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Topology Based Control of Biological Genetic Networks

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Abstract— **The traditional control scheme has been to input a signal into a plant, where the signal is derived from either an open-loop or a closed-loop. This control strategy requires that the plant be able to accept inputs or can be modified to do so. However, this situation is not always true in biological genetic networks; in these systems, there is often no input or obvious modification to allow inputs. We believe that they require a new paradigm for control. Biotechnology techniques are such that it is easier to make topological changes to a genetic network than it is to either change the states of the pathway or add more elements to the pathway. Thus, for such genetic networks it is important to develop a theory of control based on making large-scale changes (e.g. genetic mutations) to the topology of the network; we provide steps towards such a theory. We highlight some useful results from monotone and hybrid systems theory, and show how these results can be used for such a topological control scheme. We consider the cancer-related p53 pathway as an example; we analyze this system using control theory and devise a controller.**

I. INTRODUCTION

Control theory has traditionally focused on a core group of goals: to stabilize a plant, to improve plant performance, to robustify a plant, to track a reference, or to perform motion planning. In engineering systems, these goals have been achieved through an analysis-design flow; this flow is rarely linear – there is often a need to go back to previous steps and incorporate things that were missed in earlier attempts. Beginning with design specifications, we write mathematical models for the engineering system, analyze these models, and devise a controller. We also implement the controller on actual hardware.

The traditional control scheme has been to input a signal into a plant, using either an open-loop or a closed-loop controller. Such a control strategy is possible if the plant is able to accept inputs or can be modified to do so. However, this situation is not always true in biological genetic networks; in these systems, there is often no input or obvious modification to allow inputs. Instead of inputs, genetic networks are more easily influenced through large-scale modifications. Genetic networks are different from traditional engineering systems and require a new paradigm for control.

A. Topology Based Control

It is often easier to change the topology of a genetic network than it is to either change the states or elements of the network. For instance, a state could be the concentration of a protein within a cell, something which is difficult to affect to within any order of precision. Additionally, it is sometimes difficult or not feasible to modify or insert pathways by adding elements [22], [20], [8]. Thus, for genetic networks it is important to develop a theory of control based on making large-scale changes (e.g. genetic changes) to the topology of the genetic network. Fundamentally, we want to change how a cell operates and go beyond modifying the cellular environment.

Genetic networks can be modified in a variety of ways. Biotechnology techniques allow for the insertion of genetic material into bacteria, and are commonly used for alternative energy and pharmaceutical applications [13], [28]. In another technique, the genetic material of a virus is replaced with useful, genetic material. Next, the host is infected with the virus, and this inserts the useful, genetic material into the host. This control technique is being studied for use in pharmaceutical applications such as cystic fibrosis [13], [28]. Biologists continue to develop new techniques, amongst which include the use of microRNA and single interfering RNA.

Though many of these techniques are established and used in practice, there is a lack of a systematic theory or methodology to determine which modifications to make. Biological research often involves the use of intuition or trial-and-error to determine which changes are or are not beneficial for the purposes of controlling a biological system.

In this paper, we consider piecewise-affine (PWA) hybrid systems and ordinary differential equation (ODE) models of biological systems. We use two different types of models for reasons of analysis: The simpler, hybrid systems models are easier to analyze for global behavior, and the more detailed, ODE models are easier to analyze for local behavior of small components of the network. We discuss results related to hybrid systems theory, define and analyze controllers using ODE theory, and then we use these theories to analyze and build a controller for the p53 pathway – a pathway that is related to cancer.

We qualify what we mean by topological control. Our control changes the topology of the network by applying a pharamaceutical or other chemical, and the topology remains changed only in the presence of this pharamaceutical. As soon as it degrades away, the topology of the network goes back to an uncontrolled, unchanged state. Since our control is topological, it is crucial that we have a correctly identified network. The approach that we describe is unable to deal with latent variables that are unidentified, because the presence of latent variables can drastically change the

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behavior of the system.

II. PWA HYBRID AUTOMATON

The PWA hybrid systems we consider have rectangular guards, and are a simplification of general hybrid systems [19], [29]. In order to define them, we begin we a set of preliminary definitions.

