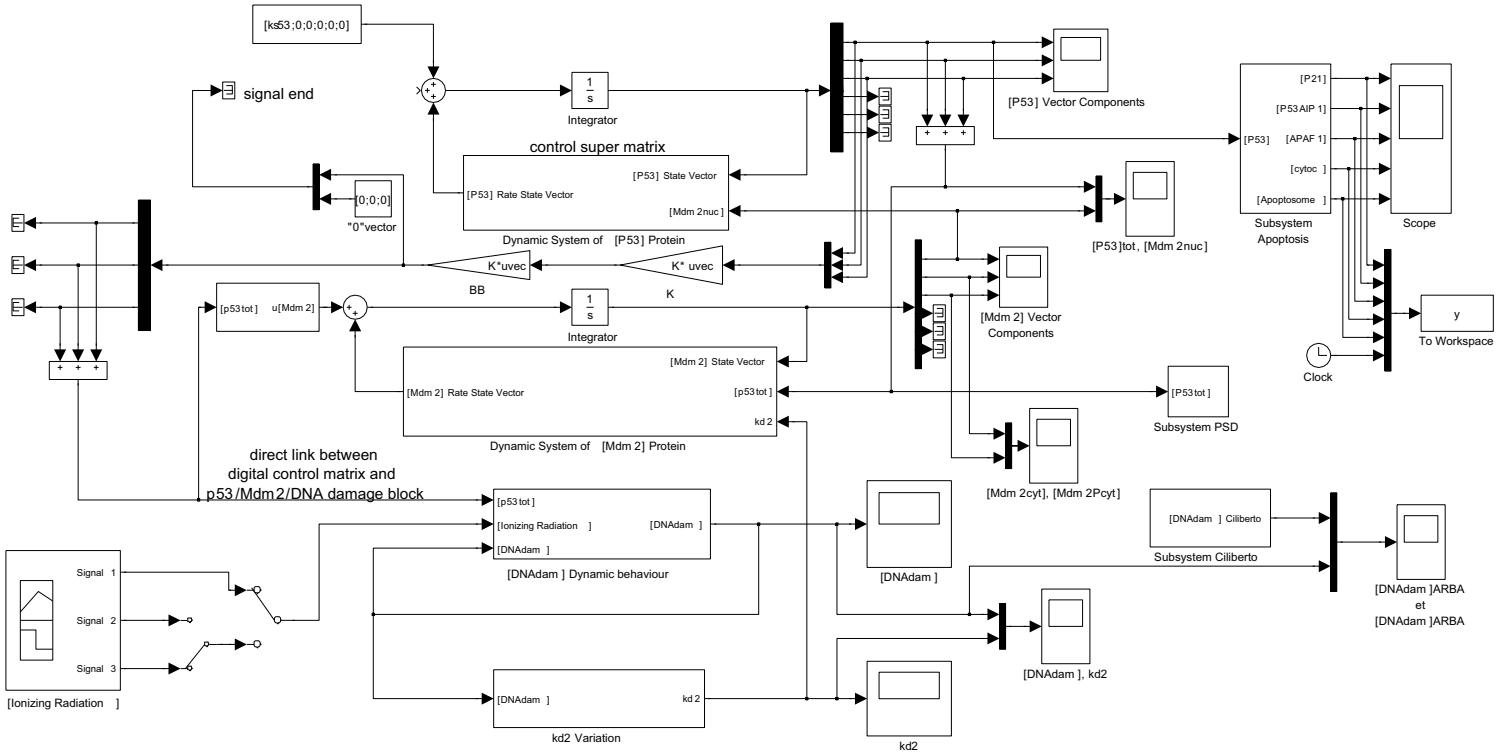
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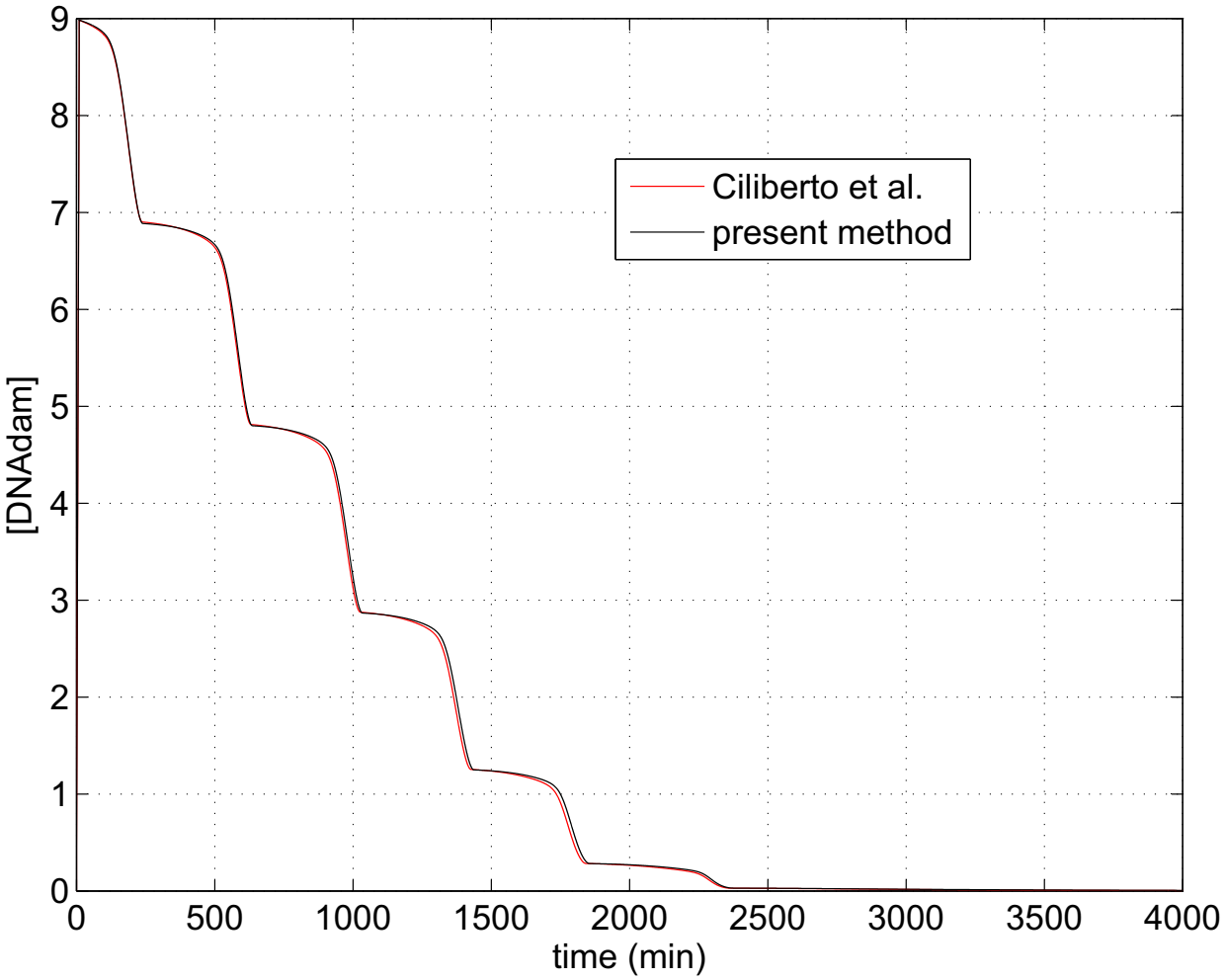
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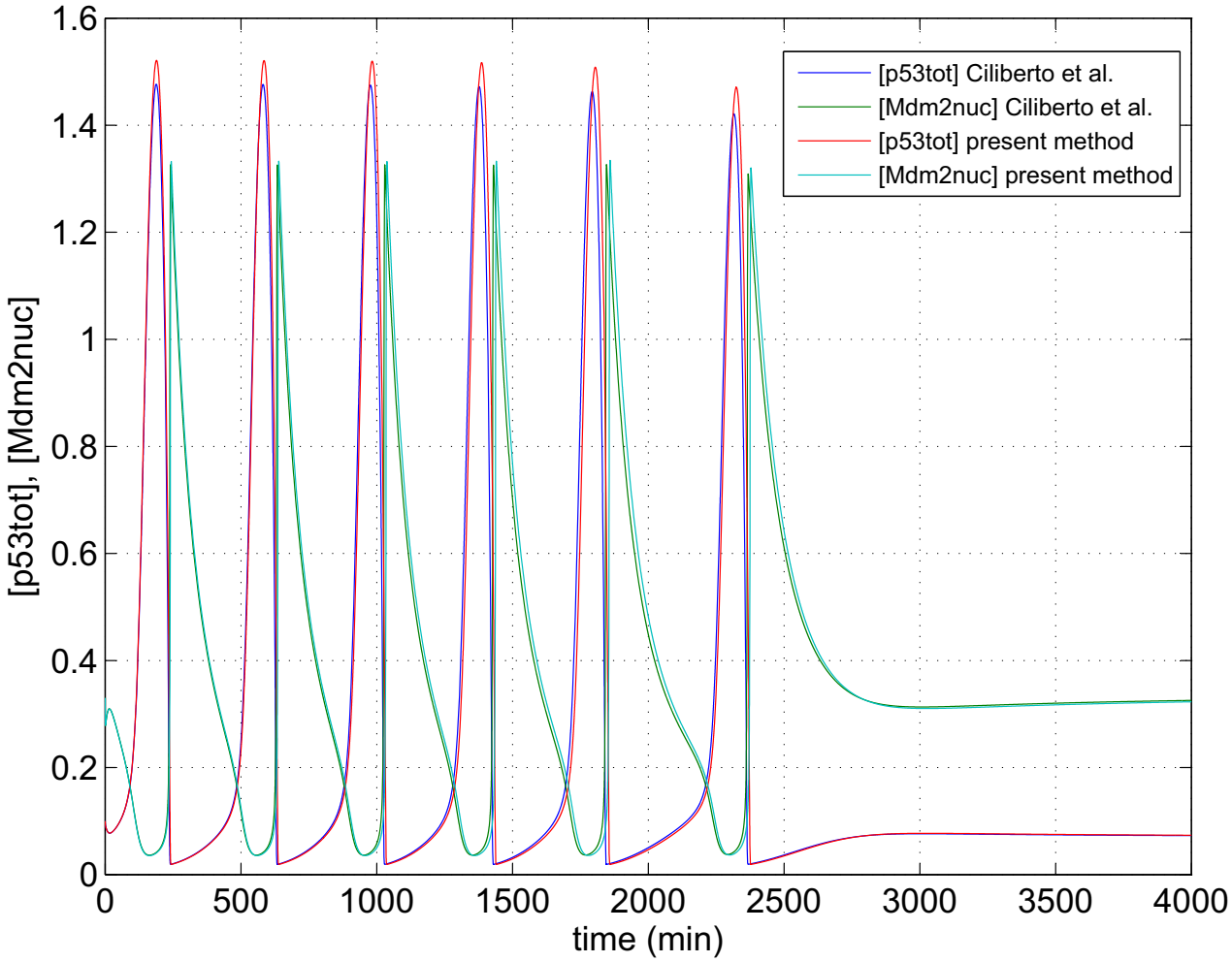
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