A. Preliminaries

We define a hypercube as

$$\mathcal{C} = \{x : l_i < x_i < u_i, \forall i \in \{1, \ldots, n\}\}, \tag{1}$$

where l_i, u_i are constants, n is the dimension of the state-space, and x_i denotes the i-th component of x. Similarly, define a hyperedge as

$$\mathcal{E} = \{x : l_i < x_i < u_i, \\ \forall i \in \mathcal{I} \wedge x_j = \gamma_j, \forall j \in \{1, \ldots, n\} \setminus \mathcal{I}\}, \tag{2}$$

where l_i, u_i, γ_j are constants and $\mathcal{I} \subseteq \{1, \ldots, n\}$ is a set of indices. Additionally, to each hypercube $\mathcal{C}^q$ we associate a total of $(3^n - 1)$ hyperedges $\mathcal{E}^q{}_k$ – by defining l_i, u_i, γ_j, and $\mathcal{I}$ for each k – such that $\bigcup_{i=1}^{3^n-1} \mathcal{E}^q{}_i = \partial\mathcal{C}$ and $\mathcal{E}^q{}_i \cap \mathcal{E}^q{}_j = \emptyset$ for $i \neq j$. An example in $\mathbb{R}^2$ of a hypercube and its associated hyperedges is shown in Figure 1.

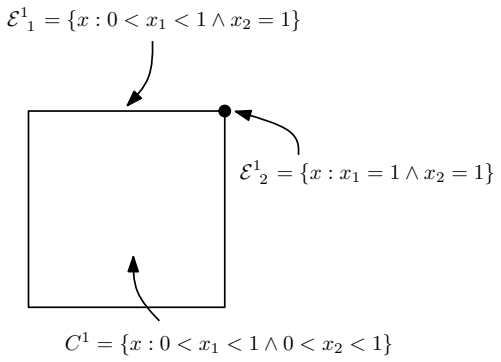


Fig. 1: A simple example of a hypercube and its associated hyperedges in $\mathbb{R}^2$ is shown. Only two of the associated hyperedges are labeled; there are eight hyperedges associated to C^1.

B. Definition of PWA Hybrid System

We define a PWA hybrid system on a domain $\mathcal{D}$ as a collection of hypercubes $\mathcal{C}^i$, with $\dot{x} = A^i x + b^i$ for $x \in \mathcal{C}^i$, such that $\mathcal{D} = \bigcup_{i>0} \overline{\mathcal{C}^i}$ and $\mathcal{C}^i \cap \mathcal{C}^j = \emptyset$ for $i \neq j$. A trajectory of this system is a solution of the vector field $f(x)$ in the sense of Filippov [9], where $f(x) = A^i x + b^i$ if $x \in \mathcal{C}^i$ and is undefined otherwise. Specifically, a trajectory of this system with initial condition $x \in \mathcal{D}$ is given by $\psi_t(x) \in \mathcal{D}$ such that

$$\frac{d\psi_t(x)}{dt} \in \bigcap_{\delta>0} \bigcap_{\mu(N)=0} \overline{\text{co}}(f(\mathcal{B}(x,\delta) \setminus N)) \tag{3}$$

almost everywhere, where $\mathcal{B}(x,\delta) = \{y : \|x-y\|_2{}^2 < \delta\}$ and the intersection is taken over all sets N with measure zero. A solution in the sense of Filippov is not necessarily unique; this property is unfortunate, because the non-uniqueness of solutions can lead to a lack of global monotonicity. For initial condition $x \in \mathcal{D}$, define $\mathcal{T}_x = [0, t_f)$ as the maximal interval such that $\psi_t(x) \in \mathcal{D}$. We can interpret t_f as the escape time at which $\psi_{t_f}(x) \notin \mathcal{D}$.

C. Trajectory Cycles

We will define the notion of trajectory cycles to describe the type of trajectories possible in a system. Intuitively, a *forward trajectory cycle* is defined as a forward trajectory of the continuous states of the hybrid system, such that the trajectory makes an infinite number of visits to a particular hypercube. Similarly, a *backwards trajectory cycle* is defined as a backwards trajectory of the continuous states, such that the trajectory makes an infinite number of visits to a particular hypercube. Note that implicit in both intuitive definitions is the inclusion of Zeno behavior.