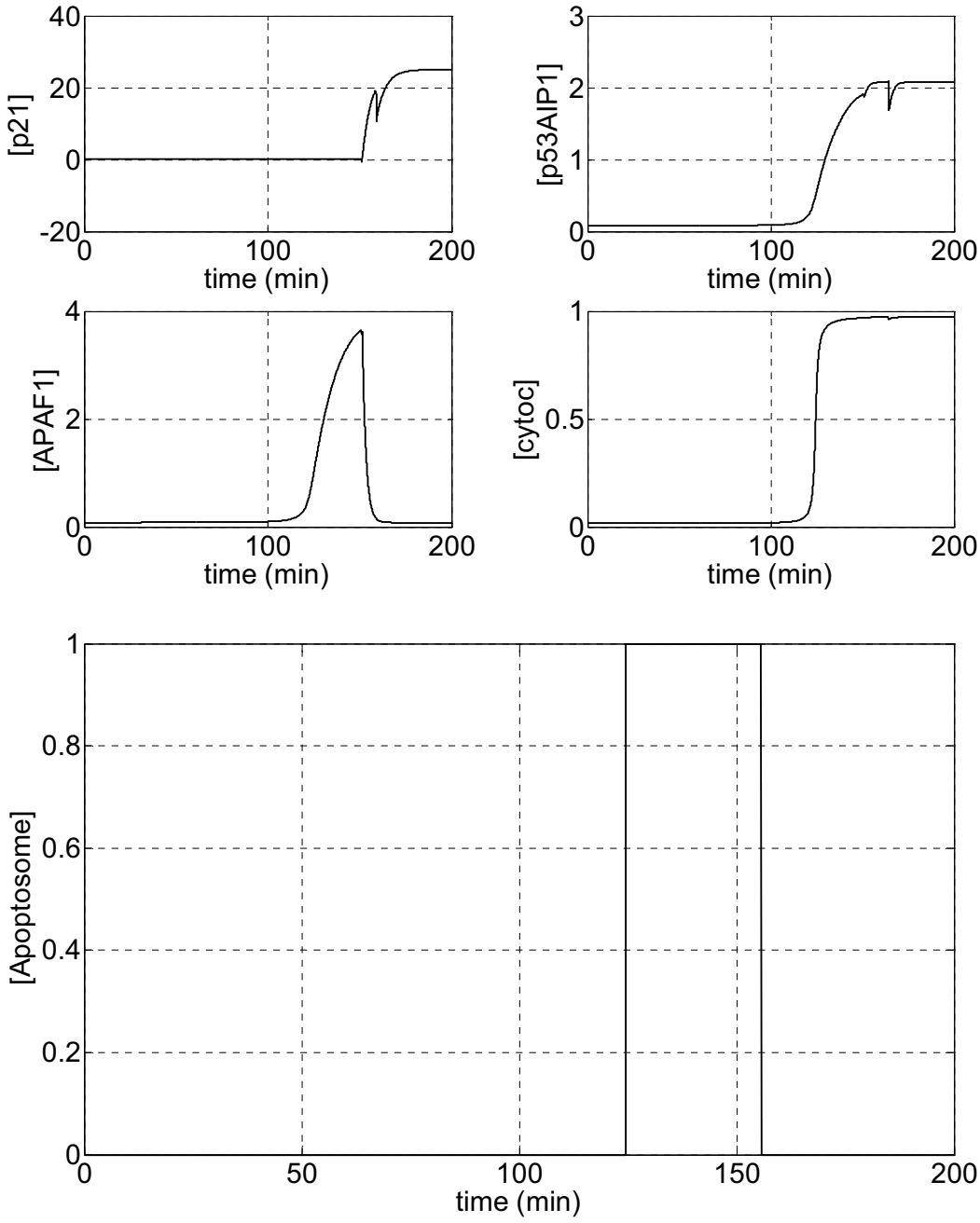
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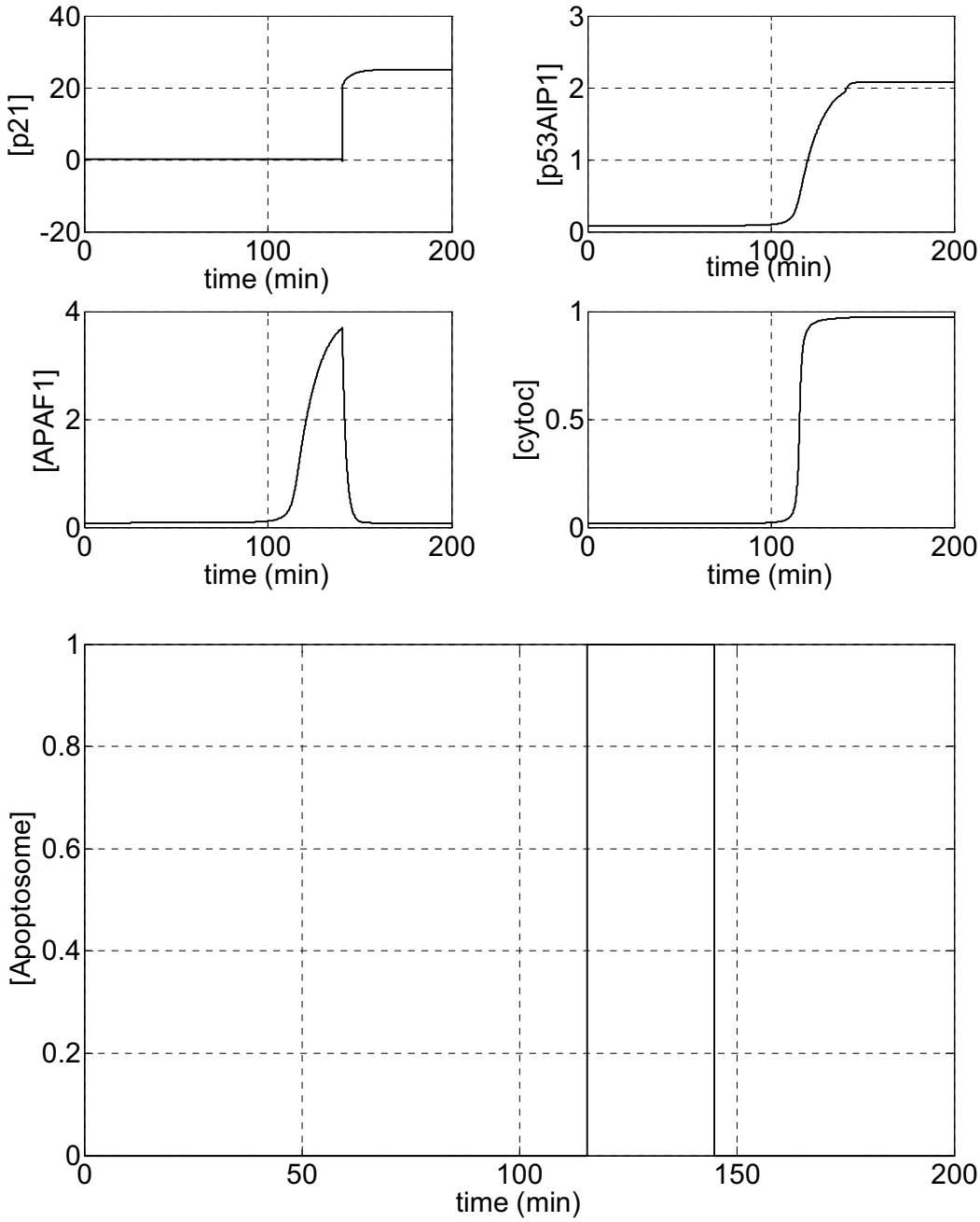
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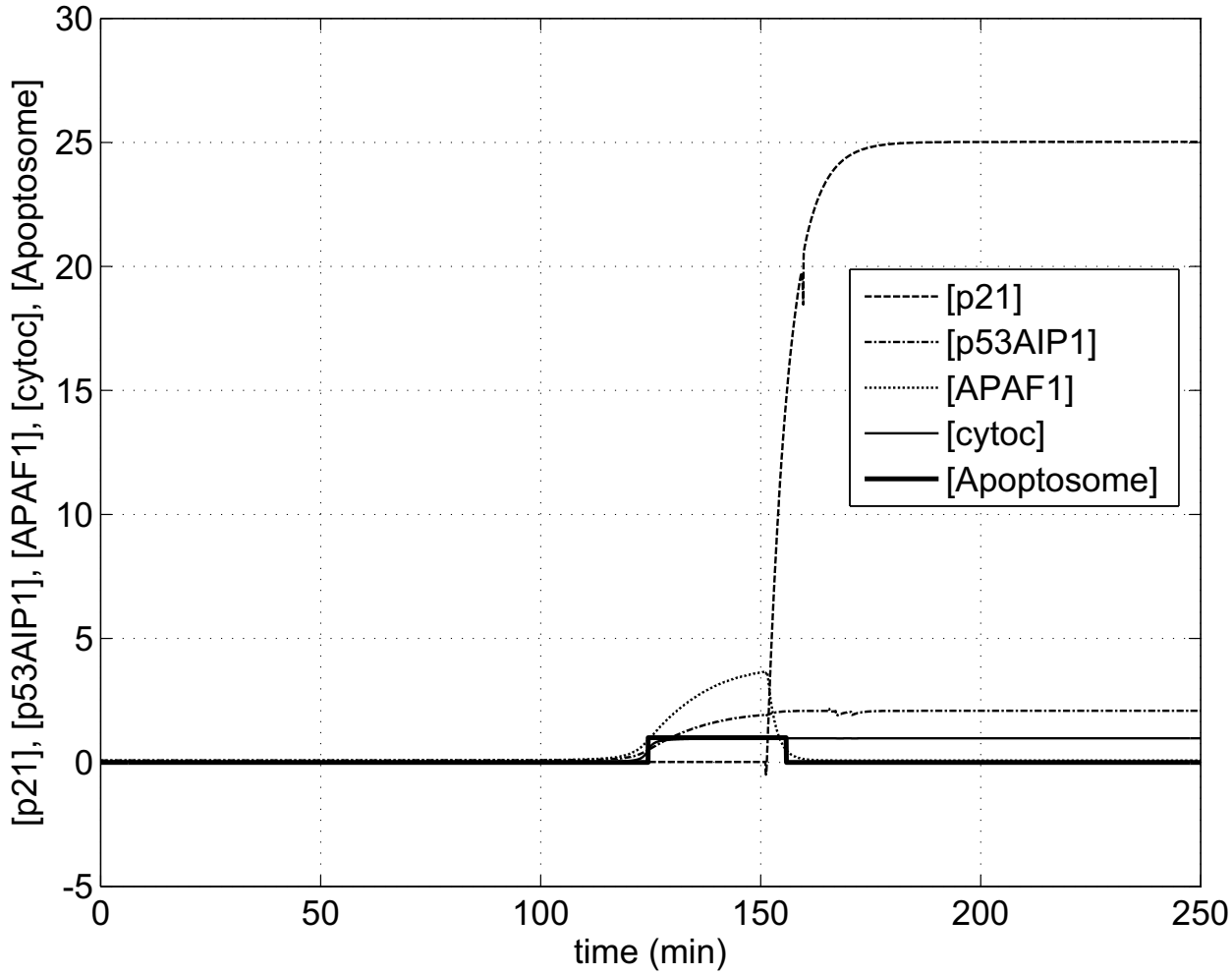
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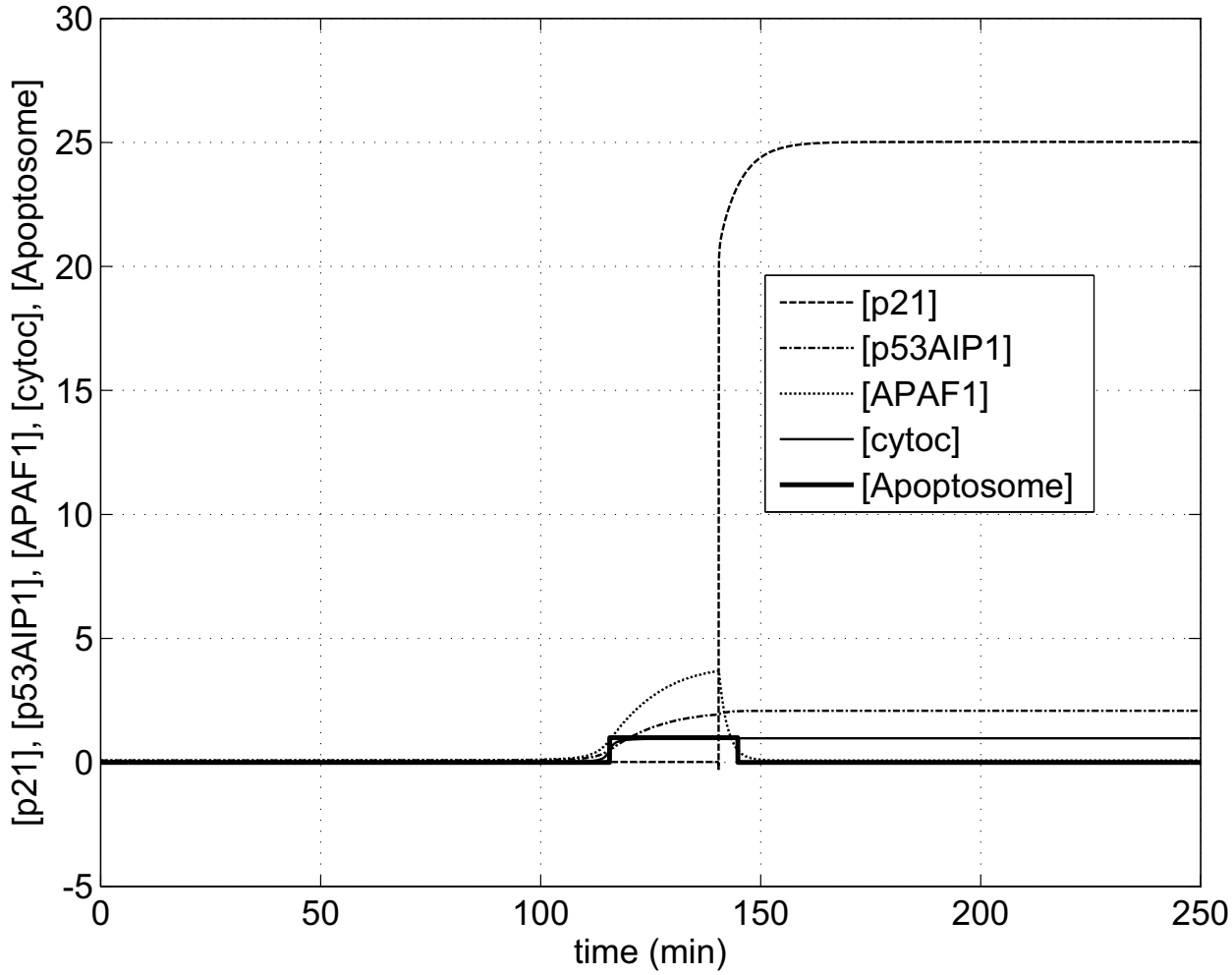
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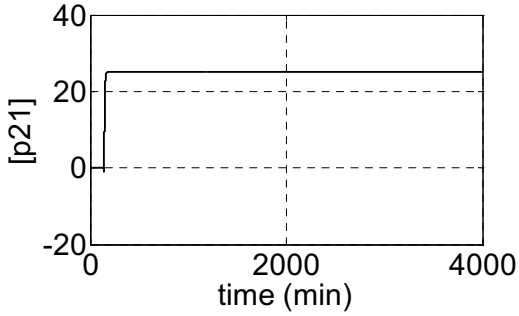
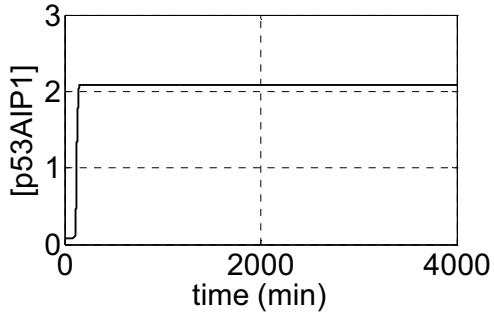
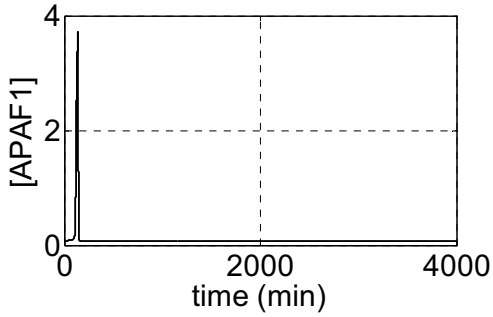
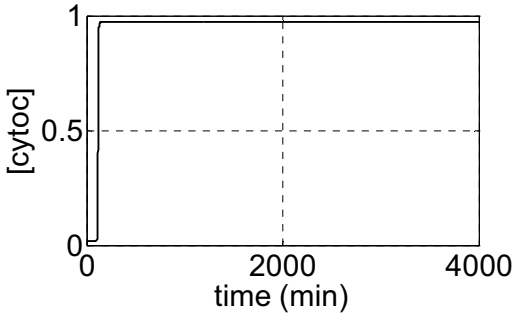

40
20
0