III. PROMOTION-INHIBITION NETWORKS

A promotion-inhibition network is a signed, directed graph $N = (V, E, S)$. $V = \{v_1, \ldots, v_n\}$ is the set of vertices, $E \subseteq \{(u, v) : u, v \in V\}$ is the set of directed edges, and $S : E \to \{-1, +1\}$ is a function that gives the sign of an edge. For an edge $e = (u, v)$: u is the direct predecessor of v, and v is the direct successor of u. A *feedback loop* is a directed cycle $L = \{e_1 = (u_1, v_1), \ldots, e_m = (u_m, v_m)\}$, where $u_i = v_{i-1}$ for $i = 2, \ldots, m$; and $v_m = u_1$. A *negative feedback loop* is a feedback loop L such that $\prod_{i=1}^{m} S(e_i) = -1$. A *monotone loop* is an undirected cycle $M = \{e_1 = (u_1, v_1), \ldots, e_m = (u_m, v_m)\}$, where either $v_i = u_{i-1}$ or $u_i = v_{i-1}$ for $i = 2, \ldots, m$; and $v_m = u_1$ or $v_1 = u_m$. A *negative monotone loop* is a monotone loop such that $\prod_{i=1}^{m} S(e_i) = -1$.

A. Relation to Biological Genetic Networks

Genetic networks are often elucidated in the form of a promotion-inhibition network, and examples are shown in Figure 2. Intuitively, a positively (negatively) signed edge between two vertices means that an increase in the direct predecessor leads to an increase (decrease) in the direct successor, and biologists term this as promotion (inhibition). These networks do not describe the underlying biological mechanism of an edge, but this information will be important when designing controllers.

We can generate a PWA hybrid system model from a promotion-inhibition network, using the techniques of [7], [12], [25], [14], [3], [11], [5]. Similarly, we can generate an ordinary differential equation model [17], [26] from these networks. There are various advantages and disadvantages to the two types of models, and we discuss this below.

IV. EXISTENCE OF TRAJECTORY CYCLES

An important class of results in the hybrid and monotone systems theories relates the topological structure of a system to the global behavior of trajectories of the system, independent of any coefficients in the system. One useful theorem

concerns a PWA hybrid system derived from a promotion-inhibition network, in the manner of [7], [12], [25], [14], [3], [11], [5]. Under technical assumptions on the PWA hybrid system, if there are no negative feedback loops in the promotion-inhibition network, then there are no trajectory cycles [4].

Under the given conditions, these systems are stable and trajectories converge to an equilibrium point in a node-like manner; that is, trajectories qualitatively look like the trajectories of a stable, linear system with purely real eigenvalues. Moreover, the presence of negative feedback is a necessary condition for the presence of limit cycles, centers, and foci. The shortcoming of this theorem is that it does not apply to many systems with self-inhibition, and this is common in biological systems.

An similar result holds for ODE systems. Under technical conditions, if the promotion-inhibition network has no negative monotone loops, then the system is a monotone system [17], [1]. Consequently, the trajectories of the system converge to an equilibrium and there are no stable oscillations [16]. These theorems apply to systems with self-inhibition, but they are stricter because negative montone loops are stricter than negative feedback loops.

V. Controllers

Through the use of results stated in Sect. IV, several different topology based controller schemes become apparent. The basic idea is to use operations, such as removing edges or nodes in the promotion-inhibition network, to change the topology of the genetic network. We want to force the genetic network into a situation such that it has no negative feedback. Because this will ensure that the system does not oscillate and has simple dynamics, it will be easier to design the controller to move the system into a desired state. Such control is quite crude in relation to traditional control techniques, but it can be used to achieve useful results in certain situations.

It is important to keep in mind that the fundamental ideas of the controllers are contained within the basic topological examples given below. Though these are examples, the examples are general since the equations model two broad classes of reactions. Based on existing biological techniques [13], [28], the controller examples that we give are hypothetically feasible. However, it is not always possible to implement the controller.