-20
[p21]
0
2000
4000
time (min)


3
2
1
0
[p53AIP1]
0
2000
4000
time (min)


4
2
0
[APAF1]
0
2000
4000
time (min)


1
0.5
0
[cytoc]
0
2000
4000
time (min)

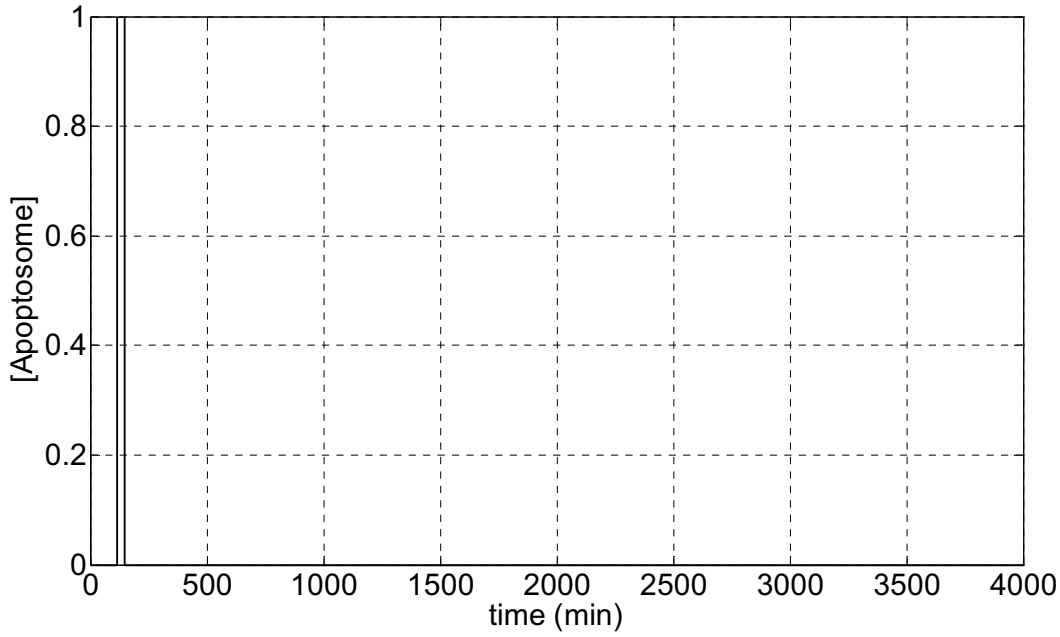

1
0.8
0.6
0.4
0.2
0
[Apoptosome]
0
500
1000
1500
2000
2500
3000
3500
4000
time (min)