Despite this, the reason that the fundamental ideas are topological is that there are multiple, biological ways to achieve the effects that we describe. For instance, a node removal can be accomplished via compounds or pharmaceuticals that bind to a protein and remove its function, instead of the antisense RNA example that we give [13]. The controller used to remove an edge or a node must correspond to the biological mechanism behind the edge or node, otherwise the controller will fail.

A. Inhibition Edge Removal Example

Gene therapy techniques can add genetic material, but they cannot remove genetic material. Thus, to remove an inhibition edge we must be more clever: we can use competitive binding to accomplish such an aim. The basic idea for the control is to add a high number of copies of a gene and its promoter region to a cell. This negates the effect of any inhibitors.

Suppose that we have a promotion-inhibition network with edge $e = (A, B)$ and label $S(e) = -1$, and we use a control that enforces $[D_0] \gg [A_0]$, where the subscript 0 is used to denote initial concentrations and the square brackets $[\cdot]$ denote concentration. One model for this is the set of reactions

$$A + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AD \tag{4}$$

$$D + E_i \underset{k_{-2,i}}{\overset{k_{2,i}}{\rightleftharpoons}} DE_i \xrightarrow{k_{4,i}} B + D + E_i \tag{5}$$

$$D + F_i \underset{k_{-3,i}}{\overset{k_{3,i}}{\rightleftharpoons}} DF_i, \tag{6}$$

where E_i are proteins which promote the production of protein B, D is the DNA which codes for protein B and has the accompanying promoter region, and F_i is a protein which binds to DNA D but does not begin transcription. Note that i indexes over multiple proteins and complexes. In these reactions, AD, DE_i, AP, and DF_i are intermediate complexes. The effect of the inhibitors A and F_i is to prevent the formation of the DE_i complex, that is either A or F_i cannot simultaneously bind with either E_i or D. Note that (5) describes the aggregate process of activators and enzymes producing a protein and (4) and (6) describe an inhibitor binding to DNA.

These reactions can be written as a set of fractal reaction equations [23], [24], [31] as

$$\frac{d[AD]}{dt} = k_1[A]^{\alpha_1}[D]^{\alpha_2} - k_{-1}[AD] \tag{7}$$

$$\frac{d[DE_i]}{dt} = k_{2,i}[D]^{\alpha_{3,i}}[E_i]^{\alpha_{4,i}} - k_{-2,i}[DE_i] \tag{8}$$

$$\frac{d[DF_i]}{dt} = k_{3,i}[D]^{\alpha_{5,i}}[F_i]^{\alpha_{6,i}} - k_{-3,i}[DF_i] \tag{9}$$

$$\frac{d[B]}{dt} = \sum_i k_{4,i}[DE_i]^{\alpha_{7,i}}, \tag{10}$$

with the following constraints:

$$[A_0] = [A] + [AD] \tag{11}$$

$$[D_0] = [D] + [AD] + \sum_i [DE_i] + \sum_i [DF_i] \tag{12}$$

$$[E_{i,0}] = [E_i] + [DE_i] \tag{13}$$

$$[F_{i,0}] = [F_i] + [FE_i]. \tag{14}$$

The constraints assume fixed initial concentrations. In these fractal equations, each k is a reaction rate, each α is an exponent that relates concentration to the speed of the reaction, and the brackets denote concentration.

From the positivity of concentrations and (11), it is clear that $0 \leq [AD] \leq [A_0]$, which implies that

$$[D_0] - [A_0] - [DE] \leq [D] \leq [D_0] - [DE]. \tag{15}$$

However, since $[D_0] \gg [A_0]$, we have that approximately

$$[D] = [D_0] - [DE]. \tag{16}$$

Consequently, if $[F_{i,0}] \sim [D_0]$ then our system is approximately the same as if reaction (4) did not occur. In the special case of $\alpha_{3,i} = \alpha_{5,i} = 1$, solving for the rate of production, using standard assumptions, gives

$$\frac{d[B]}{dt} = \sum_i k_{4,i} \left(\frac{k_{2,i}}{k_{-2,i}} ([D_0]/H)[E_i]^{\alpha_{4,i}} \right)^{\alpha_{7,i}}, \tag{17}$$

where

$$H = 1 + \sum_i \frac{k_{2,i}}{k_{-2,i}} [E]^{\alpha_{4,i}} + \sum_i \frac{k_{3,i}}{k_{-3,i}} [F]^{\alpha_{6,i}}. \tag{18}$$

Through the use of our controller, we were able to eliminate the inhibitory effect of A on B. Thus, we effectively break the inhibition edge in the promotion-inhibition network.

B. Node Removal Example

In a node removal controller, we remove a node from the promotion-inhibition network. We can use antisense RNA to prevent translation of protein through competitive binding with mRNA[13], [28]. The control is to add a high number of copies of the antisense RNA. This removes the effect of the node.

Suppose that we have a promotion-inhibition network with edge $e = (A, B)$ and label $S(e) = +1$, and we use a control that enforces $[P_0] \gg K$, where $[mAP] \leq K$. One model for this is the set of reactions

$$D + E_i \underset{k_{-1,i}}{\overset{k_{1,i}}{\rightleftharpoons}} DE_i \xrightarrow{k_{3,i}} DE_i + mA \tag{19}$$

$$mA \xrightarrow{k_4} mA + A \tag{20}$$

$$mA \xrightarrow{k_5} Z_1 \tag{21}$$

$$A \xrightarrow{k_6} Z_2 \tag{22}$$

$$mA + P \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} mAP \tag{23}$$

where E_i are proteins which promote the production of protein A, D is the DNA which codes for protein A and has the accompanying promoter region, P is the control of added antisense RNA. Also, Z_1 and Z_2 are the aggregate products of degradation. Note that i indexes over multiple proteins and complexes. In these reactions, mA is the translated mRNA for protein A, DE_i is an intermediate complex, and mAP is the complex of mRNA bound with the antisense RNA. Note that (19) describes the aggregate process of activators and enzymes translating the DNA for a protein into mRNA, (20) describes the aggregate process of translation of mRNA into protein, and (23) describes the binding of a mRNA with the added antisense RNA. Since P is the antisense RNA of the mRNA, this prevents translation of the mRNA into protein when P is bound to mA. Also, (21) and (22) describe the degradation of mA and A, respectively.

These reactions can be written as a set of fractal reaction equations [23], [24], [31] as

$$\frac{d[DE_i]}{dt} = k_{1,i}[D]^{\alpha_{1,i}}[E]^{\alpha_{2,i}} - k_{-1,i}[DE_i] \tag{24}$$

$$\frac{d[mA]}{dt} = k_{3,i}[DE_i]^{\alpha_{3,i}} - k_4[mA] + \\ - k_2[mA]^{\alpha_5}[P]^{\alpha_6} + k_{-2}[mAP] \tag{25}$$

$$\frac{d[A]}{dt} = k_5[mA]^{\alpha_4} - k_6[A] \tag{26}$$

$$\frac{d[mAP]}{dt} = k_2[mA]^{\alpha_5}[P]^{\alpha_6} - k_{-2}[mAP] \tag{27}$$

with the following constraints:

$$[D_0] = [D] + \sum_i [DE_i] \tag{28}$$

$$[E_{i,0}] = [E_i] + [DE_i] \tag{29}$$

$$[P_0] = [P] + [mAP] \tag{30}$$

$$[DE_i] \leq K_i \tag{31}$$

$$[mA] \leq K. \tag{32}$$

The last two inequalities come about through standard arguments involving nullclines. In these fractal equations, each k is a reaction rate, each α is an exponent that relates concentration to the speed of the reaction, and the brackets denote concentration.