Table 1
Parameters for the p53/Mdm2 network model

Parameters	Description	Values
f	Hill function	
$q_{ij} \in \underline{\underline{Q}}$	Bryson rule matrix diagonal elements (LQR)	1min^{-1}
$r_{ij} \in \underline{\underline{R}}$	Bryson rule matrix diagonal elements (LQR)	0.5min^{-1}
k_{s53}	Rate of overexpressed $p53_{tot}$	0.055min^{-1}
k_{d53}	Rate of $p53_{UU}$ degradation	8min^{-1}
k'_{d53}	Rate of $p53_{tot}$ degradation	0.0055min^{-1}
k_f	Rate of $Mdm2_{nuc}$-dependent $p53_U$ degradation	8.8min^{-1}
k_r	Translation rate of $p53_{UU}$	2.5min^{-1}
k_{DNA}	Rate IR-dependent DNA damage	0.18min^{-1}
k_{dDNA}	Rate of $p53_{tot}$ degradation-dependent DNA damage	0.017min^{-1}
IR	Ionizing Radiation	
J_{DNA}	State variable in Hill function for DNA repair	1
$ampl$	IR dose amplitude unit	1

Table 2
Optimal control matrix coefficients

Matrix elements	Description	Constant
k_{11}	Digital optimal control matrix element	$0.0023min^{-1}$
$k_{12} = k_{21}$	"	$0.0017min^{-1}$
$k_{13} = k_{31}$	"	$0.0004min^{-1}$
k_{22}	"	$0.0015min^{-1}$
$k_{23} = k_{32}$	"	$0.0004min^{-1}$
k_{33}	"	$0.0001min^{-1}$