Typically, the reversible reactions are much faster than the irreversible reaction, that is the reactions corresponding to $k_{1,i}, k_2, k_{-1,i}, k_2$ are much faster than those corresponding to $k_{3,i}, k_4, k_5$ [23]. Under this assumption, we can apply the quasi-steady state assumption to get

$$[DE_i] = \frac{k_1}{k_{-1}}[D]^{\alpha_{1,i}}[E_i]^{\alpha_{2,i}} \tag{33}$$

$$[mAP] = \frac{k_2}{k_{-2}}[mA]^{\alpha_5}[P]^{\alpha_6}. \tag{34}$$

Combining (28) and (34) gives

$$[mA] = \left(\frac{k_{-2}[mAP]}{k_2[P]^{\alpha_6}} \right)^{1/\alpha_5} \tag{35}$$

$$\leq \left(\frac{k_{-2}K}{k_2([P_0] - [mAP])^{\alpha_6}} \right)^{1/\alpha_5}. \tag{36}$$

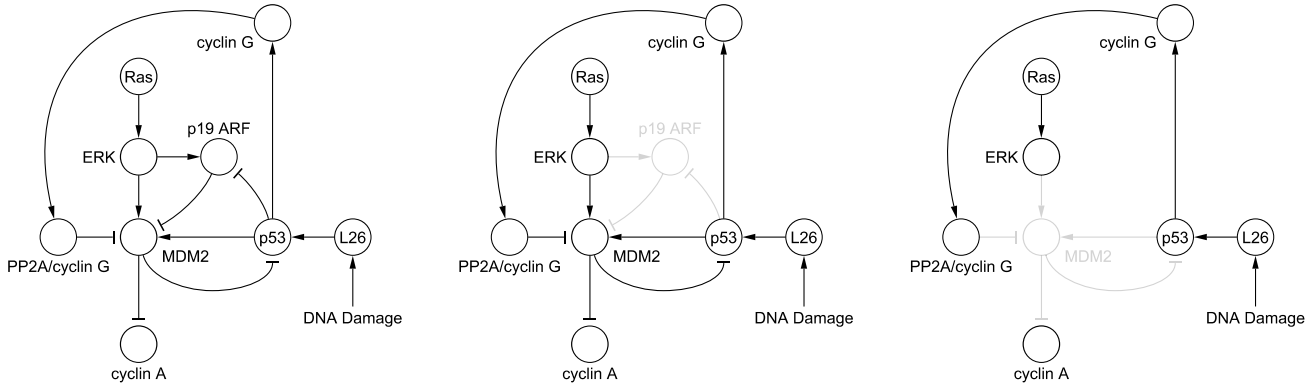
Because the controller enforces that $[P_0] \gg K$, we get that approximately $[mA] = 0$. Consequently, we approximately have that

$$\frac{d[A]}{dt} = k_5[mA]^{\alpha_4} - k_6[A] \leq 0. \tag{37}$$

Through the use of the controller, we were able to reduce the concentration of protein A to zero. Thus, we effectively remove node A from the promotion-inhibition network.

VI. P53 PATHWAY

The p53 protein is an important tumor suppressor, which reacts to stress signals and induces an appropriate cellular response [15], [10], [2], [30]. These stress signals include DNA damage, heat shock, cold shock, and spindle damage. These stress signals lead to a post-translational modification



(a) Normal p53 Pathway (b) Abnormal p53 Pathway (c) Abnormal p53 Pathway with Controller

Fig. 2: When a subsegment of the normal p53 pathway [15], [21], [27], [18] becomes abnormal, such as loss of p19 ARF function [21], the system behaves unfavorably by underexpressing p53. Using a controller, certain edges and nodes of the pathway can be removed to make the system behavior more favorably.

of p53, causing the p53 to trigger downstream pathways involved with cell cycle arrest, cell senescence, or apoptosis [15]. The inactivation of p53 can lead to tumor development [2].

A promotion-inhibition network for a subsegment of the p53 pathway is shown in Fig. 2a. In roughly 10% of human tumors, p53 is inactivated through overexpression of MDM2 [10]. MDM2 can be overexpressed through an inactivation of p19 [21], and this is shown in Fig. 2b. MDM2 works to reduce expression of p53 [15], [10], [2], [30] by increasing the degradation rate of p53 and facilitating the nuclear export of p53 [2], [30]. Thus, inhibition of MDM2 has been considered as a possible strategy for cancer treatment [30], [10].

In designing a controller for the abnormal p53 pathway, we must keep in mind the underlying biological mechanisms for the edges in the network. Here, the inhibition edge between MDM2 and p53 is due to protein-protein interaction, and so we cannot simply use the controller given in Sect. V-A. However, we can use the controller given in Sect. V-B to remove the node coressponding to MDM2. The controller is shown in Fig. 2c, and is implemented through the addition of antisense RNA that binds with the mRNA for MDM2. Based on existing techniques [13], [28], the controller is hypothetically feasible.

Time course concentrations of p53, cyclin A, and MDM2 are shown in Fig. 3a for the normal p53 pathway, Fig. 3b for the abnormal p53 pathway, and Fig. 3c for the abnormal p53 pathway with controller. These simulations come from an ODE model of the network, and in the simulations we do not remove the edges between either MDM2 and p53 or MDM2 and cyclin A. In the normal p53 pathway, concentrations of p53 and cyclin A are high, and concentrations of MDM2 are low. In the abnormal p53 pathway, p53 and cyclin concentrations are low, whereas MDM2 is in high concentration. In the abnormal p53 pathway with controller, the controller is used at times $t = 200$, $t = 250$, and $t = 300$. The controller causes cyclin A and p53 concentrations to increase to higher levels, and reduces MDM2 concentrations. The controller must be used at multiple times, because the cyclin A promoter is modeled to decay. So, the effect of the controller wains as time goes on. If the controller is not applied again, the system returns to an abnormal state.

VII. CONCLUSIONS AND FUTURE WORKS

A. Conclusions

We have presented steps towards a new paradigm for the control of biological genetic networks through topology based controllers. Such techniques may also be useful for understanding the effects of pharmaceuticals. The basic idea of the controller is two-fold. First, we abstract the use of pharmaceuticals to a graph-theoretical interpretation. Secondly, we simplify the dynamics of the system, by removing negative feedback, and then let the simplified dynamics steer the system to a desirable state. We gave derivations for two possible controllers to remove edges or nodes of a network, and used one of these controllers to treat abnormalities in the cancer-related p53 pathway.

B. Future Works

What is needed is an understanding of the biological mechanisms behind interactions, and a library of controllers to deal with and eliminate such interactions. Additionally, we need algorithms to identify what the optimal edges to remove are. Reach set algorithms from hybrid systems may be useful for this. In fact, we used PWA hybrid systems to analyze global system behavior, because efficient algorithms – for system analysis – exist for such systems [5], [6], [7], [4], [3]. These topics are the subject of our current research.

VIII. ACKNOWLEDGMENTS

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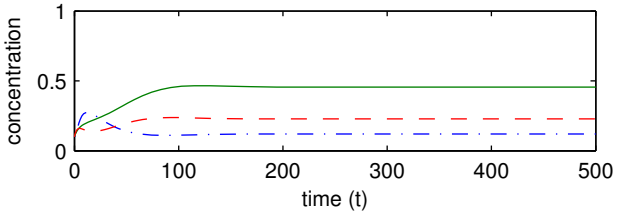
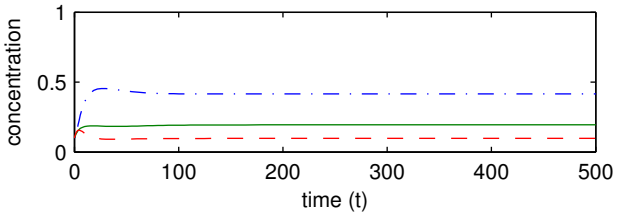
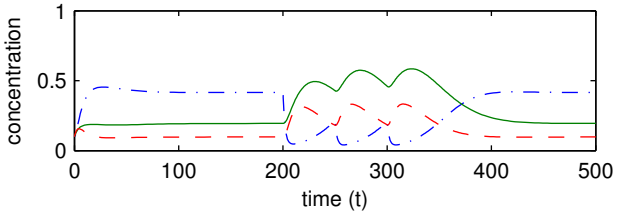


(a) Normal p53 Pathway



(b) Abnormal p53 Pathway



(c) Abnormal p53 Pathway with Controller

Fig. 3: The time course plots for the different pathways displays the effect of the abnormality and the controller. Note that p53 is solid, cyclin A is dashed, and MDM2 is dash-dotted.

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